Detection of *Demodex* species in acne vulgaris patients using different diagnostic methods

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ABSTRACT:

Demodex is a permanent ectoparasite of the pilo-sebaceous units, related mainly to the facial region. Two species are specific for humans, *Demodex folliculorum* and *D. brevis*.Some of these species appear to be associated with variable skin disorders, yet controversy persists concerning this issue. Some reports indicate a connection between acne vulgaris and demodicosis.

Aim of the work: to evaluate variable methods to expose *Demodex* mites.

Patients and Methods : A total of 60 cases enrolled in the study were divided into 2 categories; 30 patients with acne vulgaris and 30 healthy volunteers. Samples were collected by three different methods: deep skin scraping, hair epilation and Scotch adhesive tape method.

Results: According to deep scraping method that successfully diagnosed all positive cases (14 cases), occurrence of *Demodex* mites in patient with acne was significantly higher than control (40%&6.7% respectively). All detected mites were *D. folliculorum* in acne cases and *D. brevis* in control group**.** Regarding hair epilation method, one case only of *Demodex* mites was identified in patients with acne. In contrast,no mites were detected by scotch tape method within the two study groups.

Conclusions: Our result found that hair epilation and the ordinary adhesive tape methods were inferior, compared to the deep skin scraping method in diagnosing demodicosis that showed considerably higher occurrence in acne group. Further studies are recommended to explore risk factors behind such occasion.

KEYWORDS:: *Demodex*, acne, deep scraping.

INTRODUCTION:

Demodex is a genus of minute mites that live in or near pilosebaceous units of mammals. Their name, *Demodex* is originated from the Greek “demo” for lard and “dex” for boring worm. This is related to their worm-like morphology and habitat in sebaceous follicles which occasionally resulting in some skin disorders, including acne vulgaris **[1].**Acne vulgaris is a long-term skin disease that arises when hair follicles are blocked by dead epithelial cells and sebum. It is typified by black heads, whiteheads, pimples and possible scarring **[2][3].** It affects areas of the skin with high number of sebaceous glands, including the face, upper part of the chest, and the back. Yet the etiology of this

skin disorder is uncertain **[4].** *Demodex* mites may colonize skin of any individuals without causing any clinical symptoms, yet infestation of these mites was reported to be one of the factors associated with acne vulgaris **[5] [6].**In order to discover the possible association between *Demodex* mites and different skin disorders, variable methods are used to assess the presence of *Demodex* mites within or nearby the affected skin areas aided by microscopic examination. These methods involve skin scraping technique, adhesive tapes method, expressed follicular contents, comedone extraction, hair epilation, and skin biopsies**[7].**Generally, choice of the diagnostic methods depends on many variables, including the cost, feasibility of the diagnostic tool beside the technical proficiency **[8].**The main purpose of the present study was to assess the practicability of different diagnostic tools to detect *Demodex* species among acne vulgaris patients.

PATIENTS AND METHODS:

The study was a prospective cross sectional study over the period from July 2018 till October 2018. The study included a total of 60 subjects that were divided into 2categories; 30 patients with acne vulgaris and 30 healthy volunteers.Sample size was calculated using (G power version 3). Minimal sample size of patients was 30 in each group needed to get power level 0.80, alpha level 0.05 and 30.0% as an expected difference in the prevalence of *Demodex* between acne patients and control. Informed consent was obtained from all participants. Patients were enrolled from outpatient clinic at Dermatology Department of Fayoum General Hospital in addition to Dermatology and Leprosy Hospital in Fayoum. Male or female patients diagnosed as acne vulgaris by aqualified dermatology specialist and welling to participate in the study were eligible to join the study. Patients who did not have a comedone as the special sign of acne vulgaris, patients suffering from skin disorders other than acne vulgaris and patients recently under anti-parasitic therapy were excluded.

Concerning diagnostic methods, at least three samples were prepared for each method. Deep skin scraping was applied according to **The University of Bristol, (2017) [9].** Suitable sites on the individuals' skin were chosen for sampling and then one drop of liquid paraffin was spread on about 1.5 x 1.5 cm of these areas. The skin was then pinched between thumb and index finger and gently rolled, and then by using a blade scraping was continued until there was slight capillary ooze. The scraped materials then were transferred and gently mixed with a drop of liquid paraffin on a microscopic slide. A cover slip was placed over the smear and then the slide was microscopically examined.For hair epilation, a forceps was used to epilate facial hairs from 3 sites (eyebrows, mustache and beard). The epilated hairs were placed on a glass slide and gently mixed with a drop of liquid paraffin, covered with a cover slip then microscopically examined. Sample collection by Scotch adhesive tape method was done according to **Lacey et al. (2015)[1]**with modification in which a 5cm length of commercially available scotch adhesive tape was applied to the 3 different sites of the forehead and left for about 30 minutes. Then the adhesive tapes were removed from the skin and directly applied to microscopic slides with the adhesive side downwards facing the glass of the slide, then microscopically examined.

