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Evaluation efficiency and phenotypic analysis to ITS-2gene targeting sample from patient infested with scabies. (S. scabiei Var.*hominis*) in Thi-Qar province/Iraq

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Abstract---The second internal transcribed spacer region of the nuclear ribosomal gene cluster (ITS2). This cluster consists of three genes (18S rDNA, 5.8S rDNA and 28S rDNA) which have transcribed into RNA. Nevertheless, not translated into protein. It is sensitive to detect *S. scabiei* DNA even after treatment. In addition, this gene can detect genetic variations of species that are related taxonomically. This study aimed to determine and compare the sensitivity and specificity of ITS-2, S. *scabiei,* genes to the microscopic examination of scabies skin scrapings. In addition, employs, The ITS2 as a powerful tool for studies of intraspecific variation and phylogenies of closely related species. The results of the PCR for the diagnosis of S. scabiei .var .hominis was,(50%) of total sample Frome .25,(50%) mite-positive and 25(50%) mite-negative ,50%,Sensitivity 50% specificity, Positive predictive value (60%),negative predictive value (40%). The sensitivity of the scabies PCR reached between (30% and 60%) globally, ITS-2 region is suitable for phylogenetic studies of astigmatic mites. as this region shows comparatively higher variation between the diverse species of mites and lower distinction within the species. These astigmatic mites are located in the phylogenetic tree based on their genetic features>.

Keywords---sensitivity, ITS2, specificity, phenotype tree, molecular identification.

Introduction

Scabies is an important public health circumstance both in resource-poor and advanced countries. It is a state characterized by occupation of the mite *Sarcoptes scabies*, on the skin of a human or animal host after skin-to-skin interaction with a giver host. (Hengge *et al.*, 2006). Several subtypes, or varieties (var.), were reported such as *S. scabiei var. canis i*n dogs, *var. vulpes* in red fox and *var. hominis* in humans. No structures have been described to analytic morphological dependably differentiate between hypothetical changes from diverse hosts. The taxonomy of *S. scabiei* is still undefined, (Fain, 1968 ,Heo et al., 2021). Microscopic examination of skin scrapings, which recognised mites, ova or faecal pellets (scybala). To improve the outcomes, the tunnel done by the parasite by sharp blade and (Muller oil) or geliseren were applied to bring the mite at the surface. A negative microscopic outcome does not reject scabies, furthermore Factors such as remove burrows, (atypical scabies) with little mite burden , low sensitivity of the technique and inefficient method have been put survey as possible details for a negative (Muller et al., 1973,Goldsmith et al., 2012, Oakley, 2013, Dupuy et al., 2007,Chosidow & Fuller, 2017). Nuclear ribosomal DNA still provides one of the most complete tools for a multitude of molecular tasks. The main ribosomal locus in eukaryotic organisms consists of three genes encoding the (18S, 5.8S and 28S) subunits of the ribosome. Between these genes are the internal transcribed spacers 1 (ITS1, between the 18S and 5.8S gene) and 2 (ITS2, between the 5.8S and 28S gene) Sequence examination of ITS- 2 presented no host separation and topographical isolation, while 16S showed occurrence of both hosts altered and geographically isolated inhabitants of *S. scabiei(Hillis and Dixon,1991)..*(Naz et al., 2018).

Material and Method

Sample collection

The study was surveying in Neisseria middle of Thi-Qar /Iraq. In addition, prison reform management. After taking safety recommendations from the ministry of Justice, and the Thi-Qar health subdivision. Samples of inmates and civilians has been occupied with the agreement of them by the pledge without any stress. The cases of ordinary scabies were established by clinical comment, By specific dermatologist using the subsequent criteria: past of contact with an individual(s), infested by scabies; pruritus worse at nocturnal; presence of tunnels, and vesicles; occurrence of secondary lesions, (nodules, papules, crusts, folliculitis, and excoriations or) in specific sites. Out of (160), Sample from different age and sex were examined clinically, and confirm infested with scabies (see egg, nymph, mite, scybala) Ander microscope (50) specimen sent to polymerase chain reaction (PCR) laboratory. (S. Alasaad et al., 2009)(Engelman et al., 2020).

