

PROCEEDINGS

## DIAGNOSIS OF SKIN AND EYELID DISEASES ASSOCIATED WITH *DEMODEX SPP.*

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### ABSTRACT

**INTRODUCTION:** *D. folliculorum* and *D. brevis* are two types of obligate ectoparasites in humans that inhabit hair follicles, sebaceous and meibomian glands. Initially considered commensals, now there is growing evidence of their direct or indirect involvement in numerous (mainly chronic) skin and eyelid diseases.

**AIM:** The present study aims to categorize the information on the methods for diagnosing the diseases associated with *Demodex spp.* and to specify the morphological characteristics essential for the identification.

**MATERIALS AND METHODS:** Various review and meta-analysis articles from the last ten years have been studied, primarily focused on diagnosing demodicosis in humans. The specific morphological features of both species have been visualized with photomicrographic images of some of the positive results.

**RESULTS:** From the variety of skin testing procedures, the best method to use in outpatient practice is a superficial biopsy with scotch tape. If demodicosis of the eyelids is suspected, the standardized examination method after eyelash removal can be modified by fixing the material with scotch tape, which allows efficient transportation. Implementing the two methods leads to the rapid and accurate identification of these ectoparasites.

**CONCLUSION:** The introduction of two new diagnostic techniques in the parasitological laboratories reveals the involvement of *Demodex spp.* in the pathogenesis of numerous skin and eyelid diseases. This allows dermatologists and ophthalmologists to refine the therapeutic approach in each case, improving the patients' clinical condition and quality of life faster and more efficiently.

**Keywords:** *Demodex spp.*, demodicosis, ectoparasites, direct microscopy, blepharitis, rosacea

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## INTRODUCTION

The genus *Demodex* spp. includes 65 species of microscopic mites and obligate ectoparasites in the hair follicles of 11 orders of mammals (1,2,3,4). Only *D. folliculorum* and *D. brevis* colonize human skin. *D. folliculorum* is 0.3–0.5 mm long and inhabits mainly the hair follicles of the face (cheeks, nose, forehead, temples, auricle, scalp) and eyes (eyebrows and especially eyelashes) (3,5,6,7). *D. brevis* (0.15–0.3 mm) feeds primarily on sebum and therefore colonizes the sebaceous glands on the face, neck and torso. It is also found in the meibomian glands of the eyelids (1,2,5,6,8).

The colonization of humans with *Demodex* spp. varies depending on the age. *Demodex* mites are probably transmitted on the skin of newborns through close physical contact after birth, but due to low sebum production, infants and children under five years do not carry large numbers of mites (8). The highest degree of skin area/hair follicle infestation is observed in 20- to 30-year-olds, due to the most intense sebum secretion (2,9). With increasing age, this indicator declines, but the number of infected persons increases. Many studies indicate that virtually all adults over 70 are carriers of *Demodex* spp. (1,2,6,9).

First described and long considered harmless commensals, numerous current studies and meta-analyses associate the involvement of *D. folliculorum* and *D. brevis*, directly or indirectly, as pathogens in different clinical syndromes classified into two groups *facial demodicosis* and *demodicosis of the eyelids*.

The state of the humoral and especially the local cellular immune response of the skin plays an essential role in the intensity of *Demodex* reproduction (the so-called degree of colonisation). The current opinion is that the mites are commensal at a quantity below 5/cm<sup>2</sup> in standardized tests. The diagnosis *cutaneous demodicosis* is made when the number of parasites exceeds this number, there are clinical symptoms, and/or when penetration into the dermis is detected on a histological preparation (2,7,9,8). The presence of more than 3–5 mites per hair or in one follicle is considered to be significant colonization of facial hair, eyelashes, and eyebrows (4,5).

Rosacea is one of the most common chronic skin conditions. The involvement of *Demodex* spp. was associated with this disease's pathogenesis and connected with the aggravating of its course (3,7,10,11,12). Studies report significantly greater infestation in individuals with rosacea than in control groups of clinically healthy individuals of the same age. The highest degree of colonization is observed in the more severe forms—papulopustular and granulomatous rosacea (2,10,13). *Demodex* infestation is recorded in another common chronic skin condition—acne vulgaris (3,11). High infestation levels have also been found in other facial skin and scalp diseases, such as seborrheic dermatitis, pityriasis folliculorum, perioral dermatitis, and androgenetic alopecia (1,3,4,7,8,14,15).

