Dynamics of proinflammatory (TNF-α) and anti-inflammatory (IL-10) cytokines in different clinical forms and variants of children chickenpox

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Abstract

Background: Research of the levels of tumor necrosis factor -α and interleukin-10 in serum of children with chickenpox in different clinical forms and variants of the disease.
Material and methods: 84 children aged from 5 months to 14 years were tested to determine cytokine concentration. The dynamics of the content characteristics of necrosis factor tumor-α and interleukin-10 in serum of children with different clinical forms and variants of children chickenpox was studied. Statistical analysis was performed using the statistical software package "STATISTICA FOR WINDOWS 5,0" (StatSoft, USA, 1998).
Results: It was determined that the dynamics of the content characteristics of studied cytokines varied depending on the clinical form and variant of the disease. For uncomplicated varicella only necrosis factor tumor-α increase is typical. Significant increase of both studied cytokines was found in groups of children with complicated chickenpox. The imbalance in the system of cytokines with bacterial complications of varicella reflects the high activity of the inflammatory process.
Conclusions: These results confirm the feasibility of using indicators of TNF-α and IL-10 not only to assess the severity of chickenpox, but to predict the development of bacterial complications.
Key words: chickenpox, varicella-zoster virus, tumor necrosis factor-α, interleukin-10, children.

Introduction

Chickenpox is one of the most common highly-contagious airborne infectious children diseases [1]. The data of many clinical and epidemiological observations in recent years refute traditional idea of chickenpox as the classic "children infection" characterized mainly by mild course of the disease and full recovery. Not only a significant increase in the incidence of varicella [2], but also frequent severe and complicated variants of the disease draw attention [3]. Besides the specific complications of chickenpox caused directly by varicella-zoster virus, the bacterial complications developed as a result of penetration of pathogenic bacteria through damaged skin and mucous membranes require special attention. Children with immune system disorders suffer the most severe course and complications. For these children chickenpox is particularly dangerous, because the rate of complications among them reaches 30-50% [4, 5].

Considering the urgency of varicella issue in modern medicine, a detailed study of this disease represents not only academic interest, but also has important practical value. It is important to pay careful attention to still unexplored immunopathogenic mechanisms of different courses of varicella, including the development of complications.

Accumulated over recent years studies confirm the role of cytokines in the regulation of immune response, because the cytokines are responsible for the interaction between innate and adaptive immunity, acting in both directions. Clinical features and peculiarities of many infectious diseases directly depend on the level of production of proinflammatory and anti-inflammatory cytokines and their effect on immune effector and immunoregulatory mechanisms [6].

It is natural to expect that the development of severe and complicated forms of chickenpox is largely dependent on violations in intercellular connections that develop as a result of maladjustment of immune processes [7, 8].

One of the triggering mediators and cytokines of the immune response is the tumor necrosis factor-α (TNF-α), which has a key role in the inflammatory response and its progression [9, 10]. The TNF-α is directly involved in mobilizing cells in the source of infection [10], and the increase in the concentration of this cytokine is a sign of the adverse course of the disease [11]. Interleukin-10 (IL-10) plays an important role in blocking the production of inflammatory cytokines, including TNF-α [12, 13]. It also plays an important role in limiting the immune response to pathogens and their input with minimal immunopathological changes to the body [14]. In turn, it was proved that hyperproduction of IL-10 leads to reduced resistance to infectious factors [15, 16] and the development of potential complications.

Objective: research of the levels of tumor necrosis factor-α and anti-inflammatory cytokine – IL-10 in serum of children with chickenpox in different clinical forms and variants of the disease.

Material and methods

84 children aged from 5 months to 14 years were tested to determine cytokine concentration. All patients were hospitalized in the Lviv Oblast Infectious Clinical Hospital (LOICH) and/or in the surgical department of the City Clinical Hospital for Children (CCHC) during 2006-2012. Four comparison groups were formed, considering the clinical form (mild, moderate) and course of the disease (uncomplicated, complicated). The first group included 16 children with mild chickenpox and uncomplicated course of the disease, the second group – 23 children with moderate form of chickenpox, uncomplicated course, the third group – 26 children with superficial complications of varicella with skin lesions of subordinated soft tissue and oral mucosa, the fourth group – 19 patients with chickenpox with deep abscess lesions in different parts of the body.

The diagnosis of varicella was based on typical clinical manifestations of the disease, epidemiological history data and laboratory results. The changes in cytokine profile were studied by determining the levels of TNF-α and IL-10 in serum of children with chickenpox.

