

Assessment of Youth Health in Uzbekistan According to the Results of Immunological Screening

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Abstract

An assessment of the health indicators of young people in Uzbekistan was carried out based on the results of immunological screening - determining the quantitative content of antigen-binding lymphocytes (ASL), sensitized to tissue antigens (TA) of various organs. For a comparative study, male and female volunteers aged 18-25 years old living in Tashkent city of the Tashkent region (northern region) and in the city of Termez of the Surkhandarya region (southern region) of Uzbekistan was examined. Consequently, in the southern region of Uzbekistan (Termez), the frequency of manifestation of pathological processes in various organs is relatively higher than in the northern region (Tashkent).

Keywords: Blood, Lymphocytes, Determination of Antigen-binding Lymphocytes (ASL), Specifically Sensitized to Tissue Antigens (TA) of Various Organs.

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INTRODUCTION

No communicable diseases are common in all age groups, in all regions and in all countries. These diseases are most commonly seen in older people, but data collected in recent years show that 17 million of all no communicable disease-related deaths occur in people aged 30 to 69. Many factors in children, adults, and the elderly, such as unhealthy diets, physical inactivity, exposure to tobacco smoke, or alcohol abuse, further increase the risk of developing medical conditions. At the third high-level meeting of the UN General Assembly (2018), a global action plan for the prevention and control of no communicable diseases for 2018-2030 was prepared, which includes nine global goals related to the prevention and treatment of physical diseases [17].

In recent years, the head of state has signed a number of decrees and resolutions regarding the development of the healthcare sector. An example is Decree PQ-5124, which covers a wide range of issues, including the transformation of the primary care system into an early detection system, accelerating digitalization, increasing the volume and improving the quality of the provision of narrow medical services, replacing them with specialized institutions and centers [16]. This decision requires representatives of the healthcare system, first of all, to strengthen the early diagnosis and prevention of diseases, the widespread use of methods for assessing the effectiveness of treatment. Immunological screening using antigen-binding lymphocytes against tissue antigens before the onset of

symptoms can help in early detection of the disease.

One of the manifestations of immune reactions when endogenous or exogenous proteins enter the internal environment is the appearance in the peripheral blood of specific sensitized antigen-binding lymphocytes (ASL) in relation to this tissue antigen (TAG). The antigen-binding properties of lymphocytes are provided by the presence of special receptors located on the outer surface of their membrane. A continuous and prolonged increase in functional loads on organs leads to the development of dystrophy, which disrupts the intracellular process, which is a prepathological process. In the prepathological process, objective and subjective symptoms do not arise in connection with the compensatory mechanism of the body, therefore, methods for collecting and diagnosing objective data about it have not been studied.

In pre-pathological conditions, dystrophic cells have the ability to regenerate, during which time we identify the causes of dystrophy and eliminate them, the cells quickly return to their structure and prevent the development of pathology, resulting in the restoration of organ function, preventing damage to healthy cells [6, 7, 9, 10].

It has been established that an increased need for anti-inflammatory corticosteroids with an increase in the amount of toxin in rats infected with typhoid fever leads to dystrophy due to hyper function of the adrenal glands. At the end of the experiment, we observed that ASL activation against TAGs formed from glandular dystrophic proteins led to aseptic necrosis of the adrenal glands [8].

ASL studies are especially sensitive to TAG in the brain, endocardium, myocardium, lungs and kidneys, reflecting the depth of pathological changes in the organs and tissues of patients with congenital heart defects. In practice, it has been established that the level of ASL in children with heart defects is on average 4.8 times higher than in healthy children, in the endocardium – 5.9 times, in the lung tissue – 5.87 times. Apparently, this is the result of prolonged functional overstrain of the heart and lungs [11].

The negative effect of radiofrequency electron radiation on the human body was demonstrated by the results obtained in the dynamics of heart tissue sensitized with TAG-ASL. As the duration of this effect increases, the detected organic changes become more frequent and lead to deep and irreversible dystrophies. Given this fact, regular preventive examinations using the detection of ASL against TAG of organs and tissues as diagnostic and prognostic criteria can help optimize the early detection and treatment of organ lesions [12].

