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## Investigations of seborrheic dermatitis. Part I. The role of selected cytokines in the pathogenesis of seborrheic dermatitis

### Badania nad łojotokowym zapaleniem skóry. Część I. Rola wybranych cytokin w patogenezie łojotokowego zapalenia skóry

#### Authors' Contribution:

- A** Study Design
- B** Data Collection
- C** Statistical Analysis
- D** Data Interpretation
- E** Manuscript Preparation
- F** Literature Search
- G** Funds Collection

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#### Introduction:

The etiology of seborrheic dermatitis is not fully understood. It has been observed that a number of anascogenic yeasts of *Malassezia* spp. is related to the intensity of the symptoms. The aim of the study is to measure the concentration of selected inflammatory factors IL-2, IL-4, IFN- $\gamma$  and TNF- $\alpha$  in the serum by an immunoenzymatic method, as well as to confirm the relationship between the studied factors and the clinical condition of the patients (sex, the intensity of skin lesions according to the Scaparro scale) and, finally, to compare the results with the control group.

#### Material/Methods:

The total number of subjects who participated in the study was 66. The control group (C) consisted of 30 volunteers (23 females and 7 males), with no clinical disorders, aged 24–65 (37.41 $\pm$ 6.08 years). Thirty-six patients with seborrheic dermatitis (16 females and 20 males), aged 19–76 (38.61 $\pm$ 13.77), made up the study group. The determination of IL-2, IL-4, IFN- $\gamma$  and TNF- $\alpha$  was performed by ELISA using a Human High Sensitivity kit (Diaclone, France). Clinically, the intensity of the disease process was evaluated on the Scaparro et al. scale, as modified by Kaszuba.

#### Results:

We observed statistically significantly higher levels of IL-2 and IFN- $\gamma$  in patients with seborrheic dermatitis compared to the control group.

#### Conclusions:

We conclude that seborrheic dermatitis is a dermatosis characterized by a cell type immune response with an important role of IFN- $\gamma$  and IL-2.

#### Key words:

seborrheic dermatitis • IL-2 • IL-4 • IFN- $\gamma$  • TNF- $\alpha$

## Streszczenie

#### Wstęp:

Etiologia łojotokowego zapalenia skóry nie została do końca wyjaśniona. Wykazano związek między gęstością zasiedlenia skóry przez drożdżaki z rodzaju *Malassezia* a nasileniem objawów. Celem pracy była ocena poziomu wybranych cytokin zapalnych IL-2, IL-4, IFN- $\gamma$  i TNF- $\alpha$  w surowicy chorych (ocena związku z płcią i rozległością zmian skórnych wg skali Scaparro) oraz porównanie z grupą kontrolną.

#### Material/Metody:

Badania przeprowadzono u 66 osób. Grupę odniesienia (O) stanowiło 30 osób (23 kobiety i 7 mężczyzn), klinicznie zdrowych w wieku od 24 do 65 lat (37,41 $\pm$ 6,08 lat). Grupę badaną (B)

stanowiło 36 osób (16 kobiet i 20 mężczyzn) chorych na ŁZS, w wieku od 19 do 76 ( $38,61 \pm 13,77$ ). Interleukiny 2, 4, IFN- $\gamma$  i TNF- $\alpha$  oznaczano z użyciem zestawu: Human High Sensitivity Elisa (Diacclone, Francja). Klinicznie stopień nasilenia procesu chorobowego oceniano wg skali Scaparro i wsp., w modyfikacji Kaszuby.

**Wyniki:** Przeprowadzone badania wskazują na istotne statystycznie podwyższenie poziomu IL-2 i IFN- $\gamma$  u wszystkich badanych chorych na łojotokowe zapalenie skóry.

**Wnioski:** Łojotokowe zapalenie skóry jest dermatozą z dużym udziałem odpowiedzi immunologicznej typu komórkowego z istotną rolą IFN- $\gamma$  i IL-2.

