

THE PHARMA INNOVATION - JOURNAL

Clinical and Immunological Features of Rosacea in Patients of Carpathian Region

Nataliya Senyshyn^{1*}, Ludmyla Khimeychuk¹, Nina Bychkova², Sergiy Fedorov¹

1. Ivano-Frankivsk National Medical University, 76018, Ivano-Frankivsk, Ukraine.
[Email: nata_if@mail.ru]
2. National Medical University named after O. Bogomolets - 01001, Kyiv, Ukraine.

Rosacea is a chronic and potentially life-disruptive disorder primarily of the facial skin, often characterized by flare-ups and remissions. The incidence of this dermatosis has been steadily increasing from year to year and ranging from 2 - 5% of skin diseases. The 120 patients with different stages of Rosacea were observed in this study. The cellular and humoral immunity disturbance was established in patients with Rosacea.

Keyword: Rosacea, Immunity, Demodex

1. Introduction

Today, acne rosacea is a topical problem of modern dermatology. The incidence of this dermatosis has been steadily increasing from year to year and, according to the literature, ranging from 2 - 5% of skin diseases and has 7th position in frequency among skin diseases^[1,2,3].

Rosacea is polyethiological disease, based on a significant role of many exogenous and endogenous factors. Acne rosacea is severe disease in the cosmetology which localized on the face and significantly changes the appearance that often affects the performance of the patient, his position in society, personal life, reduces emotional stability, quality of life, promotes emotional disorders^[1,4,5].

1.1 The aim of study is to establish of the clinical features of the course and determine the status of immunological reactivity in patients with rosacea, residents of Ivano-Frankivsk region.

2. Materials and methods

120 people with rosacea and 30 healthy individuals (control group) were observed. Among the examined patients were 43 male individuals (36%) and 77 females (64%) aged 30 to 59 years. Patients were divided into groups according to the stage of the disease: patients with erythematous-teleangiectatic stage - 17 persons, patients with papular-pustular stage of acne rosacea - 88 persons and conglobat stage of rosacea - 15 persons. The diagnosis of rosacea, disease stage and treatment strategy was determined according to the Order of Ministry of Public Health of Ukraine № 286 from 07.06.2004 year. General clinical, laboratory and special examinations of patients conducted at the Ivano-Frankivsk Regional Dermatology clinics. The initial evaluation of patients was performed according to the European standards^[1,6,7], according to differentiated by gender and age programs that include mandatory for laboratory and instrumental investigations and medical consultations related specialties. Along with

clinical methods of examination of patients with rosacea (complaints, history of disease, history of life, objective test) and general clinical laboratory assessments (complete blood count, blood chemistry, urinalysis, coprogram, ultrasound of the abdomen and consultation of gastroenterologist) in this study we also used the following research: identification of Demodex folliculorum, bacteriological examination of skin microflora, the study of immune status, which included the tests I and II levels as required by the Memorandum WHO^[8]: quantify of the major lymphocyte populations and subpopulations, identifying subpopulations of lymphocytes (CD54 +, HLA-DR +, CD25 +), the level of pro- and anti-inflammatory cytokines.

3. Results and Discussion

Erythematous-teleangiectatic stage of rosacea was diagnosed in 17 patients (14.17%), papules and pustular stage - in 88 patients (73.33%) and conglobat stage - only in 15 patients (12.50 %); and, in both men and women dominated papules with pustular stage of disease. Lesions in the patients suffering from rosacea placed on the face. As more patients are localized on the cheeks (79.17%), led - in 20.83% and chin - in 27.50% of patients. Only 6.67% of patients with rosacea process apply to eyelids and nose. According to the Hydrometeorological Service, including Ivano-Frankivsk Regional Centre for Hydrometeorology, in Carpathians is characterized by high humidity (75-79%), mood night and day temperature (10-15°C), which contributes to chronic process. According to the literature an important role in the onset and progression of rosacea skin microbiots plays infringement and availability, in particular, Demodex folliculorum [9,10,11]. The first study found Demodex in 60 patients (50.0%). The 28 re-examined patients (23.3%) revealed Demodex. Studies have shown the presence of Demodex in 88 patients (73.3%) .

The Demodex detection dependence of disease stage is presented in Table. 1.

