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Microscopic and molecular study of Sarcoptes scabies in Iraq

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Abstract

Background: Scabies is a widespread dermatological disorder that is commonly linked with pyoderma, particularly in warm climates and is the most prevalent dermatological infection in the world. This study aimed to detect scabies by microscopical and molecular methods to confirm that they may be caused by animal mites other than humans. Methods: This study was performed in order to identify scabies of humans by the microscopical examination and polymerase chain reaction (PCR) test in the governorate of Al-Anbar in Iraq. In accordance with this purpose, 170 skin scrapings were collected from suspected patients, who attended Al-Ramadi Teaching Hospital in Iraq during the period of 1st December of 2021 to 1st April of 2022. The samples were subjected to smearing using the KOH-traditional method, which was followed by using microscopy to investigate the presence of mites, eggs, and/or egg shells. The samples also were used to DNA extraction by a special kit then the extracted DNA were used in PCR test that targeted the mitochondrial cytochrome oxidase subunit 1 (cox1) of Sarcoptes scabies. Results: The results of the direct smears showed the presence of mites in 100 (58.8%) of the tested samples. The PCR results revealed the detection of the cox1 gene of Sarcoptis scabies in 64 (64%) of the microscopically positive samples. Conclusion:The current study demonstrated higher levels of human scabies by the direct smear method than that by the PCR.

Keywords:

Scabies, cytochrome oxidase subunit 1 (cox1), Polymerase chain reaction (PCR), scrapings.

The mite Sarcoptes scabies causes scabies, a common ectoparasite illness. This ectoparasite's popular term is "mite itch," which stems from the extreme itching it causes (Puza & Suresh, 2018). Sarcoptes are often very infectious and may be readily transferred via intimate physical contact, sexual partners, and family members (Abd El-Aal et al., 2016). Infection develops when the mite Sarcoptes penetrates the corneal layer of the skin, causing irritation (Walton & Currie, 2007). Scabies laboratory confirmation has traditionally used microscopic analysis of skin scrapings. Although microscopic analysis of skin scrapings has a 100% positive predictive value and a quick turnaround time, its sensitivity varies depending on the amount and quality of the material obtained (Saraste & Castresana, 1994). Nucleic acid amplification tests (NAATs) have been extensively employed in diagnostic parasitology in recent years, giving greater sensitivities than standard microscopy and allowing the determination of parasite burden when quantitative assays are applied (Makouloutou et al., 2015). S. scabies genetic characterization utilizing a marker of subunit 1 cytochrome c oxidase (COX-1) (Roberts et al., 2005). COX-1 gene is often employed by researchers in molecular epidemiology research and is the most useful marker for the genetic characterization of S. scabies in animals and humans (Ramachandra et al., 2015). Actually, since the beginning of time, people have had scabies. Scabies may have existed as early as 494 BC, according to archaeological findings in Egypt and the Middle

