

How to Cite:

Nuraliev, N. A., & Ruzmetov, F. N. (2022). Description of antiendotoxigenic immunity immunological properties in children diagnosed with microbe etiological diseases. *International Journal of Health Sciences*, 6(S2), 13592–13601. <https://doi.org/10.53730/ijhs.v6nS2.8571>

Description of antiendotoxigenic immunity immunological properties in children diagnosed with microbe etiological diseases

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Abstract---The aim of the study was to determine the titer of antibodies against gram-negative bacterial endotoxins in diseases of various microbial etiology in children, to assess its diagnostic value and to evaluate the immunological properties of antiendotoxigenic immunity. It was found that the percentage of seropositive sera to GShPB antigens was convincingly higher in children with MEK and IID than in healthy children. The percentage of detection of antitoxin antibodies depended on the type of microorganism. The most important in YID were *Escherichia coli* i *Pseudomonas aeruginosa*, and in MEK were *Escherichia coli*. In terms of the intensity of antitoxin antibody formation, MEK differed sharply from other groups. While "medium" and "high" titers were detected mainly in the CEC, only "low" titers were detected in healthy and YID-observed children. Determination of the intensity of antibody formation against GShPB endotoxins at "medium" and "high" titers is recommended as an additional diagnostic and prognostic criterion in the diagnosis of MEC.

Keywords---endotoxin, antiendotoxigenic immunity, gram-negative bacteria, diseases microbial etiology.

Introduction

Endotoxins are components of the cell wall of gram-negative bacteria and are thermostable, most of which are released after bacterial cell death, inducing the synthesis of cytokines and other mediators. It differs from other protein toxins by its lack of organotropy and non-specific effects, endotoxins also have the property of suppressing the process of phagocytosis. The term "endotoxin" was introduced and recommended to science by R. Pfeiffer [9, 11]. An analysis of scientific sources published by domestic and foreign researchers shows that endotoxins

consist of a complex of proteins and LPS. It is known that in vivo in the body all groups of endotoxin molecules are responsible for the emergence of their biological effect [8, 13, 16].

It is known that antibodies are important humoral antiendotoxin factors. Antibodies against gram-negative bacterial lipopolysaccharides (LPS) O-chains have high neutralizing activity, but the high variability of the O-chain of gram-negative bacteria reduces the likelihood that specific antibodies meet the corresponding endotoxin. Therefore, attention is paid to the deep determinants of the LPS R-layer, especially antibodies against Re-glycolipid [4, 10, 12]. LPS enters thymus-associated antigens, polyclonally stimulates V-lymphocytes, alternatively activates the complement system, is an adjuvant.

Low levels of endotoxins in the blood of representatives of the normal microflora of the human colon, gram-negative microflora have an antigen-stimulating effect on the immune system, enhance the non-specific resistance of the human body, increase the anti-tumor activity of cells [2, 3]. Specific features of antiendotoxic immune endotoxin in inflammatory diseases of bacterial etiology in children - pyrogenic effect, activation of blood flow and intravascular thrombus formation, hemodynamic disturbances, enhanced immune humoral system, impaired renal function, weak immunogenic effects and pathogenesis of formation and development of these nosological units associated with [6]. It is known that the distal parts of the intestine are a "natural storehouse" for gram-negative microflora and their endotoxins. Normally, due to the barrier function of the intestine, less endotoxins are released into the bloodstream, which binds to Kupfer cells, macrophages, erythrocytes, lipoproteins, other proteins in the blood plasma in the portal vein system and is detoxified in hepatocytes [14, 15].

It has been found that up to 6% of portal blood is normally pumped into the systemic bloodstream through portocaval and liver anastomoses without entering the liver. Without entering the liver, control of LPS inflow of microorganisms into the general bloodstream from the intestine is mainly carried out by the hypothalamic-pituitary-adrenal system, the degree of portal hemorrhage along portocaval anastomoses depends on its functional activity. This allows us to reflect a physiological phenomenon such as the presence of endotoxin in the systemic bloodstream of healthy people in the postulates of "systemic endotoxemia" and "endotoxin aggression", a universal factor in the pathogenesis of human disease, the development of which .

