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CYTOKINES CHANGES IN THE PATHOGENESIS OF ACUTE OR EXACERBATED CHRONIC PANCREATITIS CONSIDERING THE ETIOLOGY AND GENDER

Key words: *pancreatitis, alcoholic, biliary, cytokines, IL-4, TNF-α, IL-1β.*

Abstract. *To study the content of certain cytokines in the serum of patients with acute or exacerbated chronic pancreatitis and, considering the etiological factor and sex, 181 patients have been examined. The study revealed the most pronounced increase in the production of proinflammatory TNF- α 2.47 times with a compensatory increase of anti-inflammatory cytokine IL-4 6.77 times, which is exacerbated by alcohol genesis (the content increase of IL-4 8.98 times). For acute alcoholic edematous pancreatitis the concentration increase of IL-1β with the predominant increase of IL-4 confirms the activation of humoral immunity. The course of acute biliary edematous pancreatitis is accompanied by the higher levels of systemic inflammatory reactions and is characterized by the higher contents of TNF-α by 41.4% than of alcoholic genesis, at the lower concentrations of IL-4 by 43.8%. For biliary edematous pancreatitis the persons with exceeding standards of IL-1β (χ^2=9.52, p=0.002) are more likely to occur by 16.74%, with no significant difference in the frequency of TNF-α and IL-4 considering the etiology of pancreatitis. Female gender is not associated with the increased levels of IL-1β under alcoholic or biliary acute edematous pancreatitis and is a potential factor in the protection of immune disorders.*

Introduction

Acute pancreatitis (AP) is one of the most spread diseases today. The nosology, covering 15-20% of patients with acute abdominal surgical pathology, demonstrates in recent decades a clear trend of incidence growth and prevalence (according to official Ukrainian statistics - 102 cases per 100 thousand of population [P.D. Fomin, 2012]. The increasing incidence of AP and exacerbated chronic pancreatitis (ECP) is mainly due to the increase of the incidence of gall-stone disease and alcohol as the main etiological factors of pancreatitis. At the same time, the quality of diagnostics has improved.

Recently while considering the AP or ECP pathogenesis, more attention is paid to the activation of immune system.

The regularities of cytokines interaction have become clear only in recent years, although the role of inflammatory mediators in the pathogenesis of AP was studied since 90-ies, but [9, 18]. So, the clinical studies and the experimental data of pancreatitis reproduction it has been shown that the key mediators of the inflammation start-up are preimmune cytokines TNF-α and immune (IL-1β, IL-6, IL-8, etc.). Also, the same cytokine may cause the action of different directions, depending on its concentration, a specific type of receptor on the cell and its state of activation. Alongside with this fact cytokines interacting with specific receptors of cells can stimulate or limit proliferation, differentiation, migration and effector function of immune cells [1, 4; 5, 7].

To prevent excessive inflammatory reactions, especially systemic inflammatory response syndrome, negative (feedback) control mechanisms related to the production of anti-inflammatory cytokines (IL-4, IL-10, IL-13) and soluble inhibitors of proinflammatory cytokines are activated in the body. That is, the ability to manage or control the cytokine balance can serve as another mechanism of influence on the course and the complications occurrence of AP [8, 13, 14]. Moreover, there is a number of experimental studies that have demonstrated the positive effect of cytokine blockade on the destructive forms of pancreatitis and the experience of the usage of antycytokine drugs in the clinic [2, 16].

The purpose of the study

To estimate the content of cytokines TNF-α, IL-1β, IL-4 in the peripheral blood of patients with AP and ECP considering the etiology factor and gender.

Material and methods

The study involved the patients with AP and ECP admitted to the emergency hospital of Chernivtsi during the last five years. The screening and diagnosis of AP were carried out according to the current or-

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der of the Ministry of Health of Ukraine [3] and the recommendations of the European Societies in diagnosis and treatment of acute pancreatitis [10, 11, 15].

181 patients with AP (edematous form) and ECP have passed the screening step, they have signed the informed consent of the patient to participate in the study, with the following complex of clinical-laboratory and diagnostic studies. Among the examined there were 37 (20.4%) women and 144 (79.6%) men. The mean age of patients was 45.1 ± 5.19 years for males, 53.7 ± 6.53 years for women (range 23-77). In order to fulfill the tasks, the patients were divided into two groups, depending on the etiology: alcohol or biliary genesis. Into the first group were included 109 (60.2%) patients with the alcoholic genesis of pancreatitis (AGP), among whom there were 108 men and one woman; in the second - 72 (39.8%) patients with the biliary genesis pancreatitis (BGP), among whom there were 36 (50%) men and 36 (50%) women.

The control group consisted of 37 practically healthy persons of corresponding age and sex, who during last 6 months did not have acute or the exacerbation of chronic inflammatory processes of any localization.