Dry ultra-cold freezing storage

Use a sharp sterile blade, size (15). The papule or nodule were scraped from between the fingers, the wrist. after wetting it with distilled water, and placed inside the Eppendorf tube and tightly closing, at -20 °C, to PCR detection, (S. F. Walton et al., 1999).

Microscopic examination

Skin-scraping examples from exposure areas were gained. the lesion was wet with glycerine and then shaving using a clean, sterile scalpel. The scraped skin was moved to a slide, a few droplets of (10 %) KOH and enclosed with a coverslip, addition. Potassium hydroxide (KOH) can be functional to the samples to melt extra keratosis rubbish(Alasaad et al., 2009,Micheletti et al., 2017).then We are closure the edges with apparent nail polish ,then incubation for 20 mints at 37c, till investigation. Interpretation was done within 3 hours at first (10X) then (40X) magnification. (Birnbach, 2019)

Mite DNA extraction

The sample (50) also were exposed to a (PCR) test that targeted ITS-2 of *S.scbiei* table (1) from the same patient another skin scrapings were taking from interdigital ,wrist which is confirm exist mite , as mention above Genomic DNA from skin samples were extracted by using (gSYAN DNA mini kit) (Tissue protocol) Geneaid. USA and complete compatible to company instructions as following

Table 1
primers for molecular identification *of S. Scabiei*

Primers	Sequences(5'-3')	Products' size
ITS-2	F- CGACTTTCGAACGCATATTGC	400bp
	R- GCTTAAATTCAGGGGGTAATC	

PCR amplification

The PCR reaction mixture was ready by mixing (10µl), GoTaq Green Master \Mix. (Promega, Madison, WI, U.S.A.), (7µl) water (10 mM Tris-HCl, 1 mM EDTA, pH 8.0), 1µl of mol^{-1} forward primer. 1µl of mol^{-1} revers primer. In addition, 1µl of DNA sample. PCR was lastly in a final volume of 20 µl, with the following steps: heated once for 2 min at 95 °C. Followed by 36 cycles of the 30 s at 94 °C. 30 sec annealing at 56-60 .°Flow by extension at 72 °C,for 90s.finally PCR tube were heated at 72 °C for 2min and then cool at 4°C .

Gel Electrophoresis

The PCR yields were electrophoresed in a 2% agarose gel, and pictured ,by staining with 0.2 μgmL^{-1} of ethidium bromide. (Fukuyama et al., 2010)

Sequences analysis and phylogenetic relationships

PCR produce of fifty Scabies samples and eight isolates were sequenced using ITS-2 gens, by Microgen Company (Seoul, South Korea). The gotten DNA sequences were scanned to identify the similarity with the subject sequence in the database. Alignment process with BLAST (www.ncbinlm.nih.gov/BLAST); BioEdit

software was practical, for decoration and elimination of aligned sequences. The phylogenetic tree for sequences (accession number) and linked sequences ITS-2 was found using Molecular Evolutionary Genetics Analysis (MEGA10) program with the alignment of (ClustalW) construct Maximum

Result

This study used the polymerase chain reaction (PCR) method for the molecular identification of *S. scabiei .var .hominis* for first time in Thi-Qar province, by using the second internal transcribed spacer (ITS-2) of nuclear ribosomal DNA (rDNA) as genetic marker. DNA gene have molecular length (400 base pair),The results of the PCR for the diagnosis of *S. scabiei .var .hominis* for (50) samples, of each sample contained.25 (50%) mite-positive and 25(50%) mite-negative in microscopic exam. The outcome was,50% PCR-mite positive ,50%,Sensitivity 50% specificity, Positive predictive value (60%),negative predictive value (40%), demonstrated in table (2) and table (3).

