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ABSTRACT

of the dissertation for the degree of Doctor of Philosophy

LABORATORY DIAGNOSIS AND IMMUNOLOGICAL FEATURES OF OCULAR CHLAMYDIA TRACHOMATIS INFECTIONS

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Field of science:	Medicine
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GENERAL DESCRİPTİON OF THE WORK

Relevance of the topic. Every year, 90 million people worldwide become infected with chlamydia. According to other statistics, more than 5 million people become infected with chlamydia in Eastern Europe and Central Asia every year. In 2004, it was reported that at least 5-10% of young sexually active people in Russia were infected with chlamydia[2].

According to research, in more than half of cases of urogenital chlamydia, pathological changes also occur on the mucous membrane of the eye. According to various authors, the proportion of paratra-choma ranges from 3% to 30% of the total number of conjunctivitis cases.[1]

Chlamydia trachomatis is an obligate intraocular bacterium that causes inclusion conjunctivitis and has been the leading cause of blindness worldwide in the last century. Improved socioeconomic and housing conditions, availability of antibiotics, and national trachoma control programs have reduced its prevalence in developed countries, but the disease persists in resource-poor regions of Africa, Asia, and India.[3] According to a 2016 WHO report, trachoma has been limited to 42 countries, but still causes vision loss in approximately 1.9 million people. India is among the top five countries accounting for about half of all active trachoma cases. The implementation of the Global Elimination of Trachoma 2020 program by WHO using the SAFE strategy (surgery for trachomatous trichiasis; antibiotics for C. trachomatis; facial hygiene; and environmental improvement) has significantly reduced the incidence of trachoma, but trachoma still persists in India[4]. The global increase in reproductive tract infections by urogenital serotypes (D-K) of C. trachomatis has led to an increase in eye an

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1.Elwell, C. "Chlamydia cell biology and pathogenesis".// K. Mirrashidi, J. Engel / Nature Reviews Microbiology. -2016. -14 (6): -p.385–400.

2.World Health Organization. Global WHO alliance for the elimination of blinding trachoma by 2020. Wkly Epidemiol Rec. 2013;88:241–56. [Google Scholar]

3.World Health Organization. Global WHO alliance for the elimination of blinding trachoma by 2020. Wkly Epidemiol Rec. 2013;88:241–56. [Google Scholar]

increase in eye infections caused by C. trachomatis. The study of C. trachomatis as an eye infection still requires attention and research[4].

In Azerbaijan, the most common eye infectious disease arice as ocular Chlamydia trachomatis infections. Recently, studies have been conducted and articles have been written on the topics of immune disorders in urogenital chlamydia infection among pregnant women, the state of cellular and humoral immunity in pregnant women with chlamydia, etc. However, there are still many unstudied issues related to ophthalmic chlamydia in our country[5].

Object and subject of the study; The subject of the dissertation is the epidemiological, laboratory and immunological characteristics of ocular Chlamydia trachomatis infections. The main methods used in the diagnosis of the disease:

- direct identification of the causative agent in the scab (cytological method), immunoenzyme, immunofluorescence, chain poly-merase reaction), detection of chlamydia in cell culture (using McCoy's medium),culture method (which is considered the standard), serological tests, constitute the analyzed and evaluated subject of the study.
- 125 patients, who applied to the Department of Eye Diseases of the Central Military Hospital of the Ministry of Defense of the Republic of Azerbaijan were taken as the object of the study. 95 of them were included in the main group (patients with ocular Chlamydia trachomatis infections in whom chlamydia was detected by microscopic and bacteriological methods), and 30 patients were included in the control group (patients with similar clinical symptoms).

Aims and objectives of the study.

The aim of the study is to conduct a clinical epidemiological analysis of patients with ocular Chlamydia trachomatis infections, to

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4.World Health Organization. Report of the Second Global Scientific Meeting on Trachoma. WHO/PBD/GET 1.04. Geneva: World Health Organization; 2003. http://www.who.int/blindness/2nd%20GLOBAL%20SCIENTIFIC%20MEETING.pdf. Accessed August 11, 2011.

5.Cəfərova, S.R. Hamilə qadinlarda urogenital xlamidioz və ureaplazmozun müalġcəsinin prinsipləri // Cəfərova, S.R. / - Bakı, - Sağlamlıq jurnalı, -N4, -2015,- s.68-7

analyze the immunological changes that occur depending on the clinical manifestations using various methods.

The objectives of the study include the following:

• Analysis of the clinical manifestations of ocular Chlamydia trachomatis infections.