East. Aristotle described "lice" in the fourth century BC that "escape from small pimples if they are pricked"—a description that is compatible with scabies. Ibn Zuhr, an Arab physician, is said to have given the earliest clinical description of scabies mites (Fraser et al., 2019). The term "scabies" and a description of its defining characteristics are attributed to the Roman encyclopedist and physician Aulus Cornelius Celsus (c. 25 BC − 50 AD). The Italian physician Giovanni Cosimo Bonomo (1663−1696) described the parasitic origin of scabies in his 1687 letter "Observations about the flesh worms of the human body" (Mohy et al., 2018). Scabies became one of the earliest human illnesses with a clear cause according to Bonomo's description. A sulfur-containing cream with the medical name Wilkinson's ointment was frequently used in Europe in the late 19th and early 20th century for the topical treatment of scabies. In 1945, correspondence that had been published in the British Medical Journal described the composition and history of many iterations of the ointment (Arlian et al., 2017). Scabies, along with tinea and pyoderma, is the three most prevalent skin conditions in kids. About 100 million individuals (1.5% of the population) are affected by it as of 2010, and gender has no bearing on how often it occurs. The mites are widely dispersed over the globe and equally infect people of all ages, races, and socioeconomic groups under various climatic conditions (Fraser et al., 2017). In congested places with unsanitary living circumstances, scabies is more often seen. As of 2009, there are thought to be 300 million scabies cases annually worldwide, despite claims to the contrary from different groups. Approximately 1-10% of the world's population is thought to have scabies, although, in other groups, that number may be as high as 50-80% (Matsuyama et al., 2019). However, the absence of a standardised reporting system for the condition makes it difficult to determine the severity of the issue. Epidemiological statistics are based on a variety of data sources, including institutional settings, such as schools, hospitals, or care facilities, as well as government reporting systems, military databases, and institutional contexts. The World Health Organization (WHO) added scabies to its list of neglected tropical diseases in 2013, and the International Alliance for the Control of Scabies (IACS), which unites experts from all around the world, was established in 2011 (Anderson & Strowd, 2017). Transmission occurs via direct skin-to-skin contact. Human scabies mites may survive outside of the human body for 24-36 hours in typical room settings (21 ˚C and 40-80% relative humidity); during this period, they can infest. Indirect transmission (through clothes, bedding, and other fomites) has been hypothesised; however, this has been difficult to confirm experimentally (Andriantsoanirina et al., 2015). Early Mellanby investigations demonstrated that indirect transmission is unlikely to play a substantial role, even in situations of crusted scabies if the host is extensively infested. In these studies, volunteers slept on bedding that had been used by scabies patients less than 24 hours previously. Only 1.3% of volunteers (4 out of 300) got infected when the patients had parasite rates of 20-50. When patients had parasite rates of 200 or higher, 30% of volunteers (3 out of 10) got infected (Rider et al., 2015). Sarcoptes scabiei was originally assigned to the genus Acarus and given the name Acarus scabiei DeGeer, 1778. S. scabiei classification has developed with mite nomenclature. Sarcoptes scabiei is currently classified as a member of the superfamily Sarcoptoidea and the family Sarcoptidae, like numerous other mammalian ectoparasitic mites. S. scabiei is a member of the superorder Acariformes, the order Sarcoptiformes, the suborder Oribatida, the infraorder Desmonomata, and the group (hypoorder) Astigmata (together with the house dust mites Dermatophagoides farinae, D. pteronyssinus, and Euroglyphus maynei) (Micali et al., 2016). The Sarcoptidae family has three subfamilies (Sarcoptinae, Teinocoptinae, and Diabolicoptinae) with 16 genera and 118 species that all live on the skin of mammals. The four genera Sarcoptes (1 species), Prosarcoptes (3 species), Trixacarus (3 species), and Kutzerocoptes comprise the subfamily Sarcoptinae (1 species). Sarcoptes and Trixacarus caviae have similar appearances and may be mistaken (Sunderkцtter et al., 2019). Trixacarus caviae is a guinea pig parasite that is significantly smaller than Sarcoptes. In people who touch or handle afflicted guinea pigs, Trixacarus caviae may cause pruritic dermatitis. Aside from size variations, a few additional characteristics clearly differentiate Sarcoptes from T. caviae. The dorsal setae of T. caviae females are simple, but those of S. scabiei are cone- and spine-shaped, and T. caviae dorsal scales are more widespread and reach to the idiosoma's posterior. T. caviae dorsal setae sci, l1, and d1 are not lamellate like S. scabiei (Khalil et al., 2017; Thomas et al., 2015)

Materials and Methods

Over a four-month period, 170 patients attended the dermatological clinic at Ramadi Teaching Hospital (1st December 2021 to 1st April 2022). The study was conducted in accordance with the Declaration of Helsinki and was approved by the local ethics committee of the College of medicine Anbar University, Iraq. Dermatology professionals used established criteria to pick scabies patients.