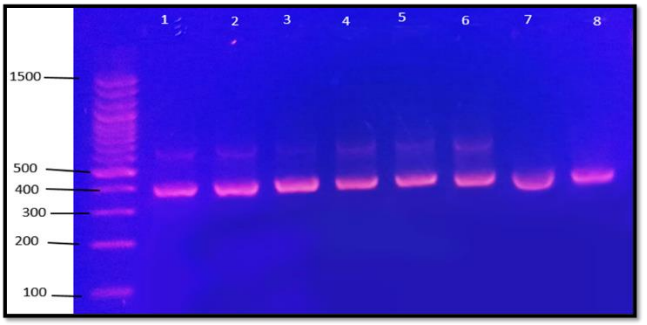

Fig 1. gel electrophoresis, by PCR products of the Sarcoptes S.cabiei.var.hominis, (ITS-2) .gene from skin scabies specimens. M: marker (100-1500bp). Lanes (1 to 8): Positive PCR products at400bp

Table 2
Data reveal different result between Microscopic exam and PCR

Microscopic exam	P.C.R exam	No (%)	Interpretation
Positive	Positive	15 (30%)	TP ,MIC TP, PCR
Negative	Positive	15 (30)	FN, MIC FP, PCR
Negative	Negative	1o (20%)	TN, , MIC TN, PCR
Positive	Negative	10 (20%)	FP, , MIC FN, PCR

Table 3
A cross-tabulation of result comparative between microscopic test and PCR test

PCR test result	Microscopic test result		total
	positive	negative	
positive	15	10	25
negative	10	15	25
total	25	25	50
Sensitivity	Specificity	p.p.v	N.p.v
50%	50%	(60%)	(40%)

ITS-2 sequence analysis

Seven novel polymorphic sites were recognised in sequence study of *S. scabiei var. hominis*. ITS-2 sequences from different regions of Thi-qar province matched with location sequence from GenBank. These sequences were categorised by two transitions (C-T) on site and 30, (G-A) on site 240, six transversion (G-T) site 220, (G-C) on site 250, (A-T) site 250, and (T-A) on site 270. (?-A) 260 on site represented deletion mutation. The represented sequences with seven polymorphic sites have 98-99 % intra- isolate identity. Characterised sequences designed a cluster on the two together with reference sequences from GenBank. First (scabies 4, 5, 6, 7, and 8), displays a fixed nucleotide, presenting (T) Instead of an (C) exchange at position 30,and (T) instead (G) on the site 220, and similarity with five transversion above except (scabies 7)which excluded by exchange nucleotide(A) instead of (G) this difference explains the cluster comprising these specimens in the UPGMA tree,. Second (scabies1, 2, and 3) which high conserve its nucleotide (G instead T which is mutated in first cluster site) on site 210 .scabiei 1, scabiei 3site (30) was identical with origin sample in GenBank, representing the wide host range and geographical dissemination of *S. scabiei* population. Fig. (4. 16). All sequences resulting from mites in human formed two cluster together with reference sequences, which includes isolates from, China, Spain, and Egypt.

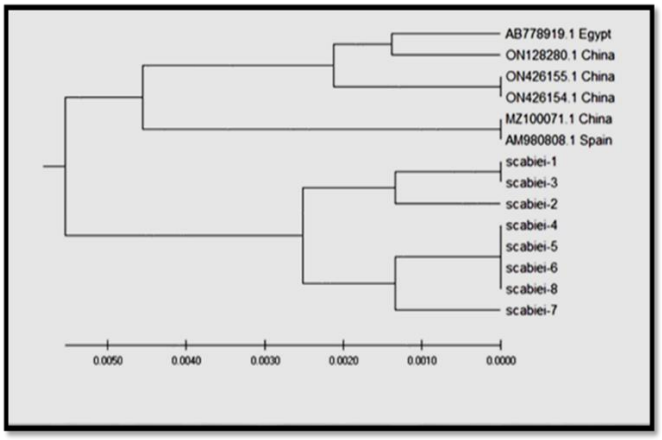

Fig 2. Relationship of identified Thi-Qar -ITS-2 sequences with selected ITS-2 sequences. The nucleotide sequences of the identified molecules were aligned using ClustalW and phylogenetic tree was constructed using MEGA

