ORIGINAL ARTICLE

Effectiveness of Cytochrome *c* Oxidase Subunit 1 Gene Nested Polymerase Chain Reaction Compared to Dermoscopy or Microscopy Alone for the Detection and Diagnosis of *Sarcoptes scabiei* var. *hominis* Infection

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Background: While microscopy (MS) evaluation of skin scrapings has a 100% positive predictive value and specificity by definition for scabies diagnosis, it has low sensitivity. Dermoscopy (DS) has not yet been widely accepted for diagnosis, and long-term clinician training is required. **Objective:** To evaluate the diagnostic validity of cytochrome *c* oxidase subunit 1 (cox1) gene nested polymerase chain reaction (PCR) as an adjunctive method for diagnosing scabies. **Methods:** This was a prospective, single institution study, conducted on a total of 302 skin lesions from 50 patients suspected of scabies at Kangdong Sacred Heart Hospital in Seoul, Korea. DS, MS, and cox1 nested PCR were performed on all patients. **Results:** Of the 302 lesions, 145 (48.0%) were obtained at first visit and 157 (52.0%) were identified in the course of follow-up visits after treatment. For all lesions, DS and MS sensitivity levels were 55.9% (73/136) and 55.2% (75/136), respectively, with cox1 gene nested PCR considered as 100%. The results of DS and MS identification showed no difference between each other and showed significant difference from that of cox1 gene nested PCR. **Conclusion:** Nested PCR detecting cox1 may be prospectively used to comprehensively diagnose lesions of scabies in clinical practice. **(Ann Dermatol 33(5) 419∼424, 2021)**

-Keywords-
Clinical laboratory techniques, Dermoscopy, Polymerase chain reaction, *Sarcoptes scabiei*, Scabies, Skin diseases, infectious

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INTRODUCTION

Scabies is a disease caused the mite *Sarcoptes scabiei* var. *hominis*. Infestation is characterized by severe nocturnal pruritus, high contagiousness, and no response to general anti-inflammatory treatment. It is estimated that 300 million cases of scabies occur every year worldwide and is most common in tropical or subtropical developing countries in association with young children, poor hygiene, homelessness, crowdedness, and poverty[1]. In industrialized countries, scabies is most common in elderly and immunocompromised individuals and causes significant public health issues due to outbreaks in long-term care facilities, schools, military barracks, and hospitals. For example, an estimated incidence of 233 to 470 per 100,000 person-years was reported in a national study conducted from 1994 to 2003 in England[2].

Scabies diagnosis is typically made by skin scraping at an appropriate site and confirming mites, eggs, or feces by microscopy (MS), which is regarded as the gold standard for diagnosis. While microscopic examination of skin scrapings has 100% positive predictive value and specificity by definition, it has a low sensitivity, which further varies ac-

cording to the quality and quantity of the skin scrapings received[3]. While dermatologists commonly use a 10× pocket handheld dermoscope to differentiate benign pigmentary lesions and/or melanoma, dermoscopy (DS) is also useful to diagnose scabies *in vivo* by observing a characteristic triangular structure with a following burrow structure that has been described as resembling a jetliner with its condensation trail[4]. Dupuy et al.[5] reported that sensitivity of DS is not inferior to microscopic examination of skin scrapings. DS-guided skin scraping with microscopic examination (DSGSS-ME) can be used to assess treatment efficacy in addition to diagnosing scabies[6]. However, use of a dermoscope has not yet been widely accepted as a routine procedure for scabies diagnosis, as long-term clinical training is needed to avoid confusing artefacts with a positive diagnosis[7].

Wong et al.[8] developed a conventional polymerase chain reaction (PCR) amplification to detect the cytochrome c oxidase subunit 1 (cox1) gene of *S. scabiei* as a useful tool for diagnosing scabies, and Hahm et al.[9] introduced a cox1 nested PCR method that was found to be more sensitive than the protocol of Wong et al[8]. However, these studies have disadvantages in that they did not present results of individual lesions because they examined multiple sites in each patient and judged them positive if only one of the samples was positive. Furthermore, since most of these studies were performed only at the first diagnosis, no results were available for diagnosis at the time of follow-up observation.

Therefore, we designed this study to present the characteristics of individual scabies lesions by examining each lesion with DS, MS, and cox1 nested PCR. Each test was conducted, and results were recorded at the both the first visit and during follow-up examinations.