The study of ASL parameters, which are especially sensitive to the corresponding TAGs in the dynamics of postoperative diseases of the sclera, cornea and cornea, can be a prognostic indicator of the consequences of the pathological process and the effectiveness of treatment [13].

The traditional method for detecting antigen-binding lymphocytes is the adhesion test (formation of a nest with antigen-loaded erythrocytes). ASL is detected under a microscope, forming erythrocyte nests and binding at least three erythrocytes associated with the antigen. With the help of other tests (ELISA, PGAR, IGF, etc.), the diagnosis is made by identifying specific antibodies. Experiments on immunization and infection of various animals have shown that ASL can be detected in the blood 12 hours after antigenic stimulation, i.e. long before the formation of antibodies. In addition to early diagnosis, the ASL test can be used to monitor the effectiveness of the treatment of infectious diseases, since ASL antibodies are also detected in the absence of antibodies and a negative PCR result [14].

The use of ASL in the diagnosis of acute renal failure, which developed as a result of acute intestinal infections, can serve as an objective indicator for assessing the stage of development of renal failure [3].

A comparative analysis of data from patients with brucellosis showed that, with the regular dynamics of ASL indicators characteristic of TAH in the brain, liver, joint capsule, kidneys, prostate gland in men and ovaries in women, they depended on the depth and severity of the lesion [1, 15]. It can also be observed that in patients treated with chronic brucellosis against the background of dysbacteriosis, ASL values increased 3.64 times compared to liver tissue antigens and 4.69 times compared to joint tissue TAG [2].

PURPOSE OF THE STUDY

Assess the health indicators of young people in Uzbekistan based on the results of immunological screening.

OBJECT AND SUBJECT OF RESEARCH

Male and female volunteers aged 18-25 living in Tashkent city of Tashkent region (northern region) and Termez city of Surkhandarya region (southern region) of Uzbekistan. As a result of immunological screening - determination of the ASL content in the blood of more than 6% to TA of organs in young people of the Republic of Uzbekistan, it was found that the frequency of detection of ASL to TA of the brain, liver, endocardium, myocardium, kidneys, pancreas and prostate in men significantly exceeds that of young people in Termez (southern region) than Tashkent city (northern region). Elevated levels of ASL to TA in the general group of surveyed are mainly due to the city of Termez.

MATERIAL AND RESEARCH METHODS

In 51 volunteers from the city of Tashkent and 49 volunteers from the city of Termez, 6-8 ml of blood was taken from the cubital vein in the morning on an empty stomach. There were 42 males and 58 females. Blood was taken into test tubes containing 2 ml of saline and 3 drops of heparin. Isolation of lymphocytes from heparinized whole blood was performed by centrifugation in a ficoll-verografin density gradient of 1.077 g/ml.

Detection of antigen-binding lymphocytes by the indirect rosette reaction method. To study organ lesions and the effectiveness of the therapy, the method of quantitative registration of antigen-binding lymphocytes (ASL), specifically sensitized to tissue antigens (TA) of the liver, was used, which is based on the use of the reaction of indirect rosette formation (PHRO) according to the method of Garib F. Yu. (1995).

With the defeat of various genesis of any organ, in its cells there is a violation of intracellular processes and the development of dystrophy. An increase in the degree of dystrophy causes destruction and necrosis of cells. Molecules or fragments of structural and functional proteins with organ specificity enter the internal environment. Tissue proteins and molecules that are “alien” for the internal environment acquire the status of tissue antigens (TA), and an immune reaction is launched aimed at their neutralization and elimination [4, 5].