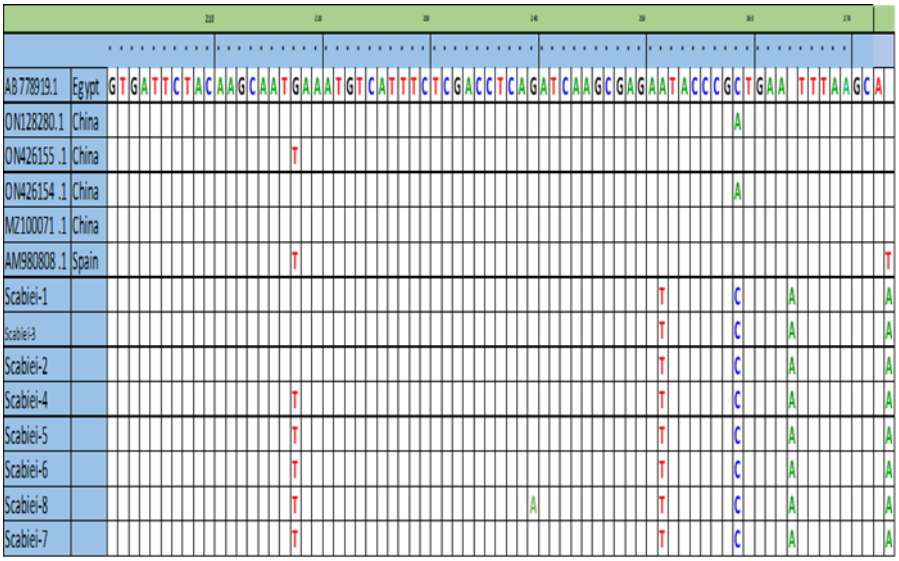

Fig 3. Summary of the nucleotide variation detected in the ITS-2 for the individual mites studied. Dots indicate the identity with the first sequence. Polymorphic sites were designated with IUPAC code

Discussion

The results of the PCR. targeting ITS-2. for the diagnosis of *S. scabiei .var.hominis.* was for (50) samples. Of each, sample contained.25 (50%) mite-positive and 25(50%) mite-negative in microscopic exam. The outcome was,50% PCR-mite positive ,50%, Sensitivity 50% specificity, Positive predictive value (60%), negative predictive value (40%). On the other hands, Delaunay et al developed a PCR assay for identification based on the ITS-2 region, that showed minor sensitivity.he fined out of (87) patients with dermatoscopically confirmed scabies, (33) had positive scabies PCRs, with confirmed scabies, negative predictive value 61.7% with a sensitivity of 37.9%. This low sensitivity, agreement with result, pointed above. Caused this low sensitivity. (atypical) lesions in place of mites or eggs, the sensitivity of the scabies PCR reached between (30% and 60%) globally .established on clinically doubted scabies Affording to previous reports, (Delaunay et al., 2020) The results of the study who joined Alkarama Teaching Hospital Wasit Province\ Iraq.. The established that 47% out of 100 patient (nearly matching with our result) of the experienced individuals were positive for the occurrence of the mite cox1 gene in the skin scrapings. (Rahi et al., 2021).

The microscopic examination yields a false negative since; scratching by the subject can cause mechanical removal of the mite; Thus, most subjects had that pus in the lesion since superinfection consider as acaricide, which decreased sensitivity (Walter et al. 2011) on the other hand, in Nested PCRs,. the ITS-2 genes were positive for *S. scabiei* DNA in 29 of 51 in mite –positive samples ,moreover targeting ITS-2 genes PCRs were achieved to detect *S. scabiei* DNA in mite-negative samples of skin scrapings from patients with recognised scabies lesions, were positive for *S. scabiei* DNA in (49 of 96)samples. (Fukuyama et al., 2010) , last survey reveal matching with our result, i.e. out of (25) skin scraping

which (positive or negative) in microscopic exam conventional PCR targeting ITS2gene detected 15 sample was positive.