Before the specimens were obtained, none of these individuals had undergone antiscabietic medication. From clinically suspicious lesions, scrapings were collected. Each item was put in a sterile plastic container and examined under a microscope as well as by PCR. The scrapings were digested with 2 drops of 10% potassium hydroxide before being inspected under a 40x microscope for the presence of mites, nymphs, or eggs (Pentinsaari et al., 2016). When mites or eggs were found on patients, they were diagnosed as confirmed scabies. A WizprepTM gDNA Mine Kit (Cell / Tissue) was used to extract DNA, which was adapted from a user-developed procedure (WizprepTM gDNA Mine Kit). In brief, skin samples were digested with 200l of GT1 Buffer and homogenised by grinding. A 250-bp segment of the S. scabies cytochrome c oxidase subunit 1 (cox1) gene was amplified using the particular forward primer scabF1 (5=-CTTATTATT CCTGGATTTGGRTA) and the specific reverse primer scabR2 (5=-CTAATTTTCCTCCTAATATTGTWGA-3=).

Statistical analysis

Data were statistically analyzed by using Statistical Package for Social Sciences (SPSS) version 15 software. Data were represented as number and percentage. when appropriate. p-Value ≤ 0.05 was considered statistically significant.

Results

General characteristics of the patients and control.

Ages of the patients and control were ranged between (1 − 70) years. The mean age of the patients was 24.050± 18.6491 which did not differ from controls which was 22.575 ± 16.52 as show in Table 1.

Table 1: Age and gender of the patients and control

Characteristics	Patients (n=100)	Controls (n=40)	p-value
Age, years Mean±SD Range	24.050 ±18.6491 1.0-70.0	22.575 ±16.5237 1.0-70.0	0.147
Gender Male Female	22.419 ± 18.1006 25.281 ±19.1190	26.118 ±17.5246 19.957 ± 15.6139	0.093

The ages of patients and control were divided into five groups (less than 15) (46%), (16-30) (21%), (31-45) (16%), (46-60) (12%) and (more than 60) (5%). The age group (less than 15 years) was increased significantly over the other groups followed by (16-30) years while the age group of (more than 60) was significantly decreased in the study. As show in Figure 1.

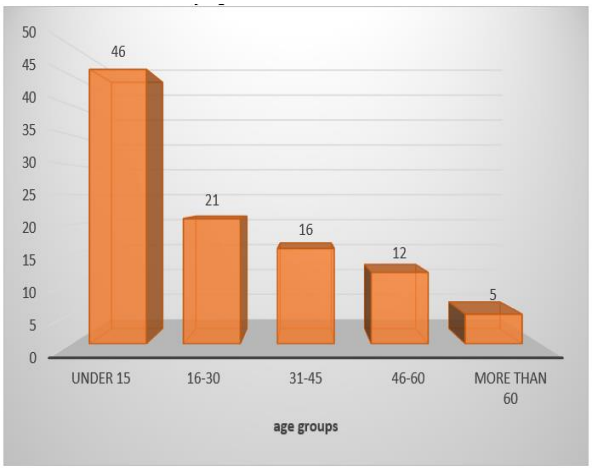

Figure 1: percentage of infection according to the age

The results of this study revealed that the percentage of females (57%) was higher than males (43%). As shown in Figure 2

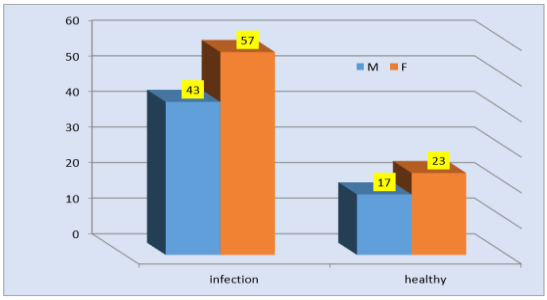

Figure 2: The percentage of males and females between patients and control

Results of microscopical examination and PCR

Out of 170 skin scrapings from suspected patients, one hundred cases of scabies (58.8%) were diagnosed microscopically by finding adult arthropod or their eggs as shown in Table-2. While according to the PCR analysis the result showed that only 64 cases 64% were positive in PCR analysis and 36% were negative. This reveals that only 37.6% of all suspected cases have Sarcoptes scabies DNA. All negative controls, including skin scrapings from normal skin, were also negative by the cox1 PCR according to the Table -3.

