



Comparative Evaluation of Methods of Diagnostics of Demodicosis

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Abstract

The article lists the main methods for diagnosing demodicosis. A study was carried out to compare two methods to determine the Demodex mites light microscopy of skin scrapings and confocal laser scanning *in vivo* microscopy. The advantage of the latter method for diagnosing demodicosis in patients with acne and rosacea is proved.

Keywords

Acne; Rosacea; Demodicosis; Demodex mites; Confocal laser scanning *in vivo* microscopy; Demodex folliculorum longus; Demodex folliculorum brevis

Introduction

Demodicosis is a disease from the group of acarosis, caused by the parasitization of a conditionally pathogenic mite - Demodex folliculorum longus and Demodex brevis. Mites measuring 0.2 - 0.5 mm live in the ducts of sebaceous and meibomian glands, in the mouths of human and mammalian hair follicles.

Despite the fact that human skin mites are part of the microflora of the skin and in the vast majority of people do not cause any clinical manifestations and complaints, nevertheless, they support the acuteness of the inflammatory process with such dermatoses as acne, rosacea, seborrheic dermatitis, perioral dermatitis, and also can cause an independent disease [1]. In the presence of Demodex mites, the clinical picture of the disease can acquire a more pronounced character with predominance of papulopustular elements, diffuse erythema, granuloma formation, nodular elements, and macroabscess [1]. Stcherbatchoff, having detected mites in the ciliary follicles of human eyelids, proved the role of the mites in the development of blepharitis and blepharoconjunctivitis [2]. It is interesting that different types of mites cause a different clinical picture, which is associated, presumably, with the size of the mites themselves. When Demodex folliculorum longus is detected, erythema and desquamation of the epithelium are more often observed, while detecting Demodex folliculorum brevis - symmetrical papulopustular elements [3]. Prolonged chronic course of demodicosis is characterized by thickening of the skin, a feeling of contraction, a decrease in elasticity and softness, the presence of serous or blood-purulent crusts.

It is possible to establish the diagnosis of "Demodicosis" only after conducting a laboratory diagnosis using which Demodex mites will be found. The most common method of laboratory diagnosis is based on the compilation of an acarogram by counting larvae, nymphs, eggs and adults. The criterion for mites activity is the number of more than 5 adults, larvae or eggs per cm². When diagnosing demodicosis eyelashes, the rule is the detection of one mite for 2-4 eyelashes. To assess the effectiveness of the therapy, repeat acarograms are done to count the number and determine the activity of mites. The activity of mite-borne invasion can be judged by the change in the number of mites per cm². It is known that during treatment Demodex mites can move to areas untreated with acaricidal agents. In such cases, most often, mites are localized at the edge of the scalp.

Technically, the procedure for detecting the mites is quite simple in execution. Demodex mites can be detected by scraping, when extracting the contents of the sebaceous gland ducts or removing eyelashes and/or eyebrows without damaging the hair follicles. Scraping of the skin is carried out with a disposable scalpel in the places where the Demodex mites accumulates most (forehead, wings of the nose, chin). The test material is placed on a slide with 10% alkaline solution (KOH), covered with a slide and viewed under a small magnification of the microscope. The advantage of the technique lies in the possibility of analysing a large area of damage immediately, as well as extracting mites not only from the surface of the skin, but also directly from the sebaceous glands. However, there is a problem - it is not always possible to detect mites in the depths of the sebaceous glands. The disadvantage is also the traumatization of the epithelium, the relative pain of the procedure and the discomfort of patients after epilation. It should be noted that scraping is not a highly informative method and, with a negative analysis of laboratory research, does not prove the absence of mite-borne invasion [4].