In the presence of TA in the internal environment of the organ, antigen-binding lymphocytes (ABL) differentiate and circulate in the blood, capable of specifically binding to TA only of this organ. The level of ASL to TA reflects the intensity of the processes of destruction and necrosis of structures in the organ: an increase in ASL in dynamics indicates an increase, and a decrease in ASL indicates a fading of the intensity of these processes, which makes it

possible to assess the degree of organ damage, as well as the effectiveness of the therapy. The value of the method for determining ASL to TA is its high sensitivity and specificity: the level of ASL reaches a diagnostic level in the early stages and long before the onset of clinical signs of organ damage, which makes it possible to predict the risk of organ failure early. Establishment of the ASL reaction with TA of several organs allows one to reveal the multi-organ damage of the body during the development of pathology.

The principle of the method for determining ASL to TA is as follows. Sensitized lymphocytes of the patient specifically bind the tissue antigen with their surface receptors. In PHPR, human erythrocytes of the O (I) blood group are used, on the membranes of which, with the help of a 3.0 % solution of CpCl₃, tissue antigens obtained from the tissue of human organs are loaded. During the incubation of a suspension of lymphocytes of the subject with erythrocytes loaded with TA, a rosette is formed, consisting of a centrally located lymphocyte and erythrocytes attached to it with TA on the membrane. Under a microscope with an immersion lens, the percentage ratio of rosette-forming lymphocytes to the total pool of lymphocytes is calculated.

To take into account the nonspecific interaction of lymphocytes with the antigen, a rosette formation reaction with erythrocytes loaded with human serum albumin is carried out in parallel.

Obtaining organ-specific antigens from organ tissues (Borisova A.M., Moskvina S.N., 1975).

The tissue of organs obtained from persons who died from accidental injuries and taken no later than 4-6 hours after death is washed from blood and ground in a homogenizer in 0.3-0.5 M Tris-ThCl-buffer (pH 7.8-8, 2) within 1.0-1.5 minutes at the speed of the homogenizer 13000 - 20000 rpm. The resulting mass is subjected to centrifugation at 3000 rpm. The resulting supernatant, containing specific structural and functional tissue proteins (TA), is resuspended in the same volume of buffer and the antigen is converted into a soluble state by treatment with papain in a protein ratio of 1:85 - 1:25, the enzyme is incubated with papain for 1 hour at 37°C. A 0.05M sodium podacetate solution is then added to deactivate the papain. The reaction mixture is centrifuged at 37.000 rpm for 1 hour. The supernatant is collected, concentrated and applied to a chromatographic column with Sephadex G-200. The purified drug is packaged in 1.5-3.0 ml.

Loading of antigen on erythrocytes through CpCl₃. To prepare an erythrocyte antigenic diagnosticum, to 0.1 ml of 50% human erythrocyte sediment of group 0 (I) add 0.1 ml of antigen and 0.1 ml of a 1.0% solution of CpCl₃ prepared by extempore in isotonic sodium chloride solution. The suspension is incubated at room temperature, then washed 3 times in a buffered solution of isotonic sodium chloride solution. Suspension of erythrocyte diagnosticum is brought to 106 erythrocytes in 1 µl. In parallel, a control diagnosticum with human serum albumin is prepared by a

similar method to account for non-specific formation of rosettes.

Technique of setting RNRO to detect ASL to TA. A suspension of lymphocytes (0.1 ml of 2x10⁶ cells/ml) is mixed with 0.1 ml of antigenic (TA) erythrocyte diagnosticum in a ratio of 1:50. The mixture is incubated for 1 hour at 40°C. The resulting rosettes are fixed by adding 0.25% glutaraldehyde solution. The mixture is centrifuged and smears are obtained from the sediment on a glass slide, the smear is stained with Romanovsky-Giemsa stain. The percentage of rosette-forming lymphocytes is counted on smears under an immersion microscope system. The content of ASL in the blood of patients is determined by the difference between the parameters of the interrosette formation of sTA and serum albumin.

STATISTICAL PROCESSING OF RESULTS

To assess the statistical significance of differences between comparable mean values, the correctness or erroneousness of the answer was determined using Student's t-test (t). The level of significance of this response (P) was determined according to the Student's distribution table (P-significance coefficient).

