Clinical picture, diagnosis and treatment of rosacea, complicated by *Demodex* mites

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Abstract

The article analyzes the clinical picture and course of rosacea in patients with *Demodex* mites. It presents the advantages of using the method of confocal laser scanning microscopy over the method of light microscopy of facial skin scrapes. The aimes were to study the influence of *Demodex* mites on the clinical picture and course of rosacea; to compare laboratory and instrumental diagnostic methods for detecting *Demodex* mites; to evaluate the effectiveness of external therapy aimed at eliminating *Demodex* mites. 212 people were examined. The study included healthy patients, patients with a diagnosis of rosacea with the presence and absence of *Demodex*. The presence of *Demodex* mites was confirmed by two methods of study (light microscopy of skin scrapes and confocal laser scanning *in vivo* microscopy). *Demodex* mites promote the development of acute-inflammatory morphological elements, increase the duration of the condition (more than 5 years, P<0.01) and the probability of recurrence (from 1 to 3 relapses in 39.5% of patients, P<0.05), resulting in a decrease in the quality of life of patients (dermatology life quality index is 12.5±4.5, P<0.05). Antiparasitic drug ivermectin, in the form of an external form, at a concentration of 1% has a high therapeutic efficacy (in 93.3% of cases). *Demodex* folliculorum shows signs of parasitism, while *Demodex* folliculorum brevis is a saprophyte. The severity of the condition does not depend on the quantitative load of the mites in the scrape. As an antiparasitic drug, it is recommended to use 1% ivertmectin.

Introduction

In connection with the growth of cultural level of the society as a whole and the increase in individual exactingness to one's own appearance, both among men and women, face dermatosis remain one of the pressing problems. Despite the large number of scientific works devoted to the pathogenesis of rosacea, the question of *Demodex* mites' role in the development of the clinical picture of the condition remains open in the contemporary literature.

According to some authors, *Demodex* mites are representatives of the conditionally pathogenic microflora of facial skin along with Propionibacterium acnes, Staphylococcus epidermidis and Malassezia fungi.[1,2] This opinion is supported by the fact that in 55-100% of cases, mites are detected, both in patients with face dermatosis and with patients having no clinical signs of dermatological illnesses.[3-5] However, there are scientific papers proving that *Demodex* mites are capable of pathogenic parasitization and are the most frequently detected microbial agents in rosacea.[4] At the same time, attention is drawn to the lack of *Demodicosis* diagnosis in the International Classification of Illnesses of X revision, which points to the fact that *Demodex* mites species act rather as an agent complicating the course of rosacea. Currently, two species of *Demodex* mites parasitize on human skin: *Demodex* folliculorum and *Demodex* brevis.[6] The modern literature has no substantiated scientific studies indicating the role of the species belonging of the causative agent in the formation of the clinical picture of rosacea. Existing assumptions are not fully proven. The available data on the parasitization of *Demodex* mites in patients with rosacea are inconsistent and, in many cases, are mutually exclusive.

The available diagnostic methods for detecting *Demodex* mites do not meet the requirements of modern medicine, do not guarantee the absolute reliability of the test results, and are often traumatic. One modern diagnostic technique in dermatology is 25 lasers scanning *in vivo* microscopy.[7] This is an innovative method, the advantages of which are non-invasiveness and high information content, however, to date, in the Russian Federation this method has not been used to detect *Demodex* mites. Thus, in order to improve the quality of diagnosis and therapy, it becomes necessary to conduct a scientific study with an analysis of the clinical picture, comparing the methods of diagnosis and treatment of patients with rosacea associated with *Demodex* mites.

The purpose of the study is to evaluate laboratory and instrumental diagnostics and therapy of patients with rosacea complicated by *Demodex* mites.

Objectives of the study

i) To study the influence of *Demodex* mites on the clinical picture and course of rosacea;
ii) To study the features of the clinical picture of rosacea associated with *Demodex* mites, depending on the species belonging to *Demodex* mites;
iii) Evaluate the effectiveness of laboratory and instrumental diagnostic methods for detecting *Demodex* mites;
iv) To evaluate the effectiveness of external therapy aimed at eliminating *Demodex* mites in patients with rosacea.

