THE LEVEL OF MEDIATORS OF IMMUNE RESPONSE IN INFANTS WITH CONGENITAL CLEFT LIP AND PALATE

57 infants were examined at age from 0 to 15 months with congenital clefts. Operative-medical actions were conducted after the checkup of infant depending on degree of deformation. 16 practically sound infants of the similar age formed the control group. Study of cytokine levels for IL-1, TNF, IFNγ in blood serum was made using IFA method. Received results showed that increasing level of pro-inflammatory cytokines was observed under congenital cleft. Depth of broken level depends on clinical form of congenital clefts. The most deflection was observed on infants with congenital double-sided cleft lip and palate (CCLP-2). The noted changes on condition of cytokine status on infants with congenital cleft lip and palate led to qualifying the secondary immunodeficient condition, requiring undertaking immunomodulating actions.

Keywords: Cleft lip and palate, immunity, cytokines

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Introduction

Genetically mediated factors, industrial and household intoxications (benzene, polymers, synthetic fibers, pesticides, etc.), the increase in background radiation, violation of the ecological balance, vaccination, bad habits, uncontrolled market of medicines and food - all this leads to the expansion of the range of factors leading to a change in reactivity of the organism.

Particular attention should be given to the infants with congenital pathology of face (CPF) - congenital cleft lip and palate (CCLP) - as one of the most frequent congenital defects of the development in person. CCLP is inherited in 15% of observations; in 85% infants pathology of maxillofacial area is formed in the first 3 months in intrauterine development under the influence of physical, chemical or biological external factors on the organism of future mother.

Congenital cleft of upper lip and palate is most common developmental anomaly. There is a trend of increase in frequency of the birth of infants with specified malformation (Belyakova et al., 1996; Dektyareva, 2000; Durnova et al., 2003). About 60% of these infants are detected at the first 7 days in obstetric facilities. Orofacial clefts occupy one of the leading places among malformations. They fall into “great five” deformities and occupy on frequency the 2nd place. Cleft of lip and palate forms 86.9% from all congenital malformations of face development. Practically any 5th usual cleft is a component of the heavy syndrome (Yakovlev et al., 2000).

Damages in different sections of immunity are one of the important causes of the pathological process of maxillofacial region is (Markina et al., 1999). The manifestations of secondary immune insufficiency in oral cavity differ in elements of specifics, including polymorphisms of primary and secondary pathological elements, prolonged paces of development of inflammatory processes, unrepresentative localization of the affects of mucous mouth (Adam, 1985).

Change of the condition of immune system of infants with CCLP is little studied though it is known that under CPF in infants secondary immunodeficiencies can exist (Yamada et al., 2002). That is why essential elaboration and improvement of treatment methods and rehabilitation of the given contingent of infants is difficult to realize without an in-
depth study of the characteristics of their immune system and justifying the use of immune-oriented preparations (Pinelis et al., 1983).

The immune status of infants in the neonatal period and in the first year of life to a considerable extent is connected with particularities of pregnancy in their mothers (Yamada et al., 2002). It is known that the immune response includes multidirectional type effector mechanisms, each of which is optimal with respect to certain pathogens. In this subpopulations of T-helpers play the key role in regulation of functions of immunocytes through production of cytokines possessing opposition (pro- and anti-inflammatory) effects. Opposition pools of cytokines - IFNγ and IL-4, IL-10 - are considered as markers of Th1- and Th2-lymphocytes, out of which IFNγ intensifies cellular-mediated immune reply, and IL-4 and IL-10 - the humoral one (Kalandarova, 2007).

In postnatal adaptation of immune systems of the newborn one of the leading mechanisms is the activation of system of cytokines which play the important role in protection of colonizing mucous shells and skin of infant by actuating phagocytosis and starting immune processes in T-lymphocytes (Yarilin, 1999).

