MOLECULAR DETECTION USING PCR IN DIAGNOSING SCABIES

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ABSTRACT

World Health Organization stated that scabies is Neglected Tropical Disease which estimates more than 300 million people are infected with scabies. This study aims to review the implementation of Polymerase Chain Reaction (PCR) as diagnostic tools for scabies. This review conducted by browsing 268 articles and found five relevant articles using eligible criteria. From 5 relevant articles, it can be concluded that PCR testing for scabies may be helpful in the improvement of sensitivity for the diagnosis of scabies by clinical criteria. This study's limitation is it only reviews the application of PCR in diagnosing Sarcoptes scabiei in human. It must be better if there is research about comparison of PCR with other diagnostic tools in Indonesia. This study can be useful in diagnosing people suffered from scabies in Islamic boarding school, prisons, orphanages, and densely populated neighborhoods in tropical country, especially in Indonesia.

Keywords: scabies, PCR, diagnosis, sarcoptes scabiei

I. INTRODUCTION

Scabies is a skin disease caused by Sarcoptes scabiei infestation and sensitization. The World Health Organization (WHO) in 2017, stated that scabies is included in the Neglected Tropical Disease (NTD), which requires large-scale control. WHO estimates that more than 300 million people, or around 3% of the world's population, are infected with scabies. (WHO, 2017) In Indonesia, according to data from the Indonesian Ministry of Health, the prevalence of scabies in 2017 is 6% of the total population. (Kemenkes, 2017)

Scabies can provide typical symptoms so that they are easily diagnosed; however, if the clinical symptoms are not typical, scabies' diagnosis is challenging to establish. Typical clinical symptoms are severe itching complaints at night (nocturnal pruritus), or when the heat is hot, and the sufferer sweats. Typical skin eruptions include tunnels, papules, vesicles, and pustules at the site of predilection. Although scabies symptoms are typical, sufferers usually come for treatment when it is already in an advanced stage and do not have any more typical clinical symptoms because excoriation has arisen, secondary infection by bacteria, and lichenification. (Sungkar S., 2016)

Another problem in diagnosing scabies is that clinical symptoms of scabies can mimic the symptoms of other skin diseases or are covered by other diseases such as eczema and impetigo so that diagnosis becomes difficult. The diagnosis relies on clinical symptoms to be less efficient and has a sensitivity of less than 50% because it is difficult to distinguish active infestations, residual skin reactions, or reinfections. Tunnel detection with Indian ink has been done for a long time, but the test is not practical, hence it is rarely used. Misdiagnosis results in falsetreatment and causes the patient not to heal, and continues to be a source of infection for the environment. (Dupuy, 2011)

The exact diagnosis of scabies is determined by finding mites or eggs in a laboratory examination, but mites are difficult to find because only a few mites infest sufferers. According to Mellanby, out of 900 scabies patients, only found 11 mites per sufferer on average, and most sufferers only found 1-5 mites per sufferer. (Mellanby, 2011) In previous study at a pesantren in Jakarta, the prevalence of scabies was 72.6%, but only 8 mites were found in all sufferers. (Sungkar S., 2016)
II. LITERATURE REVIEW AND HYPOTHESIS DEVELOPMENT

Scabies examination with polymerase chain reaction (PCR) can be one method of detection of *S. scabiei*. With the PCR technique, scabies diagnosis is made easier because it is sensitive to enzymatic amplification of gene fragments from a small amount of parasitic material. PCR is a method for accurately identifying parasites, knowing the characteristics of parasitic genes, diagnosing parasitic infections, knowing the isolation and characteristics of expressed genes, detecting drug resistance, developing recombination of DNA vaccines, and analyzing overall parasitic genomes. (Chandler & Fuller, 2019)

The disadvantage of PCR is the dependence of the method on the presence of mites or parts of mites in the preparation, so it is not possible to be widely used because the amount of mites is only small. PCR can be relied upon if other methods cannot diagnose scabies. PCR followed by ELISA detection can increase diagnostic sensitivity in patients with atypical scabies, but this method is very time-consuming and costly. (Sungkar S, 2016)