The samples were systematically screened under a low microscope magnification power of x40 and x100 to detect any parasitic stages related to *Demodex* mites. Mites were identified by the characteristic morphological details and by morphometric analysis using an eyepiece (ocular) micrometer that has been calibrated against a stage micrometer in combination with a specific objective lens.The number of mites per 1 cm2 was counted and diagnosis was considered if the number of mites on the skin was more than 5 mites /cm2.The collected data were organized, tabulated and statistically analyzed using SPSS software statistical computer package version 22 (SPSS Inc, USA). Data were presented as frequencies and percentages, chi square ($\chi 2$) or Fischer exact test, when appropriate, was used as a test of significance. For interpretation of results of tests of significance, significance was adopted at $P \leq 0.05$.

RESULTS:

Three methods were applied in this study to diagnose demodicosis in 2 categories of subjects. The first group was related to patients suffered from acne vulgaris. The second group was a control healthy cases which did not experience any dermatological problems. Microscopic examination for the 2 involved groups revealed positive findings as regard *Demodex* species in (14) samples out of the total 60 collected samples. Twelve of them were from group of cases complained of acne vulgaris and the remaining 2 positive cases were from the control group.*Demodex folliculorum* mites were identified by morphologically features (figure1) in 12 positive samples extracted from acne cases and the quantity was more than 5 mites /cm2. While *Demodex brevis* mites were observed in 2 samples only from control group with fewer quantity, less than 5 mites /cm2.

Regarding the diagnostic methods, all positive cases were detected by deep skin scrapping method and one positive case was discovered by hair epilation method, while no positive case detected by scotch adhesive tape method. According to the deep scraping method, occurrence of *Demodex* mites in patient with acne and control was 40% & 6.7% respectively, which was a statistically significant, (p=0.002) (table 1, figure 3). All detected mites in acne cases were *D. folliculorum* with all their life cycle stages (figure 2), while *D. brevis* adult mites were detected exclusively in the 2 samples related to the control group.Regarding hair epilation method, one case only of *D. folliculorum* mites was identified in patients with acne. On the other hand, no mites were detected by scotch adhesive tape method within the 2 study groups.

DISCUSSION:

According to the deep scraping method used in this work, occurrence of *Demodex* mites in patient with acne and control was 40& 6.7% respectively. All the detected mites in acne cases were *D. folliculorum* and the 2 positive cases in the control group were related to *D. brevis*. All the positive samples were identified by deep scraping method. Hair epilation method detected one case only of *Demodex* mite in patient with acne, while scotch tape method failed to detect any positive sample. In general, there are variable diagnostic approaches to detect *Demodex* mites. One of these approaches is the superficial skin surface biopsy (SSSB) which is commonly used and considered as a gold standard method by some investigators to diagnose dermatological illness generally **[10].** In this method (SSSB), high bond glue (cyanoacrylate) is basically applied on the

skin for few minutes in order to perform this diagnostic tool. By this technique, the superficial part of the horny layer was collected, beside some of the follicle contents. Therefore, SSSB detects the few mites present on the skin surface and some mites in the pilosebaceous duct and sebaceous gland **[11].**In the present work, SSSB was not among the chosen diagnostic tools to diagnose *Demodex* mites. One of the reasons behind eliminating SSSB method in this work was related to the reported cautionary concerning toxicity of cyanoacrylic. These toxicity including skin irritation, contact dermatitis, allergic skin reaction and flu-like symptoms. Besides, the sporadic mites which may penetrate into the dermis are not usually detected by this method **[12].**

The present study preferred the deep skin scraping diagnostic tool and this was in agreement with **Bunyaratavej *et al.* (2016) [11],** who favored the technique and recommended its use. The authors concluded that, the method is efficient, reliable, feasible and also time saving diagnostic tool to identify *Demodex* mites. The previous authors supported our choice and confirmed the finding of **Richard *et al.* (2012)[12],** concerning deep skin scraping, being an effective technique to diagnose demodicosis with a high degree of accuracy compared to SSSB procedure.