• Comparative study of the frequency of occurrence of clinical forms of ocular Chlamydia trachomatis infections in the main and control groups

• Comparative assessment of the specificity and sensitivity of IFA and PCR methods in patients with ocular Chlamydia trachomatis infections with reference examination methods

• Assessment of changes in CD markers of the immune system in patients with ocular Chlamydia trachomatis infections

• Assessment of pro-inflammatory and anti-inflammatory interleukins from cytokine cascades depending on the phase of the disease

Research methods: Epidemiological (observational), cytological, immunoenzymatic, immunofluorescent, polymerase chain reaction, culture method (using McCoy medium), serological, and statistical methods were used in the study.

The provisions put forward for defense:

1. Analysis of clinical forms of ocular Chlamydia trachomatis infections based on the data obtained as a result of the study
2. Comparative analysis of uveitis in patients with chlamydia with a control group.
3. Evaluation of sensitivity and specificity in typical, atypical, asymptomatic clinical forms when making a diagnosis using the IFA and PCR method
4. Evaluation of changes in the T-cell circle depending on the clinical forms in patients with ocular Chlamydia trachomatis infections
5. Results of the analysis of changes in the synthesis of pro-inflammatory cytokines in patients with ocular Chlamydia trachomatis infections **Scientific novelty of the study:**

– An epidemiological and immunological analysis of the first clinical forms of ocular Chlamydia trachomatis infections in Azerbaijan was conducted in modern times;

– For the first time, epidemiological aspects of ophthalmological manifestations of sexually transmitted infections were studied independently in the field of ophthalmology;
– The most effective methods for diagnosing ocular Chlamydia trachomatis infections were studied in a comparative manner;
– For the first time, epidemiological monitoring of chlamydia was conducted among ophthalmological patients visiting medical facilities;
– For the first time in our country, the causative agent of chlamydiosis was detected in examination materials taken from the eye.

Practical and theoretical significance:
As a result of the conducted studies, it will be possible to determine the early diagnosis of ocular Chlamydia trachomatis infections in risk groups for to chlamydial infection and the effective treatment regimen. This will ensure the sustainable and effective recovery of those infected. A scheme for the application of laboratory examination methods in combination or separately will be given. This will ensure the detection of those infected with ophthalmic chlamydia and the determination of the titer of various immunoglobulins to assess the nature and intensity of infection among the examined individuals.

Dissertation approval and application: The dissertation materials were discussed at the 02nd preliminary interdepartmental discussion meeting of the Faculty of Public Health of Azerbaijan Medical University dated October 11, 2024, and at the 02nd scientific seminar of the FD 2.28 Dissertation Council under Azerbaijan Medical University dated December 18, 2024.

The results of the dissertation are intended to be used in the curriculum of medical educational institutions in the form of methodological recommendations and in the practical work of ophthalmology departments of medical institutions of our republic.

The main results of the dissertation were published in 14 scientific articles, 7 of which were published in scientific and practical journals (1 in Belarus) and 7 in the form of theses (1 in Ankara and 1 in London) in conference materials.

Name of the organization where the dissertation was performed;

Department of Epidemiology and Biostatistics, Azerbaijan Medical University

Publications. 14 scientific works have been published on the topic of the dissertation

The volume and structure of the dissertation: The dissertation consists of 139 pages, including "Literature Review", "Materials and Methods" and three chapters dedicated to special studies, conclusion, results, practical recommendations and a list of used literature. The list of literature reflects 191 sources (160 of which are in English). The work is illustrated with 9 tables and 14 figures. The dissertation consists of 203852 characters (Introduction – 15372 characters, Chapter I – 52633 characters, Chapter II – 17193 characters, Chapter III – 22211 characters, Chapter IV – 39149 characters, Chapter V – 52615 characters, Conclusions – 2456 characters, Practical recommendations – 2223 characters).

CONTENT OF THE DISSERTATION

Materials and methods. The study used various materials and methods necessary for the laboratory diagnosis and investigation of immunological features of ocular Chlamydia trachomatis infections. The earliest and easiest method of laboratory diagnosis is the direct detection of Halberstaedter-Prowazek bodies by staining conjunctival smears with the Romanowsky-Giemsa method. Isolation of C. trachomatis on Hella medium or McCoy medium is the most specific method and is considered the "gold standard". Of the antibody detection assays, the IFA method with monoclonal antibodies has a sensitivity of 85%-90%. In addition, PCR has a higher sensitivity and specificity than antigen detection tests.