Maternal antibodies to endotoxin are detected in the umbilical cord blood of children at birth in the form of IgG to Reglicolipid. The intestines of newborns are sterile for the first 10-20 hours after birth. Transplacental transfer of maternal antiendotoxin antibodies is one of the important factors of natural immunity, which ensures the adaptation of the newborn to the conditions of life outside the uterus. Soon after birth, children develop physiological systemic endotoxemia [1, 13], which is the result of colonization of the intestine with gram-negative microflora. The amount of free endotoxin in the blood plasma of healthy infants is 1.36 ± 0.1 pkg / ml (1.9 ± 0.25 pkg / ml in adults), with endotoxin units ranging from 0 to 1 EU.

In infants, high levels of endotoxin in the blood plasma are detected against the background of a decrease in the titer of antiendotoxin antibodies in the state of adaptation to the early neonatal period. In newborns in the early neonatal period, the amount of LPS in the blood decreases and the titers of antibodies to Reglycolipids increase with the completion of the adaptation period. An increase in the amount of endotoxin antibodies (IgG) is observed with the decrease of representatives of the normal intestinal microflora, the amount of which at 5 days averaged 231 ± 21 optical density conditional units in healthy newborns, indicating the ability of infants to synthesize individual antibodies. It should also be noted that a certain increase in the titer of these antibodies does not exclude the possibility that they are provided by the parent antibody-producing cells. Transplacental transfer of maternal antiendotoxin antibodies is one of the important factors of natural immunity, ensuring the adaptation of the baby from the uterus to external living conditions [13].

Normative values of antiendotoxin antibody titers in healthy newborns in the late neonatal period were found to be 213.4 ± 5.6 units [14]. Bacterial infection in children in the first months of life, intestinal syndrome occurs against the background of weakened antiendotoxin immunity. A positive course of bacterial infections (regardless of etiology) is always accompanied by an increase in the titer of antiendotoxin antibodies, and a decrease in severe forms of infectious diseases. Detection and assessment of antiendotoxin immunity in inflammatory diseases of bacterial etiology is of great diagnostic value for early diagnosis of these diseases, to determine the prospects for disease outcome. Inclusion in health practice increases the diagnostic value of antiendotoxin immunity by establishing clinical-immunological criteria to prevent these nosological units from becoming chronic.

The purpose of the study

To determine the titer of antibodies against gram-negative bacterial endotoxins in diseases of various microbial etiology in children, to assess its diagnostic value and to evaluate the immunological properties of antiendotoxin immunity.

Research material and methods

To achieve the goal, the blood serum of children suffering from common diseases among children permanently residing in our region - acute tonsillitis, acute bronchitis and urinary tract infections was studied. The study involved 251 sick children aged 3-14, living in the Khorezm region of Uzbekistan, of whom 87 were diagnosed with acute tonsillitis, 71 with acute bronchitis, and 93 with urinary tract infections (UTIs). All of these patients were grouped into a major group as microbial etiologic diseases (MECs). In order to compare the results, the blood serum of 25 practically healthy children (comparison group 1) and 61 children with colonic dysbiosis (DID) (comparison group 2) was examined. All comparable groups were representative of the age-sex composition of the studied contingent.

Given that no "positive" and "false positive" reactions were observed in young children due to immature immune systems and low duration of antigen stimulation [8], children under 3 years of age were not included in the study. All

children were divided into age groups as follows: 3-6 years (preschool period) and 7-14 years (school period). All diagnoses were made using the 10th revised International Classification of Diseases (ICD, 1997). Ethical principles related to the involvement of children in medical research have been implemented in accordance with the Helsinki Declaration of the World Medical Association (Helsinki, 1964, final supplement Seoul, 2008).

