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Comparative study between direct skin smear and polymerase chain reaction test in the identification of human scabies in Al-Kut City, Wasit Province, Iraq

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Abstract

This study was performed in order to compare between direct skin smear and polymerase chain reaction (PCR) test in the identificationof human scabies in the City of Al-kut, Wasit Province, Iraq. In accordance with this purpose, 100 skin scrapings were collected from 100 patients, who attended Alkarama Teaching Hospital during the period of January 1 to June 30 of 2021. The samples were subjected to direct 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 exposed to a PCR test that targeted the mitochondrial cytochrome oxidase subunit 1 (*cox*1) of mites. The results of the direct smears showed the presence of mites in 8 (8%) of the tested samples. The PCR findings revealed the detection of the *cox*1 gene in 47 (47%) of the studied samples. PCR revealed 92% and 96% of sensitivity and specificity, respectively, while, the direct smear method showed 14% and 37% of sensitivity and specificity, respectively. The presence work demonstrates higher levels of detection of human scabies by the PCR method than that by the direct skin smears.

Keywords: Human scabies, KOH-skin smears, mites, PCR.

Introduction :

Sarcoptesscabiei, a mite that causes scabies, is spread from person to person by immediate skin contact. Fomites like clothes and bed linen have a limited effect in the transmission of the mites since the mites can only exist apart from skin for a short period of time (usually 24 to 36 hours). The adult female mites excavate tunnels named burrows into the skin (epidermis) and lays their eggs over a period of time. The larvae leave the eggs in a period of incubation of (2-4) days and grow into adults in ten to fourteen days. A hypersensitivity response to the mites, their eggs, or their wastes, emerges in the affected person 21 days after the initial contact to the mites. About ten to fifteen adult female mites are often found in a typical infestation(1–3).

Scabies is generally characterized by burrows, red-inflamed papules, and widespread itching, which worsens at night. When it comes to burrows, they're generally found in the interdigital spaces between fingers, in the wrist flexure, in the armpit, or in the genital or breast region. Burrows can appear as vesicle-, pustule-, or nodule-based lesions on the head and neck in newborns and the elderlies(4).

Other itchy skin rashes, including eczema, *Tinea corporis* (ringworm), impetigo, and psoriasis are sometimes mistaken with scabies because of their similar symptoms. One research in Brazil found that 18% to 43% of eczema-diagnosed youngsters really had scabies. After scratching, subsequent bacterial infections including impetigo, pyoderma by group A streptococcal microorganisms and staphylococcus aureus may occur. Poststreptococcal glomerulonephritis and heart disease are two common consequences of bacterial infections. Scabies can also lead to social isolation, anxiety, and sleep deprivation, as well as considerable financial expenditures both directly and indirectly(5–9).

When the body's immune system attempts to handle the mite, a disease called crusted scabies (Norwegian scabies) develops. This leads to massive mite overpopulation, acute inflammation and exaggerated keratosis responses. About 50% of people with crusted scabies don't have any symptoms of itching. If you have an immune system that is normal, you might have crusted scabies, but it is more common in those who have immunodeficiency disorders such as HIV or autoimmune illness. Crusted scabies is more challenging to cure than classic scabies and is frequently misinterpreted as psoriasis or eczema, particularly after the use of a topical steroids. Crusted scabies is more infectious than classic scabies due to of the large number of mites present (perhaps millions), and this can lead to large epidemics(4,10).

The diagnosis of the scabies is usually performed depending on the disease history and using some techniques, such as direct skin smearing, burrow ink test, and dermatoscopy, in which visualization in a direct manner is done on magnified skin(4). This study was performed in order to compare between direct skin smear and polymerase chain reaction (PCR) test in the identification of human scabies in the City of Al-kut, Wasit Province, Iraq.

Materials and methods :

Skin samples :

The study involved the use of 100 skin scrapings were collected from 100 patients, who attended Alkarama Teaching Hospital during the period of January 1 to June 30 of 2021. The patients were evaluated medically by dermatologists, and after, taking the consents of patients or their parents, skin scrapings were performed from the skin lesions.