The level of interleukins IL-1β, IL-4 and the tumor necrosis factor - alpha (TNF-α) were determined by the method of enzyme immuno-enzyme assay (ELISA) using a set of reagents (Interleukin-4 ELISA-BEST) and by chemiluminescence analysis (CLIA) using the Immulite F1427, Siemens. The standard indexes for these cytokines were as follows: IL-1β - <5 pg/ml; TNF-α - <8.1 pg/ml; IL-4 - 0-4 pg/ml. The concentration of the cytokines IL-1β and TNF-α was determined in 181 patients, and IL-4 - in 90.

The statistical analysis was performed using MYSTAT 12 (Systat Software Inc., USA) and Scout 2008 Version 1.00.01 (U.S. Environmental Protection Agency, USA). The reliability of data for independent samples were calculated by t-test Student (the distribution ranges close to normal) or U-criterion Wilcoxon-Mann-Whitney (with uneven distribution). The analysis of qualitative features - by the 2 criterion. The difference was considered reliable at $p<0.05$.

Discussion of the results

The study results of cytokines concentration in the serum of patients with edematous pancreatitis (EP) of different origins are shown in table 1.

An increase of proinflammatory cytokines concentrations of peripheral blood: IL-1β - by 27.25%, TNF-α - 2.47 times ($p<0.001$), as well as anti-inflammatory (second generation) IL-4 - 6.77 times ($p<0.01$) is marked in patients with EP.

The concentration growth of IL-1β - the main mediator of inflammatory reactions promotes the deepening of the inflammatory processes and the increase of TNF-α concentration indicates the activation of nonspecific proinfectious protection factors and mechanisms. The higher concentrations of IL-4 may be explained by the increased immunosuppressive action.

Table 1

The concentration of cytokines in the serum of patients with edematous pancreatitis of various genesis

Cytokines, unit of measure	Practically healthy people (n=37), M±m	Patients with edematous pancreatitis	
		Exceeding norms, n (%)	Data, M±m
IL-1β, pg/ml	4.11±0,05	27 (14,92%)	5.23±0.024 p<0.001
TNF-α, pg/ml	4.27±0,23	121 (66,85%)	10.54±0.489 p<0.001
IL-4, pg/ml	3.62±0,18	58 (64,44%)	24.51±4.9 p<0.004

A number of immuno-hematological parameters have different meanings depending on the etiology of EP. The results of studying of proinflammatory and anti-inflammatory cytokines concentration in the peripheral blood of patients with AGP are presented in Table 2.

The concentration of IL-1β increases by 28.95%, TNF-α - by 92.74% in peripheral blood of patients with AGP. In addition to the increasing concentrations of proinflammatory (IL-1β and TNF-α), the level of anti-inflammatory cytokine IL-4 increases 8.98 times which is synthesized by Th2, mast cell (basophils). IL-4 induces proliferation of B-lymphocytes and promotes the humoral immune response and also stimulates the proliferation of Th2, which form a humoral immune response.

The study results of pro- and anti-inflammatory cytokines concentrations in the peripheral blood serum of patients with BGP are shown in Table 3.

It is important that at BGP the concentration of leading preimmune proinflammatory cytokines increases simultaneously: IL-1β and TNF-α by 26.52% and 3.29 times respectively. Such growth of these cytokines in patients with BGP results in the inc-

Table 2

The concentration of proinflammatory and anti-inflammatory cytokines in the peripheral blood serum of patients with pancreatitis of alcoholic genesis

Cytokines, unit of measure	Practically healthy people (n=37), M±m	Patients with alcoholic genesis edematous pancreatitis, n=109	
		Exceeding norms, n (%)	Data, M±m
IL-1β, pg/ml	4.11±0.05	9 (8.26%)	5.3±0,041 p<0.001
TNF-α, pg/ml	4.27±0.23	76 (69.73%)	8.23±0,244 p<0.001
IL-4, pg/ml	3.62±0.18	39 (67.2%)*	32.49±5,2 p<0.01

Notes: * - of 58 examined patients with AGP

Table 3

The concentration of proinflammatory and anti-inflammatory cytokines in the peripheral blood serum of patients with pancreatitis of biliary genesis

Cytokines, units of measure	Practically healthy people (n=37), M±m	Patients with biliary edematous pancreatitis, n=72	
		Exceeding norms, n (%)	Data, M±m
IL-1β, pg/ml	4,11±0,05	18 (25,0%)	5,2±0,028 p<0,001
TNF-α, pg/ml	4,27±0,23	45 (62,5%)	14,04±1,048 p<0,001
IL-4, pg/ml	3,62±0,18	19 (59,38%)*	18,24±4,0 p<0,01

Notes: * - of 32 examined patients with BGP

reased production of acute phase inflammation proteins by hepatocytes, including B-group of acute phase proteins; increases the production of IL-2, IL-3, IL-6, IL-8, colonystimulating factors, etc. The concentration growth of these interleukins points to the inflammatory process deepening that develops into staid character.