To study the levels of cytokines the following diagnostic kits of reagents were used: ELISA TNF-alpha (TNF-α) kit of "Orgenium Laboratories" (Finland) and "IL-10 - ELISA BEST" (Vector-Best CJSC, Koltsovo, Novosibirsk Region, Russian Federation). Determining the level of cytokines was performed in dynamics – with patient's admission to hospital (2-6 days of illness) and in early convalescence period (8-12 days of illness). The control group contained 14 healthy children of the same age, with the average values of TNF-α and IL-10 that were respectively 11.59 (8,48-14,24) [9,68–12,82] and 27,32 (19,42–29,88) [22,56–29,02] pg/ml. Statistical analysis was performed using the statistical software package «STATISTICA FOR WINDOWS 5,0» (StatSoft, USA, 1998). Since the Shapiro-Wilks test testified their non-Gaussian distribution, all the obtained during the study data were processed by calculating the median (Me), minimum and maximum (Min-max), and interquartile scale (Lq – bottom quartile; Uq – top quartile). To detect statistical significance of differences between groups of indicators they used the non-parametric U-Mann-Whitney test, the comparison of ranked characteristics within individual groups at different stages of research (at the time of admission of the patient to the hospital, which coincided with 2-6 days of illness, and on 8-12 days of the disease) was performed using paired Wilcoxon test. The differences of parameters were compared for the two points that were considered statistically significant at $p < 0.05$.

Results and discussion

Depending on the intensity of the manifestations of intoxication and the nature of lesions, 16 patients were diagnosed with mild (Group 1), and 23 with moderate (Group 2) form of chickenpox. The chickenpox of 45 patients was accompanied by the development of bacterial complications, among which the most frequently encountered complication that developed as a result of direct penetration of pathogenic bacteria through damaged skin rashes and mucous membranes. These complications are: abscesses, phlegnons, bullous streptoderma, boils, gingivostomatitis,

Table 1

Dynamics of cytokines in serum of children with uncomplicated chickenpox

Comparison groups	Content of TNF-α, pg/ml., [Lq–Uq]		Content of IL-10, pg/ml., [Lq–Uq]	
	2–6 day	8–12 day	2–6 day	8–12 day
Group 1, n=16	16,195 (12,06–4,15) [14,39–21,43]* ‡	11,195 (8,24–18,02) [10,01–13,4]Δ	27,68 (18,02–2,06) [22,54–29,375]	28,715 (18,04–30,92) [24,7–29,5]
Group 2, n=23	74,11 (24,26–93,82) [44,12–148,11]*‡	22,06 (9,16–124,28) [12,04–42,75]* Δ	28,76 (19,88–34,01) [26,52–29,99]	28,04 (20,18–32,96) [24,96–29,73] Δ
Control group, n=14	11,59 (8,48–14,24) [9,68–12,82]		27,32 (19,42–29,88) [22,56–29,02]	

Notes: * - significant difference compared with those of the control group (p <0,05); ‡ - significant difference between groups comparing values; Δ - significant difference between the two time points.

etc. Considering the localization of the pathological process, patients were divided into groups with surface and deep complications of varicella (third and fourth group).

The group of surface complications included 26 patients with children chickenpox with bacterial complications (Group 3), accompanied by the development of purulent inflammatory lesions of skin surface or oral mucosa: 11 children with pyoderma, 7 patients with gingivostomatitis, 5 patients with subcutaneous abscesses and 3 patients with isolated severe boils with perifocal infiltration of tissues.

The group with deep varicella complications included 19 children with abscess lesions in different parts of the body (Group 4). The first clinical symptoms of abscess lesions of various parts of the body evolved on 3-6 days of illness when set against a sudden deterioration of general condition and fever febrile numbers appeared to intense pain in various parts of the body: chest (7 patients), abdominal (6), lumbar (4) and hips (2 patients). On examination of these areas, they observed redness, cyanotic skin tone, and significant infiltration of soft tissues. With some children the pathological process quickly spread to adjacent parts of the body.

In order to study the role of cytokine profile imbalance in shaping variant of the disease course, we determined the level of cytokines in serum of children. In conducting this study, it was found that the patients had multi-directional changes in the concentrations of TNF-α and IL-10, depending on the clinical form of the disease and its course.

In the process of studying the content of cytokines in the group of children with mild chickenpox, it was revealed that at the time of hospitalization only increase of the TNF-α level was observed, which was 1.4 times higher than the corresponding figures in the control group (p <0.001). In the dynamics of disease, the level of TNF-α was normalized and normal value did not differ from the indicators in the control group. The values of IL-10 in serum with mild form did not differ from the values in the control group.

