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THE SERUM LEVELS OF CYTOKINES IN THYROID HEART FAILURE PATIENTS

In recent years, the system of cytokines has a significant role in forming and progressing chronic heart failure of various etiologies (ischemic, inflammatory, infectious and allergic, etc.).

Aim. To study the activity of proinflammatory cytokines (interleukin-1 α (IL-1 α), interleukin-1 β (IL-1 β), interleukin-6 (IL-6) and interleukin-8 (IL-8) and anti-inflammatory cytokine (interleukin-4 (IL-4) in heart failure (HF) in thyrotoxicosis patients.

Materials and methods. 64 patients and 20 healthy volunteers were examined; they were distributed in 3 groups: group I (44 patients) patients with thyroid cardiomyopathy, group II (20) patients with no sign of cardiomyopathy and group III – 20 healthy volunteers. The serum levels of interleukines 1 α , 1 β , 4, 6 and 8 at admission, in 21 days and 6 months were measured.

Results. The level of IL-6 and IL-8 were significantly higher in patients with thyrotoxic HF compared to those without HF and healthy subjects. Correlation in IL-6 and IL-8 serum levels and severity and duration of HF were revealed. No correlation in IL-4 serum levels and HF was found.

Conclusions. It can be assumed that IL-6 and IL-8 are involved in the progression of heart failure and, less likely, in the formation of cardiomyopathy; however, for a more accurate identification of these regularities further studies are required.

Key words: interleukin; heart failure; thyroid toxicity; thyrotoxicosis; cytokines

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Рівні цитокінів у сироватці крові хворих на тиреотоксичну кардіоміопатію

Останнім часом значна роль у формуванні та прогресуванні хронічної серцевої недостатності різної етіології (ішемічна, запальна, інфекційно-алергічна та ін.) відводиться системі цитокінів.

Мета дослідження. Вивчення активності прозапальних цитокінів (інтерлейкіну-1 α (IL-1 α), інтерлейкіну-1 β (IL-1 β), інтерлейкіну-6 (IL-6) та інтерлейкіну-8 (IL-8) і протизапальних цитокінів (інтерлейкін-4 (IL-4) при серцевій недостатності у пацієнтів з тиреотоксикозом.

Матеріали та методи. Було обстежено 64 пацієнти і 20 здорових добровольців, розподілених на 3 групи: I група (44 пацієнти) з тиреотоксичною кардіоміопатією, II група (20) – пацієнти без ознак кардіоміопатії і III – 20 здорових добровольців. Були виміряні рівні сироваткових інтерлейкінів 1 α , 1 β , 4, 6 і 8 при надходженні, через 21 день і через 6 місяців.

Результати. Рівень IL-6 і IL-8 був достовірно вищим у пацієнтів з тиреотоксичною серцевою недостатністю в порівнянні з пацієнтами без серцевої недостатності і здоровими суб'єктами. Виявлено кореляцію між рівнями IL-6 та IL-8 в сироватці крові, а також тяжкістю і тривалістю серцевої недостатності. Між рівнями IL-4 в сироватці і СН кореляції виявлено не було.

Висновки. Можна припустити що IL-6 та IL-8 беруть участь у прогресуванні серцевої недостатності та з меншою ймовірністю в формуванні кардіоміопатії, але для більш точного виявлення цих закономірностей необхідні подальші дослідження.

Ключові слова: інтерлейкін; серцева недостатність; тиреотоксикоз; цитокіни

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Уровни цитокинов в сыворотке крови больных с тиреотоксической кардиомиопатией

В последние годы значительная роль в формировании и прогрессировании хронической сердечной недостаточности различной этиологии (ишемической, воспалительной, инфекционно-аллергической и др.) отводится системе цитокинов.

Цель исследования. Изучение активности провоспалительных цитокинов (интерлейкин-1 α (IL-1 α), интерлейкина-1 β (IL-1 β), интерлейкина-6 (IL-6) и интерлейкина-8 (IL-8) и противовоспалительных цитокинов (интерлейкин-4 (IL-4) при сердечной недостаточности у пациентов с тиреотоксикозом.

Материалы и методы. Было обследовано 64 пациента и 20 здоровых добровольцев, распределенных на 3 группы: I группа (44 пациента) с тиреотоксической кардиомиопатией, II группа (20) – пациенты без признаков кардиомиопатии и III – 20 здоровых добровольцев. Были измерены уровни сывороточных интерлейкинов 1 α , 1 β , 4, 6 и 8 при поступлении, через 21 день и через 6 месяцев.