Regarding, adhesive tape stripping of the skin, **Lacey *et al*. (2015)[1]**, suggested its use as it can provides a sample from the skin surface and materials from the follicular orifices. The authors mentioned that, the method can be useful to detect mites on the skin surface as the tape can be left on the skin for several hours, thus capturing emerging and migrating mites. The adhesive tape stripping method was used in this study, but unfortunately did not obtain any positive result. Three adhesive tapes were left on the three different areas of the skin for about 30 minutes. This relatively short time which was operated in this work may explain part of failure in this technique. As a modification of superficial skin surface biopsy, cellophane tape method (CTP) was performed by **Huang and Yang (2006)[13]** with only one difference between the two detection methods. CTP uses cellophane tape and acrylate glue, whereas SSSB uses slide and cyanoacrylate glue. The previous investigators acknowledged the detection rate of *Demodex* using CTP. However, the previous investigators criticized its demanding conditions. They also documented the variable challenging factors affecting success or failure of the technique which include checkpoint area, times of examination, glutinosity of the tape, and whether or not the operational steps are correct.

Regarding other diagnostic technique, the traditional skin biopsy method was not done in the current work to avoid use of sedation or anesthesia which frequently needed prior to the technical operation. In addition, it is difficult to find *Demodex* mites in skin biopsy samples because in histological preparations the mite shrinks rapidly and transforms into a translucent "ghost" sac of chitin **[14].**Moreover, the main disadvantage of this method is related to the skin trauma with the formation of skin scar. Besides, this method cannot be used to examine a large area of the skin. This is why **Kose and Borlu (2019)[15]** criticized this method and did not recommend it, being an unnecessary and invasive diagnostic tool for detecting human demodicosis. While by using deep scraping technique, the mites are found intact, alive, movable, and are easy to be totally identified.

From the result of this study, it is concluded that cautious deep skin scraping method for at least 3 smears is a feasible method to detect all the stages of *Demodex* mites in the superficial skin as well as in the deeper skin portions without need of any toxic reagents.Hair epilation and the ordinary adhesive tape methods were inferior, compared to the deep skin scraping method in diagnosing demodicosis.Researches concerning possible solutions to overwhelm the limitations of adhesive tape method are highly recommended. Further studies on larger scales are necessary to explore the definite nature of the relationship between *D. folliculorum* and acne vulgaris.

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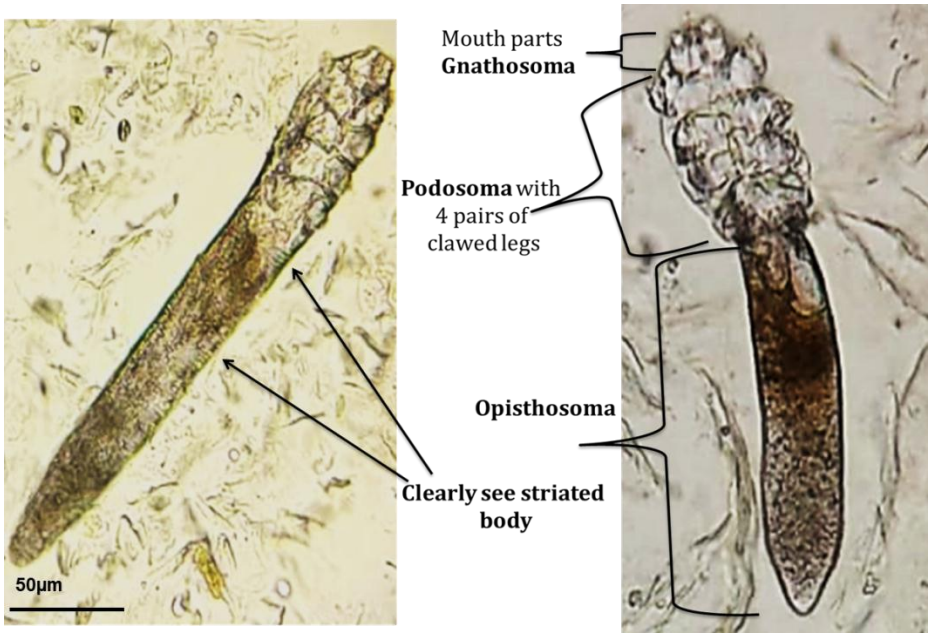

Figure(1): Photographs show ventral view of adult *Demodex folliculorum* mite, with the unique morphological features.