In the group with moderate uncomplicated form of chickenpox early in the disease course, it was marked the increase in TNF-α, which was 6.4 times higher than the corresponding figures in the control group (p <0.001). Within 8-12 days of illness, the cytokine concentration in serum decreased 3.4 times in comparison with baseline, although its values continued to differ from the values in the control group (p <0.001). In the study of IL-10 values in this group of children, only a tendency of values excess at the time of hospitalization compared to the control group was observed. The research results are presented in table 1.

In the group with surface complications of varicella early in the illness, the level of TNF-α in serum by 6.7 times exceeded the values in the control group (p <0.001). A similar pattern was observed in the study of IL-10, because its concentration in the serum at the beginning of the disease was 2.9 times higher than the values in the control group (p <0.001). On the 8-12 days of illness, the increase of TNF-α and excess of values of the control group by 7.5 times was noticed. Values

Table 2

Dynamics of cytokines in serum of children with complicated chickenpox

Comparison groups	Content of TNF-α, pg/ml., [Lq–Uq]		Content of IL-10, pg/ml., [Lq–Uq]	
	2–6 day	8–12 day	2–6 day	8–12 day
Group 3, n=26	77,415 (7,08–590,62) [29,04–112,05]*	86,7 (6,92–403,46) [43,21–132,42]* ‡	81,73 (36,54–214,19) [72,11–92,43]*	89,19 (49,16–208,72) [79,96–96,61]* ‡ Δ
Group 4, n=19	96,89 (19,46–740,39) [70,13–216,14]*	214,92 (80,92–775,55) [120,52–270,96]* ‡ Δ	124,73 (58,34–246,54) [91,02–185,46]*	162,42 (96,43–284,16) [132,96–248,14]* ‡ Δ
Control group, n=14	11,59 (8,48–14,24) [9,68–12,82]		27,32 (19,42–29,88) [22,56–29,02]	

Notes: * - significant difference compared with those of the control group (p <0,05); ‡ - significant difference between groups comparing values; Δ - significant difference between the two time points.

of IL-10 content were 3.3 times higher than the values of the control group (p <0.001).

In the group with deep complications of chickenpox in early disease, the high level of two cytokines was found: TNF-α level was 8.4 times (p <0.001) higher than the values in the control group, and the level of IL-10 – by 4.6 times (p <0.001). In the dynamics of the disease, an increase in cytokine production compared to baseline was noticed. The level of TNF-α was in 18.5 times higher that the values in the control group (p <0.001), and the level of IL-10 – by 5.9 times (p <0.001). The research results are presented in Table 2.

The analysis of the received data showed that the IL-10 level in serum did not change in groups of children with mild to moderate forms of varicella with uncomplicated course, and its values did not differ from that of children from the control group. The increase of IL-10 level in early disease was observed only in the group with moderate form of varicella without complications. But the dynamics of TNF-α in the groups of children with uncomplicated course of varicella (the first and the second group) was more expressed. The children with mild chickenpox early in the illness were observed to have a slight increase in the level of TNF-α. In moderate form of chickenpox at the time of admission to hospital it was noted a high level of TNF-α with subsequent lowering of the level of the dynamics of the disease. High rates of proinflammatory cytokine TNF-α at the onset of the disease indicate the activation of cellular factors of immunity and antiviral protection of the inflammatory reaction in response to the presence of the virus.

The changes in cytokine status observed in the groups of children with complicated course of chickenpox deserve particular attention. It was revealed a high content of both studied cytokines in early disease with increasing of their concentrations in blood serum during the second study on the 8-12 days of illness. The highest content of TNF-α and IL-10 were observed in the group of children with deep complications of chickenpox. High concentration of TNF-α in early disease with a tendency to increase its dynamics is not only an indicator of inflammatory activity, but also a factor in determining the severity of the disease. Increased level of IL-10 indicates the activation of immune antibody factor, suppression of monocyte-macrophage system, which plays a key role in the development and regulation of innate and adaptive immunity.

Conclusions

1. The study of cytokines content in blood serum of children with chickenpox detected the increased levels of proinflammatory cytokine TNF-α and anti-inflammatory cytokine IL-10 relative to the indicators in the control group.

2. The dynamics of the values of content of the studied cytokines varied depending on the clinical form of the disease and its course. In groups of children with uncomplicated course of varicella, only increase of the level of TNF-α was discovered. Instead, in complicated varicella course, the high rates of both cytokines in early disease with increasing concentration in the dynamics of the disease were observed. The highest content of TNF-α and IL-10 was registered in the group of children with deep complications of chickenpox.

3. The results prove the feasibility of studying the values of TNF-α and IL-10 as an additional criterion for assessing not only the degree of severity of the disease, but for the prediction of complications.

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