Table 2: Results of microscopy of skin scraping specimens for diagnosis of scabies

	No. of samples with the following microscopy result		
	Positive N (%)	Negative N (%)	Total (%)
Skin scrapping samples	100 (58.8%)	70 (41.2%)	170 (100%)

Table 3: Performance of PCR of skin scraping specimens for diagnosis of scabies

cox1 PCR result	Positive samples in microscope detected by PCR:		
	No from 100		
Positive	64	64%	Positive
Negative	36	36%	Negative
Total	100	100%	Total

The specimens were collected from different parts of body with different percentages according to Table-4 where arms and legs were the highly frequent sites of infection while the shoulders and elbow were the less frequent sites.

Table 4: Percentage of lesions according to the site of lesions

Site of infection	Percentage %
Finger	10.502
Elbow	3.652
Shoulder	6.393
Leg	26.485
Arm	30.136
Abdomen	22.832
Total	100

Discussion

Scabies is the most recent illness to be added to the World Health Organization's list of neglected tropical diseases (Steer, 2014). The illness is thought to be particularly frequent in resource-poor populations and is linked to overcrowding and poverty. High-income nations, on the other hand, are not immune to scabies, and outbreaks in institutions and healthcare facilities are not uncommon (Tjioe & Vissers, 2008). Nosocomial outbreaks may be exceptionally long-lasting, with a large number of secondary cases discovered in patients and healthcare employees, as well as tertiary cases detected in family members of afflicted healthcare workers (Pastenak et al., 1994). Scabies misdiagnosis is not rare, due in part to the variety of scabies presentations, but also to attending professionals' lack of clinical knowledge (Hong et al., 2010). The traditional burrows, although present, are not always easily visible and may need dermoscopy and the burrow ink test to be detected (Golant & Levitt, 2012). According to a survey of the demographics of scabietic patients, our research found that scabies is widespread among the examined communities' children and adolescents (Hay et al., 2012). While other studies have shown that it is mostly a disease of the elderly, particularly those in long-term care institutions (Lapeere et al., 2008). Overcrowding, bed sharing, under-recognition, and the absence of pruritus in infants are all possible causes of why children are disproportionately afflicted (Boralevi et al., 2014).

Nucleic Acid Amplification Tests (NAATs) have been proven to have much higher sensitivity than traditional microscopy for the diagnosis of certain parasite illnesses. The difficulty in diagnosing typical scabies stems not only from the difficulty in identifying the burrows but also from the relatively small number of mites present (Currie & McCarthy, 2010). This helps to explain why skin scraping microscopy is only sensitive in roughly 58.8% of questionable individuals (Walter et al., 2011). We found that using a microscope as the diagnostic "gold standard," the PCR test diagnosed scabies in 64% of the entire 100 microscopy-positive cases. Previous studies demonstrated the significance of PCR in the diagnosis of scabies using gene sequence data, despite the fact that only a small amount of data for these genes is accessible in GenBank (Shumaila et al., 2013). Furthermore, two investigations used mite samples or skin biopsy specimens to evaluate the PCR assay, but only a few clinical specimens, such as skin scrapings, were analysed (Bezold et al., 2001). The cox1 gene was chosen as the PCR target in our work because it is reasonably conserved, and widely separated from the cox1 genes of other frequent human ectoparasites, and there is a huge quantity of sequence data accessible. The cox1 gene seems to be a promising target for S. scabies molecular detection. The cox1 sequences of other human skin mites, pathogenic zoonotic mites, and common house dust mite species had no strong sequence similarity to the S. scabies primer sequences employed in this investigation. Because of the huge number of