There are other ways of detecting mites, for example, conducting a surface biopsy ("Scotch-probe") [4,5]. A drop of cyanocrylate glue (BF-6, sulfacrylate) is applied to the non-fatty cover slip, and then glued to the affected surface for 1 minute. After removal, a solution of alkali is applied, covered with a cover glass and viewed under a microscope at a small magnification. Modification of the technique is the use of an adhesive tape, 1 cm² in size, which, after removal, is glued to the cover glass by an alkali solution. When removing the cover glass or tape, on their surface remains the surface layer of the epidermis and the contents of the sebaceous glands with the mites that are there. The advantage of the method is its ease of use, however, the traumatization of the epithelium, the difficulty of obtaining material from the wings of the nose, and the incomplete sterility of the preparations obtained are attributed to obvious shortcomings.

A more complex method for diagnosing demodicosis is to perform a skin biopsy followed by histology of the preparations obtained. For this purpose, puncture (punch) or excision (scalpel) method takes a small area of skin; fix it during the day with 10% neutral formalin solution, seal with paraffin and stain with hematoxylin-eosin. Histological examination gives a lot of advantages; in particular, you can completely see the sebaceous gland and the surrounding areas. The main disadvantages of the method include traumatization of the skin with the formation of a scar and the impossibility of examining a large surface of the skin.

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As a diagnostic tool for detecting Demodex mites Segal et al. suggested using a dermatoscope. The method of dermatoscopy allows you to visualize mites on the surface of the skin, as well as enlarged vessels of the skin. However, in this case, also low information content is also noted, since it is impossible to detect mites during localization in the sebaceous glands and in the presence of nodular elements, macroabscesses [6].

Despite the fact that the "gold standard" for the pathomorphological evaluation of normal and affected skin in dermatology is still biopsy with subsequent histological examination, in practical medicine, informative, high-tech and non-invasive diagnostic methods will always be in demand. Such methods include confocal laser scanning *in vivo* microscopy [7].

Marvin Minsky in 1957 patented a "scanning microscope with a two-stage focusing" (the term "confocal" - based on the conjugation of foci). If a mercury or xenon lamp is used as a fluorescent light source in conventional fluorescence microscopes, then in modern confocal microscopes it is a laser. Davidovich was the first to use the laser in confocal microscopy in 1969. As a source of light in modern confocal microscopes, a laser is used to more accurately operate the optical system of the microscope, reduce the number of glare in the images, and improve the focusing of the light beam. A focused laser beam illuminates a specific point of the skin [8]. Due to a certain microscope device, the back focus of the condenser, where the "confocal" diaphragm of the photo-detector is installed, coincides with the front focus of the lens; images are obtained from a very thin layer of the object - "optical sections". The work of the confocal microscope is based primarily on the ability of various structures of the skin to refract laser radiation, thus obtaining images of the layers of the epidermis and the dermis [9] and assess the condition of the skin vessels and the dermis fibres [10]. Confocal laser scanning *in vivo* microscopy is a new method for studying the structure of the skin in the form of pictures of white-grey-black shades. Melanocytes and keratinocytes on the pictures look bright white, air, and serous fluid - black. Confocal laser scanning *in vivo* microscopy allows you to determine the thickness and visualize different layers of the skin. Thus, the method provides additional information on the composition and structure of the skin [11]. In ophthalmology, it is possible to visualize changes in meibomian glands in the form of an enlargement or obstruction, the presence of inflammatory infiltrates, and also to detect Demodex mites [12]. The method of confocal laser scanning *in vivo* microscopy can be equated to histological examination of the skin with the advantage that the study is performed non-invasively [9]. According to different data, the sensitivity of the method is 83-91%, specificity is 95-99% [13-15].

The use of confocal laser scanning *in vivo* microscopy in dermatology is today considered to be one of the most promising methods, despite the fact that it has a number of disadvantages (obtaining relatively surface images up to 200 μm , which limits the possibility of studying deeper layers of the skin, images, the high cost of equipment and its operation and, as a consequence, inaccessibility for a larger number of dermatologists) [10].