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Key words: Dermatology life quality index; *Demodex mites*; *Demodex* folliculorum; Demodex folliculorum brevis.

Contributions: AKu and YG conceived the present idea, planned the experiments and supervised the findings of this work. AKr performed the computations and verified the analytical methods. All authors together investigated all aspects of the influence of Demodex mites on rosacea patients, discussed the results and contributed to the final manuscript.

Conflict of interest: the authors declare no potential conflict of interest.

Funding: none.

Received for publication: 11 March 2018.
Revision received: 19 June 2018.
Accepted for publication: 25 July 2018.

Dermatology Reports 2019; 11:7675
doi:10.4081/dr.2019.7675

Materials and Methods

The work was performed at the databases of the Department of Dermatovenereology and Cosmetology of the Federal State-Funded Educational Institution Continuing Professional Education "Russian Medical Academy of Postgraduate Education" of the Ministry of Health of the Russian Federation and the Federal Government Budgetary Institution "Government Research Centre of Dermatovenereology and Cosmetology" of the Ministry of Health of the Russian Federation from 2013 to 2016 years. The

study protocol was approved of the Ethical Committee of the Russian Medical Academy of Continuous Professional Education. Each participant was familiarized and signed the Informed consent of the participant in biomedical research.

During the study, a total of 212 people (men and women) were examined. The study included healthy patients, patients with a diagnosis of rosacea with the presence and absence of *Demodex* mites on the facial skin. During the study, three groups of patients and healthy people over the age of 18 were formed. I group - patients with a diagnosis of rosacea with the presence of *Demodex* mites. Patients were included in group I, in which the presence of *Demodex* mites was confirmed by two methods of study (light microscopy of skin scrapes and confocal laser scanning *in vivo* microscopy in an amount of more than 5 individuals per 1 cm^2). II group is a comparison group, which was composed of patients with a diagnosis of rosacea with no *Demodex* mites. In Group II patients, two methods of study of *Demodex* mites were not found. III group comprises a comparison group, which included healthy people. Due to the fact that in 20 patients with rosacea, *Demodex* mites were detected by only one research method, they were did not include to the study, but the data of this group were used for statistical processing when comparing the effectiveness of diagnostic methods for the presence of *Demodex* mites.

Methods of the study

Anamnesis was collected and questionnaire survey according to the questionnaire of the Dermatology Life Quality Index, which included 10 items was filled by every participant.

Clinical: inspection and establishment of a preliminary diagnosis. The diagnosis of rosacea was established based on the clinical picture of the condition. To determine the severity of rosacea guided classification of the national rosacea society.[8] All patients diagnosed with rosacea were counted morphological elements on the entire surface of the facial skin. Laboratory: i) Determination of the presence and species affiliation of *Demodex* mites with the help of light microscopy of skin scrapes, the contents of the sebaceous glands, hair follicles of the eyelashes and/or eyebrows, counting of the detected individuals, larvae, eggs per unit area (1 cm^2); ii) Determination of the pH level of the facial skin is made as follows. Instrumental: i) Photographing patients before and after treatment; ii) Study of the facial skin with the help of confocal laser scanning *in vivo* microscopy to determine the presence of *Demodex* mites. Statistical: statistical processing of data was carried out by the packages of Microsoft Excel 2013 and SPSS 21. The interconnection of categorical indicators was established by Fisher's exact method. Fisher's exact test is a statistical significance test used in the analysis of categorical data when sample sizes are small. To assess the significance of the differences in the follicles, single-factor analysis of variance was used with paired comparisons. To assess the significance of the differences in the absence of a normal distribution, the Mann-Whitney test, the Kruskal-Wallis multiple comparison test, were used. Differences were considered significant at $P<0.05$.