The clinical group consisted of 125 patients, who were divided into the main and control groups. The main group consisted of 95 people, in whom inflammatory lesions of the choroid, retina, optic nerve, sclera and cornea of the eye were found to be of chlamydial etiology using informative diagnostic methods. The control group consisted of

30 patients with similar forms of the disease of other etiologies. The diagnosis of ocular Chlamydia trachomatis infections was made as a result of an ophthalmological examination, which included visometry, examination of the eye in natural light, biomicroscopy of the conjunctiva and cornea using a slit lamp, direct and inverted ophthalmoscopy, examination of the fundus, as well as laboratory examination data. Detection of Chlamydia trachomatis was carried out by the PCR method (kits from the "Litex" company) in a sample of scrapings taken from the urethra, cervical canal, tear film and by the determination of specific antibodies (IgG, IgM) in peripheral blood (SeroELISA diagnostic test system). The determination of genital microorganisms was carried out by traditional bacterioscopic, bacteriological methods, as well as cytological examination of smears taken from the mucous membrane of the urethra, cervical canal, and conjunctiva of the eye. The immunological profile included the determination of the expression of surface antigens in peripheral blood lymphocytes (CD3+, CD4+, CD8+, CD20+, CD56+, CD7+, CD38+, CD71+, CD23+, CD54+, HLA-DR+) by the method of flow laser immunocytofluorometry, the amount of serum immunoglobulins (IgA, IgM, IgG) by the method of radial immunodiffusion according to the Mancini method, total IgE (IFA method), the level of circulating immune complexes (CICs), as well as the functional activity of neutrophils in latex tests and nitro-blue tetrazolium recovery test. The mathematical processing of the obtained data was carried out by variational statistical methods.

Results of special studies.

In our study, 95 patients with confirmed ophthalmic chlamydia infection were selected as the main group

Approximately 61.05±4.57% of the patients had bilateral ocular Chlamydia trachomatis infections, and 38.94±2.32% had unilateral ocular Chlamydia trachomatis infections.

The incidence of uveitis in the main group of patients with chlamydia was 64.21%, while in the control group it was 43.33%. The odds ratio (Odd Index) was 2.34. This indicates that the incidence of uveitis among patients with ophthalmic chlamydia is 2.34 times higher than that of eye lesions of other etiologies.

Although this difference is not significantly large, it still indicates the importance of laboratory diagnostics of chlamydia in patients with uveitis.

Approximately 65.67% of the observed patients had bilateral and 34.32% had unilateral infectious-inflammatory changes.

Seven patients included in the control group were children under 15 years of age, whose symptoms differed somewhat from those of the main group. Of these, scleritis was detected in 3 patients, episcleritis in 2 patients, and uveitis in 2 patients. Keratouveitis was observed in 25% of children with uveitis, medial uveitis in 45%, and panuveitis in 30%.

Uveitis is a characteristic pathology in children with chlamydia. However, keratouveitis is considered more characteristic in the main group than at first glance. Because, although the incidence of keratouveitis in the main group was 36.36%, in the control group it was 28.57%. However, the odds ratio (Odd index) of keratouveitis between the groups was 1.27, which is approximately equal to 1. The obtained result shows that keratouveitis cannot be considered a specific symptom (pathology) among children with chlamydia.

Thus, the 95 patients with ophthalmic chlamydia, whom we included in the main group in the study, were divided into 3 groups: 1. Acute 2. Subacute 3. Chronic.

Among the many methods, PCR and IFA are distinguished by their high efficiency. If the first of them is characterized by maximum sensitivity and specificity and is aimed at the direct detection of DNA particles in the human body, then the second, by determining the specific state of human immune cells, allows us to make accurate judgments about the periods of the disease, to identify infections that are extremely important from an epidemiological point of view.

Positive results were obtained in 87.4±3.6% of infected individuals using the IFA method, and in 91.6±2.8% using the PCR method ($X2=0.24$; $p>0.05$). It should be noted that such a comparative assessment of the effectiveness of the leading methods of ophthalmic chlamydia diagnostics on such a sample material was carried out for the first time. When conducting simultaneous

examinations with these methods, it was observed that infection was detected in 93.7±2.4% (89) patients.

To assess the effectiveness of the methods, a 2x2 table used in analytical epidemiological research methods was used, and the specificity and sensitivity of the methods were calculated based on this table. As a result of the examinations conducted in the main and control groups with the IFA method, it was found that the sensitivity of the method was 87.36±3.04%, and the specificity was 93.33±4.55%.