Table 1: Demodex detection in patients with Rosacea

Stage of Disease	Quantity of patients with Demodex, n=120	
	Quantity	%
Erythematous-teleangiectatic stage, n=17	10	58,8
papules and pustular stage, n=88	75	85,9
Conglobat stage, n=15	3	20,0

As Table 1 shows, the percentage of detecting of the Demodex folliculorum in patients with rosacea is quite high. In particular, in patients with erythematous-teleangiectatic stage rosacea this mite was founded in 10 individuals (58.8%), in patients with papules and pustular stage - in 75 individuals (85.9%); and 3-rd stage disease had mite infection in 20.0% cases. Thus, its mean that Demodex folliculorum mite plays an important role in causing acne rosacea, which requires the use in the treatment of rosacea by antiparasitic agents. It should be noted that in addition to the presence of the mite Demodex folliculorum, we have found serious violations of the skin microflora in patients with acne rosacea. In particular, Staphylococcus epidermidis gave weak and strong growth in 50 patients (41.67%) who were diagnosed papules and pustular stage of rosacea. The most of patients with erythematous-teleangiectatic stage (17 patients), pustular and papules stage (38 patients) and conglobat stage (15 patients) of the disease showed I and II growth answer of Staphylococcus epidermidis. Staphylococcus aureus gave weak and strong growth in only 31 patients (25.83%) diagnosed with pustular and papules stage of rosacea. Besides this, in rare cases in patients with papules with pustular stage of acne rosacea we revealed the following microorganisms: Proteus vulgaris, Escherichia coli, Pseudomonas alcaligenes, Pseudomonas aeruginosa, Acinetobacter haemolyticus, Streptococcus haemolyticus, which gave moderate growth (III type of growth). We support the idea, that a

change of skin microflora in patients with rosacea is secondary.

After a thorough examination of patients and consultations related specialists in patients with acne rosacea we founded the following comorbidities: Lesions of the gastrointestinal tract (50% of patients), various pathologies of the cardiovascular system - in 15 patients (12.5%); illness of the respiratory system –(frequent SARS - in 46 patients with chronic bronchitis - 9 persons (7.5%), recurrent upper respiratory tract disease was observed in 27 patients (22.5%));

Diabetes mellitus was found in 2 patients, hyperplasia of the thyroid gland - 7 persons (5.8%).

Currently, there are rare data on the disturbances in the immune system in patients with rosacea, but present datas have different character and are very fragmented [1,2,5,10,12]. As shown by studies carried out by us (Table 2), in patients with erythematous rosacea stage likely observed increase in T lymphocytes and reduce the total number of B-lymphocytes compared with the control group (p <0,05).

Table 2: Cell immunity in patients with Rosacea

Immunology data	Rosacea stage			Control group, n=30
	Erythematous-teleangioectactic, n=17	papules and pustular , n=88	Conglobat, n=15	
CD 3 ⁺ cells, %	63,72 ± 0,61*	53,14 ± 0,45 *	69,17±0,36*	59,70 ± 0,43
CD 4 ⁺ cells, %	36,04±0,57 *	39,85 ± 0,74	49,9±0,45*	40,80 ± 0,82
CD 8 ⁺ cells, %	27,82 ± 0,49	26,97 ± 0,53	20,25±0,48*	26,55 ± 0,56
CD 19 ⁺ cells, %	10,05 ± 0,43 *	10,54 ± 0,43 *	12,32±0,36	13,20 ± 0,56
CD 4 ⁺ / CD 8 ⁺	1,29 ± 0,09 **	1,48 ± 0,07	2,44±0,39*	1,55 ± 0,04
CD 16 ⁺ cells, %	16,82 ± 0,83	17,98 ± 0,72	18,44 ± 0,63	17,80 ± 0,86

Notes: 1. Probability difference between the compared with control: * - p <0.05, ** - p <0.01. 2. n - number of patients studied.

However, among the subpopulation of T lymphocytes was observed certain imbalance: loss of T-helper cells in the background improbable increase in T suppressor / cytotoxic, accompanied by a decrease in the immunoregulatory index 16,78% (p <0,01). However, in patients with rosacea in papules with pustular stage was marked (Table 2) reducing the total as CD 3 + lymphocytes and CD 19 + cells. The level of CD 4 + and CD 8 + T cells did not differ significantly from similar data in healthy subjects (p <0,05). Patients with conglobat stage of the disease was observed increase the number of CD 3 + cells and CD 4 + T cells both in relation to data in healthy individuals and other groups of patients. Similarly, there were changes

in the subpopulation composition of immunoregulatory cells. Thus, the contents of CD 8 + T cells in patients with acne rosacea in conglobat stage was rather less similar data controls and patients with other stages. The above changes in the level of immunoregulatory subpopulations of lymphocytes in the conglobat stage of rosacea accompanied by increasing immunoregulatory index to 2,44 ± 0,39, which is higher than the corresponding levels in healthy individuals and in other stages of rosacea. It indicates of development at this stage of autoimmune disease changes will certainly aggravate the course of the underlying disease and require treatment optimization with the optional inclusion of basic therapy immunotropic

drugs. Level of CD 16 + lymphocytes in patients with rosacea with different stages of disease did

not differ significantly from those in healthy subjects.