Laboratory signs of systemic inflammation (increased serum levels of IL-1β, TNFα, acute phase proteins) are noted in healthy newborn infants on the background of the high antigenic load. In physiological conditions this is not accompanied by the development of clinical signs of system inflammations and polyorganic insufficiency. The maintenance of immune homeostasis in new-born infants is provided by the complex feedback mechanisms (proliferation and differentiation of lymphocytes) and increased concentration of anti-inflammatory cytokines (IL-4, TGF-β) in blood serum. The balance of pro- and anti-inflammatory cytokines may be the key that contributes to the clinical condition of the child (Ketlinskiy and Simbirsev, 2008).

The certain amount of cytokines is required for identical immune reply (Ketlinskiy and Simbirsev, 2008). Impaired production, secretion and reception of anti-inflammatory cytokines leadsto profound defects in defense, until the development of “immunological paralysis”, and exacerbates a direct damaging effect of various pathological factors (Kalandarova, 2007).

Increased secretion of proinflammatory cytokines and an imbalance ratio of the opposition pools may play an important role in the pathogenesis of the disease through increased aggregation of leukocytes to vascular endothelium, stimulation of its procoagulant activity, engaging in an excess of inflammatory effector cells which ultimately enhances pathoimmunological cascade (Yarilin, 1999) and leads to cytokine-mediated damage (Ketlinskiy and Simbirsev, 2008).

In connection with aforesaid, the purpose of the study was the study of the condition of cytokine status (IL-1, TNF, IL-6, IL-4, IFNγ) in the infants with congenital cleft lip and palate.

Material and methods

We observed 57 infants with congenital cleft of lip and palate who were operated at the surgical department of the Republican hospital in Bukhara city. Given that early (before the age of 1.5-3 years) surgical treatment leads to an early restoration of communicative functions, prevents the psycho-emotional disorders, age of the children surveyed were in the range from 0 to 14 months. The survey consisted of collection of clinical and anamnestic data and laboratory studies (general blood test and determination cytokines level).

Complex examination by specialists (surgeon, pediatrician, orthopedist, otolaryngologist, psycho neurologist) was carried out for the infants with jaw-facial pathology to detect abnormalities of somatic nature and accompanying malformations and carry out the necessary correction of deviations in the preoperative period.
Operative medical actions were conducted after the examination of infant depending on degree of available deformations. 16 practically healthy infants of the similar age made up the control group.

The study of the level of cytokines: IL-1, TNF, IL-6, IL-4, IFNγ in blood serum was conducted by the IFA method (OPO “Cytokine”, Saint Petersburg) in the Institute of Immunology in Uzbekistan.

Statistical processing of the data was carried out on personal computer using standard package of applied software.

**Results and their discussion**

From 57 examined infants girls were 68.4% (39), and boys - 31.6% (18). The majority of infants with congenital cleft and palate were born on the 2nd or 3rd pregnancy and accordingly 2nd or 3rd births. 29.8% (17) infants were born on the first pregnancy, 12.3% (7) infants - on 4th and more pregnancy. The majority examined infants were born in unfavorable course of pregnancy with toxicosis, 26.3% (15) of the women had different delivery complications, 28% (16) of the women had recovered influenza in the first trimester (Figure 1).

**Figure 1. Data of mothers’ anamnesis of the examined infants**

![Figure 1](image)

**Figure 2. Congenital clefts (%)**

![Figure 2](image)
64.9% (37) of the women were diagnosed with mild or moderate-severe iron deficiency anemia in the period of pregnancy; 24.6% (14) - a threat of miscarriage. Herpes virus infection was revealed in 22.8% (13) of the women in the first trimester of pregnancy.

Study of clinical data of infants with congenital cleft found that 22.8% (13) infants had unilateral fissures of palate; 15.8% (9) infants had the double-sided fissures of palate; 28% (16) infants had the clefts of the soft palate; 14% (8) infants had the clefts of soft and partly hard palate and 19.3% (11) infants had medial clefts of palate (Figure2).

![Figure 3: Accompanying Pathology (%)](image)

Among the accompanying diseases (Figure3) basically prevailed dysbacteriosis (in 53 infants) and allergic diseases (diathesis, stomatitis) (at 43 infants).