RESULTS AND ITS DISCUSSION

The content of ASL in the blood was studied in relation to the AH of the brain, liver, lungs, endocardium, myocardium, kidneys, pancreas, prostate in men and endometrium in women. The content of ASL to TA in the blood up to 2% was taken as the norm, from 3% to 6% - for prepathological processes, from 7% or more were taken for pathological processes in the organs.

In the general group of examined patients, the average content of ASL to TA from 3% to 6%, that is, the presence of pre-pathological processes in the organs, was: to TA of the brain - in 22% of the examined, to TA of the liver - in 21%, to TA of the lungs - in 21%, to endocardial TA - in 21%, to myocardial TA - in 24%, to kidney TA - in 22%, to endometrial TA - in 39.7% of women, to pancreatic TA - in 19%, to prostate TA - in 33.3% men.

When studying the regional features of the frequency of occurrence of prepathological processes among young people in Tashkent and Termez, the following was obtained. In Tashkent, the frequency of detection of ASL to TA from 3% to 6% was: to TA of the brain - in 23.5% of the examined, to TA of the liver - in 17.6%, to TA of the lungs - in 21.5%, to TA endocardium - in 19.6% of women, to myocardial TA - in 25.5%, to renal TA - in 23.5%, to endometrial TA - in 2.6% of women, to pancreatic TA - in 19.6%, to prostate TA - in 47.0% of men (Table 1).

In Termez, the frequency of detection of ASL to TA from 3% to 6% was: for brain TA - in 20.4% of the examined, for liver TA - in 24.5%, for lung TA - in 20.4%, for TA

endocardium - in 22.4%, to myocardial TA - in 22.4%, to renal TA - in 20.4%, to endometrial TA - in 58.3% of women, to pancreatic TA - in 18.4%, to prostate TA - in 20.0% of men (Table 1).

Table 1: Comparative assessment of the frequency of prepathological processes in young people (18-25 years old) in Tashkent and Termez based on the results of immunological screening

Organs	Blood levels of ASL to tissue antigens of organs		
	General group (n = 100)	Tashkent (n = 51)	Termez (n = 49)
	ASL from 3% to 6%	ASL from 3% to 6%	ASL from 3% to 6%
Brain	22%	23.5%	20.4%
Liver	21%	17.6%	24.5%
Lungs	21%	21.5%	20.4%
Endocardium	21%	19.6%	22.4%
Myocardium	24%	25.5%	22.4%
Kidney	22%	23.5%	20.4%
Endometriosis (n= 58)	39.7%	26.5% (n= 34)	58.3% (n= 24)
Pancreas	19%	19.6%	18.4%
Prostate (n= 42)	30.9%	47.0% (n= 17)	20.0% (n= 25)
	24.78	22.27	25.24

In the general group of examined patients, the average content of ASL to TA was 6% more, that is, the presence of pathological processes in the organs, was: to brain TA - in 7% of the examined, to liver TA - in 7%, to lung TA - in 6%, to TA endocardium - in 8%, to myocardial TA - in 2%, to renal TA - in 3%, to endometrial TA - in 3.48% of women, to pancreatic TA - in 4%, to prostate TA - in 4.76 % of men.

When studying the regional features of the frequency of occurrence of pathological processes among young people in Tashkent and Termez, the following was obtained. In Tashkent, the frequency of detection of ASL to TA of 6% or more was: to TA of the brain - in 4.0% of the examined, to TA of the liver - in 0%, to TA of the lungs - in 4.0%, to TA of the endocardium - in 4.0%, to myocardial TA - in 0%, to TA of the kidneys - in 0%, to TA of the endometrium - in 0% of women, to TA of the pancreas - in 4.0%, to TA of the prostate - in 0% of men.