Table 3: The activated lymphocyte subpopulations in peripheral blood of patients with rosacea (M ± m)

Immunological data	Stage of Rosacea			Control group, n = 30
	Erythematous-teleangiectatic, n = 17	papules and pustular, n = 88	Conglobat, n = 15	
CD 25 ⁺ cells,%	13,27 ± 0,68 *	17,91 ± 1,43 *	21,45 ± 1,65 *	8,8 ± 0,19
HLA-DR ⁺ cells,%	17,68 ± 1,81 *	22,53 ± 1,79 *	24,82 ± 1,94 *	12,3 ± 1,27
CD 95 ⁺ cells,%	7,50 ± 0,81 *	12,62 ± 1,31 *	17,64 ± 1,47	3,04 ± 0,02
CD 54 ⁺ cells,%	12,60 ± 1,37	19,81 ± 1,63 *	23,15 ± 1,68 *	11,07 ± 1,65

Notes: Probability difference in compared with the control: * - P <0.05, ** - p <0.01.

Analysis of the data presented in Table 3 shows, that immune system of patients with rosacea is observed the early T-cell activation and late activation of T-and B-lymphocytes, increasing relative level of CD 25 + lymphocytes and evidence of significant prevalence of peripheral blood T lymphocytes - namely, T-helper type I. A similar trend was for the level of HLA-DR + lymphocytes (activated T-and B-lymphocytes); its level increased in parallel with increasing severity of disease. Based on this findings, it is clear that the reason for the activation of T and B lymphocytes in peripheral blood is a disease itself, but as far as its progression and occurrence of complications related activation of immune cells increases, and most likely due to the appearance in the blood of autoantigens specific to large of comorbidity various entities that skin colonization of pathogenic and conditionally pathogenic microflora and the presence of Demodex, which also contribute to both complications of rosacea. The high content in the peripheral blood of patients with different stages of rosacea of the lymphocytes wick expressed

FAS - receptor is predictor of reading to enter into apoptosis. The high level of CD 95 + lymphocytes in patients with all stages of rosacea is due, in our opinion, especially symptoms of inflammatory reaction and performance of the main proinflammatory cytokines - TNF-α, which is an active inducer of apoptosis of immune cells.

As can be seen from the data presented in Table. 4, in patients with acne rosacea revealed significant changes in the level of cytokines in the direction of the advantages of proinflammatory activity. Thus, the level of TNF-α in patients with erythematous stage of rosacea was above data in healthy subjects at 49.91%, exceeding the data of the control group as far as complications of the disease - from 133.81% at papules with pustular stage to 116.38% - with pustular nodular stage of rosacea. In support of inflammatory activity than TNF-α is involved and the proinflammatory cytokines IL-1 β, whose level of rosacea also increases as far as complications of the disease, exceeding data in healthy subjects by 32.34% with erythematous stagem, to 80.82% in patients

with papules and pustular stage and 88.46% - with pustular stage of rosacea. Increased proinflammatory cytokines - TNF- α and IL-1 β in

patients with rosacea showed their important role in the genesis of target organ damage and formation of complications.

Table 4: The level of pro-and anti-inflammatory cytokines in the serum of patients with rosacea (M \pm m).

Data	Stage of Rosacea			Control group, n=30
	Erythematous-teleangiectatic, n=17	papules and pustular, n=88	Conglobat, n=15	
TNF- α , pg/ml	63,41 \pm 3,52 *	98,90 \pm 4,27 *	91,53 \pm 4,68 *	42,30 \pm 2,90
IL-1 β , pg/ml	52,17 \pm 2,98 *	71,28 \pm 3,06 *	74,29 \pm 3,18 *	39,42 \pm 2,10
IL- 6, pg/ml	15,45 \pm 0,65 *	16,94 \pm 0,41 *	13,47 \pm 0,51	10,31 \pm 0,73
IL- 4, pg/ml	17,93 \pm 0,57	19,22 \pm 0,17	18,56 \pm 0,34	15,42 \pm 0,84

Notes: Probability difference in compared with the control * - P <0.05.