**Słowa kluczowe:** łojotokowe zapalenie skóry • IL-2 • IL-4 • IFN- $\gamma$  • TNF- $\alpha$

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## INTRODUCTION

Seborrheic dermatitis is a chronic recurrent disorder that affects mainly areas of the skin where numerous sebaceous glands are located. It occurs in 1–3% of the population not affected by any immunological disorders. The etiology of seborrheic dermatitis is unclear. It has been observed that a number of anascogenic yeasts of *Malassezia* spp. is related to the intensity of the symptoms. This relationship has been partly confirmed; the symptoms of the disease retreat after oral and topical application of antimycotic drugs. However, anascogenic yeasts of *Malassezia* spp. appear on the skin but do not produce any symptoms of inflammation. Seborrheic dermatitis can be compared to atopic dermatitis, which is characterized by the epidermal permeability barrier being impaired to such a great extent that such symptoms appear [6,8,9].

There is also an interesting relationship between seborrheic dermatitis and psoriasis. A common type of psoriasis is seborrheic psoriasis. The areas affected and morphology of lesions are almost identical to those typical of seborrheic dermatitis. This type of psoriasis may accompany lesions characteristic of psoriasis affecting other skin areas or may be an isolated lesion and therefore difficult to identify [4]. Arican et al. determined the levels of selected cytokines (TNF- $\alpha$ , IFN- $\gamma$ , IL-6, IL-8, IL-12, IL-17, IL-18) in the plasma of patients with psoriasis and compared the obtained results with the clinical condition of the skin [1]. They observed an increased concentration of the cytokines in the serum of the patients with psoriasis, which allowed them to classify the disease among systemic diseases. The aim of this study was to measure the concentration of selected inflammatory factors IL-2, IL-4, IFN- $\gamma$  and TNF- $\alpha$  in the serum of healthy volunteers and patients with seborrheic dermatitis by an immunoenzymatic method, as well as to confirm the relationship between the studied factors and the clinical condition of the patients (sex, the intensity of skin lesions according to the Scaparro scale) and, finally, to compare the results with the control group.

## MATERIALS AND METHODS

The total number of subjects who participated in the study was 66. The control group (C) consisted of 30 volunteers (23 females and 7 males), with no clinical disorders, aged 24–65 ( $37,41 \pm 6,08$  years). Thirty-six patients with seborrheic dermatitis (16 females and 20 males), aged 19–76 ( $38,61 \pm 13,77$ ), made up the study group. The age in both the groups was not significantly different. Tables 1 and 2 present detailed data on the studied subjects. The Bioethics Committee of Lodz Medical University gave its consent to perform this study.

Adult subjects with an active disease process were included in the study. Material for laboratory purposes was isolated exclusively from subjects who, in the period of three months prior to the study, had not undergone any topical or systemic treatment with antimycotic, anti-inflammatory or steroid preparations or had taken part in a blood transfusion. Clinically, the intensity of the disease process was evaluated on the Scaparro et al. scale, as modified by Kaszuba [7].

### Scaparro scale

- I. Characteristics of seborrheic dermatitis: erythema, desquamation, itch,
- II. A four-grade scale for evaluation of the intensity of the following symptoms:  
0 – no symptoms, 1 – mild symptoms, 2 – moderate symptoms, 3 – severe symptoms.

The number of affected areas ranged from 1 to 4. The areas were the following: the scalp, face, décolleté, interscapular area:

- 1 – one area affected, 2 – two areas affected, 3 – three areas affected, 4 – four areas affected.

The total number of points a patient could receive was 13. IL-2, IL-4, TNF- $\alpha$  and IFN- $\gamma$  were determined in all the patients and healthy subjects.

Table 1. Characteristics of the groups

Groups	Diagnosis	Sex	Number		Age Min-max	Years $\bar{x}$	SD
			N	%			
Control group (C)	Clinically healthy subjects	F	23	76.66	25–61	38.19	6.91
		M	7	23.34	24–65	36.16	5.99
		Total	30	100.00	24–65	37.41	6.08
Study group (S)	Seborrheic dermatitis	F	16	44.44	19–76	35.69	13.18
		M	20	55.56	19–48	41.35	14.30
		Total	36		19–76	38.61	13.77
Total			66	100			

Legend: C – control group, S – study group, F – females, M – males.

Table 2. Comparison of the groups – statistical analysis of the results of Table 1

Age – years		Student's t-test for independent groups	
Compared groups		Test value	Significance of differences
C: F vs M		t=1.19	p>0.05
S: F vs M		t=1.77	p>0.05
C vs S	FM Total	t=1.42 t=1.38 t=1.17	p>0.05 p>0.05 p>0.05

Legend: C – control group, S – study group, F – females, M – males.

