THE CONDITION OF IMMUNE SYSTEM OF INFANTS WITH CONGENITAL CLEFT LIP AND PALATE

The material on registration of malformation has been studied in Bukhara region for 2010. Out of 159 infants, born with different intrauterine anomalies the 16.35% (26) were found with the cleft lip and palate in different variants. After the complex examination by specialists (surgeon, pediatrician, orthopedist, otolaryngologist, psychoneurologist) operative treatment was conducted depending on degree of the defect. The study of the condition of immune system in infants was conducted after operative treatment. The examined were divided into 2 groups - infants without postoperative complications and infants with postoperative complications. We studied the quantitative determination of lymphocytes with phenotype CD3, CD4, CD8, CD16, concentration IgG, IgA IgM, phagocytic activity of neutrophils and the level of CIA. It was found that in infants with congenital cleft lip and palate exist the deflections on the condition of immune system, which were more denominated in infants with postoperative complications.

Keywords: Congenital cleft lip and palate, infants, immune system

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Introduction

The severity of facial malformations is conditioned not only by external disfigurement, expressed by functional breaches, but also by the somatic frustrations bringing to braking of the growth and nongrowth of child’s organism as a whole (Abdusalyamov et al., 2000; Alimskiy et al., 2006a; Danilova and Gvozdeva, 2009; Gvozdeva, 2010a; Kim et al., 2000).

The maintaining anatomical distortions cause the breach of functions in directly struck and associate with them organs. Harmony of the development of several areas will be broken, so called combined secondary deformations appear. Observed breaches of immunological reactivity are mediated by effects of acute phase proteins, cytokines, non-specific protection factors, mechanisms of specific resistance, hereditary factors and the other systems of the organism (Alekseev, 2002; Alimskiy et al., 2006b; Gvozdeva et al., 2009; Gvozdeva, 2010b; Mirzonov and Jurikhina, 2008; Dushanova, 2003).

The way and efficiency of the treatment of cleft palate mostly depends on localization, sizes of the defect, also condition of immune system (Alimskiy et al., 2007; Gvozdeva et al., 2009). The purpose of the work was the study of some immune system parameters in infants with congenital cleft lip and palate.

Material and methods

For revealing the existence frequency of congenital cleft lip and palate (CCLP), we studied material on registrations of malformation in Bukhara region for 2010. It was revealed that out of 159 infants, born with different intrauterine anomalies, the cleft of lip and palate in different variants was noted in 16.35% (26).

For all infants with maxillofacial pathology there was organized complex examination by specialists (surgeon, pediatrician, orthopedist, and otolaryngologist, psychoneurologist) in order to reveal the deflections of somatic nature and accompanying vices of the development and undertaking the necessary correcting of deflections in preoperative
period. Operative-medical actions were carried out after the checkup of infant depending on degree of the deformation.

Depending on way of the operations all infants were divided into 3 groups: 1st group - 9 infants operated by the method L.E. Frolova- E.U.Mahkamov (UFM); 2nd group - 8 infants operated in 2 stages - veloplastics and uranooplastics on method of L.E.Frolova (VUF); 3rd group - 9 infants operated by the method of J.Bardach. The age of infants was within from 8 to 16 months. 12 practically sound infants of the same age formed the control group.

Study of the condition of immune system was conducted in 2 weeks after operative actions. Venous peripheral blood served as the material for immunological studies. Mononuclear cells were selected by the standard method in gradient of density of ficoll-verografin. Immunological studies were conducted by the study of the quantitative determination of lymphocytes with phenotype CD3, CD4, CD8, CD16, CD20 by means of monoclonal antibodies of LT serieses (“Sorbent” LTD, Moscow, Russia). The concentration of immunoglobulins was defined by the well-known method of radial immunodiffusion on Manchini (the monospecific serum of N.F.Gamaley Institute, Moscow), phagocytic activity of neutrophils was defined with the use of latex particles (1.5mcr) on the V.P.Kudryavtseva method. The concentration of circulating immune complexes was defined in blood serum with the use of PEG-6000 (“Nihol”, Tashkent).