Direct skin smearing :

Mites, eggs and/or their shell debris were visualized in the skin scrapings for the actual detection of the scabies. These scrapings were from papules and other suspected scabies skin lesions such as theextremities of the burrows. For taking the samples, mineral oil, (1-2) drops, wasplacedon the target lesion that was then scalpel-scraped. After that, 10% KOH was applied to the specimens forclearing, and, at a low power, a light microscope was used to examine these samples (11).

Molecular diagnosis :

Mite DNA extraction :

The mite mitochondrial DNA was extracted using gSYAN DNA Extraction Kit (Geneaid, Taiwan) and constructed depending on the protocol paper accompanied within the kit box. Here, samples based on 200mg weight of skin scrapings wereplacedin sterile 1.5ml tubes, and 200µl of the kit solution, GST, was applied to these tubes followed bymicropestle-based homogenizationof the mite tissues.The kit steps were followed to the end of the extraction process. The final products were estimated for the quantity and quality using a NanoDrop.

Polymerase chain reaction :

The master mix solution was prepared for a total volume of PCR reaction of 25µl. The master mix was produced using GoTaqTM Green PCR Master Mix kit (Promega, USA). The steps of the protocol that belonged to the kit were followed for this section. The *cox*1 gene at a piece of 570bp was targeted for the PCR amplification process. This process was conducted using the primer set of F: TCAGTTGTAACCGCCCATGC and R: AATGTAAACTTCCGGGTGTCCA, which were designed using the NBI-related websites and the Primer 3 Plus software. The primer set was placed in the GeneBank repository under the No. (MF083742.1). Later, these primers were purchased via (Scientific Reseracher Co. Ltd, Iraq). The following components and their amounts were used for the master mix preparation; 5µLDNA template, 2µL (10pmol) of each primer direction, 12.5µL of Go taq Green Master mix, and 3.5µL of PCR water. After brief vortex, the tubes were place in a thermocycler for the PCR reaction at one cycle of 5min-95˚C of initial denaturation, followed by 35 cycles of (0.5min-95˚C denaturation, 0.5min-58˚C annealing, and1min-72˚C extension), and one cycle of 5min-72˚C final extension. The final PCR products were electrophoresed-passed through a 1.5% agarose gel maintained with the use of ethidium bromide. In each well, 10µl of the PCR product was loaded. The ladder was loaded into a well at 3µl. The process was performed under the conditions of 100 volts and 80 Amp for one hour. After finishing the electrophoresis, the gel was visualized using a UV-based screener and imager.

Statistical analysis :

The GraphPad Prism Software (v7.0) was used to analyze the data and generate graphs. Chi-square test was employed as part of the analysis.

Results :

The results of the direct smears showed the presence of mites in 8 (8%) of the tested samples. The PCR findings revealed the detection of the *cox*1 gene in 47 (47%) of the studied samples (Figure 1).

Figure 1: **Images of agarose-gel-electrophoresis of mite PCR products of the *Sarcoptes scabiei cox*1 gene from skin scabies specimens. M: marker (1500-100bp). Lanes (1 to 19): Positive PCR products at 570 bp.**

A comparative analysis was done between the direct smear method and the PCR showed higher levels of detection by the PCR than that by the direct smear test (Table 1 and Figure 2).

Table 1: Comparison between skin smear method and PCR in the detection of scabies.

Test	Incidence		Sensitivity (%)	Specificity (%)	Chi-square	Odds Ratio	*P* value
	No	%					
Skin smear	8	8	14	37	0.098	0.098	<0.0001
PCR	47	47	92	96			