MATERIALS AND METHODS

Study population and samples

This study was performed at Hallym University Kangdong Sacred Heart Hospital, Korea, between August 2018 and February 2019. Skin scraping samples were prospectively collected from patients suspected of clinical scabies, pruritus for a long time that did not respond to usual treatment, characteristic papules and nodules, or contact history with scabies patients. Written informed consent was obtained from all patients and their parents and guardians if necessary. This study was approved by the Institutional Review Board of Kangdong Sacred Heart Hospital (IRB no. 2018-07-004-001).

DS inspection and MS examination were performed as previously described by Park et al[7]. On DS, a character-

istic structure described as above was considered positive. The scrapings of this structure which were indicated 'dermoscopy-positive (DS+)' sites, and scrapings without it which were designated as 'dermoscopy-negative (DS−)' sites were examined under the microscope at 100× magnification by a dermatologist with more than 5 years of experience in both DS and MS identification of scabies. The samples were termed 'microscopy positive (MS+)' when at least one mite or egg was seen under the microscope and 'microscopy negative (MS−)' when nothing was found. Each sample was placed in a sterile microcentrifuge tube and stored at −80°C for cox1 nested PCR analysis.

We prescribed patients with 5% permethrin cream. Patients were instructed to apply the cream at 30 g/day from neck-to-toe once or twice weekly. Patients were instructed to visit our clinic after 1 week to undergo the same procedures. Treatment and evaluation were repeated every week until DS− and MS− results were obtained.

DNA extraction from samples

Total DNA was extracted from samples using QIAamp DNA mini kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions with modifications. Samples were lysed with 180 μl buffer ATL and 20 μl proteinase K at 56°C for 1 hour. Then, 200 μl buffer AL was added to the samples and incubated at 70°C for 10 minutes. Then, 200 μl 100% ethanol was added to the samples and DNA was eluted with 60 μl buffer AE after washing out with buffer AW1 and AW2.

Nested PCR amplification and sequencing

Nested PCR was performed using two primer pairs that amplify the outer 322-bp and inner 262-bp fragment of the *cox1* gene of *S. scabiei* var. *hominis*. The primers used were the same as in our previous study[9]. No homologies were found between the primer sequences for *S. scabiei* var. *hominis* and the sequences of the *cox1* genes from *Demodex folliculorum*, *Demodex brevis*, *Dermatophagoides farinae*, *Dermatophagoides pteronyssinus*, *Microsporum canis*, or *Trichophyton rubrum* as available in the National Center for Biotechnology Information (NCBI) nucleotide sequence database.

The PCR program was the same as described in the previous study[9]. A 4 μl aliquot of each amplified product was resolved on a 1.7% (w/v) agarose gel with a 100-bp DNA ladder in parallel, and electrophoresed in 1x Tris-acetate-EDTA buffer (BIOFACT, Dajeon, Korea) at 120V for 40 minutes. The gel was stained with a fluorescent dye and photographed under ultraviolet light illumination. Positive and negative controls were paralleled in each PCR run. The positive control included scraping samples

from patients with microscopically-proved scabies and the negative controls included scrapings from patients with other dermatological diseases, such as atopic dermatitis, allergic contact dermatitis, demodecidosis, etc. DNA extracted from house dust mites (*D. farinae* and *D. pteronyssinus*; offered by the Arthropods of Medical Importance Resource Bank, Yonsei University, Seoul, Korea) and pure cultures of dermatophytes (*M. canis* and *T. rubrum*; clinical isolates donated by Professor Jong-Soo Choi, Yeungnam University, Daegu, Korea) were also used to test specificity of the cox1 nested PCR primers. Standard precautions were taken to prevent PCR contamination, and no false-positive results were obtained for the negative controls.

For sequencing, 1 μl cleaned nested PCR product, 4 μl terminator ready reaction, 1 μl 10 pM primer, and 4 μl sterile water were mixed and subjected to PCR under the following conditions: 30 cycles of 96°C for 10 seconds, 50°C for 5 seconds, and 60°C for 4 minutes. Both strands of the PCR products were mixed with 15 μl loading buffer, highly deionized (Hi-Di) formamide, and then sequenced using an ABI PRISM 3730xl DNA analyzer according to the manufacturer's instructions. The DNA sequences obtained in this study were analyzed using a BLASTn search (megaBLAST) against highly similar sequences in the online nonredundant nucleotide collection (nr/nt) database of NCBI to confirm product identity.