The growth of anti-inflammatory (immune) IL-4 in 5.04 times in the serum of patients with BGP shows the possible stimulation of immunity system humoral link, the growth of mast cells (tissue basophils), which demonstrate a protective effect and also activates the switching processes of plasma cells to the synthesis of immunoglobulin E and other processes that show immunosuppressive effect.

To determine the influence of etiology on pre-immune and immune (inflammatory and anti-inflammatory) cytokines production we analyzed these cytokines concentrations in the serum of peripheral blood (Table 4).

It has been found that in the development of AGP there is a more significant concentration growth of IL-1β by 1.92% (p<0.05) and anti-inflammatory IL-4 by 78.13% (p<0.05) than in BGP. However, higher

Table 4

Pre-immune and immune cytokines concentration in the peripheral blood serum of patients with alcoholic and biliary pancreatitis

Cytokines, unit of measure	Patients with alcoholic edematous pancreatitis (n=109)		Patients with biliary edematous pancreatitis (n=72)	
	Exceeding norms, n (%)	Data, M±m	Exceeding norms, n (%)	Data, M±m
IL-1β, pg/ml	9 (8.26%)	5.3±0.041	18 (25.0%)	5.2±0.028
TNF-α, pg/ml	p=0.046			
IL-4, pg/ml	76 (69.73%)	8.23±0.244	45 (62.5%)	14.04±1.048
IL-1β, pg/ml	p=0.0018			
TNF-α, pg/ml	39 (67.2%)*	32.49±5.2	19 (59.38%)**	18.24±4.0
	p=0.033			

Notes: * - of 58 examined patients with AGP, ** - of 32 examined patients with BGP, p – reliability of differences between the AGP and BGP groups

by 70.60% ($p<0.05$) concentration of TNF-α was observed at BGP.

Such multidirectional changes, in our opinion, can be explained as follows: first, alcohol limits the synthesis of TNF-α, and secondly, for BGP is available biliary-pancreatic reflux, in which the production of proinflammatory cytokine is supported and depends directly on the getting of bile in Wirsung duct [7].

Thus, the AGP is accompanied by the increased concentration of the proinflammatory IL-1β and anti-inflammatory IL-4 that improve the function of T-CD4+ lymphocytes, realize the proliferation of activated B-lymphocytes and regulate the function of monocytes and macrophages. BGP is characterized by the increased concentration of TNF-α, which regulates inflammatory processes depending on its concentration in blood. For low concentrations, TNF-α increases the synthesis of adhesion molecules of epithelial cells, allowing neutrophil granulocytes attach to the walls of blood vessels at the inflammation site, followed by diapedesis in inflammation place. For high concentrations it is an important mediator, indicating a high level of systemic reactions associated with inflammation and can lead to endotoxin-induced septic state [5, 12, 17, 19].

The analysis of exceeding norm frequency of the selected cytokines in patients with EP showed that the content of TNF-α was exceeded in 121 (66.85%) patients (of them - 62.81% of patients with AGP and 37.19% with BGP) of 181 patients. Moreover, the excess of standards of TNF-α was observed in 69.73% of patients with AGP and in 62.5% of patients with BGP. The gender distribution revealed the following: among the patients with AGP exceeding standards were observed in 69.4% of men and the only one woman (100.0%), whereas among the patients with BGP the exceeding of the normal range of TNF-α was observed significantly more frequently among the men (75%), than women (50%) (χ^2=4.8, p=0.0285).

The exceeding standards of levels of anti-inflammatory cytokine IL-4 in the peripheral blood of patients with EP was found in 64.44% of patients. And if among the patients with AGP the excess of the normal range was observed in 67.2% of patients, among the patients with BGP - only in 59.38% of individuals. The gender distribution revealed the following: among the patients with AGP the exceeding standards were observed in 66.7% of men and the only one woman (100.0%), whereas among the patients with BGP the excess of the normal range of IL-4 was observed only in 52.9% of men and 66.7% women (χ^2 2=0.62, p=0.4302).

A somewhat different situation was observed in the frequency of high concentrations of proinflammatory cytokine IL-1β: the excess concentration of IL-1β was found only in 14.92% of patients. The excess of the normal range among the patients with AGP was observed in 8.3% of patients (all male), and among the patients with BGP - in 25% of patients (all male), representing 50% of all men with BGP. It should be noted that in no woman was observed an increase in the concentration of IL-1β by BGP or AGP. The results, in our opinion, can be explained by the fact that TNF-α induces the synthesis of some neurotrophic factors, including IL-1β. Therefore, it is logical to assume that the synthesis of IL-1β is slightly behind in time, as to the initial reaction of the increase of TNF-α.

