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## *Demodex folliculorum* in rosacea based on a modified standardized skin surface biopsy

### Nużeniec ludzki w trądziku różowatym na podstawie zmodyfikowanej standaryzowanej biopsji powierzchni skóry

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#### Key words

rosacea, *Demodex folliculorum*, etiology

#### Słowa kluczowe

trądzik różowaty, nużeniec ludzki, etiologia

#### Summary

**Introduction.** Rosacea is a chronic disease characterized by (depending on the subtype) facial prolonged erythema, sometimes pustules or nodules. Rosacea affects all ages and sex with four subtypes. Pathophysiology aims to many different trigger factors like sun exposure, emotional stress or changed intestinal flora. Studies about *Demodex* mites according to different authors revealed that they may play a role in rosacea exacerbation in particular along with other triggers.

**Aim.** An attempt to determine the role of *Demodex* mites among patients with rosacea.

**Material and methods.** The study included patients with rosacea from 22 to 63 years of age. The patient status and content of hair follicles were assessed during two visits in our outpatient clinic. During the first visit, detailed medical history was taken, physical examination and hypoallergenic adhesive were applied (nose, chin, cheeks and forehead) in order to pursue the content of the sebaceous glands. During the second visit, patients qualified to the research were again examined while adhesives have been removed. By using stereoscopic microscope Stemi 2000, *Demodex* mites from hair follicles were analyzed.

**Results.** Initially, contents from hair follicles of 38 patients with rosacea have been examined. The presence of *Demodex* was confirmed in 11 patients. Most cases of confirmed *Demodex* infestation concerned patients with papulopustular rosacea. Live subjects were collected only from the nose and the decrease in their motility was observed over the course of time after the removal of plasters from the skin.

**Conclusions.** Obtained results confirming the infestation of 11 out of 38 patients with erythematotelangiectatic rosacea indicates *Demodex folliculorum* as a direct or indirect pathogen. Based on the results, we can state that among our patients *Demodex* mites were not a main trigger factor. Standardized skin surface biopsy is not a sufficient screening test.

#### Streszczenie

**Wstęp.** Trądzik różowaty jest przewlekłą chorobą skóry charakteryzującą się (zależnie od podtypu) przedłużającym się rumieniem oraz obecnością teleangiektazji i/lub guzków i krost. Dotyka wszystkich grup wiekowych obu płci. Patofizjologia zakłada udział wielu różnych czynników wyzwalających, w tym promieniowanie UV, stres lub zmieniona flora jelitowa. Badania nad nużeniem ludzkim wykazały, że mogą one odgrywać rolę w patogenie zaostrenia, działając jako kofaktor z innymi czynnikami.

**Cel pracy.** Próba określenia roli nużenia ludzkiego w grupie pacjentów z trądzikiem różowatym.

**Materiał i metody.** Do badania włączono pacjentów z trądzikiem różowatym w wieku od 22 do 63 lat. Stan dermatologiczny oraz ocenę zawartości mieszków włosowych oceniano podczas dwóch wizyt w naszej przychodni. Podczas pierwszej wizyty zbierano wywiad oraz przeprowadzono badanie przedmiotowe z kwalifikacją do odpowiedniego stadium choroby. W celu pobrania zawartości gruczołów łojowych na nos, podbródek, policzki oraz czoło nakładano hipoalergiczny przyklep. Podczas drugiej wizyty u pacjentów

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zakwalifikowanych do badania przylepce usuwano wraz z zawartością mieszków włosowych. Oceny dokonywano przy użyciu mikroskopu stereoskopowego STEMI 2000.

**Wyniki.** Oceniono zawartość mieszków włosowych od 38 pacjentów z trądzikiem różowatym pod kątem obecności nużeńca ludzkiego. Obecność nużeńca potwierdzono u 11 chorych. Większość przypadków (9) dotyczyła pacjentów w stadium grudkowo-krostkowym trądziku różowatego. Żywe osobniki obserwowano jedynie w obrębie płatka nosa. Ruchliwość nużeńca zmniejszyła się w miarę upływu czasu od momentu usunięcia plastrów ze skóry pacjenta.

**Wnioski.** Uzyskane wyniki potwierdziły kolonizację u 11 z 38 pacjentów z trądzikiem różowatym, co może wskazywać na jego udział w patogenieze opisywanej jednostki chorobowej. Na podstawie wyników można stwierdzić, że nużeniec nie jest głównym czynnikiem zaostrzającym przebieg choroby. Ponadto standaryzowana powierzchowna biopsja skóry nie jest w pełni zadowalającym badaniem przesiewowym.

## INTRODUCTION

Rosacea is a chronic, inflammatory facial skin condition occurring in adults and is characterized by periods of exacerbation and remission (1). The primary symptom of rosacea is persistent erythema, which with the secondary formation of telangiectasia and papules and pustules in the later stages of the disease becomes a permanent condition (2). It is a very common dermatosis which, according to the epidemiological data, occurs in the range from 2% in Germany to 10% in Sweden (3). A prevalent type of rosacea is an erythematotelangiectatic rosacea occurring in 81% of dermatology clinic patients (4). The etiopathogenesis of rosacea is still unknown but according to numerous hypotheses, it is determined by vascular disorders, immune disorders, degeneration of the connective tissue elements, pilosebaceous follicles disorders, as well as climatic, chemicals and dietary factors. Infection related etiopathogenesis becomes more popular. This is supported by an effective form of therapy aimed at unspecified microorganism (2, 5).

