Skin scrapings versus standardized skin surface biopsy to detect *Demodex* mites in patients with facial erythema of uncertain cause - a comparative study

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**ABSTRACT**

**Background:** Standardized skin surface biopsy (SSSB) is considered to be the gold standard technique to evaluate the density of *Demodex* mites for the diagnosis of demodicidosis. Potassium hydroxide (KOH) preparation of skin scrapings is a much simpler procedure that can be used to detect pathogens in the superficial skin. **Objective:** To evaluate the reliability of potassium hydroxide preparation of skin scrapings as compared to the standard skin biopsy technique with regard to capacity to detect *Demodex* mites, time consumed and technician satisfaction. **Methods:** One hundred outpatients presenting with facial erythema of uncertain cause were enrolled. Standardized skin surface biopsy and potassium hydroxide preparation of skin scrapings were undertaken in adjacent areas on the patients' right cheek. **Limitation:** Patients with normal facial skin were excluded from the study. **Results:** The accuracy of *Demodex* mite detection by potassium hydroxide preparation of skin-scrapings when compared to the standard procedure is 82%. The sensitivity, specificity, positive and negative predictive values of this method are 75%, 84.2%, 60% and 91.43%, respectively. There was no statistically significant difference between the standard and skin scraping techniques ($P = 0.238$) with regard to mite detection. Mean preparation time while using the skin scraping technique was 6 times less than that of the standard technique. For interpretation also, skin scraping technique (3.6 min) consumed much less time than the biopsy technique (9.8 min). Moreover, experienced technicians were more satisfied with skin scraping. **Conclusion:** Potassium hydroxide preparation of skin scrapings is an effective, time saving and practical technique to detect *Demodex* mites with accuracy comparable to the standard biopsy method.

**Key words:** *Demodex* mites, demodicidosis, skin scraping technique, standardized skin surface biopsy

**INTRODUCTION**

*Demodex* mites are common ectoparasites of mammals that are found in or near hair follicles. These mites are 0.3–0.4 mm in length and in the anterior third of their bodies, there are four pairs of short legs.[1] There are around 65 species of *Demodex*. Two species, *Demodex folliculorum* and *Demodex brevis*, have been identified from human skin.[2] They feed on skin cells and the cheek is the most frequently and heavily infested facial area.
region.\textsuperscript{[3]} On average, the density of \textit{Demodex} mites has been $\leq 5$ mites/cm$^2$ in normal skin. They may cause skin disease when they are present in excessive numbers in a pilosebaceous unit. A large amount of \textit{Demodex} mites can activate an inflammatory response causing folliculitis and dermatitis. That increased mite density activates inflammation is suggested by the correlation of mite density and severity of facial erythema.\textsuperscript{[4]} Thus, evaluation of the density of \textit{Demodex} mites is useful for diagnosis as well as appropriate, timely and effective management of patients presenting with facial erythema of uncertain cause. Standardized skin surface biopsy (SSSB) technique with cyanoacrylic adhesion has been accepted as the gold standard to evaluate the density of \textit{Demodex} mites.\textsuperscript{[5,6]} This procedure is considered to be non-invasive. Nevertheless, it consists of many steps; each step is complicated and thus takes a long time. Moreover, the actual density and number of \textit{Demodex} mites is difficult to assess by this method due to tight adhesion among groups of \textit{Demodex} mites sticking to each other or to the keratin (Figure 1). Further, cyanoacrylate may cause skin irritation in some patients. On the other hand, potassium hydroxide preparation of skin scrapings is a basic procedure in dermatology that is used to detect superficial skin pathogens such as fungi. This technique is easily performed in a few steps. Our study aimed to compare potassium hydroxide preparation of skin scrapings with the standard technique with regard to three aspects: \textit{Demodex} mite detection, time consumed in preparation and interpretation, and laboratory technicians' satisfaction.

METHODS

We undertook a cross-sectional study of 100 patients presenting at a dermatologic clinic with facial erythema with inconclusive diagnosis, between October 2012 and June 2013. The study was approved by the institutional review board and informed written consent was obtained from patients. All 100 patients were subjected to both standardized superficial skin surface biopsy and potassium hydroxide preparation of skin scrapings on adjacent areas of their right cheek.\textsuperscript{[7]} The next step in both techniques was to examine the sample slides under the microscope for quantitative assessment of mite density per square centimeter. Sample collection and interpretation were both done under the supervision of a doctor. Topical medications or cosmetics were forbidden for one month before these tests were undertaken. Moreover, patients on systemic antibiotics were excluded from this study. Laboratory data, preparation time and results/interpretations were collected and analyzed. In addition, this study

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\caption{The comparison of the microscopic view of standardized skin surface biopsy (SSSB) with skin-scraping techniques on two magnifications as follows: (a and b) original magnifications 40 of SSSB technique and skin-scraping with KOH technique respectively (c and d) original magnifications 100 of SSSB technique and skin-scraping with KOH technique respectively.}
\end{figure}
assessed the opinions of experienced laboratory technicians about both these techniques with the help of questionnaires exploring their satisfaction about the preparation and interpretation processes.