In Termez, the frequency of detection of ASL to TA from 6% or more: to TA of the brain - in 13.2% of the examined, to TA of the liver - in 14.3%, to TA of the lungs - in 8.1%, to TA of the endocardium - in 12.2%, to myocardial TA - in 4.1%, to renal TA - in 6.1%, to endometrial TA - in 8.4% of women, to pancreatic TA - in 4.1%, to TA prostate - in 8.0% of men (Table 2).

Table 2: Comparative assessment of the frequency of pathological processes in young people (18-25 years old) in Tashkent and Termez based on the results of immunological screening

Organs	Blood levels of ASL to tissue antigens of organs		
	General group (n = 100)	Tashkent (n = 51)	Termez (n = 49)
	ASL >6%	ASL >6%	ASL >6%
Brain	7.0%	4.0%	13.2%
Liver	7.0%	0	14.3%
Lungs	6.0%	4.0%	8.1%
Endocardium	8.0%	4.0%	12.2%
Myocardium	2.0%	0	4.1%
Kidney	3.0%	0	6.1%
Pancreas	4.0%	4.0%	4.1%
Prostate (n= 42)	4.76%	0 (n=17)	8.0% (n=25)

We studied the content of ASL in the blood up to 6% or more to TA of various organs, that is, the presence of emerging or formed pathological processes in the organs. So the content of ASL in the blood to TA of the brain more than 6% in the general group of examined was found in 7.0% of the examined, while in Tashkent this figure was 4%, and in Termez the figure was 13.2%. That is, in the city of Termez, the frequency of elevated levels of ASL to TA of the brain is detected more than 3 times more often than in the youth of Tashkent.

The frequency of detection of ASL to liver TA of more than 6% in the general group of patients was 7%, while in Tashkent this figure was 0%, and in Termez it was significantly higher and amounted to 14.3%.

The detection of ASL to TA of the endocardium and myocardium over 6% in the total sample of the examined was 8.0% and 2.0%, respectively, in those examined from Tashkent - 4% and 0%, and in young people from Termez, the frequency of detection of these ASL was 12.2% and 4.1%.

The incidence of ASL to renal TA of more than 6% was 3.0% in the general group, 0% in Tashkent, and 6.1% in Termez. The frequency of detection of elevated blood levels of more than 6% in the general sample, in Tashkent and Termez, ranged from 4.0% - 4.1%.

The frequency of detection of ASL more than 6% to prostate TA in male youth revealed the following: in the general sample - 4.76%, in Tashkent - 0% in Termez - 8.0%.

CONCLUSION

As a result of immunological screening - determination of the ASL content in the blood of more than 6% to TA of organs in young people of the Republic of Uzbekistan, it was found that the frequency of detection of ASL to TA of

the brain, liver, endocardium, myocardium, kidneys, pancreas and prostate in men significantly exceeds that of young people in Termez (southern region) than Tashkent city (northern region). Elevated levels of ASL to TA in the general group of surveyed is mainly due to the city of Termez.