### Methods of isolating the serum for laboratory purposes

The blood for biochemical analysis was taken in the morning, on an empty stomach. After complete coagulation of the blood at room temperature, the serum was isolated by centrifugation for 10 min at 1000 × g. The serum was removed and added to Eppendorf tubes. All the serum samples were stored at –75°C in a freezer.

### Determination of IL-2, IL-4, TNF-α and IFN-γ

The determination was performed by ELISA using a Human High Sensitivity kit for IL-2, IL-4, IFN-γ and TNF-α (Diacclone, France). The range of sensitivity was from 1.87 to 60 pg/ml. The result was measured with a Pointe 1800 spectrophotometer (Pointe Scientific, Poland) at a wavelength of 450 nm and a wavelength correction of 650 nm. The procedure was performed twice for all the results.

### Statistical analysis

N – the total number of subjects, n – the partial number of subjects, min–max – range of characteristic variety,  $\bar{x}$  – arithmetic mean, SD – standard deviation, Me – median, % – percentage, t – Student's t-test, p – significance of differences: < significant difference, > insignificant difference

The concentrations of interleukin in the control and study groups were compared according to the following pattern: C: F vs M C: F vs S C vs S: F C vs S: M, C vs S: Total

Legend: C – control group, S – study group, F – females, M – males.

## RESULTS

### Clinical evaluation of the skin condition of patients with seborrheic dermatitis

According to the Scaparro scale, modified according to Kaszuba, the mean intensity of skin lesions in the patients with seborrheic dermatitis was 8.50±3.28 in the females and 8.55±2.64 in the males; for both the sexes it was 8.51±3.19.

### IL-2 concentrations in the serum of subjects

The mean serum concentration of IL-2 was 13.91±0.96 pg/ml in the control group, and 17.94±2.88 pg/ml in the group of patients with seborrheic dermatitis prior to treatment. The difference is statistically significant (p<0.005). The male and female groups did not demonstrate any statistically significant differences regarding the mean concentration levels of IL-4.

### IL-4 concentrations in the serum of subjects

The mean concentration of IL-4 was 4.13±1.41 pg/ml in the control group, and 5.27±1.41 pg/ml in the study group. There were no statistically significant differences between these two groups. The male and female groups did not demonstrate any statistically significant differences regarding the mean concentration levels of IL-4.

### IFN-γ concentrations in the serum of subjects

The mean concentration of IFN-γ in the serum of patients with seborrheic dermatitis was 10.12±3.23 pg/ml. The concentrations of IFN-γ in the serum of both female and male

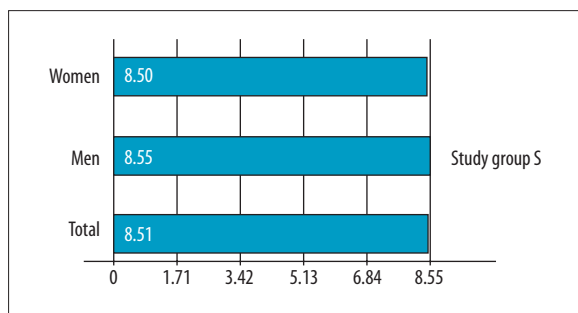


Fig. 1. Mean intensity of skin lesions in the study group with seborrheic dermatitis

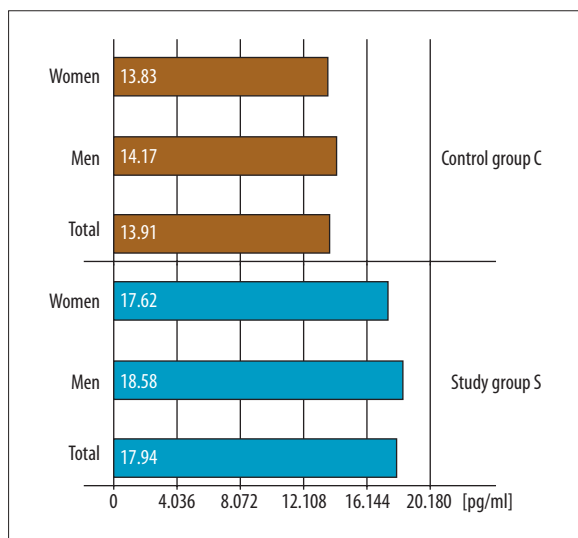


Fig. 2. Mean concentrations of IL-2 in the serum of the study and control group subjects

patients were higher than those of the control group. The difference was statistically significant ( $p < 0.05$ ).