Results

Clinical characteristics of patients included in the study

A total of 192 respondents aged 18 to 79 (mean age 30.0±11.9) were under supervision. There were 82 male participants and 110 female participants. The diagnosis of rosacea was exposed to 120 patients.

According to the method of the study, the patients were divided into two groups. Group I included patients with rosacea (60 people) who had a *Demodex* mite detected by two methods of study: laboratory – light microscopy of scrapes and instrumental – confocal laser scanning *in vivo* microscopy in an amount of more than 5 individuals per 1 cm^2, the second group included rosacea patients (60 people) who had a negative analysis for the presence of *Demodex* mites (Table 1). When comparing the data of the anamnesis of Groups I and II, the factors statistically significantly more frequent in patients with rosacea associated with *Demodex* mites were found. Statistical processing of data revealed that the frequency of detection of *Demodex* mites significantly differs depending on the factor triggering the development of the condition ($P=0.001$). All cases of mites' detection were recorded with the following predisposing factors: emotional stress (n=60; 100%), inadequate nutrition (n=24; 40%), exacerbation of concomitant diseases (n=2; 3.3%). There were no statistical differences in the exacerbations of diseases, depending on the season.

In the comparison of the duration of the rosacea in patients of groups I and II significant difference was found ($P<0.01$). The presence of *Demodex* mites in rosacea promotes a longer course of the condition of I group patients (1-5 years duration of rosacea was observed in 10 patients (16.7%); 44 patients (73.3%) had a more than 5 years duration of rosacea). At the same time, in the II group 24 patients (40%) had a less than one year duration of rosacea, the same number of patients had 1-5 years (24; 40%) and only 12 patients had more than 5 years of rosacea duration (20%).

Comparing the frequency of rosacea recurrence between patients of groups I and II, a statistically significant difference was revealed ($P<0.05$). In patients with absence of *Demodex* mites, in most cases the condition recurred only once (20; 33.3%), whereas in group I the highest number of patients had 1-3 recurrence a year (30; 50%). Thus, *Demodex* mites complicate the clinical picture of the condition and contribute to a more frequent recurrence of the process.

Evaluation of the Dermatology Quality of Life Index

When comparing the average indices of the dermatology life quality index, a statistically significant difference was revealed. In Group I patients, the mean value of the index was 12.5±4.5 (min=5.0, max=19.0). In Group II patients, the mean value of the dermatology life quality index was 8.0±3.4 (min=2.0; max=19.0), ($P<0.05$). The average value of the dermatology life quality index in the presence of *Demodex* brevis is 10.5. In the presence of *Demodex* folliculo-

Table 1. The distribution of patients by sex, age, diagnosis and the presence of *Demodex* mites.

	Group I (with the presence of *Demodex* mites)		Total (n; %)
Sex	Male n=28; 46.7%	Female n=32; 53.3%	60 (100%)
Age	47±10.4	51±17.6	
Group II (with no *Demodex* mites)			
Sex	Male n=24; 40.0%	Female n=36; 60.0%	60 (100%)
Age	41±6.3	33±2.9	
Group III (healthy people)			
Sex	Male n=30; 41.7%	Female n=42; 58.3%	72 (100%)
Age	26±1.4	31±3.1	

rum and the combined cases of simultaneous detection of two mites species - 15.5 and 13.5, respectively.

Clinical picture of patients with rosacea

The distribution of patients by severity of rosacea is presented in Table 2.

Determination of species affiliation of *Demodex* mites

The greatest number of rosacea cases in Group I patients was associated with parasitizing *Demodex* folliculorum (n=40; 66.7%), *Demodex* brevis was found in 14 patients (23.3%); both mites were found in 6 patients (in 10.0% of cases). When examining 72 healthy people by light microscopy of scrapes, *Demodex* mites were detected in six cases (n=6; 2.8%), in the remaining 66 cases (97.2%), mites failed to identify. In determining the species of *Demodex* mites in healthy people, the parasitism of *Demodex* brevis was revealed in all 6 cases. *Demodex* folliculorum (n=40; 66.7%) is statistically significant in the structure of species affiliation in patients with rosacea. The number of detection cases of this species of mite prevails over the frequency of detection of *Demodex* brevis and associated parasitism by two species of mite.