The blood taken from children for the research was authorized by their parents. Immunological studies were conducted at the Institute of Immunology of the Academy of Sciences of the Republic of Uzbekistan.

Statistical processing of results of studies was conducted with the use of standard methods of the variation statistics.

**Results**

The analysis found that out of the gross amount of the sick, boys formed 23.0% (6), and girls - 77% (20). Comparing of clinical forms of pathology found that the congenital cleft palate (CCP) occupied leading positions - 42.3% (11) (Figure 1).
Congenital unilateral cleft lip and palate (CCLP-1) is on the second place - 30.8% (8). Congenital double-sided cleft of the upper lip and palate (CCLP-2) is on the third place – 26.9% (7). The study of anamnesis and collection of the complaints showed the presence of the pathological condition of many organs and systems. Questioning of the parents of infants with CCP indicated on signs of the gastrointestinal tract disease in 26.9% (7) children, constipations - in 15.4% (4), diarrhea - in 30.8% (8), pains in the field of belly - in 3 (11.5%). Coprology of infants with CCP found different forms of coprological syndromes in 53.8% (14). Among them there were steatorrhea of the first, second and the third type (on Vinogradova, 1971) developing due to absorption alteration or violation of small intestine and hepatobiliary system. These changes are indicative of presence of malabsorption which can cause development of hypotrophy, anemia and immunodeficient condition. Development of the inflammatory process in postoperative wound was found in 34.6% (9) of operated infants. In 23.0% (6) of them a rationally conducted postoperative care lead to epithelialization of the wound on 8-10 days; 3 patients needed repeated the following surgical interference to eliminate local complications.

**Figure 2. Some of the symptoms of wound process without complications (in days)**

In figuring out factors of the risk for development of postoperative complications there was revealed adverse form of the cleft existed in these infants - broad, horseshoe shaped, hiding with expressed hypotrophy of the palate muscles.

Figure 2 shows data on dynamics of symptoms of wound process in infants without complications at early postoperative period. Table 1 indicates on longer duration of symptoms in infants with complicated course of the postoperative period. Considering the results of the study there was conducted correction of the deviations by evidence - heparin, vitamin E, etc.

While undertaking the examination of immune system the sick infants were divided into 2 groups - 1st group consisted of 17 infants without complications at postoperative period; 9 infants entered into the 2nd group with postoperative complications. As can be seen from presented given data immunological factors under congenital cleft differ from parameters of the control group (Table 2).

The comparative feature of parameters of immune system indicated on the deficit in contents of T-lymphocytes in circulating blood of the examined sick infants (P<0.01).
However, the deep deficit existed in infants having complications in postoperative period (P<0.001).

**Table 1. The Duration of Some Symptoms of Wound Healing, Depending on the Severity of Defect (in Days)**

<table>
<thead>
<tr>
<th>Signs of early process</th>
<th>Ponderosity of pathology</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>CCP</td>
</tr>
<tr>
<td>Pallor of soft palate</td>
<td>3±0</td>
</tr>
<tr>
<td>Edema of tissues of soft palate</td>
<td>3.41±0.3</td>
</tr>
<tr>
<td>Hyperemia of tissues of soft palate</td>
<td>4.1±0.3</td>
</tr>
<tr>
<td>Hematoma of tissues of soft palate</td>
<td>3.7±0.4</td>
</tr>
<tr>
<td>Fibrin coating in the tissues of soft palate</td>
<td>6.1±0.2</td>
</tr>
<tr>
<td>Necrosis of tissues of hard and soft palate</td>
<td>7.5±0.5</td>
</tr>
<tr>
<td>Beginning of granulation of lateral wound</td>
<td>8.7±0.3</td>
</tr>
<tr>
<td>Divergence of stitches</td>
<td>5.2±0.4</td>
</tr>
</tbody>
</table>