**Mean concentrations of TNF- $\alpha$  in the serum of subjects**

The mean concentrations of TNF- $\alpha$  in the serum of patients with seborrheic dermatitis was  $55.11 \pm 7.99$  pg/ml. There were no statistically significant differences between the study and control groups. The males, females and the whole study group did not demonstrate any statistically significant differences.

**DISCUSSION**

Seborrheic dermatitis is a common, chronic dermatosis with periods of exacerbation and remission. Despite numerous studies, its pathogenesis remains unclear since the analyses that have been conducted so far are incomplete and their results are contradictory. Endogenous and environmental factors are taken into consideration by the studies and some authors point out the role of immunological deficiencies [5,10,11,12].

Studies on inflammatory factors, including proinflammatory cytokines, both in the skin and serum, might seem a reasonable idea. While determining the concentrations of

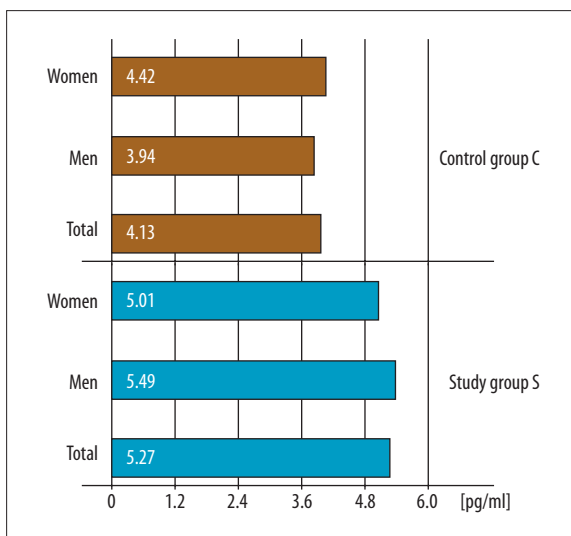


Fig. 3. Mean concentrations of IL-4 in the serum of the study and control group subjects

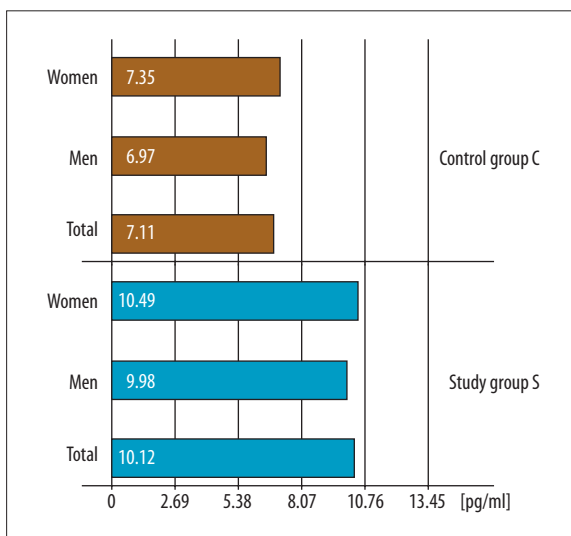


Fig. 4. Mean concentrations of IFN- $\gamma$  in the serum of the study and control group subjects

cytokines in the serum, we attempted to answer the question whether inflammatory skin diseases, including seborrheic dermatitis, affect only one organ (in this case the skin) or are systemic diseases. Therapeutic success in treating psoriasis with antibodies against proinflammatory factors in the serum would be a strong reason to replicate this procedure in the treatment of diseases whose course is often very similar to that of seborrheic dermatitis: seborrheic psoriasis, for example.

**Interleukin-2**

Interleukin-2 is released mainly by Th1 lymphocytes. Its prominent function is defense against autoimmunization through the regulation of lymphocyte activity. It also plays a role in the differentiation of T lymphocytes into Tc lymphocytes. Together with IL-4 and IL-5 it induces differentiation and proliferation of B lymphocytes. IL-2, together with TNF- $\alpha$ , enhances IFN- $\gamma$  expression through NK cells. Moreover, it activates and enhances the proliferation of NK cells.