A further study showed that *Demodex* folliculorum accompanies heavier forms of the condition (pustulous and infiltrative-productive forms of rosacea) (n=16; 26.7% and n=22; 36.7%, respectively). Combined cases of simultaneous detection of two species of mites (*Demodex* folliculorum and *Demodex* brevis) on the scrapes of facial skin by light microscopy also correlated with severe forms of rosacea (n=4; 6.7% and n=2; 3.3%, respectively).

Comparative analysis of diagnostic methods for detecting *Demodex* mites

In this step we compared all patients with rosacea included those, in which cases *Demodex* mites were revealed by only one method. To assess the validity of the confocal laser scanning *in vivo* microscopy method, the survey was conducted in all three groups of subjects. A comparative study was performed on the effectiveness of confocal laser scanning *in vivo* microscopy and scrape methods followed by microscopic study. The data obtained are presented in Table 3.

Positive results for the detection of *Demodex* mites by the method of light microscopy scrapes were 60 patients with rosacea (28.3%), confocal laser scanning *in vivo* microscopy - 80 patients (37.7%). Using confocal laser scanning *in vivo* microscopy, it was also possible to identify *Demodex* mites in healthy people (n=12; 5.7%), and in the light microscopy of scrapes of *Demodex* mites in the number of 5 individuals per 1 cm^2, only 6 healthy persons (n=6; 2.8%); in the remaining 66 healthy people (31.2%), the light microscopy of the scrapes was negative.

Quantification of *Demodex* mites

As a result of the study, we found that it is difficult to detect the mite by light microscopy of scrape per 1 cm^2 of skin (Table 4).

Table 2. Distribution of patients with rosacea I and II groups according to the severity of the condition.

Form of the condition	Group I (n; %)	Group II (n; %)	Total (n)
Erythematous-telangiectatic	6; 10	28; 46.7*	34
Papular	8; 13.3	22; 36.7	30
Pustulous	22; 36.7*	10; 16.6	32
Infiltrative-productive	24; 40*	0	24
Total	60; 100	60; 100	120

*P≤0.05.

Table 3. Comparative analysis of study methods for the presence of *Demodex* mites in diagnostically significant amounts.

Method	Identification of *Demodex* mites (>5 individuals per 1 cm^2)	Patients diagnosed with rosacea (n=140; 66%)	Healthy people (n=72; 34%)	Total (n=212; 100%)
Scrape with subsequent	+	60; 28.3%	6; 2.8%	66; 31.1%
light microscopy	-	80; 37.7%	66; 31.2%	146; 68.9%
Method	**Identification of *Demodex* mites (>5 individuals per 1 cm^2)**	**Patients diagnosed with rosacea (n=140; 66%)**	**Healthy people (n=72; 34%)**	**People (n=212; 100%)**
Confocal laser scanning	+	80; 37.7%	12; 5.7%	92; 43.4%
in vivo microscopy	-	60; 28.3%	60; 28.3%	88;56.6%

Table 4. Detection of *Demodex* mites by the method of light microscopy of scrapes.

Patients with rosacea with the presence of *Demodex* mites (n; %)	Patients with rosacea with no *Demodex* mites (n; %)			Healthy people (n; %)		Total (n; %)
Presence of *Demodex* mites >5/cm^2	Presence of *Demodex* mites <5/cm^2	Absence of *Demodex* mites	Presence of *Demodex* mites >5/cm^2	Presence of *Demodex* mites >5/cm^2	Absence of *Demodex* mites	
60; 28.3	22; 10.4	58; 27.4	2; 0.9	4; 1.9	66; 31.1	212; 100
60; 28.3	80; 37.8			72; 33.9		

Demodex mites in a diagnostically significant amount (>5 individuals per 1 cm^2) were revealed in 60 patients with rosacea (28.3%), included in group I. In 80 patients with rosacea (37.8%) with *Demodex* mites were detected in an amount of less than 5 individuals per 1 cm^2 or were absent altogether with a developed clinical picture of the condition.

