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THE PRODUCTION OF PRO-INFLAMMATORY CYTOKINES AND THE STATE OF CELLULAR IMMUNITY IN PATIENTS WITH PULMONARY TUBERCULOSIS WITH AND WITHOUT RELAPSE

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Summary.

The purpose of the study was to conduct a comparative study of the synthesis of pro-inflammatory cytokines, the state of cellular immunity, free radical oxidation reactions and antioxidant protection in patients with pulmonary tuberculosis with and without relapse.

Material and methods. The study was carried out on two groups of patients: the first group - basic which included 39 patients with relapsed pulmonary tuberculosis (TBR); 2nd control group - 39 patients with non-relapsed pulmonary tuberculosis (TB). Pro-inflammatory cytokines (TNF-α, IL-2 IL-6), CD-3 lymphocyte content, lymphocyte blast transformation response to phytohemagglutinin and tuberculin, NBT-test, AOPP, SOD, and catalase were determined in patients of both groups and healthy people.

Results. In patients with tuberculosis with relapses, compared with patients without relapses, complaints of cough and dyspnea were more often presented, a longer duration of hospitalization, late abacillation. In patients with relapses, a pronounced suppression of the functional and specific activity of lymphocytes and neutrophils was noted, which is confirmed by high levels of lipid peroxidation (Advanced Oxidation Protein Products - AORP) and low levels of the antioxidant system (SOD and catalase). The high content of pro-inflammatory cytokines in patients with relapses indicates a higher activity of the inflammatory process in these patients and corresponds to a low blast formation rate for both phytohemagglutinin and tuberculin.

Conclusion. Changes in the content of pro-inflammatory cytokines, the state of cellular immunity and the characteristics of reactions of free radical oxidation and antioxidant protection indicate a higher activity of the inflammatory process in patients with pulmonary tuberculosis with relapses compared to patients without relapses, which led to a longer duration of hospitalization, late abacillation.

Keywords: Proinflammatory cytokines, tuberculosis, relapse, cellular immunity

Rezumat. Producția de citokine proinflamatorii și starea imunității celulare la pacienții cu tuberculoză pulmonară cu și fără recidivă.

Scopul a fost realizarea unui studiu comparativ al sintezei citokinelor proinflamatorii, al stării imunității celulare, al reacțiilor de oxidare a radicalilor liberi și al protecției antioxidante la pacienții cu tuberculoză pulmonară cu și fără recidivă.

Material si metode. Studiul a fost realizat pe două loturi de pacienți: I lotul de bază - 39 de pacienți cu tuberculoză pulmonară cu recidivă (TBR); al 2-lea - lot de control - 39 de pacienți cu tuberculoză pulmonară fără recidivă (TB). Citokinele proinflamatorii (TNF-α, IL-2 IL-6), conținutul de limfocite CD-3, răspunsul la transformarea blastică a limfocitelor la fitohemaglutinină și tuberculină, testul NBT, AOPP, SOD și catalaza au fost determinate la pacienții din ambele loturi și persoane sanatoase.

Rezultate. La pacienții cu tuberculoză cu recidive, comparativ cu pacienții fără recidive, s-au prezentat mai des plângeri de tuse și dispnee, o durată mai lungă de spitalizare, abacilare tardivă. La pacienții cu recidive s-a observat o suprimare pronunțată a activității funcționale și specifice a limfocitelor și neutrofilelor, ceea ce este confirmat de niveluri ridicate de peroxidare lipidică (Advanced Oxidation Protein Products - AORP) și niveluri scăzute ale sistemului antioxidant (SOD și catalaza). Conținutul ridicat de citokine proinflamatorii la pacienții cu recidive indică o activitate mai mare a procesului inflamator și corespunde unei rate scăzute de formare a blastului atât pentru fitohemaglutinină, cât și pentru tuberculină.

Concluzie. Modificările conținutului de citokine proinflamatorii, starea imunității celulare și caracteristicile reacțiilor de oxidare a radicalilor liberi și protecție antioxidantă indică o activitate mai mare a procesului inflamator la pacienții cu tuberculoză pulmonară cu recidive comparativ cu pacienții fără recidive, ceea ce a condus la o durată mai lungă de spitalizare, abacilare tardivă..

Cuvinte cheie: Citokine proinflamatorii, tuberculoză, recidivă, imunitate celulară

Резюме. Продукция провоспалительных цитокинов и состояние клеточного иммунитета у больных туберкулезом легких с рецидивом и без рецидива.

Цель исследования – провести сравнительное исследование синтеза провоспалительных цитокинов, состояния клеточного иммунитета, реакций свободно-радикального окисления и антиоксидантной защиты у больных с туберкулезом легких с рецидивом и без рецидива.