**Table 2. Some Parameters of Immune System in Infants with Congenital Cleft Lip and Palate, (M±M)**

<table>
<thead>
<tr>
<th>Immunological factors</th>
<th>Checking group, n=14</th>
<th>1-group, n=17</th>
<th>2-group, n=9</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD3+, %</td>
<td>50.6±1.0</td>
<td>46.8±0.8*</td>
<td>40.9±0.9*</td>
</tr>
<tr>
<td>CD4+, %</td>
<td>31.3±0.8</td>
<td>27.4±0.6*</td>
<td>24.5±0.4*</td>
</tr>
<tr>
<td>CD8+, %</td>
<td>20.1±0.6</td>
<td>18.1±0.8</td>
<td>15.6±0.5*</td>
</tr>
<tr>
<td>CD16+, %</td>
<td>11.6±0.8</td>
<td>6.8±0.8*</td>
<td>15.2±0.7*</td>
</tr>
<tr>
<td>CD20+, %</td>
<td>22.3±0.8</td>
<td>27.1±0.7*</td>
<td>35.5±0.9*</td>
</tr>
<tr>
<td>Phagocytosis,%</td>
<td>49.4±1.4</td>
<td>46.9±0.8*</td>
<td>40.3±0.8*</td>
</tr>
<tr>
<td>CIC, cond.un.</td>
<td>28.6±1.1</td>
<td>34.2±1.8*</td>
<td>61.8±1.9*</td>
</tr>
</tbody>
</table>

Note: *- essence is reliable to checking group (P<0.05 - 0.001)

Analysis of subpopulational composition of lymphocytes showed that reduced level of T-helpers/inductors and T-suppressor/cytotoxic lymphocytes is characteristic for congenital cleft. The deeper deficit of essence of CD4+ and CD8+ existed in 2nd group (P<0.01). CD4+ lymphocytes, executing their helper function, help B-cells to change into antibody producing plasmatic cell; they also help CD8+ lymphocytes to transform in mature cytotoxic T-cell; and macrofags to realize the effects of hypersensitivity. The specified functions of T-lymphocytes的帮助者 are realized due to the fact that they are distributed on two subpopulations - Th1 and Th2 types, executing miscellaneous helper functions to account of the producing various cytokines-interleukins (Ketlinskiy and Simbirtsev, 2008).

Cytotoxic immune response plays important role in protection of the organism from intracellular pathogens and includes the non-specific section - NK-cells, and antigen specific section of adaptive immune response - cytotoxic T lymphocytes (CTL) (Yarilin, 1999).

Realistically low expression of CD16 antigens on lymphocytes in the examined infants comparing to the control group can be indicative of weak resistance of organism.
However, increased level of killer activities is indicative of presence of the infectious agent.

Studying the non-specific factor of protection there revealed that functional insufficiency of phagocytes was wide-spread in sick infants (P<0.001). Phagocytic reaction initiates the immune response. Reduction of activities of phagocytic protection, naturally, provides the low level of immune response, including humoral one, delay of the products of assimilations, breaches of the balance and tolerance to autoantigens.

Reserve deficit in immunocompetent cells and deficit of humoral protection condition the risk of the accumulation of abnormally high concentration of circulating immune complexes (CIC). The study of CIC level showed that in the sick infants with complications the CIC concentration was in 2.1 times above the control values (P<0.01).

It is known that CIC is formed by interaction of specific antibodies formed in organism - immunoglobulins and antigens that induced the immune response and production of these antibodies. The interaction of specific antibodies with antigens is the most important mechanism of anti-infectious protection: antibodies neutralize bacterial exotoxins, neutralize the extracellular viruses, opsonize bacteria, facilitating their phagocytosis and intracellular destruction. During CIC shaping, the structure and biological activity of the antigen change.