mitochondria and hence a large number of gene copies present in each arthropod cell, using a mitochondrial gene target may enhance PCR sensitivity (Pisani et al., 2013). Using a skin swab for PCR instead of the traditional scraping technique for microscopy may be advantageous because it avoids the need for high-quality skin scrapings, the collection of which is dependent on the expertise of healthcare workers in identifying the most likely sites of infection and obtaining sufficient skin samples. While microscopy depends on the detection of mites and/or eggs inside skin scrapings, PCR does not rely on the presence of these arthropod structures. We hypothesise that mite excreta and cellular DNA are integrated into stratum corneum cells, which eventually emerge in the squamous and are detectable by PCR. Based on this hypothesis, the increased percentage of microscopy over PCR demonstrated in this study can be explained by the difficulties in taking samples and obtaining the parasite, which is not found in all borrows or was found in a deep layer, decreasing the probability of obtaining its genome by PCR. The procedure of screening patients for scabies should be streamlined, particularly during epidemic investigations when a high number of patients may need to be checked. It also helps with the discovery of burrows, which is required for the traditional technique of clinical diagnosis. DNA may survive in clinical specimens for some time after effective treatment, as in the case of molecular identification of other diseases. The existence of detectable DNA cannot be used to indicate treatment failure (Wong et al., 2015). S. scabies generally lives in the skin's stratum granulose layer (Arlian, 1989). In normal skin, cells move from the stratum Basle to the stratum Corneum in approximately 14 days and are desquamated in another 14 to 20 days. (Wong et al., 2015). The elderly has a longer period for stratum corneum rejuvenation, which sometimes takes more than 30 days (Wong et al., 2015). Because obtaining skin specimens for scabies PCR, whether scrapings or swabs, often entails sampling of the stratum corneum, we may anticipate mite DNA to remain for at least 2 weeks and potentially longer in the elderly. In the future, the temporal persistence of mite DNA in instances of common scabies should be examined to better understand the dynamics and help in the interpretation of PCR data. Previous research has found mites in dust samples collected around scabies patients, and our sampling technique may not have been sensitive enough to identify the minimal quantity of mite DNA in the environment (Arlian, 1989). Further advancements in collection procedures that enhance the amount of skin in the samples may boost the sensitivity of the PCR tests for the early detection of scabietic patients, where prompt treatment and control measures are critical for outbreak prevention. A limited number of papers have shown the use of PCR for scabies diagnosis (Fukuyama et al., 2010). In particular, microscopic inspection was positive for several individuals who had clinically comparable scabies symptoms but were PCR negative, as this investigation demonstrated because to the high specificity of the employed primer (Ken et al., 2014). Although Sarcoptes is the most frequently known mite in the United States as a cause of human skin illness, various additional mite-associated dermatoses have been discovered. Various mite species found in animals may infest humans briefly. Such agents should be regarded as a probable cause of erythematous and sometimes pruritic skin responses of unknown origin. Pseudoscabies is a prevalent condition among those who work with animals, such as farmers, veterinarians, and pet owners. These self-limiting dermatoses are often misdiagnosed. Sarcoptes scabiei, Notoedres cati, Cheyletiella spp., Dermanyssus gallinae, Ornithonyssus bacoti, Ophionyssus natricis, and Neotrombicula autumnalis are among the species that may infest human skin and cause symptoms (Beck & Pfister, 2006). This approach may be used as an additional method for scabies diagnosis, particularly in epidemic investigations or when collecting large numbers of specimens. The comparatively limited number of individuals analysed and the absence of sequential examinations in ordinary scabies patients are possible limitations of our research. Different specimens from patients with ordinary and crusted scabies might be explored further. The possible use of swabs in addition to scrapings should be investigated in a greater number of individuals with both ordinary and crusted scabies.

Conclusion

We found that the percentage of scabies in females is more than that of males, and the highest percentage is in the age group less than 15 years. The present study demonstrated higher levels of detection of human scabies by the direct skin smears than PCR method.

Conflict of interest

The authors have no conflict of interest.

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