We established the high correlative relationship between the content of activated HLA-DR + lymphocytes and the concentration of TNF- α (r = +0,91), HLA-DR + lymphocytes and the concentration of IL-1 β (r = +0,78), and between relative number of activated CD 95 + lymphocytes and the level of TNF- α (r = +0,83). Dynamics of proinflammatory cytokine IL-6 in patients with rosacea showed that the maximum activity of the inflammatory process to its final stages observed in patients with papules with pustular rosacea stage when its level prevailing data on healthy individuals for 64.31%.

4. Conclusions

1. Changes in the microflora of the skin of patients with rosacea are secondary in nature.
2. The detection rate of Demodex folliculorum in patients with rosacea is 73.33%, which confirms the important role of mites in causing disease, and as a result, requires the use in the treatment of rosacea anti-parasitic drugs.
3. Dermatoses in patients studied were accompanied by certain changes in the cellular and humoral immunity, which were

recorded at all stages of rosacea, and depended on the severity of the disease.

4. In patients with rosacea immunity established as an early activation of T cells and late activation of B-lymphocytes.
5. In patients with rosacea is present cytokine' dysbalance: probable changes in the level of cytokines in the direction of the advantages of proinflammatory activity - TNF- α , IL-1 β and IL-6 levels against stored anti-inflammatory IL-4.

5. References

1. Адаскевич В. П. Акне и розацеа / В. П. Адаскевич - С-П. : Ольга, 2000. - С. 98-124.
2. Вислобоков А. В. Опыт лечения розацеа и периорального дерматита / А. В. Вислобоков // Российский журнал кожных и венерических болезней. - М., 2003. - №1. - С. 75-76.
3. Коган Б.Г. Етіопатогенетичні та клінічні аспекти розацеа, дерматиту періорального і демодикозу на сучасному етапі. Раціональні клініко-лабораторні алгоритми діагностики та диференційної діагностики цих дерматозів / Б.Г. Коган, В.І. Степаненко // Укр. журн. дерматол., венерол., косметол. - 2005. - №4 (19). - С. 22-36.

4. Степаненко В.І. Діагностика та диференційна діагностика вугрової хвороби (акне), розацеа і демодикозу з урахуванням аналізу клінічних ознак та симптомокомплексів на засадах доказової медицини. (Повідомлення 1) / В.І. Степаненко, А.В. Клименко // Укр. журн. дерматол., венерол., косметол. - 2009. - №1 (32). - С. 44-56.
5. Федотов В.П. Особенности дифференцированной терапии больных розацеа, сочетающейся малассезийной инфекцией кожи с учетом общего, местного иммунного статуса и факторов неспецифической защиты организма / В.П. Федотов, В.А. Джибриль // Успехи медицинской микологии. - Т.4. - Москва: Национальная академия микологии. - 2004.- С. 288-290.
6. Фицпатрик Т. Дерматология. Атлас-справочник / Т. Фицпатрик, Р. Джонсон, К. Вулф. - Москва : Практика, 1999. - 1088 с.
7. Хэбиф Т. П. Кожные болезни: диагностика и лечение / Т. П. Хэбиф; [пер. с англ. ; под общ. ред. акад. РАМН, проф. А. А. Кубановой]. - М. : МЕДпресс-информ, 2007. - [2-е изд.]. - 672 с.
8. Laboratory investigations in clinical immunology: methods, pitfalls and clinical indications: a second IUIS WHO report // Clin. Immunol. Immunopathol. - 1988. - Vol. 49. - P. 478-497.
9. Клинические рекомендации. Дерматовенерология / [под ред. А. А. Кубановой]. - М. : ГЭОТАР-Медиа, 2006. - 320 с.
10. Коган Б.Г. Порушення імунного статусу організму хворих на розацеа, демодикоз і періоральний дерматит з урахуванням патогенетичного значення інвазії шкіри кліщами демодіцитами / Б.Г. Коган, В.І. Степаненко // Укр. журн. дерматол., венерол., косметол. - 2005. - №1 (16). - С. 33-39.
11. Клименко А.В. Диференційна діагностика демодикозу, розацеа і вугрової хвороби (акне) з урахуванням аналізу результатів лабораторних тестів на наявність кліщів-демодіцит та прикметних симптомокомплексів, притаманних цим дерматозам на засадах доказової медицини. (Повідомлення 2) / А.В. Клименко, В.І. Степаненко // Укр. журн. дерматол., венерол., косметол. - 2009. - №2 (33). - С. 75-86.
12. Коган Б.Г. Стан судинного тонуусу, показники системи гомеостазу та імунного статусу організму хворих на розацеа, демодикоз і дерматит періоральний / Б.Г. Коган, В.І. Степаненко // Укр. журн. дерматол., венерол., косметол. - 2005. - №2 (17). - С. 20-25.