In comparison with conventional light microscopy, the advantages of the method are high-contrast images with high resolution, their three-dimensional reconstruction, and digital processing of the data obtained [10]. One of the advantages of the method is the ability to detect and quantify Demodex folliculorum on the facial skin of patients with rosacea and acne by counting mites and follicles per unit

area [16]. Sattler et al., examining the skin of patients with rosacea, described the presence of Demodex in the form of rounded or long conical structures [16]. Kojima et al. demonstrated the use of confocal laser scanning *in vivo* microscopy for the diagnosis of eye demodicosis [17]. The authors managed to find mites in the terminal of the bulb of the eyelashes, inflammatory infiltrates around the meibomian glands and conjunctiva.

Thus, according to the scientific literature, confocal laser scanning *in vivo* microscopy is a non-invasive and rapid method of detecting Demodex mites [18].

Given the relevance of this topic, we conducted a survey of healthy volunteers and patients with acne, rosacea using confocal laser scanning *in vivo* microscopy.

The aim of the study was to evaluate the effectiveness of demodicosis diagnosis in patients with acne, rosacea with the help of confocal laser scanning *in vivo* microscopy.

Materials and Methods

60 patients with acne and rosacea complicated by demodicosis (group I), 60 patients with acne and rosacea, not complicated by demodicosis (group II) and 30 healthy volunteers (group III) were observed. The diagnosis of acne and rosacea was exhibited on the basis of the clinical picture of the diseases.

For the diagnosis of acne, the classification of the European Guidelines for the Treatment of Acne (EU Guidelines group, 2012) [19]:

- Comedonal acne,
- Mild - moderate papulopustular acne,
- Severe papulopustular acne, moderate nodular acne,
- Severe nodular acne, acne conglobate

The diagnosis of rosacea was exposed using the improved classification of the American National Rosacea Society, proposed in 2002, according to which the following rosacea subtypes are distinguished:

- Subtype I - erythematous-telangiectatic,
- Subtype II - papulo-pustular,
- Subtype III - fimatose,
- Subtype IV - ophthalmic [20].

All respondents were examined for Demodex mites by scraping the contents of the sebaceous glands, eyebrow and eyelash epilation. On a VivaScope 1500[®] confocal laser scanning *in vivo* microscope (Lucid Inc., Rochester, NY), the study was conducted at three points (2 cheeks and forehead).

Criteria for inclusion in groups I and II were the presence of patients diagnosed with acne and rosacea; age over 18 years; signing informed consent to participate in the study. Exclusion criteria were associated somatic diseases of severe course or neoplastic nature; the presence of alcohol or drug dependence; lack of desire for the patient to continue research; the occurrence of allergic reactions, as well as the development of pronounced side effects on the background of treatment; pregnancy and lactation. Criteria for inclusion in group III: age over 18; absence of skin diseases and somatic diseases of severe course or neoplastic nature.

The presence of the mites was confirmed by the method of scraping the contents of the sebaceous glands, epilation of the eyebrows and eyelashes. Material was taken with the help of a sterile scarifier from the places of the greatest accumulation of sebaceous glands on the face - forehead, nose, chin, cheeks. The material was placed on a slide in a drop of KOH solution, and then microscopies. The number of mites was calculated as 1 cm². The diagnosis of demodicosis was considered eligible for contamination by skin mites more than 5 per 1 cm², eyelashes more than 4 individuals. During microscopy, a species assessment of Demodex mites was carried out: Demodex folliculorum longus and Demodex folliculorum brevis.

The VivaScope 1500® confocal laser scanning *in vivo* microscope (Lucid Inc., Rochester, NY) was examined at three points (2 cheeks and forehead), in two modes of the VivaBlock and VivaStack microscope, the skin of the patients was visualized as squares measuring 5x5 mm, the laser power was 21.7 mW. Using a confocal microscope, the number of mites in the follicles was calculated, the average size of the follicles and mites and the depth of the mites were determined.