Concentrations of gram-negative bacterial cell wall LPS antitoxic antibodies in serum were determined by IFA method using an experimental test system [7, 10]. Cultures of microorganisms were obtained from the National Collection of Human Infectious Microorganisms of the Research and Practice Center for Epidemiology, Microbiology, Infectious and Parasitic Diseases of the Ministry of Health of the Republic of Uzbekistan (2006, based on Contract 2). Passports of strains with inactive cultures with basic descriptions of bacteria were obtained. Collection strains of *Klebsiellae pneumoniae*, *Escherichia coli*, *Citrobacter freindii*, *Proteus vulgaris* and *Pseudomonas aeruginosa* were used in the study. Strains were used in the form of an inactivated bacterial mass at a concentration of 1×10^9 microbial body / ml.

Antigenic microorganisms were isolated by Buaven using extraction of diurnal culture in trichloroacetic acid. This method is part of the chemical method of antigen extraction. Statistical processing of the results was performed using generally accepted variational statistical methods for medical-biological research. The organization and conduct of research was based on the principles of evidence-based medicine.

Results and discussion

It is known that one of the etiological criteria for gram-negative conditionally pathogenic bacteria (GSHP) is the detection in the serum of antigens of microorganisms, which are antibodies specific to endotoxin [5]. Therefore, in the first part of the study, the titer of antibodies against GShPB antigens was determined. The titers of specific antibodies to GShPB antigens in the serum of most of the children examined by MEK and YID were convincingly higher than those of healthy children ($R < 0.001$). The degree of detection of antibodies depended on nosological units and the type of microorganism ($R < 0.001$).

Percentage of antibody detection against *Escherichia coli* (88.0%, $n = 221$), *Citrobacter freindii* (79.3%, $n = 199$), *Pseudomonas aeruginosa* (70.2%, $n = 176$) antigens in children detected by MEK *Proteus vulgaris* (56.2%, $n = 141$), were significantly higher than the titer of antibodies against *Klebsiellae pneumoniae* (51.4%, $n = 129$) antigens ($R < 0.05$). A high percentage of seropositive samples relative to *Escherichia coli* antigens in children detected by MEK was associated with multiple detection of antibodies in SYI (Figure 1). A similar trend was observed in children with YID: *Escherichia coli* 80.3% ($n = 49$); *Citrobacter freindii* 52.5% ($n = 32$); *Pseudomonas aeruginosa* 39.3% ($n = 24$); *Proteus vulgaris* 59.0% ($n = 36$); *Klebsiellae pneumoniae* 41.0% ($n = 25$). The difference was in the percentage of antibodies detected against the antigens *Proteus vulgaris* and *Pseudomonas aeruginosa*.

Thus, the percentage of antibody encounters against GShPB antigens in blood serum was convincingly higher in children observed with MEK than in both comparison groups. The percentage of antibody detection of microorganisms compared to each other was MEK and in healthy children, respectively, *Escherichia coli*, *Citrobacter freundii* and *Pseudomonas aeruginosa* larvae, respectively, in children with YID, respectively, *Escherichia coli*, *Proteus vulgaris*, *Citrobacter freundii* antigen. The incidence of seropositive and seronegative samples differed in the comparable groups: seronegative samples were more common in healthy children ($R < 0.001$), seropositive in children with RID ($R < 0.001$), seropositive samples in children diagnosed with MEC were more than control but less than dysbiosis ($R < 0, 05$).