The clinical presentation in the eye is due to the mite's reproduction in the eyelash follicles or meibomian glands of the eyelids. The mildest clinical presentation is bilateral redness of the lid margin with crusts and circular, waxy dandruff of the eyelashes. Considered a pathognomonic sign (2,5,6,12,16), it leads to irregular growth and more frequent loss of eyelashes. Acute blepharitis with swelling, redness, tearing, and conjunctival injection may develop, but ocular demodicosis is most often associated with chronic inflammation of the eyelids (2,5,6,17,18). In addition to cylindrical dandruff, other symptoms observed are redness, lacrimation, thickened eye secretion, feeling of a foreign body, and blurred vision related to another problem—the dry eye syndrome (5,6,18). It is found especially when the meibomian glands are infested with *D. brevis*, which decreases their secretion (19). Those two processes progress in parallel for a long time, leading to a deterioration of the patient's quality of life. Chronic blepharitis is often observed in persons with problems with the local immune response of the skin, and working in dusty and dry conditions is considered an occupational risk. The most severe cases of colonization with *Demodex* spp. have been registered in persons with general immunosuppression—on systemic corticosteroids, children and adults after cytostatic therapy for hematological diseases and other neoplasms, patients with uncontrolled HIV/AIDS, with chronic liver and kidney failure, diabetes (1,2,3,6). In the most severe cases of chronic blepharitis, the protracted inflammation and superimposed bacterial infec-

tion can lead to keratitis, corneal vascularization, and vision loss (5,6).

### AIM

The purpose of the present study is to systematize the information regarding the methods of diagnosis of skin and eyelid diseases associated with *Demodex spp.* and to specify the morphological characteristics of the mites, which are essential for their identification after the implementation of two new methods for the diagnosis of skin and eyelashes in two specialized parasitological laboratories in the city of Varna.

### MATERIALS AND METHODS

A systemic study was performed on review articles and meta-analyses from the last ten years investigating the clinical syndromes associated with *Demodex spp.*, especially those describing the diagnostic procedures. Selected methods implemented in two parasitological laboratories in Varna city were surface impressions with scotch tape, eyelash epila-

tion, and fixation with scotch tape. A retrospective analysis of data from the two laboratory systems was performed from 01.10.2019 to 31.05.2022 with 227 examined individuals. Relative proportions of the positive results by age and sex were estimated. Given the study's retrospective nature and the patients' preserved anonymity, the study does not require ethical approval.

To visualize the morphological features of *Demodex* mites, several positive findings are documented as photomicrographic images using a digital camera on a Nikon Eclipse E100 light microscope. For a detailed visualization of the ultrastructure of the mites, representative photomicrographic images were prepared using a scanning light microscope Axio Imager Z2 (Carl Zeiss).

### RESULTS AND DISCUSSION

The information on current diagnostic methods in dermatological and ophthalmological practice regarding diseases associated with *Demodex spp.* is presented in Table 1. In order to differentiate which

*Table 1. Methods for diagnosing Demodex spp. and assessment of the suitability of their application in a specialized outpatient parasitological laboratory.*

Methods (source)	Advantages	Disadvantages	Assessment
<b>I. Methods for skin</b>			
<i>Superficial scotch-tape impression/biopsy</i> (1,2,7,8,9)	<ul style="list-style-type: none"> <li>• Not invasive</li> <li>• Suitable for all regions of the face</li> <li>• Safe and easy (can be done at home)</li> <li>• Does not require immediate microscopy (allows transport)</li> <li>• Can be performed in outpatient conditions (at home)</li> <li>• Fast and cheap</li> </ul>	Only reaches the superficial layer (less informative for active pustules and <i>D. brevis</i> )	<b>Suitable</b>
<i>Direct microscopy after skin scraping</i> (1,7,8)	<ul style="list-style-type: none"> <li>• Reaches deeper into the skin</li> <li>• Useful for pustules</li> <li>• Suitable for all regions of the face</li> <li>• Standardized for 1 cm<sup>2</sup></li> </ul>	Only performed by a dermatologist Requires immediate microscopy (does not allow transport)	Not suitable
<i>Superficial skin biopsy with cyanoacrylate adhesive</i> (1,7,8,9,11)	<ul style="list-style-type: none"> <li>• Reaches deeper skin layer</li> <li>• Standardized for 1 cm<sup>2</sup></li> </ul>	Inflicts epithelial trauma Depth is achieved with repeated application Difficult to perform in some regions of the face (nose) Allergic reactions to the adhesive are possible Requires microscopy immediately (does not allow transport)	Not suitable