Statistical analysis

The Student's t-test and χ^2-test were utilized to analyze patient demographics. McNemar's test and Cohen's κ were used to compare the outcomes of DS, MS, and cox1 nested PCR. The 95% confidence interval (95% CI) of the difference between two groups was calculated using a Student's t-test. Non-inferiority margin was estimated as 10 percentage points. Statistical analyses were performed using IBM SPSS software (version 24.0; IBM Corp., Armonk, NY, USA) and a *p*-value less than 0.05 was considered statistically significant.

Table 1. Demographics based on dermoscopic findings

| Variable | Results of dermoscopy | | *p*-value |
	Positive site (n=86)	Negative site (n=216)	
Mean age (yr)	54.8±25.2	54.4±22.0	0.89
Male	35	101	
Female	51	115	
Male:female	1:1.46	1:1.14	0.34

Values are presented as mean±standard deviation or number only.

Nucleotide sequence accession numbers

The sequences obtained in this study were deposited in GenBank under accession numbers MK609346~MK609481 and showed 98% similarity to the reference nucleotide sequence of the *S. scabiei* var. *hominis cox1* gene (GenBank accession number AY493388.1).

RESULTS

From August 2018 to February 2019, a total of 50 patients suspected of having scabies were enrolled and underwent DS, MS, and cox1 nested PCR. The mean age of patients was 54.5±22.9 years (range, 1~86 years), and the male to female ratio was 1:1. When dividing by site, a total of 86 sites were positive and 216 were negative for DS. There was no statistically significant difference in age (*p*=0.89) and sex ratios (*p*=0.34) between the DS+ and DS− sites (Table 1). The DS, MS, and cox1 nested PCR tests were performed on a total of 302 skin lesions in these patients and the results were recorded, respectively (Table 2). A total of 145 samples were obtained at the first visit (at diagnosis) and 157 were obtained in the course of follow-up visits after treatment. Assuming cox1 nested PCR as the standard criterion, the sensitivities of DS and MS at first visit were 64.6% (51/79; 95% CI, 53.0%~75.0%) and 62.0% (49/79; 95% CI, 50.4%~72.7%), respectively, which were higher than 43.9% (25/57; 95% CI, 30.74%~57.64%) and 45.6% (26/57; 95% CI, 32.36%~59.34%) during follow-up. For all lesions, sensitivities of DS and MS were 55.9% (76/136; 95% CI, 47.1%~64.4%) and 55.2% (75/136; 95% CI, 46.4%~63.7%), respectively, using the cox1 nested PCR as 100% (95% CI, 98.8%~

Table 2. Results of MS and cytochrome *c* oxidase subunit 1 (cox1) nested polymerase chain reaction (PCR) according to DS findings

| Variable | DS+ | | DS− | | Sum |
	MS+	MS−	MS+	MS−	
First visit					
nPCR+	48	8	6	22	79
nPCR−	3	3	2	58	66
Follow-up visit					
nPCR+	19	6	7	25	57
nPCR−	3	1	4	92	100
Total					
nPCR+	62	14	13	47	136
nPCR−	6	4	6	150	166
Sum	68	18	19	197	302
Total	86		216		302

Values are presented as number only. DS: dermoscopy, MS: microscopic examination of skin scraping, nPCR: cox1 nested PCR.

Table 3. Results of DS and MS when cytochrome *c* oxidase subunit 1 (cox1) nested polymerase chain reaction (PCR) is considered as the gold standard

Variable	First visit (%) (95% CI)	Follow-up visit (%) (95% CI)	Total
DS			
Sn	64.56 (52.99~75.00)	43.86 (30.74~57.64)	55.88 (47.12~64.38)
Sp	90.91 (81.26~96.59)	96.00 (90.07~98.90)	93.98 (89.20~97.07)
PPV	89.47 (78.48~96.04)	86.21 (68.34~96.11)	88.37 (79.65~94.28)
NPV	68.18 (57.39~77.71)	75.00 (66.58~82.23)	72.22 (65.74~78.08)
Acc	76.55 (68.80~83.18)	77.07 (69.70~83.39)	76.82 (71.65~81.46)
MS			
Sn	62.03 (50.41~72.72)	45.61 (32.36~59.34)	55.15 (46.39~63.68)
Sp	92.42 (83.20~97.49)	93.00 (86.11~97.14)	92.77 (87.71~96.21)
PPV	90.74 (79.70~96.92)	78.79 (61.09~91.02)	86.21 (77.15~92.66)
NPV	67.03 (56.39~76.53)	75.00 (66.43~82.34)	71.63 (65.10~77.55)
Acc	75.86 (68.06~82.57)	75.80 (68.33~82.27)	75.83 (70.59~80.55)