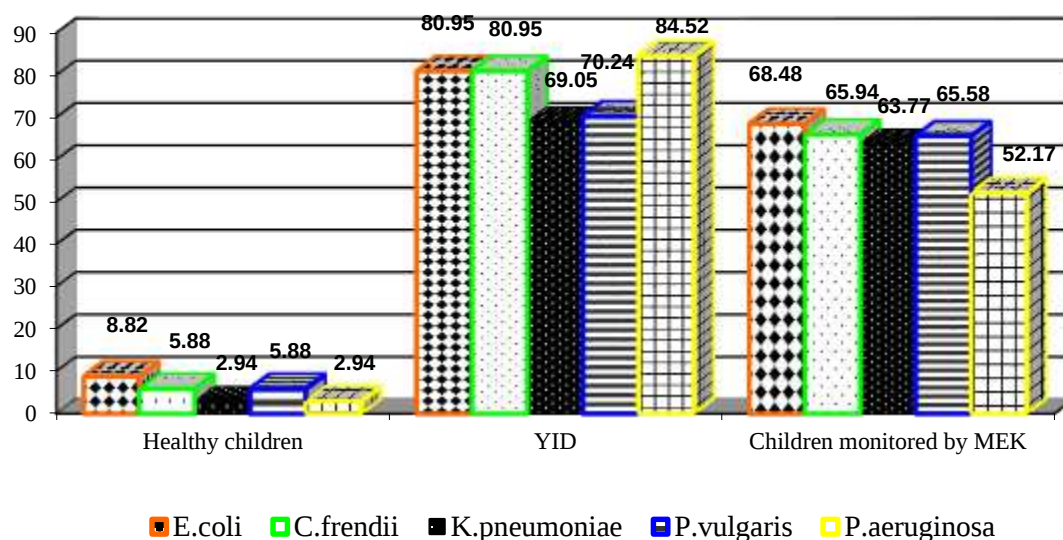


Figure 1. Comparative results of IFA with antigens of conditionally pathogenic enterobacteria of children's serum,% (YID - colonic dysbiosis, MEK - diseases of microbial etiology)

The next part of the study was devoted to the assessment of antiendotoxic immune status in the examined patients and healthy children. The results showed that the rate and titer of antibody antibodies to GShPB endotoxins depend on the nosological unit, body condition, and age of the subjects. Therefore, we found it necessary to describe the age groups (3-6 years and 7-14 years) separately. While a positive titer of antibodies to GShPB endotoxins (seropositive samples) was detected in $10.0 \pm 6.9\%$ of healthy children aged 3-6 years (Table 1), positive results decreased with increasing age of children (Table 2) ($6.7 \pm 4, 8\%$), but no convincing difference was observed between the numbers ($R > 0.05$). The fact that no differences in the detection of antibodies to GShPB antigens were found in practically healthy children differed from the results obtained by some researchers [12].

Table 1
Percentage of detection of seropositive samples in the serum of sick and healthy children aged 3-6 years

Groups	Indicators, %
1 comparison group (1 in 10 positive)	10,0±6,9
Comparison group 2 (15 out of 20 positive)	75,0±5,1* ↑
Main group - MEK (88 out of 97 positive)	90,7±2.4* ↑

Note: * - Sign of a reliable difference from comparison group 1, ↑ - direction of change of indicators.

Among children diagnosed with IID, the rates differed significantly compared to healthy children, with antiendotoxin antibody levels detected in $85.4 \pm 3.9\%$ of subjects. Detection of antibodies to GShPB endotoxins in children with MEC reached $90.7 \pm 2.4\%$, which was significantly higher than in both comparison groups ($R < 0.001$ and $R < 0.05$, respectively). With the age of the subjects, the percentage of antibody detection increased in the main group and in the 2nd comparison group, although not convincingly ($R > 0.05$) (Table 2).

Table 2
Percentage of detection of seropositive samples in the serum of patients aged 7-14 years and healthy children

Groups	Indicators, %
1 - comparison group (1 in 15 positive)	6,7±4,8
2 - comparison group (35 out of 41 positive)	85,4±3,9* ↑
Main group - MEK (137 positive out of 154 people)	89,0±2.3* ↑

Note: * - Sign of a reliable difference from comparison group 1, ↑ - direction of change of indicators.