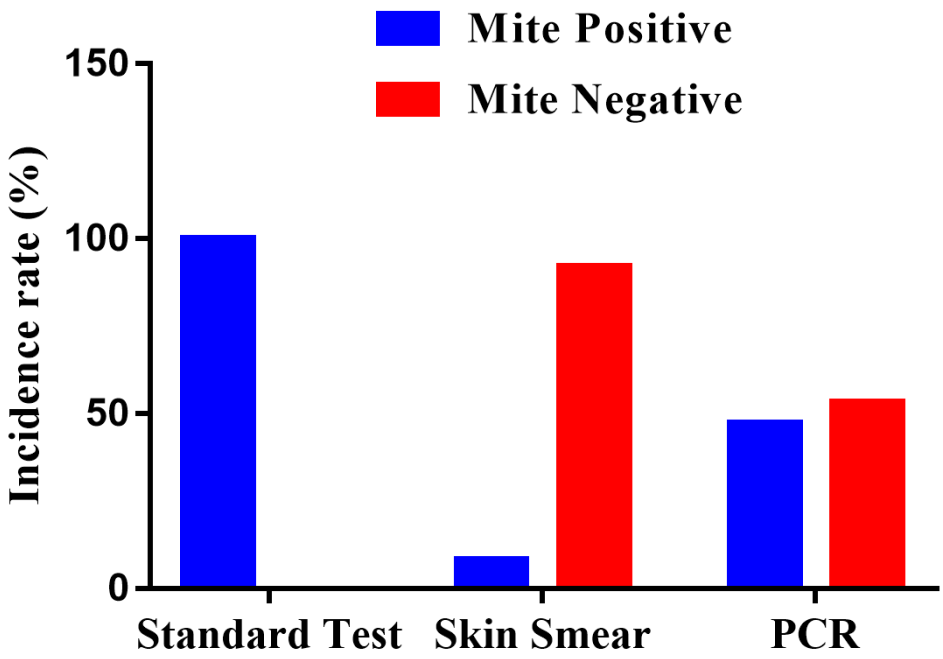

Figure 2: Comparison between skin smear method and PCR in the detection of scabies.

Discussion :

Skin scabies of humans is a contagious skin disease caused by the mite *Sarcoptes scabiei*. Sometimes, the disease undergoes misdiagnosed as eczema, psoriasis, or impetigo due to similarity of the signs and symptoms. In addition, usual skin smears may reveal false negative presence of the mites in the skin lesions (4). Therefore, finding a better diagnostic tool may help overcome such unwanted results and enhance the treatment process.

The study, here, revealed, using the direct skin smear, that 8% of the patients were infected by the skin mites. Our results agree with Mero and Hassan, (2014) (11), who found that 522 (5.5%) patients were infected by the scabies from a community sample of 9450 individuals in Duhok Province, Iraq, performed in the period between 2012 and 2013. This matching between our findings and with those

by Mero and Hassan, (2014) (11) indicates that the process of diagnosis using direct skin smear was performed without any erroneous process to eliminate any unplanned variables. Alsamarai, (2009) (12) had detected the presence of scabies in patients using direct smearing by oil drops or by a ink method. The author has found that the mites infested 132 (1.1%) of 1194 patients, who visited a dermatological clinic in Tikrit, Iraq. In the period between 2018 and 2019, in Duhok Province, Iraq, AlBerfkani and Mero (13) used direct skin smearing in the detection of scabies and found that the mites were present in (46-48%) of the tested individuals from a united Nation Camps in Zakho City, Duhok Province, Iraq. In a study performed in Diyala Province, Iraq, in 2016, Alzobydy (2018) (14) found that the incidence rate of the scabies, according to the use of direct smear microscopy, was 344 (2.16%) from 15891 of skin lesion suffering patients.

For the PCR, the outcomes of the current study demonstrated that 47% of the tested individuals were positive for the presence of the mite *cox*1 gene in the skin scrapings. Bae *et al*. (2020) (15) found that *cox*1-targeted PCR was effective in the diagnosis of 22 (66%) of 33 patients. This piece of result is higher than our PCR finding indicating higher levels of incidence in Korea than that from our studied city. This could be explained due to the presence of high humidity in Korea in compared with that in the current study city. Mnati *et al*. (2020) (16) detected that the incidence of scabies in Basra, Iraq, correlated positively with moisture. Wong *et al*. (2015) (17) showed that PCR was highly effective than the direct smear method in the detection of mites in the skin scrapings of their patients. Delaunay *et al*. (2020) (18) revealed that PCR was 28.4–48.4% sensitive in the identification of the skin scabies. These studies may show higher preferences to use PCR over skin smearing in the diagnosis of mites of skin. These data from the above mentioned literatures come into agreements with our results, in which PCR has higher sensitivity rates than that from direct smear methods.

Conclusion :

The present study demonstrated higher levels of detection of human scabies by the PCR method than that by the direct skin smears. PCR has 92% and 96% of sensitivity and specificity, respectively, which are higher than those from the direct smear method used in the current study.

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