Материал и методы. Исследование проводили на двух группах больных: 1-я основная группа – 39 больных туберкулезом легких с рецидивом; 2-я контрольная группа – 39 больных туберкулезом легких без рецидива. У больных обеих групп и здоровых определяли провоспалительные цитокины (TNF-α, IL-2 IL-6), содержание CD-3 лимфоцитов, реакцию бласттрансформации лимфоцитов на фитогемагглютинин и туберкулин, NBT-test, AOPP, SOD и каталаза.

Результаты. У больных туберкулезом с рецидивами по сравнению с больными без рецидивов чаще предъявлялись жалобы на кашель и одышку, больший срок госпитализации, поздняя абацилляция. У больных с рецидивами отмечено выраженное подавление функциональной и специфической активности лимфоцитов и нейтрофилов, что подтверждается высокими показателями перекисного окисления липидов (Advanced Oxidation Protein Products - AOPP) и низкими показателями антиоксидантной системы (SOD и каталаза). Высокое содержание провоспалительных цитокинов у больных с рецидивами свидетельствует о более высокой активности воспалительного процесса у этих больных и соответствует низкому показателю бластообразования как на фитогемагглютинин, так и на туберкулин.

Заключение. Изменения содержания провоспалительных цитокинов, состояния клеточного иммунитета и особенностей реакций свободнорадикального окисления и антиоксидантной защиты свидетельствуют о более высокой активности воспалительного процесса у больных с туберкулезом легких с рецидивами по сравнению с больными без рецидивов, что обусловило большую продолжительность лечения в стационар, позднее абацилирование.

Ключевые слова: Провоспалительные цитокины, туберкулез, рецидив, клеточный иммунитет.

Introduction.

Tuberculosis is classified as a cytokine-dependent disease with a pronounced imbalance of T-lymphocyte subpopulations and changes in the cytokine network. The course of tuberculosis at the present stage is characterized by a variety of clinical and morphological features, which may be associated with the emerging secondary immunodeficiency, which is detected in the vast majority of patients with pulmonary tuberculosis (Erokhin V.V., 2009).

Like other mediators, cytokines serve for intercellular signaling during the development of the inflammatory process. In its initial stages, local tissue cells can secrete cytokines such as IL-6, but as soon as lymphocytes and mononuclear phagocytes appear in the focus of inflammation, they can, when activated by the action of an antigen, secrete their own cytokines (TNF-α, INF-γ) , which, acting on the endothelium of local vessels, further enhance cell migration. (Kitaev M.I., et al. 2016; Shovkun L.A. et al. 2016). Cytokines act according to the relay principle, in the form of a cytokine cascade. They are secreted by cells only under external influences, providing homeostasis and immune protection. The main activity of cytokines is the regulation of the immune response. The cytokine system plays an important role in the regulation of inflammatory processes. Tuberculosis is accompanied by pronounced changes in the cytokine system (Urdahl K.B., et al. 2003). One of the reasons for the unfavorable course of tuberculosis may be the high production of pro-inflammatory cytokines. Anti-inflammatory cytokines are able to suppress the cytotoxic activity of immunocompetent cells [Bowssiotis V. et al., 2000].

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The human immune system uses oxidative stress to fight pathogens, and some reactive oxygen species can serve as mediators in cellular signaling pathways. However, the most dangerous component of oxidative stress, the formation of reactive oxygen species (ROS), is very unfavorable for the immune system. To combat this adverse factor, there is an antioxidant system. Antioxidant levels and ensuring good protection from antioxidant systems are very important in preventing oxidative stress. Enzymes such as superoxide dismutase and catalase are involved in the complex mechanisms of antioxidant defense, which are released to reduce ROS levels.

Changes in the system of free radical oxidation and antioxidant protection also affect the course of the tuberculosis process (Shepelev A.P. et al., 2012).

The purpose of the study was to conduct a comparative study of the synthesis of pro-inflammatory cytokines, the state of cellular immunity, free radical oxidation reactions and antioxidant protection in patients with and without relapse of pulmonary tuberculosis.

Material and methods. The study was carried out on two groups of patients: the first group - basic which included 39 patients with relapsed pulmonary tuberculosis (TBR); 2nd control group - 39 patients with non-relapsed pulmonary tuberculosis (TB), and 100 healthy people.

In patients of both groups and healthy people, the content of pro-inflammatory cytokines (TNF-α, IL-2 IL-6) was determined in an enzyme immunoassay using kits from Vector-Best, the content of CD-3 lymphocytes, the severity of the reaction of blast transformation of lymphocytes to phytohemagglutinin (RBTL -fga) and tuberculin (RBTL-tub) according to the method of Ghinda S.S. (1982). NBT-test, as an indicator of neutrophil activity according to the Park B.H. et al. (1968). Marker AOPP (end products of deep oxidation of proteins) according to the method of Witko-Sarsat V. et al. (1996). SOD and catalase according to the method of Gudumac V., et al., (2010).