It was found that the level of detection of antiendotoxin antibodies in the serum of children depends on the age of the children being tested, the development of IUD in children, the diagnosed nosological unit and the type of GShPB. In order to identify the nosological unit that had the greatest impact on the main group indicators, we performed a comparative analysis of MEK parameters by disease. The results showed that the highest rates of detection of antibodies to endotoxin were found in children aged 3-6 years with SYI, and differed significantly not only from comparison groups ($R < 0.001$), but also from children diagnosed with acute tonsillitis and acute bronchitis (Table 3).

Table 3
Indications for the detection of antiendotoxin antibodies in the serum of children aged 3-6 years by nosological units,%

Groups	Indicators,%
1 - comparison group, n = 10	10,0±6,9
2 - comparison group, n = 20	75,0±5,1* ↑
Acute tonsillitis, n = 21	76,2±3,1* ↑
Acute bronchitis, n = 28	89,3±2,7* ↑

Urinary tract infections, n = 20	95,0±2,4* ↑
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Note: * - Sign of a reliable difference from comparison group 1, ↑ - direction of change of indicators.

We observed a similar trend in the 7–14-year-old children studied, although the numbers were different (Table 4). Thus, the detection of antibodies to GShPB antigens in sick children with MEK and YID differed significantly from the parameters of healthy children, but when compared, a reliable difference was found in 3–6 years ($R < 0.05$), no statistically significant difference in 7–14 years ($R > 0.05$). A convincing difference from nosology comparison groups 1 and 2 was observed in acute bronchitis and SYI in 3–6-year-olds, whereas in 7–14-year-olds, such a difference was found only in SYI.

Table 4

Indications for the detection of antiendotoxin antibodies in the serum of children aged 7–14 years by nosological units, %

Groups	Indicators, %
1 - comparison group, n = 15	6,7±4,8
2 - comparison group, n = 41	85,4±3,9* ↑
Acute tonsillitis, n = 66	83,3±2,9* ↑
Acute bronchitis, n = 43	90,7±2,9* ↑
Urinary tract infections, n = 73	97,3±2,0* ↑

Note: * - Sign of a reliable difference from comparison group 1, ↑ - direction of change of indicators.

After a comparative evaluation of the detection rate of antiendotoxin antibodies, the intensity of their formation was studied comparatively. For the convenience of describing the results obtained, the intensity (titer) of antitoxin antibody formation was divided into the following groups [12]: "negative" (titer was not determined); "Past" (titer 1: 2 - 1: 8); "Medium" (titles 1:16 - 1:64); "High" (titer 1: 128 and higher) titers. The results showed that the serum antibody titers in healthy children aged 3–6 years (comparison group 1) were in most cases (90.0 ± 7.8%) "negative" (Table 5). The intensity of antibody detection in MEK differed sharply relative to the comparison groups. The difference in intensity was evident when the percentage of detection of antiendotoxin antibodies did not differ in MEK among children aged 3–6 years who observed IID. The same indicators were observed in children aged 7–14 years (Table 6).

Table 5

Indicators of the intensity of the formation of antiendotoxin antibodies in the serum of children aged 3 - 6 years, %

Groups	"Negative"	"Past"	"Medium"	"High"
1 - comparison group, n = 10	90,0±7,8	10,0±6,9	0	0
2 - comparison group, n = 20	25,0±6,4*	75,0±8,6*	0	0
Acute tonsillitis, n = 21	23,8±5,1*	19,0±4,7*	28,6±4,9	28,6±4,9
Acute bronchitis, n = 28	10,7±6,8*	17,9±3,8*	35,7±3,6	35,7±3,6
SYI, n = 73	5,0±2,5*	10,0±2,5*	25,0±2,5	60,0±2,0

Note: * - Sign of a reliable difference from the 1st comparison group

Table 6
Indicators of the intensity of the formation of antiendotoxin antibodies in the serum of children aged 7 - 14 years, %