Результаты. Уровень ИЛ-6 и ИЛ-8 был достоверно выше у пациентов с тиреотоксической сердечной недостаточностью по сравнению с пациентами без сердечной недостаточности и здоровыми субъектами. Выявлена корреляция между уровнями ИЛ-6 и ИЛ-8 в сыворотке крови, а также тяжестью и продолжительностью сердечной недостаточности. Между уровнями ИЛ-4 в сыворотке и СН корреляции выявлено не было.

Выводы. Можно предположить, что ИЛ-6 и ИЛ-8 принимают участие в прогрессировании сердечной недостаточности и с меньшей вероятностью в формировании кардиомиопатии, но для более точного выявления этих закономерностей необходимы дальнейшие исследования.

Ключевые слова: интерлейкин; сердечная недостаточность; тиреотоксикоз; цитокины

The most important problem of modern medicine is diseases that are accompanied by the development of heart failure. The prevalence of heart failure in the human population is 1-2 % [1] and is projected to reach 7 % in the near future [2]. In 60 % of patients with cardiovascular disease, asymptomatic cardiac dysfunction is observed, which is further transformed into heart failure [3]. In this case, the mortality after the diagnosis of heart failure during the first year reaches 15 %, and in the subsequent six years dies more than 60 % [4]. The steady increase in the incidence of heart failure, high costs of treatment and high mortality rates determine the issues of prevention and treatment as the most important socio-medical problem [1].

Among various pathologies, which are complicated by heart failure, a special place is occupied by the diseases of the thyroid gland [5]. Changes in the size, consistency and structure of the thyroid gland in 1995 were found in 59.2 % of the eastern Ukrainian population and 60 % of the Chernobyl accident liquidators; it exceeded the control figures in 1980 by more than 10 times. An increase in the incidence of thyroid diseases is noted up to this time, both as among the liquidators of the accident, and among those who have not been exposed to ionizing radiation [6]. Both reduction and increase in the concentration of thyroid hormones adversely affects the cardiovascular system. In thyroid toxicity, cardiac symptoms are the main, and sometimes the only manifestation of thyroid disease [7].

Immune-inflammatory mechanisms of development of heart failure are of great interest. At a certain stage of the development of many diseases, including the thyroid gland, there is the activation of the immune system, which may be the cause of the development of the systemic inflammatory response. The pro-inflammatory and anti-inflammatory cytokines play a special role in these processes; it has direct cardiotropic and vasotropic effects [8] and may contribute to the formation and progression of heart failure [9].

In recent years, the system of cytokines has a significant role in forming and progressing chronic heart failure of various etiologies (ischemic, inflammatory, infectious and allergic, etc.) [9]. At the same time, some studies report the activation of the cytokine system in thyrotoxicosis, which often occurs as a result and on the background of an autoimmune

pathology of the thyroid gland [8]. Thus, the study of the activity of proinflammatory cytokines (interleukin-1 α (IL-1 α), interleukin-1 β (IL-1 β), interleukin-6 (IL-6) and interleukin-8 (IL-8) and anti-inflammatory cytokine (interleukin-4 (IL-4) in heart failure in thyrotoxicosis patients is the aim of our research.

Materials and methods

All studies were conducted in full accordance with the Helsinki Declaration and Ukrainian legislation. The informed consent of all participants was obtained prior to any medical procedures. We examined 64 patients who were treated for uncomplicated and complicated thyrotoxicosis.

The patients examined were divided into groups depending on the presence of signs of cardiomyopathy (heart failure): group I (main) consisted of 44 patients with thyrotoxic cardiomyopathy, which was determined on the basis of general clinical signs. Group II (experimental) consisted of 20 patients with thyrotoxicosis and without cardiac symptoms, with the exception of systolic tachycardia, and clinically relevant concomitant cardiac pathology of other etiology and extracardial pathology. In addition, 20 practically healthy persons were examined – group III (control group).

The average age of those surveyed in group I was 42.0 ± 12.5 years (from 16 to 63 years old); in group II – 42.0 ± 7.3 (from 29 to 56 years old); in group III – 43.3 ± 7.5 (from 29 to 58 years old). Statistically significant intergroup differences in age were not detected ($p \geq 0.05$).

Among the patients examined in all groups, women predominated – 66 % and 65 % in the main group and the experimental group, respectively.