In the examination of healthy people, 66 people (31.1%) had a negative analysis for the presence of *Demodex* mites, while *Demodex* mites were found in a diagnostic amount (more than 5 individuals per cm^2) in two subjects (0.9%), in four respondents, the mites were found in an amount of less than 5 individuals per cm^2 (1.9%). *Demodex* mites in an amount of <5 individuals per 1 cm^2 were detected much more often (n=80; 37.8%, respectively). While study the same respondents using confocal laser scanning *in vivo* microscopy, the following data were obtained (Table 5). When analyzing the data in Table 6 attention is drawn to the fact that there were no <5 mites per cm^2 in any case By using confocal laser scanning *in vivo* microscopy, *Demodex* mites were found in patients with rosacea (n=80, 37.8%) and in healthy people in a larger number of cases (n=12; 5.7%).

Using a confocal laser scanning *in vivo* microscope allowed determining the average size of *Demodex* mites. When determining the size of mites from 100 to 200 μm, it was believed that in this case *Demodex* brevis was observed, while the average length of the mite was 125 μm; from 200 to 400 μm – *Demodex* folliculorum with an average length of 293 μm. The average size of the width of *Demodex* mites was 24 μm.

When measuring the size of the follicular aperture and estimating their number per unit area, for which a randomly chosen site of 25 mm^2 was taken, statistically significant differences between the three groups were revealed (P<0.01). It was established that the size of the follicular aperture and their number per unit area in all three groups differ significantly. It was found that the largest size of the mouths of the hair follicles and the excretory ducts of the sebaceous glands were patients of group I with *Demodex* mites (0.125±25 μm), whereas in group II the size of the hair follicles and sebaceous glands was 0.89±32 μm, in group III - 0.072±29 μm.

Patients of group I had the highest number of follicles and excretory ducts of sebaceous glands in study groups per 25 mm^2 (324±11), in group II - 114±6 and in group III - 28±7.

Antiparasitic therapy in patients with rosacea

After obtaining a positive result of the study for the presence of *Demodex* mites, the patients were randomized according to treatment regimens into two equal subgroups (A and B, respectively) for 30 people each. Patients enrolled in subgroup A received only external therapy with a drug containing 1% ivermectin in the form of a cream 1 time per day for 30 days. Patients enrolled in subgroup B received a drug containing 250 mg of metronidazole systemically 2 times a day, externally 1% metronidazole in the form of a gel 1 time per day for 30 days. A repeat visit of the patients took place after 30 days of continuous therapy. Subjectively, treatment regimens of patients were well tolerated, no side effects were noted, no patient was excluded from the study. When comparing the efficacy of the therapy, it was found that statistically significantly more *Demodex* mites were found after treatment with confocal laser scanning *in vivo* microscopy (P≤0.05) (Table 7). For a comparative evaluation of the effectiveness of treatment methods in subgroups A and B, the clinical picture was compared in the next stage in patients with rosacea associated with *Demodex* mites before and after treatment.

Complaints of patients before and after the treatment are given in Table 8.

As can be seen from Table 8, after treatment statistically reliably decreased complaints of patients on rashes, burning sensation, pain, pruritus, and rubeosis. Moreover, in patients with subgroup A, complaints of greasy lusters of skin gloss decreased, which is an additional advantage of topical therapy. An objective analysis of the clinical picture of patients with rosacea associated with *Demodex* mites after the therapy showed that the following morphological elements in subgroups A and B significantly regressed: papules, pustules, excoriation (P≤0.05) compared with the original data.