REFERENCES

- Akhmedova H. Yu., Gulyamov N.G., Mirrahimova N.M., Temirova S.Yo. Comparative analysis of indicators of antigen-binding lymphocytes in acute and subacute forms of brucellosis// *Infection, Immunity and Pharmacology* 5/2019, article 55-60.
- Akhmedova Kh. Yu., Tillaboeva D. Sh., Mirrahimova N.M., Temirova S. Yo. dysbacteriosis aniklangan brucellosis bemorlardagi immunological kʻyrsatkichlar // *Infection, Immunity and Pharmacology* 5/2019, pp 61-66.
- Akhmedova M.D., Imamova I.A. Early prediction of the risk of developing acute renal failure in acute intestinal infections in children // *Preventive and Clinical Medicine* No. 1 (34), 2010 pp., 140-144
- Garib F. Yu., Gurarii N.I., Afanasiev Yu. I. Clinical value of determination of antigen-binding lymphocytes in patients with typhoid fever and other diseases: Method. Recommendations. - Tashkent. -1983 - 4 p.
- Garib F. Yu., Gurarii N.I., Gornina L.G. A method for the quantitative registration of antigen-binding rosette-forming lymphocytes in humans // *Tez. report V Rep. conf. TsNIL med. Universities of Uzbekistan.* -Tashkent, 1981.-pp. 22-24.
- Gulyamov N.G., Akhmedova Kh.Yu., Dalimov T.K., Imamova I.A. Diagnostic value of indicators of antigen-binding lymphocytes in the assessment of organ damage in infectious and non-infectious pathology // *Info., Immunity and Pharmacology.*- 2005.-№3.-S. 115-118.
- Gulyamov N.G., Akhmedova M.D., Dalimov T.K. Indicators of antigen-binding lymphocytes as a criterion for early detection of adrenal lesions and the appointment of hormonal therapy with corticosteroids in typhoid fever // *Infectious Diseases: Problems of Public Health and Military Medicine: Russia. scientific-practical. Conf., dedicated to the 110th anniversary of the department. Inf. diseases of the VMA, St. Petersburg, March 22-24, 2006 - St. Petersburg, 2006, P. 90.*
- Gulyamov N.G., Dalimov T.K., Mirzahanova D.B. Functional insufficiency of the adrenal cortex in typhoid infection: hormonal and immunological aspects of pathogenesis // *Preventive and Clinical Medicine* No. 1 (34), 2010 pp., 142-146.
- Gulyamov N.G., Imamova I.A., Dalimov T.K. Clinic, diagnosis and treatment of renal failure in acute intestinal infections // *Info., immunity and pharmacology.*- 2006.-№2.-pp. 41-43.
- Gulyamov N.G., Safarova D.D., Dolimov T.K., Imamova I.A. Immunological diagnosis of multiple organ lesions in infectious and non-infectious pathology // *Infectious Diseases: Problems of Public Health and Military Medicine: Russia. scientific-practical. Conf., dedicated to the 110th anniversary of the department. inf. diseases VMA, them. S.M.Kirova.-SPb., Vmed A.-2006, pp. 91-92.*
- Ibadov R.A., Alimdzhanova N. Yu., X.I.O.Axmedova, X.K.Aбролов, Клиникоиммунологические параллели оценки эффективности периперативной фармакотерапии легочной гипертензии у пациентов с врожденными пороками сердца// *Трудный пациент* №8-9, ТОМ 13, 2015, стр., 37-44.
- Khamidova G.M., Kayumov U.K., Maksudova L.M., Tashpulatova G.A., Prognostic value of antigen-binding lymphocytes in individuals at occupational risk of exposure to electromagnetic radiation of the radio frequency range//*Infection Immunity and Pharmacology*, 4/2019, pp. 148-154.
- Kamilov Kh. M., Maksudova L.M. Evaluation of the effectiveness of the acetylation phenotype as a criterion for predicting the course and outcomes of eye burn injuries // *Bulletin of modern clinical medicine* vol. 9, issue, 1, 2016 pp., 36-40.
- Kuzmin Yu. A., Ispaeva Zh. B., Zhankalova Z.M., Maemgenova G.N. Detection of antigen-binding lymphocytes using IF and detection of autoantibodies and autolymphocytes to the skin antigen in patients with psoriasis//*Bulletin of KazNMU* No. 2-2019, pp. 292-293.
- Mirrahimova N.M., Akhmedova Kh. Yu. Dynamics of indicators cellular immunity and antigen-binding lymphocytes in patients acute brucellosis // *Journal of Infectology. Materials of the IV Congress of the Euro-Asian Society for Infectious Diseases.* - St. Petersburg, 2016. - Volume 8. - No. 2. - P. 70.
- PQ - 5124-son. Sog'liqni saqlash sohasini kompleks rivojlantirishga doir qo'shimcha chora tadbirlar to'g'risida. May 25, 2021. SSV.uz.
- WHO. Global action plan for the prevention and control of non-infectious diseases at the 66th session. 2018.