CI: confidence interval, DS: dermoscopy, Sn: sensitivity, Sp: specificity, PPV: positive predictive value, NPV: negative predictive value, Acc: accuracy, MS: microscopic examination of skin scraping.

100%; Table 3). There was no statistically significant difference between the outcomes of DS and MS ($p = 1.00$; Table 4). However, the results of DS and MS were significantly different from that of cox1 nested PCR ($p < 0.001$, $p < 0.001$, respectively; Table 4). These differences were more pronounced during follow-up. Sensitivity of MS performed on samples from DS+ sites was 81.6% (62/76; 95% CI, 71.0%~89.6%), and there was no statistically significant difference between the results of MS and cox1 nested PCR in samples from DS+ sites ($p = 0.12$). Sensitivity of MS from DS− sites was 21.7% (13/60; 95% CI, 12.1%~34.2%), and there was a statistically significant difference between the results of MS and cox1 nested PCR ($p < 0.001$). If the non-inferiority margin was regarded as 10 percent points ($-\Delta = -0.1$), the 95% CI of difference between MS and cox1 nested PCR in DS− sites was lower than -0.1 (-0.44 to -0.29) and statistically significant ($p < 0.05$). The positive rates of cox1 nested PCR were 88.4% (76/86; 95% CI, 79.7%~94.3%) in samples from DS+ sites and 27.8% (60/216; 95% CI, 21.9%~34.7%) from DS− sites. The 95% CI of difference between them was lower than -0.1 (-0.71 to -0.50) and statistically significant ($p < 0.05$). Similarly, sensitivity of DS performed on samples from MS+ sites was 82.7% (62/75; 95% CI, 71.8%~90.3%), and there was no statistically significant difference between the results of DS and cox1 nested PCR in samples from MS+ sites. Sensitivity of DS performed on samples from MS− sites was 22.6% (14/62; 95% CI, 12.6%~35.3%), and there was a statistically significant difference between the results of DS and cox1 nested PCR. The positive rates of cox1 nested PCR were 86.2% (75/87; 95% CI, 78.4%~91.7%) in samples from MS+ sites and 28.8% (62/215; 95% CI, 24.1%~34.8%) from

Table 4. Statistical analyses among procedures

Lesion	Methods	McNemar	κ	$-\Delta$
All lesions	DS vs. MS	1.00	0.70	−0.08, 0.07
	DS vs. nPCR	<0.001*	0.51	−0.24, −0.09
	MS vs. nPCR	<0.001*	0.49	−0.24, −0.09
DS+ lesions	MS vs. nPCR	0.12	0.16	−0.20, 0.02
DS− lesions	MS vs. nPCR	<0.001*	0.23	−0.44, −0.29[†]
MS+ lesions	DS vs. nPCR	0.17	0.26	−0.34, 0.20
MS− lesions	DS vs. nPCR	<0.001*	0.25	0.13, 0.27

DS: dermoscopy, MS: microscopic examination of skin scraping, nPCR: cytochrome *c* oxidase subunit 1 nested polymerase chain reaction, Δ: 95% confidence interval of the difference. *$p < 0.05$; [†]$-\Delta < -0.1$.

MS− sites.

By analyzing the data for each patient collectively, the sensitivities of DS and MS were 78.8% (26/33; 95% CI, 61.1%~91.0%) and 75.7% (25/33; 95% CI, 57.7%~88.9%), respectively, with the cox1 nested PCR is considered as the gold standard.

DISCUSSION

Dupuy et al.[5] conducted *in vivo* DS mite identifications and *ex vivo* MS examinations of skin scrapings for 238 patients. They reported that sensitivities were 91% for DS and 90% for MS ($p = 0.005$ for non-inferiority), and specificities were 86% for DS and 100% for MS; however, this study did not present the test results of individual lesions because scabies was diagnosed with only one positive result despite the fact that they examined all skin lesions of a patient.