III. RESEARCH METHODOLOGY

Systematic searches were carried out in August 2020 on PubMed, Proquest, Science Direct, and Clinical Key. The search term was "Scabies AND Polymerase Chain Reaction." The inclusion criteria for articles were in English; there was information on scabies and PCR, articles were published in past 5 years. The exclusion criteria were duplication and could not be accessed entirely. Publications selected for the title and abstract were extracted using a standard format table and processed using a Microsoft Excel spreadsheet. The data extracted were in the form of author, year of publication, journal, and conclusion. The results are then presented qualitatively.

IV. RESULTS AND DISCUSSIONS

Of the 268 articles obtained, only five articles met the inclusion and exclusion criteria and passed the exclusion criteria. Figure 1 shows the flow of article selection. A critical review was conducted of four selected articles published by journals in the Q1 and Q2 categories based on the Scimago Journal and Country Rank. Then an analysis was carried out to review the implementation of PCR in diagnosing scabies. (Table 1).

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<th>No</th>
<th>Title</th>
<th>Subject</th>
<th>Methods</th>
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<td>1</td>
<td>Validation of PCR Assay for Identification of <em>Sarcoptes scabiei</em></td>
<td>The mite samples were collected from scabies patients by visiting government hospitals of twin City, Pakistan</td>
<td>Preparation of <em>Sarcoptes</em> mite DNA by commercial DNA extraction kit method. Two primers i.e. Sarms 15 F/R and 16S D1/D2 were used to amplify the target sequence using PCR. The amplified products were then separated by primer</td>
<td>The PCR product analysis showed specific band of 178 bp with primer</td>
<td>(Naz et al., 2016)</td>
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<td>var. hominis</td>
<td>agarose gel, electrophoresis, and analyzed after staining and visualizing in UV transilluminator.</td>
<td>Sarms 15 F/R, while with primer 16S D1/D2 bands of 460 bp and 600 bp were observed on 2% agarose gel. The appearance of a different band of 600 bp revealed that it might be due to the heteroplasmy state present in the Pakistan Sarcoptes mites population.</td>
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<td>Universal conventional and real-time PCR diagnosis tools for Sarcoptes scabiei</td>
<td>The first time two universal mitochondrial-based diagnosis methods: (i) conventional end-point PCR and (ii) TaqMan real-time PCR. These universal PCR-based diagnosis methods are highly specific, technically sensitive, and simple, and are based on the amplification of 135 bp from the mitochondrial 16S rDNA. The method (Angelone-Alasad et al., 2015)</td>
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<td>Scabies polymerase chain reaction with standardized dry swab sampling: an easy tool for cluster diagnosis of human scabies. From a total of 183 suspected cases of scabies, 164 patients were sampled, 87 had confirmed scabies and 77 did not. The dry swab was systematically rubbed across the front of both wrists, the eight interdigital spaces, and any suspected scabies lesions in all patients referred for scabies. A new PCR-based diagnostic test was run on the samples. All patients underwent clinical and dermoscopic examination. Scabies diagnosis was confirmed when a dermoscopic examination was positive or the patient had typical clinical signs of scabies. Of the 87 patients with proved scabies, 33 patients had positive scabies PCR, resulting in a 37.9% [95% confidence interval (CI) 28.4–48.4%] sensitivity and a 61.7% (95% CI 52.4–72.7%) negative predictive value. None of the 77 patients ruled out for scabies had positive PCR results.</td>
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<td>Development of Conventional and Real-Time Quantitative PCR. Skin scrapings were prospectively collected from 100 patients with suspected scabies in the Hong Kong West Hospital Cluster and the United Christian Hospital. During the study period, 100 skin scrapings were examined by microscopy and PCR for scabies. S. scabiei was detected in 29 skin scraping samples by PCR, while it was detected in only 17 of these by microscopy. None of the specimens was microscopy positive and both assays were specific and more sensitive than microscopy. (Wong et al., 2015)</td>
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<td>5</td>
<td>Diagnostic value of the molecular detection of <em>Sarcoptes scabiei</em> from a skin scraping in patients with suspect</td>
<td>Adult patients with suspected scabies, unrelated diseases, or healthy volunteers were enrolled at a tertiary hospital in Seoul, South Korea,</td>
<td>PCR was performed on the skin scrapings to target the cytochrome c oxidase subunit 1 (<em>cox1</em>) gene of <em>Sarcoptes scabiei</em>. A total of 47 participants, 33 with suspected scabies, 10 with unrelated diseases, and 4 healthy volunteers were enrolled. Of the 33 patients, 22 were classified as confirmed scabies, 2 as clinical scabies, 6 as suspected scabies, and 3 as no scabies.</td>
<td>The sensitivities of PCR were 86%, 83%, and 80% in confirmed scabies, confirmed and clinical scabies, and (Bae et al., 2020)</td>
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Anamnesis and physical examination from the suspected scabies patient are clinically used to diagnose scabies, however, definitive diagnosis of a scabies infection is based on the identification of mites, eggs or feces in skin scrapings or biopsies. This situation is problematic since it can lead to false diagnoses and mistreatment of the patient. In addition, the use of inappropriate antibiotics can cause resistance.