When we look at the results of PCR examinations, we see that the sensitivity of the method was 93.68±2.94%, and the specificity was 96.66±3.28%. This also indicates that the PCR method is considered a more sensitive method for Chlamydia trachomatis than the IFA method. However, the specificity of both methods is considered high, which allows the IFA method to differentiate chlamydia infection from other clinically similar infections by laboratory methods.

One of the interesting pictures that emerges when evaluating the effectiveness of the methods depending on the clinical stages of the disease is that the sensitivity and specificity of the IFA method in the acute and chronic periods are not inferior to the PCR method. This indicates that there is a sufficient antibody titer in the patient's blood both in the acute and chronic stages.

Such a high detection rate, especially considering that the examined patients can be classified as a risk group, indicates that the current trends in various forms of chlamydia infection in the population can be fully detected with the help of the IFA method.

As can be seen, the recommendation regarding the need to use several methods simultaneously for the correct diagnosis of ocular Chlamydia trachomatis infections or for re-examination within 5-20 days is fully confirmed. For example, 83 infected patients identified only by IFA were re-examined by PCR, as a result of which infection was confirmed in all women, and vice versa, 87 infected people with a positive result by PCR were confirmed to be infected during re-examination by IFA. At the same time, positive results were obtained mainly when these methods were repeated after 10-15 days. Thus, for the complete detection of ophthalmic chlamydial infection, IFA

and PCR should be determined simultaneously or one of them twice with an interval of 10-15 days.

In the effectiveness of both methods, a tendency is observed to decrease in the effectiveness of the methods as the duration of the disease increases, which is expressed by a very strong negative correlation. The correlation coefficient for IFA is r=-0.93±0.01, for PCR r=-0.95±0.09. If the decrease in the effectiveness of PCR with the duration of the disease is not so noticeable - from 100.0±0.0 to 91.4±4.6% (X^2=1.34; p>0.05), the decrease in the effectiveness of IFA - from 91.8±4.5 to 86.9±7.0% (X^2=6.11; p<0.01), is statistically significant. As can be seen, immunosuppressive phenomena with a duration of infection of up to 1 year activate the vital activity of chlamydia, which maximizes the effectiveness of diagnostic methods. As the duration of the disease increases, the body's immune system is restored and even strengthened under the influence of immunocorrective therapy, which weakens the activity of chlamydia and thereby reduces the effectiveness of diagnostic methods to some extent.

Among patients with asymptomatic ocular Chlamydia trachomatis infections, the effectiveness of IFA was lower than the effectiveness of PCR - 87.5±5.7% and 93.8±4.2%, respectively (X^2=3.28; p>0.05). However, the duration of the disease does not affect the effectiveness of both methods and varies from 86.9±7.0 to 91.8±4.5% with IFA (X^2=0.32; p>0.05), and from 91.4±4.6 to 100.0±0.0% with PCR. (X^2=0.87; p>0.05).

Thus, the diagnosis of ocular Chlamydia trachomatis infections should be based on the results of clinical and laboratory research methods, a comparison of clinical manifestations and anamnestic data. However, the lack of specific symptoms and the pronounced polymorphism of clinical manifestations significantly complicate the clinical diagnosis of ocular Chlamydia trachomatis infections. The almost identical effectiveness of IFA and PCR opens up broad prospects for improving the diagnosis of ophthalmic chlamydia, the main requirement for which is timely and accurate diagnosis of the infection.

One of the most pressing problems in medicine is the problem of immunodeficiency and its explanation. By explanation, we mean determining, assessing and studying the cause of immunodeficiency. Immuno-ophthalmology also helps modern medicine to explain the problem of immunodeficiency by studying new information about immunoreactivity in the eye. Various factors - stress, pathologies of infectious and non-infectious etiology, social, environmental and other factors - can be considered the causes of secondary immunodeficiency (SID). Secondary ID occurs in 30-80% of cases among general immunodeficiency, which is of great interest, since this condition forms the basis of a picture that can change the clinical course and prognosis of both infectious and non-infectious diseases.

The solution of the problem in the direction of studying the secondary immunodeficiency state is connected with the implementation of the main directions of experimental immunology: immunodiagnostics, immunoprophylaxis and immunorehabilitation. All this fully applies to immunophthalmology. It is known from many literatures that many eye diseases - pathologies of the retina, glaucoma, uveitis, myopia and a number of other diseases are characterized by the development of a secondary immunodeficiency state, which is reflected in general, comprehensive approaches to treatment using non-specific immunobiological means.