<i>Skin biopsy with histological preparation</i> (2,7,8,9)	<ul style="list-style-type: none"> <li>• The most informative</li> <li>• Assesses penetration into the dermis</li> </ul>	Not suitable for examining large skin areas or multiple areas Performed by a trained specialist Requires local anaesthesia Causes trauma and can leave scars Requires histological staining Time-consuming	Not suitable
<i>Dermatoscopy</i> (7,13,15)	<ul style="list-style-type: none"> <li>• Not invasive</li> <li>• Suitable for all regions of the face</li> <li>• Can be standardized for 1 cm<sup>2</sup></li> </ul>	Examines only the surface (less informative for active pustules and for <i>D. brevis</i> ) Performed by a dermatologist Requires specialized equipment	Not suitable
In vivo confocal laser or refractive microscopy (2,7,9,11,14,19)	<ul style="list-style-type: none"> <li>• Not invasive</li> <li>• Reach deeper skin layers</li> <li>• Can be standardized for 1 cm<sup>2</sup></li> </ul>	Performed only by a qualified specialist Requires highly specialized and expensive equipment	Not suitable
<b>II. Methods for eyelids and eyelashes</b>			
<i>Native microscopy after epilation of eyelashes</i> (1,5,6,12,16,17,19)	<ul style="list-style-type: none"> <li>• Damaged eyelashes with circular dandruff can be selected</li> <li>• Mites remain alive</li> <li>• Standardized</li> <li>• Can be performed in outpatient conditions (at home)</li> <li>• Fast and cheap</li> </ul>	Invasive Sensitivity upon extraction Difficult for transportation (in its original form)	<b>Suitable</b>
<i>Epilation of eyelashes with the addition of solutions</i> (1,5,6,12,19)	<ul style="list-style-type: none"> <li>• Damaged eyelashes with circular dandruff can be selected</li> <li>• Break down detritus (KOH, 100% alcohol) and/or make mites easier to distinguish (methylene blue, fluorescein)</li> <li>• Standardized</li> </ul>	Invasive Require microscopy immediately (does not allow transportation) The sample can be dispersed and/or lost Can change the morphology of the mites	Not suitable
<i>Confocal scanning laser microscopy</i> (5,6,7,17,12,18)	<ul style="list-style-type: none"> <li>• Not invasive</li> <li>• High informativeness deeper in the follicles</li> <li>• Standardized</li> </ul>	Performed by a qualified specialist Difficult for examination of the eyelids Requires highly specialized and expensive equipment	Not suitable

methods are suitable to be used and introduced in the working conditions in a specialized outpatient parasitological laboratory, we performed an analysis according to the following criteria: invasiveness, informativeness, safety, the possibility of standardization, requirement for the use of specialized equipment, conditions for transportation.

The analysis revealed that most methods of skin examination are invasive or require an examination at the moment, i.e., during a dermatological examination (Table 1). For these reasons we chose to introduce as a method of skin examination for the presence of *Demodex* mites the surface biopsy/

scotch tape impression, despite the impossibility of its standardization and its relatively low sensitivity (1,2,7,8,9). The method allows taking multiple prints from different areas of the face without causing superficial trauma. It makes it easy to examine (repeatedly and painlessly) the regions with a predilection for *Demodex* mites, the more difficult-to-reach areas, such as the folds of the nose, and the places with pathological changes for rosacea, acne, seborrhea, etc. An advantage is that impressions can be taken by the patients themselves at home, in the evening, synchronically with the nocturnal activity of the mites (1,2) or in the morning immediately after waking up.