Results

Demodex mites were more often detected in patients with rosacea than in patients with acne. In the course of the analysis, it was found that patients with demodicosis (group I) were dominated by heavier clinical forms of acne and rosacea. In most cases in this group there were III; IV degree of acne (34; 46%), papular and pustular forms of rosacea (14, 31%), in two patients infiltrative-productive form of rosacea (2; 4%) was diagnosed. While in group II the surface forms of the disease - I predominated; II degree of acne (23; 31%) and erythematous form of rosacea (18; 39%).

When determining the species of Demodex, it was revealed that different species and combination of two species of mite were observed in acne patients: Demodex folliculorum longus was detected in more than half of cases (n = 33, 82.5%), in 10% (n=4) - Demodex folliculorum brevis and both types of mites were found in 7.5% (n=3). In patients with rosacea, Demodex folliculorum longus (n=14; 70%) predominated in most cases.

To assess the validity of the confocal microscopy method, the survey was conducted in all three groups. The data obtained in Table 1 demonstrate not only high information value of the method, but also its superiority over microscopic diagnostics.

As can be seen from the table, on a confocal laser scanning *in vivo* microscope, mites were found in all patients with demodicosis. Demodex mites were defined as rounded or long conical formations in the mouths of the hair and sebaceous glands. In the study of Group

II patients, mites were identified in 10 (13%) patients who had a negative scraping result (Table 1).

In the study of healthy group III volunteers, Demodex mites in follicles were detected by confocal laser scanning *in vivo* microscopy in 6 people (8%).

Discussion

The presence of demodicosis complicates the course of acne and rosacea, contributing to the development of inflammatory elements. The predominance of severe forms of acne and rosacea in the group of patients with concomitant diagnosis "Demodicosis" confirms the need for microscopic diagnosis of Demodex mites in patients with acne and rosacea.

Since the type of mites Demodex folliculorum longus was more often detected in patients with acne III; IV degree and papular and pustular forms of rosacea, it can be assumed that Demodex folliculorum longus provokes the development of more severe clinical forms of the disease.

Thus, the study proves the high informative of the method, since it makes it possible to detect mites at a depth of occurrence not accessible to scarification.

With the help of a confocal laser scanning *in vivo* microscope, when scanning different layers of skin, we established an average depth of mites of about 46.63 microns, which corresponds to the level of the granular layer of the epidermis. This method allowed to calculate the average number of mites in the follicle (n=3.37) and the average Demodex mites size, which is 0.024 microns.

Conclusion

Analysis of the clinical picture of patients with acne and rosacea showed that demodicosis complicates the course of acne and rosacea, contributing to the development of inflammatory elements. This once again confirms that in order to achieve effective therapy of these dermatoses, there is a need for conducting diagnostics of Demodex mites.

The obtained data proved the high information content of confocal microscopy in the diagnosis of demodicosis and its superiority over light microscopic diagnosis. Confocal laser scanning *in vivo* microscopy makes it possible to visualize mites located in deeper layers of the skin that are not accessible for scarification. This method has a high potential of diagnostic capabilities, namely, it makes it possible to scan various layers of the skin, which allows determining the depth of the mites (≈ 46.63 μm); counting the number and setting

Table 1: Identification of Demodex mites by various diagnostic methods.

Identification Demodex mites	I group (number of patients; %)	II group (number of patients; %)	III group (number of patients; %)	Total (number of patients; %)
Detected Demodex folliculorum longus	47 (62%)	-	-	47 (62%)
Detected Demodex folliculorum brevis	8 (10.5%)	-	-	8 (10.5%)
Both types of mites	5 (6.5%)	-	-	5 (6.5%)
Detected on a confocal laser scanning <i>in vivo</i> microscope	60 (79%)	10 (13%)	6 (8%)	76 (100%)
Total	60 (79%)	10 (13%)	6s (8%)	76 (100%)

the size of the mites. The absence of traumatization of the epithelium and the painfulness of the procedure are additional advantages of the method.

Thus, as a result of the study of patients with acne, rosacea and healthy volunteers, high information content of confocal laser scanning *in vivo* microscopy in the diagnosis of demodicosis was established, and its advantages over conventional light microscopy.

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