### *Demodex folliculorum*

*Demodex folliculorum* is an arachnid belonging to the order of mites (6). They are obligatorily bound to their hosts. Various species of *Demodex folliculorum* may occur on different parts of the skin of a single host (7). Two species of *Demodex* are typical to human, namely *Demodex folliculorum* and *Demodex brevis* (6). The former is of an elongated shape and its length ranges from 0.3 to 0.4 mm. It resides in hair follicles. The latter and the smaller one measures from 0.2 to 0.3 mm. It is typically spindle-shaped with shorter legs and is usually found in sebaceous glands of the entire body or in Meibomian gland (8). Due to its inconvenient location, *Demodex brevis* is difficult to pull out and its role in the pathogenesis of skin diseases is not fully known (9). *Demodex folliculorum* outnumbers *Demodex brevis*, however the latter inhabits a larger area. Regardless of its development phase, *Demodex* mainly feeds on skin cells and the components of sebum, which explains why it resides in the seborrheic areas including nose, cheeks,

forehead and chin. In terms of anatomy, *Demodex* is composed of gnathosoma with oral apparatus and podosoma and opithosoma (10). Gnathosoma comprises the chelicerae used to suck food and pedipalps, which are used to hold the food. Prosoma has four pair of legs (10). All the *Demodex* mites avoid sunlight. They leave their initial location and emerge to the skin surface only at night to mate. The life cycle of *Demodex* lasts from 14 to 18 days (7, 11). Transmission of *Demodex* from human to human occurs during direct contact (common toiletries, towels or dust) and increases with age. According to the literature, the proportion of colonised patients ranges from 20 to 80% with the peak between second and sixth decade of life (12). The authors assume that increased infestation of facial skin with *Demodex folliculorum* may contribute to the development of the symptoms. Forton et al. proved that an average density of *Demodex* in the facial skin equals 10.8 mites per cm<sup>2</sup> (papulopustular type), and 0.7 mites per cm<sup>2</sup> in the group of healthy people. Diagnosing the *Demodex* mites include skin scrapings or standardized skin surface biopsy (SSSB). The pathogenic role of *Demodex* is still a starting point for further experimental research on their role in the direct and indirect induction of local inflammation.

### AIM

The main objective of this article is to present the current state of knowledge on *Demodex folliculorum* and their correlation to the pathogenesis of rosacea, based on the literature review and modified standardized skin surface biopsy in patients diagnosed with rosacea.

### MATERIAL AND METHODS

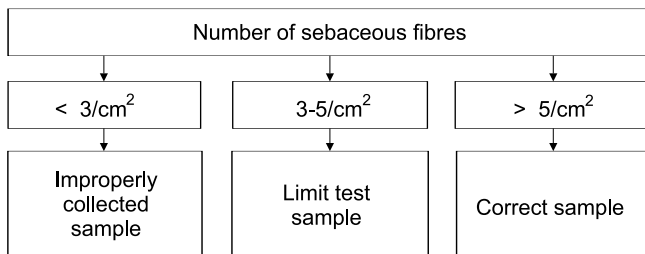
A standardized skin surface biopsy was used in the study. In the reports published so far, *Demodex* mites have been diagnosed by means of standardized skin surface biopsy (SSB). A glass slide with a drop of cyanoacrylate glue was applied to the affected areas of the facial skin. The specimen was then examined under the light microscope. The method aimed to determine

a parasite density per cm<sup>2</sup>, thus enabling to detect live *Demodex* mites. In this article, the authors present modifications to the above described method. During the first visit, a detailed medical history and physical examination of patients considered for the study were undertaken together with the assessment of their dermatological and clinical condition. Patients who met the requirements were enrolled in the study. A hypoallergenic, round plaster of 1 cm<sup>2</sup> in diameter was pressed onto the nose, chin, both cheeks and the middle of the forehead. The plasters were left for a night and then removed together with hair follicles. They were then covered with another glass slide. The next step consisted in the quantitative analysis of the collected material by means of the stereoscopic microscope Stemi 2000. The specimens were analysed according to the algorithm (fig. 1): three times, always by the same researcher, in a period not exceeding 4 hours after the collection of the material. Only moving live organisms, identified based on their anatomical characteristics, were taken into account. Information on the number and distribution of *Demodex* was recorded in a patient's personal file. The study included 38 patients with clinically diagnosed rosacea and admitted to the Dermatology Clinic of Medical University of Silesia in Katowice. Age of patients ranged from a minimum (22 years) to maximum (63 years) with a mean age 48 +/- 10 years. As stated in the medical history, due to their dermatological condition, patients use, among others, metronidazole skin gel, azelaic acid and oral tetracycline. Based on the observed clinical signs, patients were divided into two groups: of those with erythematotelangiectatic rosacea consisting of 27 patients and of papulopustular one comprising 11 patients. The experimental group consisted of 34 female patients, which was 89% of all the subjects. Of 4 male patients, 3 were in the erythematotelangiectatic stage and 1 in the papulopustular one.