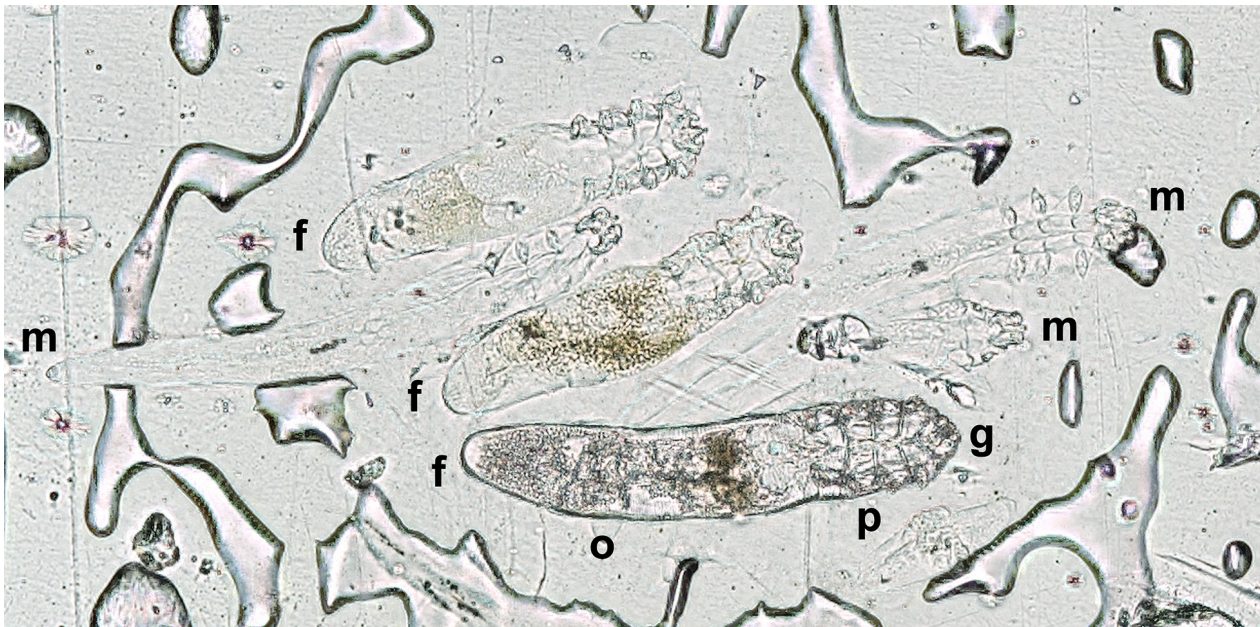
This also allows them not to disturb their daily routine and quality of life since, after taking the samples, they can apply the necessary cosmetic or therapeutic skin care. For maximum information and to reduce the probability of false negative results, patients are instructed in advance about the conditions for the correct collection of the material, and it is recommended that the prints be taken repeatedly, from different areas of the face (including the lesions), for several days. Another positive aspect is that scotch tape microscopic examination is a highly well-known and daily used method in parasitological laboratories, as it is a routine procedure for the examination of the perianal region for the presence of *Enterobius vermicularis* eggs. This makes specialist parasitologists and experienced laboratory technicians highly skilled in this unique field of diagnostics and allows them to easily distinguish biological elements from the multitude of artefacts.

To date, a total of 25 adult patients (22 women and three men) have been examined with the scotch tape impression method in both laboratories, with 44.0% (n = 11) positive results. *D. brevis* was identi-