Results. In both groups there were patients comparable in sex and age. In both groups (table 1) there were significantly fewer women ($p<0.05$). Compared to TB patients, TBR patients had a significantly longer duration of hospital treatment ($p<0.01$), abacillation ($p<0.01$) occurred significantly later.

Compared to TB patients, TBR patients significantly (Table 2) more often complained of cough ($p<0.05$) and dyspnoea ($p<0.05$).

The content of CD-3 lymphocytes (table 3) in patients with TBR was 56.3±1.33%. In patients with TB - 61.1±1.28%, that is, significantly more ($p<0.001$). The content of CD-3 lymphocytes in both groups was significantly lower than in healthy people (from $p<0.05$ to $p<0.001$).

In RBTL, the rate of blast formation (table 3) for phytohemagglutinin in patients with TBR was 56.8±0.96%, and for tuberculin 3.0±0.19%. In patients with TB, phytohemagglutinin was 64.1±0.77%, and tuberculin was 4.4±0.28%, that is, in both cases it was significantly higher ($p<0.001$). The indices of blast formation for phytohemagglutinin and tuberculin in all cases significantly differed from those of healthy individuals (from $p<0.05$ to $p<0.001$).

NBT-test as an indicator of neutrophil activity, in patients with TBR was 0.11±0.003, which is significantly less than in patients with TB - 0.12±0.004 ($p<0.05$). The NBT-test indicators in both groups were significantly lower than in healthy people ($p<0.001$).

Thus, in patients with TBR, both the activity of lymphocytes (functional and specific) and the activity of neutrophils are suppressed.

Table 1

Distribution of patients by sex, age and duration of treatment (M±m)

Indicators	Group 1 - TBR	Group 2 - TB
Age (years)	42,2±1,73	44,3±2,00
Gender: men (abs./%) women (abs./%)	25 / 64,1±7,85 14 / 35,9±7,85●	25 / 64,1±7,85 14 / 35,9±7,85●
Duration of treatment (days)	93,9±7,81	64,6±3,88□
Abacillated (days)	79,6±9,19	49,2±3,66□

● - statistically significant difference between the indicators of men and women
□ - statistically significant difference between the indicators of groups 1 and 2

Table 2

Symptoms (M±m)

Indicators	Group 1 - TBR	Group 2 - TB
Cough (days)	38,8±6,23	22,9±2,82□
Sputum (days)	23,2±4,10	20,0±2,97
Shortness of breath (days)	30,9±6,27	15,2±2,71□
Chest pain (days)	9,2±2,80	4,11±1,61

□ - statistically significant difference between the indicators of groups 1 and 2

Table 3

Peculiarities of cellular immunity in the examined groups (M±m)

Indicators	Healthy	Group 2 - TBR	Group 1 - TB
CD-3 (%)	26,7±1,37	56,3±1,33Δ	61,1±1,28Δ□
РБТЛ (фга) (%)	78,4±0,72	56,8±0,96Δ	64,1±0,77Δ□
РБТЛ (туб) (%)	1,34±0,080	3,0±0,19Δ	4,4±0,28Δ□
NBT-test (усл.ед)	0,14±0,006	0,11±0,003Δ	0,12±0,004Δ□

□ - statistically significant difference between the indicators of groups 1 and 2
Δ- statistically significant difference between the indicators of healthy and sick people

Table 4

Peculiarities of the content of proinflammatory cytokines in the examined groups (M±m)

Indicators		Healthy	1st group - TBR	2nd group - TB
IL-2	(pg/ml)	4,2±0,23	23,6±1,25Δ	28,8±2,60Δ
IL-6	(pg/ml)	6,2±0,42	46,3±2,23Δ	36,8±2,17Δ□
TNF-α	(pg/ml)	26,7±1,37	221±30,1Δ	118±10,5 Δ□

□ - statistically significant difference between the indicators of groups 1 and 2
Δ- statistically significant difference between the indicators of healthy and sick people

Table 5

Lipid peroxidation and the antioxidant system

Indicators		Healthy	1st group - TBR	2nd group - TB
AOPP	(μM/l)	25,6±1,03	42,5±2,57Δ	33,5±2,76Δ□
SOD	(u.c.)	831 ±29,2	717±101,3Δ	1086±146,9Δ□
Catalase	(μM/l)	13,6±0,60	9,2±0,59 Δ	16,6±2,18Δ□

□ - statistically significant difference between the indicators of groups 1 and 2
Δ- statistically significant difference between the indicators of healthy and sick people

The content of IL-2 in patients with TBR (Table 4) was 23.6±1.25 pg/ml, and in patients with TB it was 28.8±2.60 pg/ml, that is, it did not differ significantly. The content of IL-6 in patients with TBR was 46.3±2.23 pg/ml, and in patients with TB it was 36.8±2.17 pg/ml, i.e. significantly lower ($p<0.05$). The content of TNF-α in patients with TBD was 221±30.1 pg/ml, and in patients with TB it was 118±10.5 pg/ml, i.e. significantly lower ($p<0.05$). The content of TNF-α, IL-2, IL-6 in all cases significantly differed from that of healthy people (from $p<0.05$ to $p<0.001$).