10 women (8 with erythematotelangiectatic rosacea and 2 in the papulopustular stage) and in one man with papulopustular rosacea (tab. 1). Most cases of confirmed *Demodex* infestation concerned patients with papulopustular rosacea (9). In 4 cases the material was collected properly (number of sebaceous threads > 5 cm<sup>2</sup>), whereas in other cases the material was assessed as limit. The number of detected *Demodex* mites ranged from 4 to 25 (13 +/- 6 on average). Live subjects were collected only from the ales and the decrease in their motility was observed over the course of time after the removal of plasters from the skin.

**Table 1.** Diagnostic algorithm for modified standardized skin surface biopsy.

No.	Gender	Age	RT	GK	Test sample	Number of <i>Demodex</i>
1	female	22	yes		limit	10
2	male	27		yes	limit	9
3	female	47		yes	limit	18
4	female	51		yes	limit	15
5	female	36	yes		correct	25
6	female	38		yes	correct	16
7	female	60		yes	limit	11
8	female	38		yes	correct	23
9	female	59		yes	limit	12
10	female	49		yes	limit	4
11	female	56		yes	correct	8



**Fig. 1.** Results of modified standardized skin surface biopsy from patients with diagnosed rosacea.

**RESULTS**

The material collected in the group of 38 patients, was classified as follows: 4 cases of incorrect intake, 14 cases of limit intake and 20 cases of correctly collected sebaceous thread. The presence of *Demodex* was confirmed in 11 patients including

**DISCUSSION**

Rosacea is a chronic and recurrent inflammatory disease that predominantly affects people aged between 25-35 or 45-55 and is characterised by such symptoms as erythematous lesions, telangiectasias, inflammatory eruptions (papules and pustules), as well as subjective symptoms including itching and burning of the facial skin. Female patients with rosacea constitute the majority of the study group, accounting for 89% (n = 34), whereas male patients represented 11% (n = 4). The obtained results tally with the literature, according to which the majority of patients are women or as pointed out by others, they seek dermatologist advice earlier and more frequently (4, 13). The age of patients included in the study also does not differ from the literature. The average age of patients was 48 and the stated age range is 30-50 (13). Most of the investigated patients, that is 67% (n = 23), were diagnosed with an erythematotelangiectatic rosacea which was prevalent among both women (58%; n = 20) and men (75%; n = 3). A papulopustular rosacea was diagnosed in 28% of patients (n = 11) including 10 women and one man. Also these results does not differ from the literature data, according to which an erythematotelangiectatic rosacea is the most frequently (approx. 81%) diagnosed type of this illness (4). The pathogenesis of this disease has not been fully explained yet as it is assumed that there

are predisposing factors such as vascular disorders, immune disorders, degeneration of connective tissue extracellular components and the impact of external factors (14). A pathogenic factor which is being analysed more and more often is the role of the *Demodex folliculorum* infestation. Discovered in 1841 by Henle and Berger and described in details by Simons in 1842, *Demodex folliculorum* is considered by many authors as a saprotrophic organism not involved into disease process of the human skin. The human immune system seems to tolerate *Demodex* and reacts with a local inflammatory response only in the case of an increased population of the mites. According to the literature, *Demodex* colonization rate reaches 100% and increases with age. In the carried out research, among 38 patients diagnosed with erythematotelangiectatic rosacea there were only 11 *Demodex* carriers. It may mean that the method of standardized skin surface biopsy is not a sufficient screening test, though fulfilling the requirements of a diagnostic test. The factors affecting the growth of *Demodex* population on the human skin are surface skin condition including an increased seborrhea (15). The skin of patients with rosacea, especially in the case of papu-

lopustular type, differs significantly from a healthy skin (reduced hydration, increased pH) (16). Although, the composition of sebum in patients with rosacea differs slightly, the location of skin lesions is closely related to seborrheic areas, particularly in the case of papulopustular rosacea (17). What is more, oral retinoids reducing sebum are efficient in the treatment of papulopustular rosacea, which additionally confirms the seborrhea's role in the pathogenesis of rosacea (15). The results of the research regarding the density of *Demodex* mites on the facial skin corresponds to the literature data (18). However, it is required to increase a target research group and to compare the degree of infestation with a healthy population. An average density of *Demodex folliculorum* per cm<sup>2</sup> of the skin in patients with rosacea (which is 13.72 organisms/cm<sup>2</sup>) corresponds to the values presented by other authors (18). Affected facial skin microenvironment of the patients with papulopustular rosacea may contribute to the development of mites, which according to numerous authors, only transmits other pathogenic factors (16). Obtained results confirming the infestation of 11 out of 38 patients with erythematotelangiectatic rosacea indicates *Demodex folliculorum* as a direct or indirect pathogen.

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