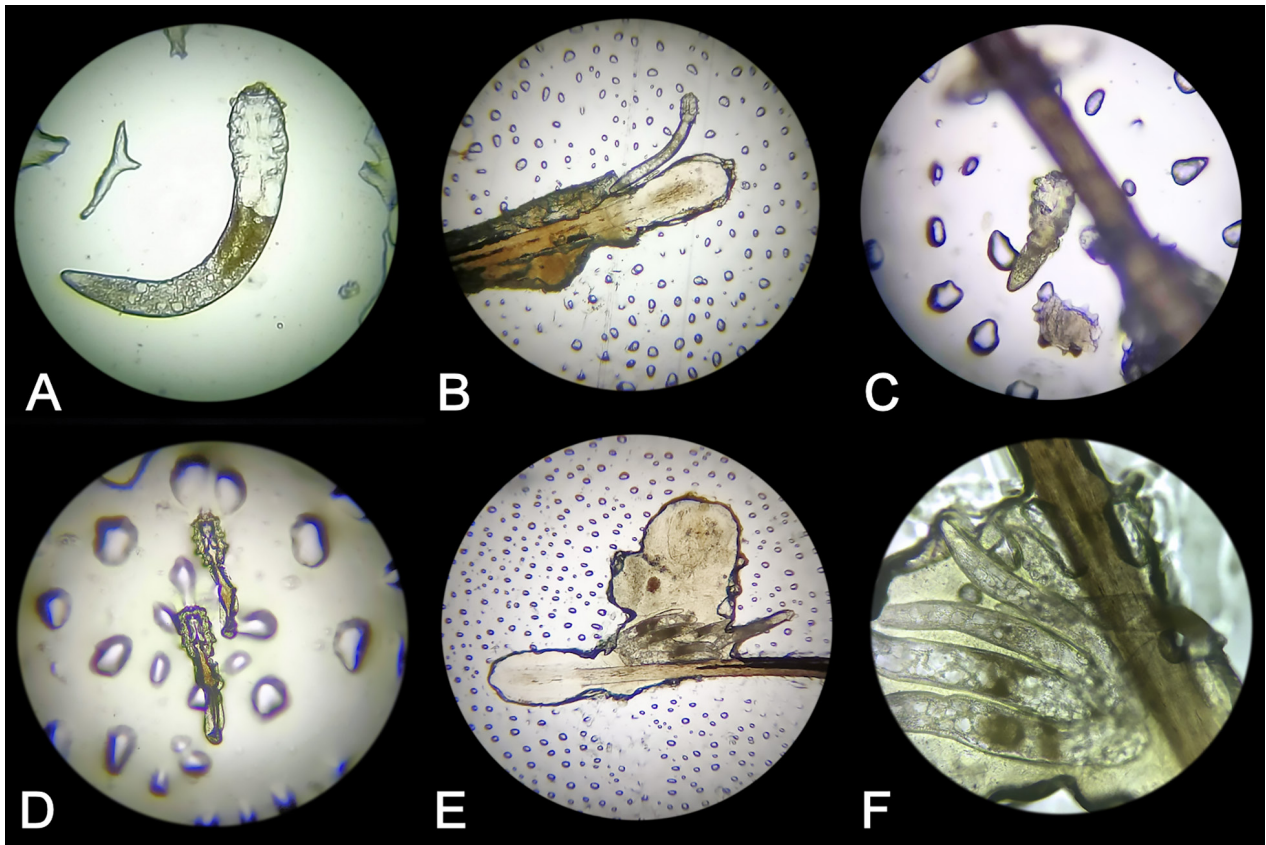
fied in two individuals, and *D. folliculorum* was detected in the rest of the cases.

Microscopy of skin prints is performed in a bright field with small objective magnifications (5x, 10x). Under these conditions, an entire mite can be observed, and species differentiation is possible (Fig. 1, Fig. 2). Good knowledge of the *Demodex* ultrastructure is extremely important because the parasites die within a few hours (1) and their morphology changes due to rapid desiccation (Fig. 2D). Sometimes, taking prints can damage the integrity of the body. The separate anterior segment is easily identified due to the presence of the mandible and limbs. In contrast, the posterior segment is more difficult to distinguish, and it is necessary to use a higher objective magnification (40x) to look for the characteristic transverse striation of the chitinous sheath (Fig. 1).

The clinical signs of chronic inflammation of the eyelids and the presence of problems in the eyelash hair follicle, including the characteristic circular dandruff, are easily seen during a standard ophthalmological examination with biomicroscopy. The desire of the ophthalmologists from the city of Var-



**Fig. 1.** Morphological characteristics of *D. folliculorum* mites on a native scotch-tape preparation; panoramic image taken with an Axio Imager Z2 light microscope, Carl Zeiss (10x; 0.25). The body of *D. folliculorum* is elongated, transparent, and composed of two fused segments. The shield (podosoma) (p) contains eight limbs. At the anterior end is the mandible (gnathosoma) (g). The posterior part (opisthosoma) (o) is elongated, transversely striated, and contains the digestive system and the organs of the male or female reproductive systems. The sexual dimorphism of the *Demodex* mites is visualized—the three female mites (f) are shorter, with a rounded posterior end. Males are elongated (m), pointed, and almost entirely transparent.