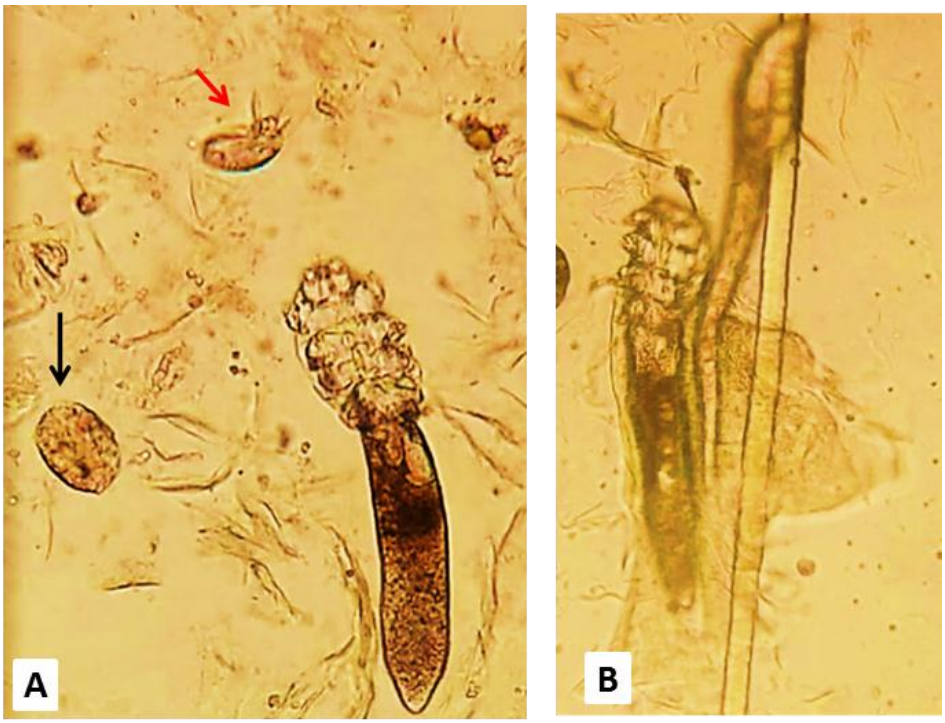

Figure (2): A:*Demodex folliculorum* mite with egg appears beside it (black arrow), adjacent to another egg that possibly starting hatching (red arrow). **B:** Nymph of *D. folliculorum* identified by skin scrapping method.

Table (1): Table signifies the occurrence of *Demodex* mites within the study groups.

	Acne group (N=30)		**Control group (N=30)**		**P-value**
	N	**%**	**N**	**%**	
Deep scraping method					
Yes	12	40.0%	2	6.7%	**0.002***
No	18	60.0%	28	93.3%	
Hair epilation method					
Yes	1	3.33%	0	0.0%	----
No	29	96.66%	30	100.0%	
Scotch tape method					
Yes	0	0.0%	0	0.0%	----
No	30	100.0%	30	100.0%	

*Significant

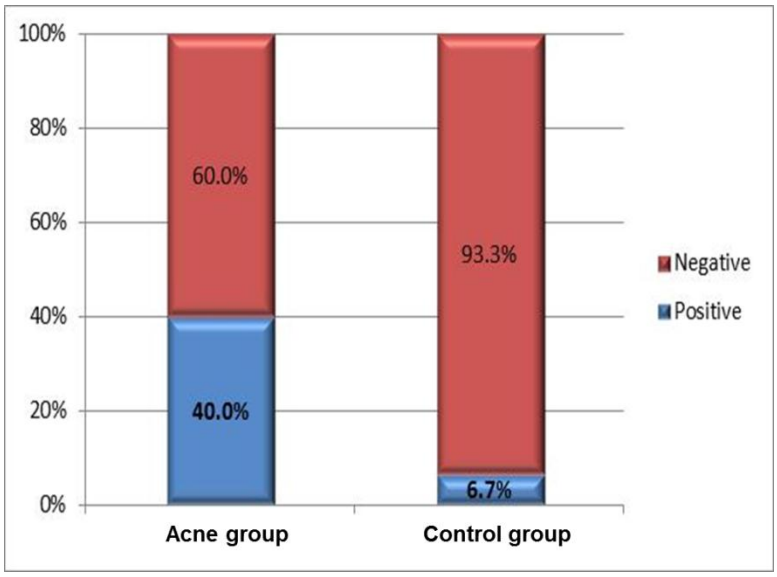

Figure (3): Bar chart expresses the occurrence of *Demodex* mites within the study groups.