Measurement of the level of serum interleukins was performed by solid phase enzyme immunoassay using standard ProCon IL-1 α , ProCon IL-1 β , ProCon IL-2, ProCon IL-6, ProCon IL-8 reagents manufactured by “Protein Contour”, RF.

Tablets were used as a solid phase, with antibodies sorbed with them to interleukins. The determinations were performed as follows: 1) the serum to be examined was introduced into the well of the tablet with antibodies sorbed on them; 2) during the incubation, antigens bound to antibodies; 3) the plate was washed off from the unbound antigen and added antibodies to the immunoglobulins (secondary antibodies) labeled with the enzyme; 4) the plate was

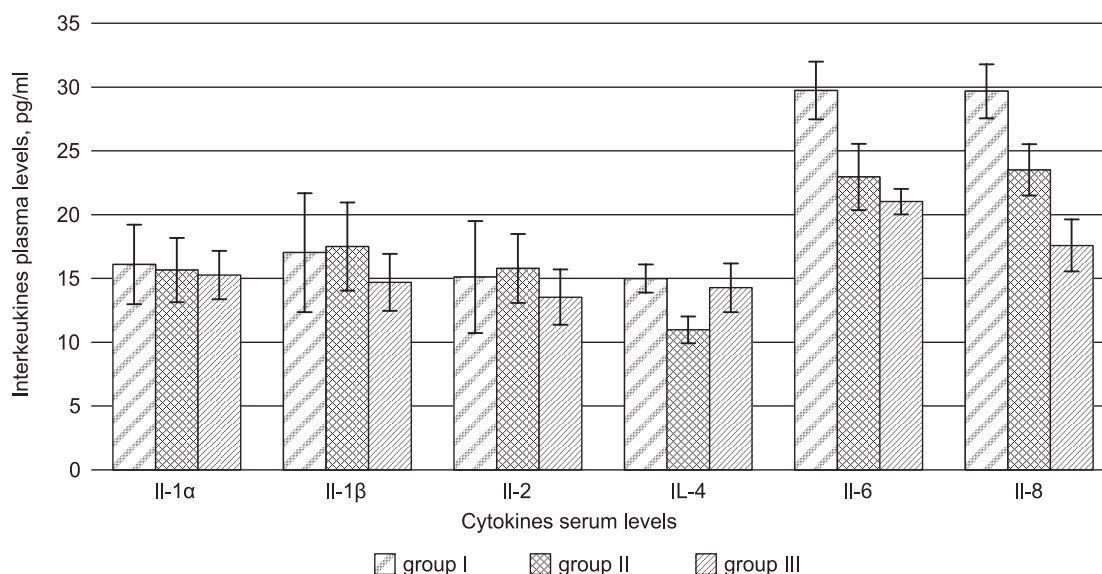


Fig 1. The serum levels of cytokines

washed again, and the enzyme substrate and chromogen were added; 5) under the action of the product of the enzymatic reaction chromogen changed its color. The more antibodies labeled with the enzyme bound to antigen-antibody complexes, the enzyme activity and the color intensity of the solution were higher. The concentration of antigen in the sample was determined spectrophotometrically at a wavelength of 450 nm based on the optical density of the colored solution. The calibration curve was constructed, and the interleukin concentration was calculated.

The study was conducted in the dynamics of the pathological process: at admission to the hospital, in 21 days and 6 months.

The data obtained was processed using variation statistics and the standard office suite Microsoft Office 2010 and SPSS, taking into account the recommendations for medical and biological research. Nonparametric methods were used for comparison of data – the Kruskal-Uollis criterion for inter-group comparisons and Rank-Mann-Whitney's criterion for comparing two choices. The correlation analysis was performed using a nonparametric method for calculating the Spearman rank correlation coefficient. The results were considered reliable at 95 % significance level ($P < 0.05$).

Results and discussion

The results obtained are presented in Fig. 1. The concentration of IL-1α in patients of group I was 16.09 ± 3.12 pg/ml, which was slightly higher than the control ($p \geq 0.05$), with a minimum value of 9.12 pg/ml and a maximum of 24.49 pg/ml (the difference between the minimum and maximum values was almost 2.7 times – both normal values and values higher and lower than the values of group III were observed). The concentration of IL-1β was