*Fig. 2. Morphological characteristics of Demodex spp. on native scotch-tape preparations; images taken with a digital camera on Nikon Eclipse E100 light microscope.*

*A. D. folliculorum on skin surface impression (x40);*

*B. D. folliculorum in the characteristic circular detritus around an eyelash follicle (5x);*

*C. D. brevis near eyelash (x10);*

*D. Two desiccated D. folliculorum, the picture is taken 48 hours after the initial microscopy (10x);*

*E. Eyelash colonisation with D. folliculorum (n=8) and excessive hyperkeratosis (5x);*

*F. Eyelash colonisation with D. folliculorum (n=7) (40x).*

na for a precise diagnosis with etiological confirmation of the presence of *Demodex* mites necessitated the introduction of this new type of diagnosis. The analysis of literature sources (Table 1) indicates that the epilation of eyelashes and their native microscopy is the method of first choice for the diagnosis of demodicosis of the eyelids (1,6,16,17,19). The procedure can be done after coating with immersion oil or with reagents that break down the detritus and make the mites easily distinguishable (1,5,6,12,19). In both cases, however, the preparation must be examined immediately, which complicates and considerably increases the time of a specific ophthalmological examination. So the question on how to transport the material (eyelashes with pathological chang-

es) to a parasitological laboratory arose. Using our experience working with scotch tape preparations, we modified the method by suggesting that immediately after plucking at least four or more eyelashes from each eye, which is the requirement to assess the degree of colonization (4,5), they should be fixed on a microscopy slide with adhesive tape. Since the scotch tape is entirely transparent, it does not hinder microscopy and does not change the morphology of the mites. Additional benefits are that once the material is collected and sealed, it cannot be misplaced/lost, ensuring safe transportation and eliminating the need for on-the-spot testing.

After introducing this method in October 2019, 202 patients were examined in the two laboratories,



with 81.68% positive results. *D. folliculorum* was detected in the majority of cases, and *D. brevis* was identified in only one person (Fig. 2C).

Most of the patients were adults, and among the few children examined (n=9), we found only two positive findings of *D. folliculorum*. In men, the prevalence of *Demodex*-positive samples is 83.33%, and 80.51% for women, which does not represent a statistically significant difference. The reason for the extremely high levels of positive results is that, in most of the studied persons, eyelash epilation was carried out during a specialized eye examination in patients with symptoms of acute or chronic blepharitis and is an indicator of the highly effective collaboration between the parasitological laboratories and the ophthalmology centers of Varna city.

Microscopy of the eyelashes sealed with scotch tape is carried out natively in a bright field at low magnifications (5x, 10x). For the presence of *Demodex* mites, the full extension of the eyelashes and the remaining part of the preparation are screened, counting the number of mites or nymphs found. In most cases, the parasites are found in or near the eyelash follicles (Fig. 2B, C, E, F). They can be single (Fig. 2B, C) or many in number, and within 24 hours of their removal, a characteristic movement of the limbs and mandible is observed. Difficulty in identifying them can occur if they are hidden in the detrital sleeve around the hair, requiring higher magnification to distinguish the details of the front part or the striations of the distal part of the body. We observed the highest degree of colonization (over 5–10 parasites in one follicle) in patients with chronic immunosuppression (with lymphomas, and autoimmune diseases requiring systemic corticosteroid therapy) (Fig. 2. E, F).

## CONCLUSION

*Demodex* mites accompany human hosts throughout their life and, under certain circumstances, can be the cause or may facilitate various pathological conditions of the skin and hair. In the present study, we describe the introduction for the first time in Bulgaria of two new methods for morphological examination in specialized parasitological laboratories. Superficial skin impressions with scotch tape and native microscopy of eyelashes after epilation are easily performed, fast and effective meth-

ods that allow revealing the involvement of *Demodex* spp. in the etiopathogenesis of many diseases of the skin and eyelids. This allows dermatologists and ophthalmologists to refine the therapeutic approach and include a specific ethnological acaricidal therapy only in cases of proven need. Otherwise, treating the described chronic conditions is often lengthy, expensive, and without the desired effect. The introduced diagnostic procedures also provide objective control over the effect of the treatment by tracking the degree of colonization over time. Creating an affordable opportunity for timely and accurate detection of *Demodex* spp. for both skin and eyelid conditions is a prerequisite for timely and effective treatment, quick improvement of the clinical condition and quality of life, and prevention of various dermatological and ophthalmological complications.

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