B-system is presented by quantitative existence of B-lymphocytes with CD20 molecule and the level of immunoglobulins of IgG, IgA, IgM classes.

**Figure 3. Concentration of immunoglobulins of main classes in the examined infants (mg/%)**

![Figure 3](image)

The comparative feature of the content in circulating CD20+-cells showed that under CCP the level of these cells realistically increases (P<0.01) with maximum values in infants with complications (P<0.001). The achieved results allow to consider that for congenital clefts is characteristic the activation of B-cellular link of immune system on background of imbalance in population of T-lymphocytes, particularly at presence of the complicated condition. The study of concentrations of the main classes IgG, IgA and IgM showed that the reduction of the syntheses of IgG and IgA occurs at the sick infants (P<0.05) (Figure 3). As to IgM, its content was characterized by reliable increasing (P<0.01). As it is well
known, this type of antibodies is produced against infectious agents, actuates complement and intensifies phagocytosis (Penelis, 1983). Possibly, an increased synthesis of IgM in group of the sick infants is connected with occurrence of infectious process.

For activation of T-lymphocytes it is necessary existence at least of two consequent processes. The first signal is provided by joining MNS-associated peptides with T-cellular receptor. The second activating signal causes the expression of IL-2 (CD25) receptors on T-lymphocytes that promotes cells to S-phase of the cellular cycle with the following cellular replication. The analysis of the received results showed that under CCLP the reliable increase of expressions of activation markers exists, both early activation of CD25+ cells and late activation of HLA-DR+. Moreover, at presence of the complications the level of lymphocytes with receptor to IL-2 is increased in 1.55 times (P<0.05)

Thereby, expression of markers of activations of HLA-DR and CD25 on lymphocytes of the sick infants is increased in contrast with such in healthy infants. Increase of the amount of lymphocytes, expressing CD25 and HLA-DR antigens, testifies on stability in the process of activation and transition to proliferation.

Apoptosis - one of the forms of programmed cellular death, which is characterized by damage of DNA under influence of endonuclease. Forming then apoptotic bodies are subjected to phagocytosis. Apoptosis is so important component of immunological processes as proliferation and differentiation. The level of lymphocytes with receptor to apoptosis in our studies in the examined group of infants was realistically increased. Moreover, in infants with complications the increase was significant (P<0.01).

Conducted study has shown that process of the development of immunopathological conditions in infants with congenital cleft lip and palate has their own features and in greater degree is conditioned by functional insolvency of immunocompetent cells. It is shown that different clinical conditions correspond to the immune disorders defining severity and degree of progressing of process. We cannot exclude also important role of the functional disorders on the part of such effector cells, as macrophages, participating in processing of antigen and its presentation to naive T-helper lymphocyte, since, exactly at this stage the chain of regulating events occur, defining direction of immune response (Yarilin, 1999).

These data show that certain combination of factors leading to pathology is created already on early stage of immune response in infants with CCLP. These factors influence on the development of the pathological response, bring to the condition of areactivity, may cause the apoptosis of reacting cells and distortion of activation processes in regulating cells, as well as the initiation of proliferation and differentiation of lymphocytes, and absence of amplification of immune response.

**Conclusion**

The presented data point to significant pathogenic role of immune disturbances in process of the shaping of the inflammatory process under surgical interference in infants with congenital cleft lip and palate. The difficulty of pathogenesis, variety of pathological processes and diversity of mechanisms of realization, as well as the depth of immune damages point to need of the undertaking in preoperative period long and intensive immunocorrigating therapy for achievement of steadfast clinic-immunological remissions. The successful decision of this problem is connected with proper approach to recovering the operation of immune system. Consequently, it is necessary to include complex immunorehabilitation, including not only medicamentous but also non-pharmacological treatment, rendering plural actions and promoting processes of the reconstruction of damaged sections in the systems of the organism.
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