Analysis of the clinical picture showed a positive dynamics of therapy, which manifested itself in a significant decrease in the number of morphological elements characterizing the severity of inflammation (P≤0.05). The effectiveness of the therapy was confirmed by a reduction in subjective complaints of patients after the treatment, and patients who received only external therapy had no complaints of a feeling of lusters of skin and the appearance of greasy lusters, which is an additional advantage. Thus, clinical observations demonstrated a lack of superiority in combined antiparasitic therapy using a systemic drug compared to external therapy using a preparation containing 1% ivertmectin as a cream, as confirmed by statistical analysis.

Discussion

Association of *Demodex* mites identification with predisposing factors such as emotional stress, inadequate nutrition, exacerbation of concomitant diseases are correlated with the data of already available studies, in which the role of trigger factors in the development of demodecosis is discussed.[9-12] In addition, the effect of heat, cold, spicy food on the development of rosacea wasdetailed in a study by Aubdool.[13]

Our findings confirm the hypothesis of Turgut Erdemir *et al*., that the *Demodex* mites affect the severity of the disease and contribute to the progression of the pathological process. In addition, the authors

Table 5. Detection of *Demodex* mites by confocal laser scanning *in vivo* microscopy.

Patients with rosacea with the presence of *Demodex* mites (n; %)	Patients with rosacea with no *Demodex* mites (n; %)	Healthy people (n; %)		Total (n; %)
Presence of *Demodex* mites >5/cm^2	Absence of *Demodex* mites	Presence of *Demodex* mites >5/cm^2	Absence of *Demodex* mites	
80; 37.8	60; 28.3	12; 5.7	60; 28.3	212; 100
80; 37.8	60; 28.3	72; 34		

have proved that the density of mites increases depending on the severity of the disease.[14]

The effect of rosacea on the life and social activity of patients is still debated.[15] In a meta-analysis of Bewley *et al*., which included 1,624 patients diagnosed with rosacea, 26.4% complained of anxiety and depression, and 43% had a quality of life violation. 62% of respondents indicated that rosacea affected their work and social life, and 26.1% began to avoid social contacts because of the disease.[16] When comparing the average indicators of the Dermatology Quality of Life Index, it was revealed that in patients of group I the condition has a *very strong effect* on the life of the patient, in patients in group II; rosacea had a *moderate effect* on the life of patients. Rosacea, complicated by the presence of *Demodex* brevis, has a *moderate effect on life* of patients. In the presence of *Demodex* folliculorum and combined cases of simultaneous detection of two species of mites, the condition *greatly affects* the life of patients. In patients with the presence of *Demodex* folliculorum, the dermatology life quality index is statistically significantly different from the index of patients with *Demodex* brevis (P<0.05), which is logically logical, since we established that *Demodex* folliculorum is found in patients with heavier forms of diseases.

The detection of *Demodex* mites is not only statistically more significant in patients with rosacea than in the rest of the population,[17] but also as can be seen from the Table 2, *Demodex* mites were more often found in patients with more severe clinical forms of rosacea (pustulous, infiltrative-productive forms).

Such pathological formations as deep papulopustulous elements and nodes, facial skin erythema, greasy lustre are more pronounced in patients of group I, which again confirms that the presence of *Demodex* mites predisposes to the emergence of acute inflammatory morphological elements, contributing to the development of more severe clinical forms of diseases. Our results coincide with the results of a study by Moravvej *et al*., where it was proved that mites play a role in the development of rosacea, stimulate the formation of an inflammatory process, which subsequently leads to tissue damage and the formation of telangiectasias.[18]

The data obtained in determining the species belonging to *Demodex* mites show that *Demodex* folliculorum is more often detected in the analyses than *Demodex* brevis and *Demodex* brevis is detected more often than the combined cases are recorded. *Demodex* folliculorum is statistically significantly more common in patients than simultaneous parasitization of two species of mites (P<0.01). This suggests that in the absence of a clinical picture of rosacea, it is significantly more significant that *Demodex* mites` species will not be found in scrape (P<0.01). In studies by Erbağci *et al.* (1998), Divani *et al*. (2009) and Gonzalez-Hinojosa *et al*. (2018) demonstrated that statistically significantly more people in the clinical manifestations of rosacea will be found to have *Demodex* mite than in healthy volunteers.[19-21]