Groups	"Negative"	"Past"	"Medium"	"High"
1 - comparison group, n = 15	93,3±3,1	6,7±4,8	0	0
2 - comparison group, n = 41	14,6±4,2*	85,4±3,9*	0	0
Acute tonsillitis, n = 66	16,7±3,7*	24,2±3,1*	28,8±3,5	30,3±3,1
Acute bronchitis, n = 43	9,3±4,9*	20,9±4,5*	27,9±4,4	41,9±4,1
SYI, n = 73	2,7±1,9*	16,4±2,6*	28,8±2,5	52,1±2,2

Note: * - Sign of a reliable difference from the 1st comparison group

It can be seen that in this age group, the trend of changes in the performance of children aged 3-6 years has been maintained. Thus, a significant convincing difference was found in the MEK (acute tonsillitis, acute bronchitis, and SYI) parameters for both comparison groups on the parameters of the intensity of antibody formation against GShPB endotoxins. There were no differences in the trend of change among the age groups. Among the tested children with IUD, the rates differed convincingly compared to healthy children, with antiendotoxin antibody levels detected in the majority of subjects ($R < 0.001$). The percentage of antibody detection increased with the age of the subjects. Antibodies were found in $76.92 \pm 4.5\%$ of children aged 3 to 7 years with IBD (20 out of 26 children), while in children aged 7 to 15 years they increased by $87.93 \pm 4.3\%$ (51 out of 58 children).

The convincing difference between the MEK and YID groups is particularly pronounced in children aged 3 to 7 years ($R < 0.05$). A reversal was observed in the comparison groups of MYAK and healthy children. The detection rate of antiendotoxin antibodies in children's serum was determined by: the age of the children being tested; development of III-IV level of IBD in children; to the diagnosed nosological unit; Depending on the type of ShPE. The age differences of the children examined were as follows: first, negative titers in children aged 3 to 7 years were observed only in children with SYI, and in other nosologies in children aged 7 to 15 years; second, the reverse was observed in the detection of high titers of antiendotoxin antibodies; third, the percentage of healthy children with a positive titer was higher in children aged 7 to 15 years.

Application of the method of determining the titer of antibodies against endotoxins of gram-negative bacteria in various microbial etiologies in children, assessing its diagnostic value and assessing the clinical and immunological properties of antiendotoxin immunity systematizes the treatment and prevention of bacterial etiological diseases in children. Reduction of diseases of bacterial etiology among children reduces the percentage of their overall morbidity, ensures a high level of health, high medical efficacy of the results of research. The method of assessing the clinical and immunological properties of antiendotoxin immunity in children with microbial etiology increases the effectiveness of this service by systematizing medical care for children, which reduces the incidence among children, as well as has a positive impact on children's quality of life. The method

of assessing the clinical and immunological features of antiendotoxic immunity in children with microbial etiological diseases The method of assessing the incidence of microbial diseases, the systematization of medical services The method of assessing the clinical and immunological status of these diseases showed savings of up to 32,000 soums per case.

Conclusions

1. The percentage of seropositive sera to GShPB antigens was convincingly higher in children with MEK and IID than in healthy children. The percentage of detection of antitoxin antibodies depended on the type of microorganism.
2. The percentage of detection of antitoxin antibodies in blood serum was statistically higher than in other comparable pathological conditions in children with SYI. The most important in YID were *Escherichia coli* i *Pseudomonas aeruginosa*, and in MEK were *Escherichia coli*.
3. Antitoxin antibodies differed sharply from MEK comparison groups in terms of intensity (titer) formation. While "medium" and "high" titers were detected mainly in the CEC, only "low" titers were detected in healthy and YID-observed children.
4. Determination of the intensity of antibody formation against GSHPB endotoxins at "medium" and "high" titers is recommended as an additional diagnostic and prospective criterion in the diagnosis of MEC.

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