While undertaking the immunological studies the examined infants were split into the following groups: congenital cleft of palate (CCP) - 19 infants; congenital unilateral cleft lip and palate (CCLP-1) - 23 infants; and congenital double-sided cleft of the upper lip and palate (CCLP -2) - 15.

<table>
<thead>
<tr>
<th>Cytokines (pkg/ml)</th>
<th>Checking group</th>
<th>Infants with congenital clefts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CCP</td>
<td>CCLP-1</td>
</tr>
<tr>
<td>TNFα</td>
<td>32.4 ± 15</td>
<td>89.3 ± 17*</td>
</tr>
<tr>
<td>IL-1β</td>
<td>27.8 ± 12.6</td>
<td>68.6 ± 9.3*</td>
</tr>
<tr>
<td>IFNγ</td>
<td>31.7 ± 5.1</td>
<td>17.5 ± 4.3*</td>
</tr>
</tbody>
</table>

Note: * - Reliable difference from the data of control group (P<0.05-0.001)

The results of research of cytokines levels in the sick infants are presented based on clinical manifestations of congenital clefts (Figure 4 and Table 1). As can be seen from the given data, in the sick infants the change of concentrations of cytokines are noted, particularly expressed in the infants with CCLP-2. The sick patients demonstrated significant differences between the concentrations of cytokines from the values obtained in the control group.

In infants with CCLP-2 TNF-α serum level was vastly increased (123±36 pkg/ml) in contrast with the control group (32.4±15 pkg/ml, P<0.001), then under CCP the increased level made 2.7 times (89.3±17 pkg/ml, P<0.01), and under GFLP-1 TNFα level made 3.6 times. So, TNF-α serum level made in average 118.2±32 pkg/ml (P<0.01). In infants with CCLP-2 IL-1β level in blood serum increased 4.1 times (114.7±23 pkg/ml,
As it is well known, IFNγ is produced by activated Th1-cells and NK-cells. In our study the decreased level of IFNγ was noted in the sick infants, in contrast with the data of the control group (31.7±5.1 pg/ml). This fact existed in all groups and did not depend on degree of disease severity: in CCLP-2 - 17.4±3.5 pg/ml (P<0.05), in CCLP-1 - 17.8±4.1 pg/ml (P<0.05), and in CCP - 17.5±4.3 pg/ml (P<0.05).

Thus, while analyzing a row of the level of inflammatory cytokines in blood serum of infants with congenital cleft we noted significant increase of TNF-α and IL-1β in CCLP-2 and moderate increasing of their serum content in CCLP-1 in contrast with control group. The received results confirm the presence of negative correlation between concentration of IFN-γ and IL-1β that is indicative of damage of immunoregulatory mechanisms in congenital anomaly.

In physiological conditions IL-1 is capable to intensify the production of IFNγ through activation of Th1-cells. Consequently, reduced concentration of IFNγ serum points to the destruction of IL-1-mediated product of IFNγ by Th1-cells. It is not excluded that activation of macrophagal link of immunity can promote increasing macrophage production of substances inhibiting syntheses of IFNγ.

The inflammatory reaction under congenital cleft is characterized by primary activation of Th2 cells. The key cytokine, responsible for polarization of immune reply on Th2-type, is interleukin-4, which together with interleukin-12 and molecular complex CD40-CD40L participates in starting the B-lymphocytes synthesis of antigen-specific immunoglobulins of E (IgE) class (Yarilin, 1999).

IgE-dependent activation of high affinity receptors (FcεRI) of the obese cells and basophils, as well as low affinity receptors (FcεRII) of eosinophils brings to deliverance in the centre of the inflammation integer a row of preformed and synthesized de novo biologically active substances with broad spectrum of pro-inflammatory features providing influx of actuated effector cells from peripheral blood into the other systems and responsible for development of the allergic inflammation (Dektyareva, 2000).