Comparing the results obtained by light microscopy and confocal laser scanning *in vivo* microscopy in patients with rosacea and healthy people, in more cases *Demodex* mites are detected by confocal laser scanning *in vivo* microscopy, whereas scrape in these patients were negative. The obtained data demonstrate not only high information content of the confocal laser scanning *in vivo* microscopy method, but also its superiority over microscopic diagnostics. This was also demonstrated in studies by Gonzalez *et al*. and Rajadhyaksha *et al.*[22,23]

In the examination of healthy people by light microscopy, *Demodex* mites were detected in 6 cases (2.8%). Given the ability of the mites to move over the surface of the skin at a speed of 8-16 mm/h,[9,10] as well as random selection of the study site, this fact does not prove the absence of mites. Given the species belonging to *Demodex* mites in healthy people (all had *Demodex* brevis); the absence of a clinical picture of the condition confirms that this species belongs to saprophyte of facial skin. By using confocal laser scanning *in vivo* microscopy, *Demodex* mites were defined as rounded or long conical formations in the mouths of the hair and sebaceous glands with the presence of peripheral contouring in the number from one to 25 individuals (an average of 3.37). Sattler *et al*. showed the possibility of using confocal laser scanning *in vivo* microscopy to determine *Demodex* mites and described

Table 6. Analysis of the clinical picture of patients with rosacea associated with *Demodex* mites, before and after the therapy.

Elements	Sub-group A		Sub-group B	
	Before treatment	After treatment	Before treatment	After treatment
Papules	100*	43	74*	48
Pustules	82*	30	63	42
Open comedons	87*	42	87*	47
Milium	60*	23	41*	14
Teleangiectasias	41*	14	56*	12
Perifocal erythema	56*	20	75*	36
Excoriations	45*	11	68*	21
Pigmentation	74	53	90*	63
Greasy lusters of skin	65*	25	72	56

*P≤0.05.

Table 7. Comparative detection table of *Demodex* mites after the therapy in subgroups A and B.

	Scrape of the skin, squeezing the contents of the sebaceous glands followed by microscopy		Confocal laser scanning *in vivo* microscopy	
	Presence of *Demodex* mites (n; %)	Absence of *Demodex* mites (n; %)	Presence of *Demodex* mites (n; %)	Absence of *Demodex* mites (n; %)
Sub-group A	2; 3.3	28; 46.7	4; 6.7*	26; 43.3
Sub-group B	4; 6.7	26; 43.3	10; 16.7*	20; 33.3

*P≤0.05.

them as rounded and long conical structures.[7] A comparison of the effectiveness of the performed therapy once again demonstrates the superiority of confocal laser scanning *in vivo* microscopy over the subsequent light microscopy (Table 7). When analyzing the data obtained in Table 7, it can be stated that both methods of treatment had high antiparasitic efficacy. Considering the fact that there was no statistically significant difference in negative analyzes for the presence of *Demodex* mites in both laboratory and instrumental diagnostics, we can speak of a high antiparasitic efficacy of a topical medicine containing 1% ivertmectin in comparison with combined treatment with the systemic drug metronidazole and a topical agent containing 1% metronidazole. The relevance of ivermectin for the treatment of rosacea has been shown in a study by Cardwell *et al*.[24] When comparing 1% ivermectin and 0,75% metronidazole, ivermectin showed greater efficacy. This was expressed in the quality of life of patients, reducing the number of inflammatory elements and the absence of side effects.[24]

Analysis of the clinical picture showed a positive dynamic of therapy, which manifested itself in a significant decrease in the number of morphological elements characterizing the severity of inflammation (P<0.05). The effectiveness of the therapy was confirmed by a reduction in subjective complaints of patients after the treatment, and patients who received only external therapy had no complaints of a feeling of lustre of skin and the appearance of a greasy lustre, which is an additional advantage. Thus, clinical observations demonstrated a lack of superiority in combined antiparasitic therapy using a systemic drug compared to external therapy using a preparation containing 1% ivertmectin as a cream, as confirmed by statistical analysis. Stein *et al*. showed that after 12 weeks of ivermectin treatment, the skin of patients was defined as *clean* or *almost clean*. There was a significant reduction in the percentage of inflammatory lesions in the ivermectin treatment group. The results of the study showed that 1% ivermectin is an effective and safe treatment for inflammatory lesions in patients with rosacea.[25]