The high content of proinflammatory cytokines in patients with TBR indicates a higher activity of the inflammatory process in these patients.

The indicator (Table 5) of lipid peroxidation (AOPP) was most pronounced in patients with TRR 42.5±2.57 μM/l, which was significantly higher than in TB patients 33.5±2.76 μM/l ($p<0$ .05). The indicator of the system of antioxidants (SOD), on the contrary, was significantly higher in patients with TB - 1086±146.9 u.c. vs. 717±101.3 u.c. in patients with TBR ($p<0.05$). The indicator of the antioxidant system (catalase), on the contrary, was significantly higher in patients with TB - 16.6±2.18 μM/l, versus 9.2±0.59 μM/l in patients with TBR ($p<0.05$).

The values of AOPP, SOD, catalase in all cases significantly differed from those of healthy people (from $p<0.05$ to $p<0.001$).

Conclusion. In patients with relapses, a pronounced suppression of the functional and specific activity of lymphocytes and neutrophils was noted, which is confirmed by high levels of lipid peroxidation (AOPP) and low levels of the antioxidant system (SOD and catalase).

The high content of proinflammatory cytokines in patients with relapses indicates a higher activity of the inflammatory process in these patients and corresponds to a low blast formation rate for both phytohemagglutinin and tuberculin.

Changes in the content of pro-inflammatory cytokines, the state of cellular immunity and the characteristics of reactions of free radical oxidation and antioxidant protection indicate a higher activity of the inflammatory process in patients with pulmonary tuberculosis with relapses compared to patients without relapses, which led to a longer duration of hospitalization, late abacillation.

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Bibliography.

1. Gudumac V., Tagadiuc O., Rîvneac V. et al. *Investigaţii biochimice.* Elaborare metodică. Micrometode, Vol. II. Tipografia Elena V. SRL. Chişinău, 2010, 98 p.

2. Bowssiotis V., Tsai E., Ynis E. *IL-10 – producing T-cells suppress immune responses in anergic tuberculosis patient.* Y. Clin. Invest. 2000. V. 105. № 9. P. 1317–1325.

3. Park B.H. et al. *Infection and Nitroblue–tetrazolium Reduction by Neutrophils.* The Lancet, vol 11, 1968, N 7567, p. 532–534.

4. Urdahl K.B., Liggit D., Bevan M.J. et al. *CD8+ T cells accumulate in the lungs of Mycobacterium tuberculosis-infected mice, but provide minimal protection.* J. Immunol. 2003. Vol. 170. № 2. P. 1987–1994.

5. Witko-Sarsat V, Friedlander M, Capeillère-Blandin C, et al. *Advanced oxidation protein products as a novel marker of oxidative stress in uremia.* Kidney Int. 1996 May;49(5):1304-1313].

6. *Гинда* С.С. *Модификация микрометода реакции бласттрансформации лимфоцитов. Лабораторное дело*. – 1982. – № 8. – С. 23–25.

7. *Ерохин В.В. О некоторых механизмах патогенеза туберкулеза. Проблемы туберкулеза*. – 2009. – № 11. – С. 3–9.

8. *Китаев М.И., Алишеров* А.Ш., *Дуденко* Е.В., *Сыдыкова* С.С., *Чоноров*а О.А. *Цитокиновый профиль больных лекарственно-устойчивым и лекарственно-чувствительным туберкулезом легких. Вестник КРСУ. 2012. Том 12. № 2, С. 75-79.*

9. *Шепелев А. П., Шовкун Л. А. Перекисное окисление липидов и система антиоксидантов в норме и при патологии: монография. – Ростов*-на-Дону: ГБОУ ВПО *РостГМУ Минздравсоцразвития России, 2012. – 364с.*

10. *Шовкун Л.А., Кампос Е.Д., Константинова А.В., Франчук* И.М. *Дифференциально-диагностические признаки инфильтративного туберкулеза легких в зависимости от характера воспалительной тканевой реакции (продуктивной или экссудативной). Медицинский вестник Юга России. № 2, 2016. С. 79-81.*