Based on recent findings, it can be possible to sequence some mite DNA to check the presence of different strains of *Sarcoptes scabiei*, if any, in Pakistani population. Additionally, genotypic differentiation among the populations of *Sarcoptes scabiei* and worldwide genotypic data comparison could also be done in order to construct a phylogenetic tree. (Naz et al., 2016)

A study successfully designed and applied two universal PCR based diagnostic methods for *S. scabiei*, one based on conventional end-point PCR and the other on TaqMan real-time PCR. These new methods were standardized and found to have high specificity and technical sensibility in 23 host species from 14 counties. They successfully diagnosed (based on skin scrapings) different clinical degrees of sarcoptic mange affecting several animal species. This study recommends further testing and the application of these new universal methods worldwide. (Angelone-Alasaad et al., 2015)

This article should propose a new diagnostic method for scabies based on a new, specific PCR marker protocol and an original, standardized, and nontraumatic sampling method. This scabies management protocol could be adapted for large clusters as it is easily implementable, nontraumatic, repeatable, and does not require expert evaluation. The aim is to detect at least one scabies-infested person. In one study, this method shows a sensitivity of 90% with five samplings and 99% with 10 samplings in patients with proven scabies. (Delaunay et al., 2020)

One study showed that *cox1* PCR of skin swab samples has better sensitivity for scabies diagnosis than microscopic examination. Although PCR may not replace microscopy as the diagnostic test of choice, it may have a role in aiding the diagnosis of scabies in patients with atypical presentations, outbreak investigations, and environmental studies. (Wong et al., 2015)

Scabies PCR was shown to offer an improvement in assay sensitivity compared to that of microscopy examination for the diagnosis of scabies by clinical criteria. Furthermore, standardized skin sampling method also need to be evaluated to increase the sensitivity of the molecular examination. Therefore, this technique can be considered an adjunct method for the diagnosis of scabies, particularly in microscopy-negative suspected cases. Further larger-scale studies will be needed to evaluate the scabies PCR's diagnostic performance and validate the new IACS criteria by using more sensitive diagnostic tests. (Bae et al., 2020).
V. CONCLUSION

The cox1 gene is relatively well conserved. Its sequence has no high levels of similarity to the sequences of other human skin mites, pathogenic zoonotic mites, or common house dust mite species. This mitochondrial gene is also present in large quantities in arthropod cells, potentially improving a PCR-based assay's sensitivity. The specificity of the scabies PCR in the no scabies control was 100% regarding from some study. PCR testing for scabies may help improve sensitivity and specificity for scabies diagnosis by clinical criteria.

REFERENCES