Conclusions

1.Edematous acute pancreatitis is accompanied by the growth of production of proinflammatory TNF- α 2.47 times with a compensatory increase of anti-inflammatory cytokine IL-4 6.77 times, which is exacerbated with alcohol genesis (increase of the content of IL-4 8.98 times).

2.With alcoholic acute edematous pancreatitis the increase of the concentration of IL-1β with a predominant increase of IL-4 confirms the activation of humoral immunity. The course of biliary acute edematous pancreatitis is accompanied by the higher levels of systemic inflammatory reactions and is characterized by the higher contents of TNF-α by 41.4% than with alcoholic genesis, at the lower concentrations of IL-4 by 43.8%.

3.With biliary edematous pancreatitis significantly more often by 16.74% are met persons with the excess of standard norms of IL-1β (χ^2=9.58, p=0.002), with no significant difference in the frequency of TNF-α and IL-4 considering the etiology of pancreatitis. Female sex is not associated with the increased levels of IL-1β neither alcoholic, nor biliary acute edematous pancreatitis and it is a potential factor in the protection of immune disorders.

Prospects for further research

The determination of the possible genetic mechanisms of immunological disorders formation for acute pancreatitis considering the etiology and gender.

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ИЗМЕНЕНИЯ НЕКОТОРЫХ ЦИТОКИНОВ В ПАТОГЕНЕЗЕ ОСТРОГО ИЛИ ОБОСТРЕНИЯ ХРОНИЧЕСКОГО ПАНКРЕАТИТА С УЧЁТОМ ЭТИОЛОГИИ И ПОЛА

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Резюме. С целью изучения содержания отдельных цитокинов в сыворотке крови больных с острым панкреатитом и обострением хронического, с учётом этиологического фактора и пола, обследован 181 больной. Выявлен наиболее выраженный рост продукции провоспалительного TNF- в 2,47 раза с компенсаторным увеличением противовоспалительного цитокина IL-4 в 6,77 раза, что усиливается при алкогольном генезе (рост содержания IL-4 в 8,98 раза). При алкогольном остром отёчном панкреатите рост концентрации IL-1β с преимущественным увеличением IL-4 свидетельствует об активации показателей гуморального звена иммунитета. Течение билиарного острого отёчного панкреатита сопровождается более высоким уровнем системных воспалительных реакций и характеризуется более высоким содержанием TNF-α на 41,4%, нежели при алкогольном генезе, и более низкой на 43,8% концентрации IL-4. При билиарном отёчном панкреатите достоверно чаще на 16,74% встречаются лица с превышением нормы IL-1β (χ^2 =9,52, p=0,002), без достоверной разницы в частоте TNF-α и IL-4 с учётом этиологии панкреатита. Женский пол не ассоциирует с повышением уровня IL-1β ни при алкогольном, ни при билиарном остром отёчном панкреатите и есть потенциальным фактором защиты иммунных нарушений.

Ключевые слова: панкреатит, алкогольный, билиарный, цитокины, IL-4, TNF-α, IL-1β.

ЗМІНИ ЦИТОКІНІВ У ПАТОГЕНЕЗІ ГОСТРОГО ЧИ ЗАГОСТРЕННЯ ХРОНІЧНОГО ПАНКРЕАТИТУ З УРАХУВАННЯМ ЕТІОЛОГІЇ ТА СТАТІ

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Резюме. З метою вивчення вмісту окремих цитокінів у сироватці крові хворих на гострий панкреатит і загострення хронічного, з урахуванням етіологічного чинника та статі, обстежено 181 хворий. Виявлено найбільш виражене зростання продукції прозапального TNF-α у 2,47 раза із компенсаторним збільшенням протизапального цитокіну IL-4 у 6,77 раза, що посилюється за алкогольного ґенезу (зростання вмісту IL-4 у 8,98 раза). За алкогольного гострого набрякового панкреатиту зростання концентрації IL-1β із переважним збільшенням IL-4 засвідчує активацію показників гуморальної ланки імунітету. Перебіг біліарного гострого набрякового панкреатиту супроводжується вищим рівнем системних запальних реакцій і характеризується вищим вмістом TNF-α на 41,4%, ніж за алкогольного ґенезу, за нижчої концентрації IL-4 на 43,8%. За біліарного набрякового панкреатиту вірогідно частіше на 16,74% зустрічаються особи із перевищенням норми IL-1β (χ^2=9,52, p=0,002), без вірогідної різниці у частоті TNF-α і IL-4 з урахуванням етіології панкреатиту. Жіноча стать не асоціює з підвищенням рівня IL-1? ні за алкогольного, ні за біліарного гострого набрякового панкреатиту і є потенційним чинником протекції імунних порушень.

Ключові слова: панкреатит, алкогольний, біліарний, цитокіни, IL-4, TNF-α, IL-1β.

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