In this regard, along with clinical and anamnestic factors are important the laboratory findings on level of IgE in blood and concentrations of IL-4 in different biological liquids.
The received data on IL-4 levels in blood serum and in saliva, as well as the level of general IgE in blood serum of the examined infants are presented in Table 2.

<table>
<thead>
<tr>
<th>Group of infants</th>
<th>IL-4 in saliva</th>
<th>IL-4 in blood serum</th>
<th>IgE in blood serum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Checking group</td>
<td>6.7 ± 0.3</td>
<td>2.85 ± 0.15</td>
<td>103 ± 6.8</td>
</tr>
<tr>
<td>CCLP-2</td>
<td>310 ± 11.5</td>
<td>15.0 ± 2.23*</td>
<td>328 ± 26.8*</td>
</tr>
<tr>
<td>CCLP-1</td>
<td>76 ± 8.4 *</td>
<td>12.1 ± 2.12*</td>
<td>262 ± 23*</td>
</tr>
<tr>
<td>CCP</td>
<td>28.3 ± 3.6*</td>
<td>8.6 ± 1.6</td>
<td>173 ± 15*</td>
</tr>
</tbody>
</table>

Note: *- Reliable difference from the data of control group (P<0.05-0.001)

As can be seen on Table 2, increasing level of general IgE is found in periphery blood of the sick infants. The most high level was noted in infants in group with CCLP-2 (P<0.001). In CCLP-1 the IgE in blood increased 2.5 times in contrast with control data (P<0.01). For the infants with CCP was typical the 1.7 times increase of IgE (P<0.05).

Study of IL-4 level in blood serum in infants revealed the similar pattern: the most high IL-4 level is typical for infants with CCLP-2 (P<0.01). Unlike periphery blood, in liquid greatly drawn near to the centre of the inflammation (saliva), the IL-4 level in infants with CCLP-2 is realistically above (P<0.01) in contrast with data of infants with CCLP-1 (P<0.001), and in infants with CCP IL-4 level - 2.7 times below the factors of infants with CCLP-1 (P<0.05).

The received data are indicative of more expressing activation of Th2 type cells in infants with CCLP-2. In the sick infants in microenvironment of Th0-cells the cytokines are present, providing development of population of Th2 cells; important role in this process plays IL-4, released by activated mast cells. Inflammatory infiltrate in mucous shells contains the big amount of Th2-lymphocytes and cytokines, attracting eosinophils, basophils and neutrophils into the centre of inflammation (Yarilin, 1999).

Polyfunctionality expects the participation of cytokines in different physiological and pathological processes. In combination with the variety of the sources of cytokines it is possible to expect the reactive realignment of cytokine network in many (unless all) diseases. In the same time, functional specificity inherent each of cytokines allows a peculiarity of their imbalance depending on pathology.

One of the reasons of immunodeficient conditions is a destruction of immunoregulatory processes, supported by Th1- and Th2-lymphocytes. As it is well known, the first synthesize the cytokines stimulating cellular immunity (IL-1, IL-2, IL-6, IL-8, IL-12, IFN, TNFα and oth.), the second synthesize the cytokines stimulating humoral immunity (IL-4, IL-5, IL-10, TGF-β, etc.). In normally functioning organism the balance of the interaction between Th-1 and Th-2-lymphocytes is determined. But profound change of their activities can lead to serious adverse consequences in the functioning of immune system as a whole.

It is established that the allergic process causes the activation of Th2-cells and the synthesis of cytokines that have a suppressive effect on cellular immunity. The cytotoxic destruction mechanism, connected with T-killers, is activated (Ketlinskiy and Simbirtsev, 2008). Therefore, the received results indicate on pronounced immunological changes which contribute to the development of complications of this disease.
Conclusion

Summarizing the presented data, it should be noted that development of intrauterine anomaly is very important problem already due to their broad spreading at present. And the described changes in condition of cytokine status can be qualified as secondary immunodeficient condition requiring undertaking of immunomodulating actions.

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