Conclusions

As a conclusion, the following is stated:

i) *Demodex* mites complicate the clinical picture and the course of rosacea. When analyzing the clinical picture and the course of the condition in patients with rosacea associated with *Demodex* mites, it is established that *Demodex* mites, promote the development of acute-inflammatory morphological elements (deep papular, pustulous elements, nodes, perifocal erythema), increase the duration of the condition (more than 5 years, P<0.01) and the probability of recurrence (from 1 to 3 relapses in 39.5% of patients, P<0.05), resulting in a decrease in the quality of life of patients (dermatology life quality index 12.5±4.5, P<0.05). The severity of the clinical manifestations of rosacea does not depend on the number of individuals detected by the method of light microscopy of scrapes of facial skin. The most significant factors predisposing to the development of complications are: morpho-functional characteristics of the skin (high greasiness, reduced moisture, alkaline pH shift, and larger pore size), emotional impact, including stress, inadequate nutrition and exacerbation of concomitant diseases.

ii) In patients with severe manifestations of the condition (pustulous and infiltrative-productive forms of rosacea), the species of the mites *Demodex* folliculorum (P<0.01) is more often detected. *Demodex* brevis is found in mild forms of the condition and in healthy people, without showing signs of parasitism (P<0.01).

iii) Confocal laser scanning *in vivo* microscopy is an effective diagnostic method to detect *Demodex* mites that does not require preliminary preparation for analysis and allows detecting *Demodex* mites at the level of the spiky epidermis layer, which is not accessible for scarification, to identify the species belonging to the size of *Demodex* mites (from 100 up to 200 μm - *Demodex* brevis, 200 to 400 μm – *Demodex* folliculorum).

iv) Antiparasitic drug ivermectin, in the form of an external form (cream), at a concentration of 1% (1 time per day, the general course of 30 days) has a high therapeutic efficacy in patients with associated with *Demodex* mites (in 93.3% of cases). The effectiveness of external therapy with a drug containing 1% ivermectin (course of 30 days) is comparable to the combined treatment with the systemic drug metronidazole 250 mg per os 2 times a day and the external application of 1% metronidazole (gel) 1 time per day for 30 days.

v) All patients diagnosed with rosacea pustulous and infiltrative-productive form are examined for the presence of *Demodex* mites with the definition of their species.

vi) When *Demodex* folliculorum is detected, regardless of its quantitative load, treatment with antiparasitic drugs is indicated.

vii) When *Demodex* brevis is found, given its weak possibility of parasitism, treatment with antiparasitic drugs is not indicated.

As an antiparasitic drug in the treatment of rosacea associated with *Demodex* mites, it is recommended to use 1% ivermectin in the form of a cream for 30 days externally. The drug should be applied a thin layer on the previously cleaned facial skin at night. The subsequent treatment should be carried out in accordance with the main diagnosis.

Table 8. Comparison of subjective complaints of patients with rosacea associated with *Demodex* mites before and after treatment.

Complaints	Sub-group A		Sub-group B	
	Before treatment	After treatment	Before treatment	After treatment
Rashes	100%	70%*	100%	65%*
Pain	68%	34%*	75%	30%*
Burning sensation	59%	40%	64%	30%*
Rubeosis	69%	40%*	70%	40%*
Pruritus	70%	20%*	54%	10%*
Chromatosis	42%	25%	36%	17%
Presence of crusts/excoriations	45%	30%	39%	20%
Starburst veins	60%	40%	58%	40%
Greasy lusters of skin	70%	40%*	65%	40%

*P≤0.05.

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