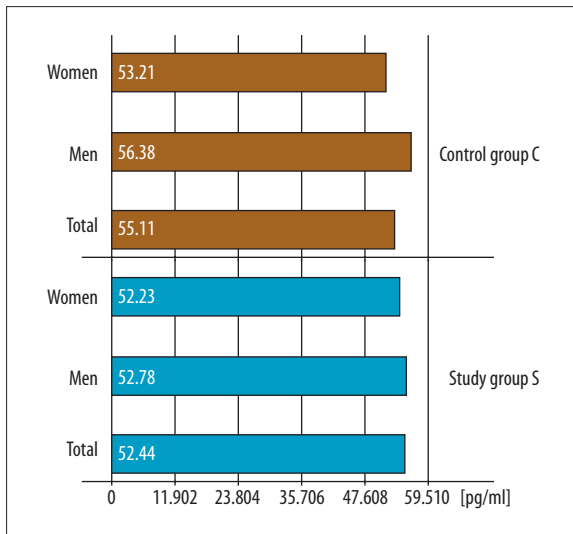


Fig. 5. Mean concentrations of TNF- $\alpha$  in the serum of the study and control group subjects

#### Interleukin-4

IL-4 affects the immune system in many ways. It can directly inhibit the Th1 cell response in a similar way to IL-10, IL-11 or antibodies against TNF. Unlike the mentioned factors which have suppressive properties, IL-4 can directly differentiate Th cells. It is responsible for transformation of naive cells and possibly for modeling the proliferation of cells already induced into Th1 so that they will finally proliferate into Th2 cells, which have anti-inflammatory properties.

#### Interferon- $\gamma$

IFN- $\gamma$  is a cytokine released by T lymphocytes after they are induced by antigens, cytokines or mitogen. Moreover, it is produced by NK cells. It is a cytokine released in the course of the cell response (Th1) and is induced mainly by IL-12 [2]. Together with TNF- $\alpha$ , it induces the production of inflammatory cytokines – IL-6, IL-8, IL-12, IL-18 [1].

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#### Tumor necrosis factor alpha

TNF- $\alpha$  is a mediator of the inflammatory response, both systemic and topical. With other cytokines it enhances the proliferation of B lymphocytes (with IL-6) and T lymphocytes (with IL-2 and IL-8). Together with IL-2, it can also stimulate the cytotoxicity of NK cells and production of Tc lymphocytes.

#### A high level of TNF- $\alpha$ has been observed many times in autoimmune diseases

The professional literature does not contain any information on the evaluation of the concentrations of IL-2, IL-4, IFN- $\gamma$  and TNF- $\alpha$  in the serum of patients with seborrheic dermatitis, or any information on changes in their levels in the course of either the described dermatosis or other diseases traditionally regarded as seborrheic dermatoses. Faergeman et al. investigated the role of skin inflammatory cells in seborrheic dermatitis and inflammatory factors released by the cells by immunohistochemical methods. They demonstrated that the skin of the patients has a greater number of cells releasing inflammatory factors such as lymphocytes, macrophages, monocytes, Langerhans cells and granulocytes, both in the areas affected and unaffected by lesions, in comparison with the skin of healthy subjects. Intracellular staining, both in the areas of skin affected and unaffected by lesions, was much more visible than in the skin of healthy subjects. The patients with seborrheic dermatitis demonstrated an increased number of intracellular inflammatory factors such as IL-1 $\alpha$ , IL-1 $\beta$ , TNF- $\alpha$ , IFN- $\gamma$ , IL-12 and IL-14 [3].

The findings of the study indicate statistically significantly increased levels of IL-2 and IFN- $\gamma$  in our patients. We can hence suppose that seborrheic dermatitis is a dermatosis which is characterized by a cell type immune response.

The concentrations of IL-4 and TNF- $\alpha$  did not show statistically significant differences. It can therefore be concluded that these cytokines do not play an important role in the pathogenesis of the disease. Further studies are needed to explain the pathomechanism of seborrheic dermatitis.

The authors have no potential conflicts of interest to declare.