18.02 ± 1.65 pg/ml, it was slightly significant above the control values; however, the difference between the minimum and maximum values reached 3.2 times – from 8.29 to 26.12 pg/ml. The concentration of IL-2 was 15.12 ± 4.39 pg/ml, i.e. not significantly higher than the similar values in the control group ($p \geq 0.05$), and the difference in individual values was greater than 4 times – from 7.36 pg/ml to 29.58 pg/ml; IL-6 – 29.73 ± 2.27 pg/ml (from 22.15 pg/ml to 38.84 pg/ml – difference more than 75 %); IL-8 – 29.67 ± 2.12 pg/ml (from 21.64 pg/ml to 37.59 pg/ml, the difference was almost 75 %). The anti-inflammatory cytokine IL-4 concentration, on the contrary, was 14.99 ± 1.10 pg/ml (from 6.39 pg/ml to 24.36 pg/ml – the difference between the maximum and minimum value was in 3.8 times), it was slightly higher than the control value and credibly higher than in group II ($p < 0.05$).

Compared to group II significant differences were found in the concentrations of IL-4 ($p < 0.05$), IL-6 ($p < 0.001$) and IL-8 ($p < 0.001$), and the rest did not show significant differences.

As can be seen from the data presented, the individual values of the concentrations of IL-1α, IL-1β, IL-2 in the main group had more variety, any clear regularity was not detected, despite the tendency of their increase. In this regard, the focus of further analysis was given to IL-4, IL-6 and IL-8.

To determine the relationship between the concentration of IL-4, IL-6 and IL-8 and clinical data, a correlation analysis was performed, which resulted in a positive correlation between the severity of heart failure and the concentration of IL-6 and IL-8 ($r = +0.783$ and $+0.610$ respectively, $p < 0.001$), as well as no correlation with the concentration of IL-4 ($r = -0.157$, $p \geq 0.05$). Similar results were obtained for the analysis of the relationships between the content of cytokines and the duration of the disease:

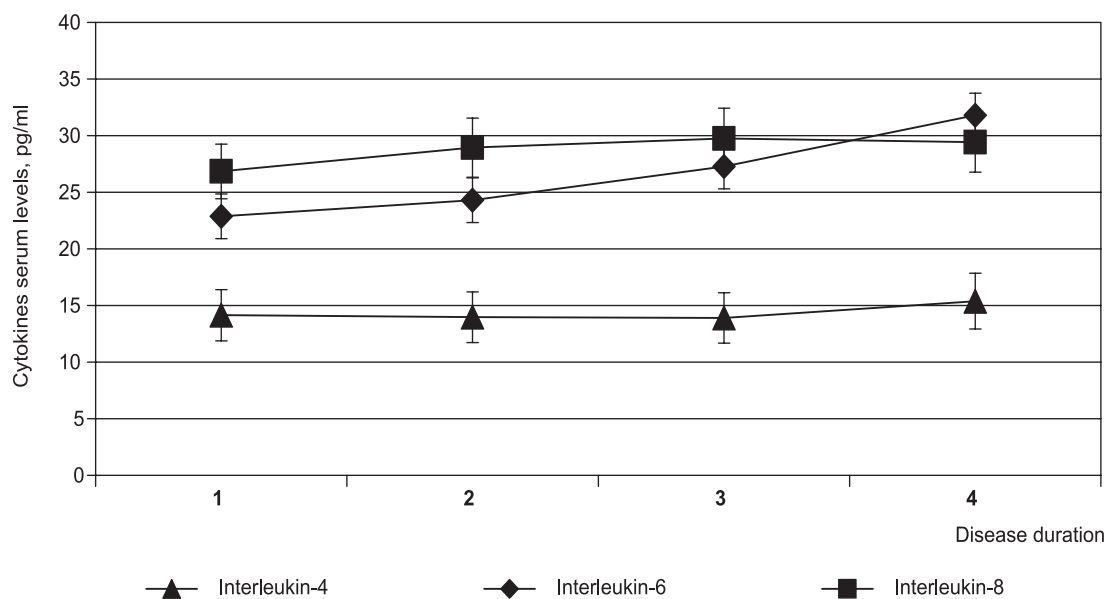


Fig. 2. The concentration of cytokines depending on the duration of the disease: 1 – up to 6 months, 2 – from 6 to 12 months, 3 – 1-2 years, 4 – more than 2 years

positive correlation $r = + 0.689$ and $+0.597$, respectively, for $Il-1\beta$, $Il-6$, $Il-8$ ($P < 0.001$), the correlations with the concentration of $Il-4$ were not found.

These data became the basis for comparing the concentrations of $Il-6$, $Il-8$ and $Il-4$ cytokines in patients with different duration of the disease (Fig. 2) and with different severity of heart failure (Fig. 3).