**Standardized skin surface biopsy technique**
Subjects cleaned their faces with gentle cleanser and water before the test. After drying the skin, a drop (about 0.05 ml) of cyanoacrylate glue was applied to an area of 1 cm² at one end of the slide and the glue was spread out to a homogeneous thickness and then the slide was put against the right cheek. The slide was left in place for around 5 minutes until the cyanoacrylate changed in consistency and then gently removed.

**Skin scraping with potassium hydroxide preparation**
Subjects cleaned their faces with gentle cleanser and water before the test. After the skin dried, a sterile sharp blade was scraped on a one-square centimeter area of the right cheek. The accumulated debris or scale was collected on a glass slide, a drop of 20% potassium hydroxide was added and the coverslip was applied.

**Questionnaires**
Laboratory technicians were administered questionnaires dealing with both standardized skin surface biopsy as well as skin scraping with potassium hydroxide preparation. The questions pertained to four aspects including (1) steps of preparation, (2) preparation and interpretation time, (3) difficulties in interpretation and (4) technician and patient satisfaction. Patient discomfort during sample collection was also carefully monitored by the technicians.

**Statistical analysis**
All statistical procedures were performed using statistical package for the social sciences (SPSS, Inc., Chicago, IL, USA) version 18. McNemar’s test was used to make comparisons between the two techniques. A P value less than or equal to 0.05 was considered significant.

**RESULTS**
In 100 patients with facial erythema of uncertain cause, the accuracy of potassium hydroxide preparation of skin scrapings in detection of Demodex mites was 82%, when compared to the standardized skin surface biopsy technique. The sensitivity, specificity, positive predictive value and negative predictive value of the scraping method were consequently 75%, 84.2%, 60% and 91.4%. There was no statistically significant difference (P = 0.238) [Table 1] between the two studied techniques with regard to detection of Demodex mites. On the other hand, the biopsy technique was found to be much more time-consuming for preparation and interpretation. The mean preparation time involved in the scraping technique (1.1 min) was 6 times less than that of the biopsy technique (6.7 min). Moreover, the mean time needed for interpretation of scrapings (3.6 min, standard deviation [SD] =0.47) was also 2 times less than that required for biopsy interpretation (9.8 min, SD = 0.56). The analysis of the questionnaires showed that technicians universally agreed that the skin scraping method was less time consuming and easier to perform and interpret than the biopsy technique. Technicians were more satisfied with the skin scraping technique, and 80% of technicians observed that patients had less anxiety and pain during the scraping procedure as compared to the biopsy method.

**DISCUSSION**
Askin and Seçkin found that standardized superficial skin biopsy was the best technique for detecting Demodex mite density.[8] However, steps of preparation and time and ease of interpretation of this technique were not assessed in their study which was conducted with fewer patients than our study. In our study, 100 patients with facial erythema of uncertain cause were enrolled. Potassium hydroxide preparation of skin scrapings is an accepted method to detect Demodex mites.[4] For our comparative study, potassium hydroxide preparation of skin scrapings was chosen because this is a basic dermatologic procedure that can be easily performed in under 5 minutes. Comparison with the standard technique demonstrated that the skin scraping technique was able to evaluate the density of Demodex mites effectively and reliably with high accuracy (82%). Moreover, this method has high sensitivity and specificity values (75%, 84.5% respectively) for Demodex mite detection which
supports its ability to correctly identify Demodex mites. The high negative predictive value (91.4%), indicates that the possibility of false negative outcomes is small therefore, demodicidosis can be excluded when there is a negative result with the skin scraping technique (Demodex mite density < 5 mites/cm²). Unlike another earlier comparative study,[8] we also focussed on preparation and interpretation time of the skin scraping technique and recorded the opinion of experienced laboratory technicians. Our results demonstrate that the skin scraping technique is time saving. Experienced laboratory technicians were unanimous in their opinion that the skin scraping technique is more practical and satisfactory than the standard technique. However, one limitation is that this study was conducted in a tertiary care hospital where procedures are performed by experienced technicians. Moreover, patients with normal facial skin were excluded from the study.

CONCLUSION

Potassium hydroxide preparation of skin scrapings is an effective technique to diagnose demodicidosis with a high degree of accuracy that is comparable to the standard biopsy procedure. The skin scraping method is reliable, time saving and practical. It will thus enhance general practitioners’ ability to identify and manage demodicidosis.

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Conflicts of interest
There are no conflicts of interest.

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