Wong et al.[8] performed MS and conventional cox1 PCR on 100 skin scraping specimens collected from 29 patients. Sensitivity of MS was 58.6% (17/29) if the cox1 PCR was considered the diagnostic standard. In our study, the detection rate of scabies by nested PCR in patients who were MS− was 28.4% (61/215), which was similar to the outcome of our previous study (25.7%, 9/35), but was higher than conventional PCR (14.5%, 12/83) in the Wong et al.[8] study. There may be high false-negative rates in MS in the Wong et al.[8] study because they did not include DS and the efficacy of a single round PCR assay was overestimated. A previous study used DSGSS-ME and cox1 nested PCR to test samples from 63 patients[9]. When the cox1 nested PCR sensitivity was considered 100% (95% CI, 90.5%∼100%), then the sensitivity of MS was 75.7% (95% CI, 58.8%∼88.2%) and the difference between them was significant ($p = 0.004$), which is consistent with our study (78.79% for DS and 75.76% for MS). However, this study also fails to present the test results of individual lesions.

Therefore, we performed DS individually on a total of 302 skin lesions from 50 patients suspected of having scabies, and MS and the cox1 nested PCR for samples obtained from each lesion in our study. For all lesions, sensitivities of DS and MS were 55.9% (95% CI, 47.1%∼64.4%) and 55.2% (95% CI, 46.4%∼63.7%), respectively, when the cox1 nested PCR was considered 100% (95% CI, 98.8%∼100%). The outcomes of DS and MS were not statistically different ($p = 1.00$), consistent with those of Dupuy et al[5]. The results of DS and MS were significantly different from that of cox1 nested PCR ($p < 0.001$, $p < 0.001$, respectively), consistent with those of our previous study ($p = 0.004$)[9]. There was a statistically significant difference ($p < 0.001$) between MS and cox1 nested PCR, and inferiority ($-\varDelta = -0.44$ to $-0.29 < -0.1$) of MS to cox1 nested PCR was found only in DS− sites. We therefore concluded that MS is not inferior to cox1 nested PCR in DS+ regions, but inferior in DS− sites. The 95% CI of the difference between the positive rates of the cox1 nested PCR of DS+ and DS− sites was less than −0.1 (−0.71 to −0.50); therefore, more sensitive results can be obtained using DS even if cox1 nested PCR is performed. The sensitivity of MS in samples from DS− sites was only 21.7% (95% CI, 12.1%∼34.2%), indicating that MS could be expected when examining any lesion without DS. The specificity and positive predictive values of MS are theoretically 100%, but they were 92.8% (95% CI, 87.7%∼96.2%) and 86.2% (95% CI, 77.2%∼92.7%), respectively, in this study. It is possible that eggs or mite bodies observed in the microscopic examination were false-positive, or there might have been errors in transferring the sample on the glass slide to a microcentrifuge tube.

For the 145 samples obtained at first diagnosis, the false negative rates of DS and MS were 35.4% (28/79; 95% CI, 25.0%∼47.0%) and 38.0% (30/79; 95% CI, 27.3%∼49.6%), and the false positive rates were 9.1% (6/66; 95% CI, 3.4%∼18.7%) and 7.6% (5/66; 95% CI, 2.51%∼16.80%), respectively. At follow-up, the false negative rates were 56.1% (32/57; 95% CI, 42.4%∼69.3%) and 54.4% (31/57; 95% CI, 40.7%∼67.6%) and the false positive rates were 4.0% (4/100; 95% CI, 1.1%∼9.9%) and 7.0% (7/100; 95% CI, 2.9%∼13.9%), respectively. The reason for the increase in the false negative rate at follow-up examinations may be due to the fact that dead mite bodies and DNA still remain in the epidermis even though the signs on the skin surface, as indicated by mites, were reduced after first after treatment.

In conclusion, the cox1 nested PCR test can be utilized as an adjunctive method for diagnosing scabies, and it is more sensitive and accurate than DS or MS alone at first diagnosis as well as at follow-up examinations. Furthermore, it might be better to make use of DS to increase the sensitivity of diagnosis even if cox1 nested PCR is performed.

ACKNOWLEDGMENT

CONFLICTS OF INTEREST

The authors have nothing to disclose.

FUNDING SOURCE

DATA SHARING STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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