Analysis of the data allowed us to reveal that the concentration of $Il-6$ begins to increase with the duration of the disease more than 1 year. The concentration of $Il-8$, increasing already from the second period, in the future remains relatively stable. The serum level of anti-inflammatory cytokine $Il-4$ has no significant differences in all disease histories of patients analyzed.

Comparing the concentrations of cytokines in patients with varying degrees of heart failure it was found that the most indicative dynamics was revealed in the serum levels of $Il-6$ and $Il-8$. Thus, the concentration of $Il-6$ was 26.70 ± 2.96 pg/ml; 30.16 ± 4.33 pg/ml and 35.86 ± 2.21 pg/ml in the absence of HF, at HF 1 st. and at HF 2a, respectively.

The concentration of $Il-8$ had less clear dynamics, but there was still a tendency to increase during the progression of heart failure: 29.47 ± 3.99 pg/ml; 28.30 ± 3.89 pg/ml and 32.16 ± 4.81 pg/ml in the absence of HF, with HF 1st and with HF of 2nd a st., respectively.

It is interesting to note that in patients with ophthalmopathy a significantly higher concentration

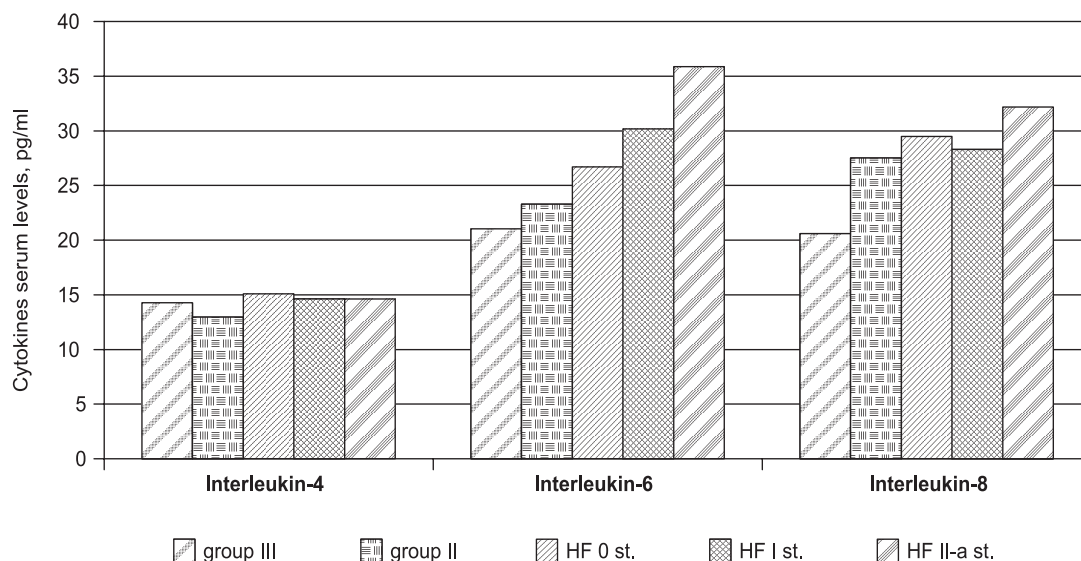


Fig. 3. The concentration of cytokines depending on the degree of heart failure

of Il-8 was observed compared to patients who had no ophthalmopathy (23.93 ± 1.87 pg/ml and 29.55 ± 1.12 pg/ml, respectively, $p < 0.05$).

CONCLUSIONS

The Il-4 content did not have statistically significant differences in patients with varying degrees of heart failure although there was a tendency to decrease the concentration compared to patients who had no signs of heart failure ($p \geq 0.05$).

Thus, in patients with thyrotoxic cardiomyopathy an increase in the concentration of cytokines Il-6 and Il-8 was found, both in comparison with the control group and the experimental group. To a greater extent, the severity of deviations compared to the control group performance is noted in the pheno-

mena of heart failure, as well as in patients with the disease duration of more than 1 year. Il-4 has significantly less variation compared to the control group and the experimental group, with increased concentrations more pronounced in patients with the disease duration of more than 1 year. It can be assumed that Il-6 and Il-8 are involved in the progression of heart failure and, less likely, in the formation of cardiomyopathy since significant differences in the control values and the value of the indicators of the experimental group, as well as patients in the main group with duration of the disease less than 6 months were not detected.

Conflict of interests: authors have no conflict of interests to declare.

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