Bae and his colleagues found, three patients (10%) showed positive outcomes for microscopic check, but negative effects for scabies PCR. This disagreement could have been due to the genetic variety of s. scabiei. In a modern study, it was recommended that *S. scabiei* mites in humans. Were mostly disseminated into three genetically distinct clades. (Andriantsoanirina, Ariey, Izri, Bernigaud, Fang, Charrel, Foulet, Botterel, Guillot, et al., 2015), The authors achieved sequencing of cox1 gene got in mites from 60 humans and exhibited that mites could belong to diverse clades hereditarily even if they were composed in the same area. (Bae et al., 2020). The finding rate of scabies by PCR tended to be great than that of the microscopy examination even with no significant difference, proposing the great sensitivity of scabies PCR.(Bae et al., 2020) .Fukuyama and his colleagues, was notes, in skin lesions from seven patients with scabies *S. scabiei* DNA was not detected at one month after effective cure with oral ivermectin. Therefore, determining residual mite infestation in previously treated patients. may be useful in these nested PCRs (Fukuyama et al., 2010).this clarify low sensitivity in our study since most physician (dermatologists) recommended use oral ivermectin in out-clinical inside Thi-Qar province.

Nucleotide sequence and phylogenetic analyses

ITS-2 region is suitable for phylogenetic studies of astigmatid mites. as this region shows comparatively higher variation between the diverse species of mites and lower distinction within the species. These astigmatid mites are located in the phylogenetic tree based on their genetic features. (Naz et al., 2018). Outcomes of the studies carried out in Thi-Qar province showed that ITS-2 sequences from Sarcoptes mites high intra-sequence changeability with seven polymorphic sites. The represented sequences with seven polymorphic sites have 98-99 % intra-isolate identity. Previous study by Ochs and his colleagues . have defined epidemiological clear-cut in mites for two populations of *Psoroptes spp*. as of sympatric rabbits and sheep from Switzerland. These populations were establish to be natively separate and characterised by a lone nucleotide difference in rDNA ITS2.

In addition, GU and Yang reported Sarcoptes as a single heterogeneous species using ITS-2 marker. While a high degree of genetic polymorphism has been described in diverse survey in individual mite separates, there was no characteristic clustering arising owing to diverse hosts and ecological localities(Gu & Yang, 2008) (Distributed, 2002),(Alasaad, Soglia, et al., 2009). Walton et al based on microsatellite data, defined a similar epidemiological status in human-derived and dog-derived *S. scabiei* from Australia. These authors(Walton et al, Ochs et al), reflected these two groups to be likely distinct species.(Ochs et al., 1999)(S. F. Walton et al., 1999) (Noge et al., 2005). However, their (Walton and his colleagues) study involved only seven human no crusted cases. Instead Andriantsoanirina et al. when studied the same club, the association of *S. scabiei* mites composed from dogs and humans via cox1, they establish that mites from dogs and humans grouped in the same cluster. Using the microsatellite approach

on the same samples(Andriantsoanirina, Ariey, Izri, Bernigaud, Fang, Charrel, Foulet, Botterel, & Guillot, 2015).

(The results of Andriantsoanirina with his colleagues), study not only support the refusal of the theory of panmixia for *S. scabiei* in humans which Fain believe its, but also propose that mites going to different clades are genetically isolated. Furthermore, the consequences recommend that the division of *S. scabies* in varieties giving to animal or human hosts is not acceptable. Currently, *S. scabiei* is taxonomically separated into diverse Diversities based on host origin (FAIN, 1978). Giving Andriantsoanirina with his colleagues. results and other recent studies, such variations are no longer justified. A generally acknowledged(this phrases belong to Andriantsoanirina et al) cause of protohumans were the original a animal scabies; dogs and other native animals were then infected .and were themselves hypothesis proposes that people and basis for other species of wildlife (S. F. Walton et al., 2004), (Currier et al., 2011). (Zhao et al., 2015).

Conclusion

A positive microscopic test has recorded while negative PCR examination occurs. Due to the different genotypes of the species, even if they are from same area and one host. moreover Negative DNA extraction were recorded for recovery people with ivermectin. A drawing of a phenotypic tree of the parasite has found to be referenced in the gene bank of animal models such as (garola and pig.) and the study agree with , Zhao et al. via cox1 for phylogenetic study described that mites from dogs in Australia, China, and the United States grouped with mites collected from Australian persons. Those authors determined that humans could be infested with mites from dogs. ITS-2 region is suitable for phylogenetic studies of astigmatic mites. as this region shows comparatively higher variation between the diverse species of mites and lower distinction within the species. These astigmatic mites are located in the phylogenetic tree based on their genetic features.

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