A decrease in the total number of T- and B-lymphocytes was detected. Significant changes were recorded in the T-cell circle of the immune system: in half of the patients the ratio of immunoregulatory subpopulations of T-lymphocytes (CD4/CD8) changed, in 1/3 of the patients this was associated with a decrease in the number of T-helpers/inducers (CD4+). Modern laboratory-immunological diagnostic methods open up new opportunities for the etiological diagnosis of hematogenous forms of ocular Chlamydia trachomatis infections and allow for the timely appointment of targeted specific and immunoregulatory treatment. Thus, a comprehensive examination of patients with chlamydia has revealed the fact that in chronic forms of chlamydial infection, the majority of patients have background immune changes. The immunopathogenesis of chlamydial infection and background immunodeficiency mainly

cause the activation of the Th-2-conditioned humoral response (with hyperproduction of serum immunoglobulins) and an increase in circulating immune complexes (CICs). In this case, a significant increase in activated B lymphocytes (CD23+) and, in connection with this, increased production of IgE is considered a normal phenomenon. These symptoms, together with eosinophilic infiltration of the conjunctival mucosa in acute and subacute forms of chlamydial conjunctivitis, as well as the frequency of developing allergies to cold, which accompany chlamydia, indicate the possibility of an allergic component in the immunopathogenesis of chlamydial infection, including ocular Chlamydia trachomatis infections.

The diagnosis was confirmed by the detection of Chlamydia trachomatis in tear fluid by PCR (kits from the “Litex” company) and the detection of specific antibodies (IgG, IgM) in peripheral blood by IFA (diagnostic SeroELISA test system). Immunity was studied by immunofluorescence analysis of membrane markers of various clones of immunocompetent cells using monoclonal antibodies of Russian production. Population and subpopulation analysis of lymphocytes was performed on a flow cytometer FACScalibur ("Becton Dickinson"). Its indicators: CD5 (mature T-lymphocytes and immature B-lymphocytes), CD25 (B cells with receptors for interleukin 2), CD71 (proliferating cells with receptors for transferrin) were calculated using the regression analysis method. The mathematical processing of the obtained material was carried out using variational statistical methods.

We conducted a comparative analysis of the state of cellular and humoral immunity in patients with CI (chlamydia infection) and could not detect significant changes here. However, absolute rod-nucleated neutrophilia, an increase in the level of circulating immune complexes (CICs) were recorded. Therefore, we considered it important to assess the immune response in patients with CI depending on the nature of the course of infection. The nature of the course of CI was determined based on the following data

We recorded acute CI in 39 (41.1±5.1%) people. The following signs are characteristic of acute CI:

1. General blood test indicators - this includes the presence of an inflammatory process and its activation indicators, in which the role of the following nonspecific defense factors is enhanced:

- increase in the lymphocyte index;
- decrease in the lymphocyte/ESR ratio index;
- increase in the neutrophil/lymphocyte ratio index.

2. Absence of changes in the cellular immunity. Changes in the humoral circuit were accompanied by many features, for example, an absolute increase in the number of CD5 B-cells (2.1 times higher than in the control group). This subpopulation of cells appears in the early stages of the anti-infective process. These cells produce antibodies mainly against polysaccharides (Ch. trachomatis lipopolysaccharide-charidin), i.e., against non-thymocyte antigens of type 2. The response of CD5 B-cells to the antigen manifests itself early - only 48 hours after the antigen enters the body. Many features, such as the rapid development of the response, limited specificity and response only to the most common common bacterial antigens, lack of memory, characterize CD5- cells as participants in the non-specific immune response rather than the specific immune response. A 1.6-fold increase in the number of circulating immune complexes and a corresponding increase in the need for complement were observed. The efferent loop of the immune response is not quantitatively changed, but functional deviations are noted: an increase in the functional activity of leukocytes in both spontaneous and stimulated tests of nitro-green tetrazole recovery is noted.

Chronic CI was recorded in 21 (22.1±4.3%) people. The following signs are characteristic of chronic CI.

1. Changes in the leukocytogram:

– relative lymphopenia;

– a decrease in the elevated lymphocytic index below normal, an increase in the leukocyte change index; this indicates a change in immune reactivity;

– an increase in the leukocyte intoxication index by 2.9 times; it increases during tissue destruction and activation of endogenous intoxication processes.

2. An increase in the number of T-memory cells is noticeable in the cell loop, on which the speed and intensity of the secondary response depend. In addition, the increase in the number of cells from this population indicates the presence of a constant antigenic stimulus - a specific marker of chronic infection.

3. Changes in the humoral circuit of immunity:

– Massive formation of IgG antibodies - witnesses; they do not have protective significance due to the production of antibodies to variable lipopolysaccharides of chlamydia;

– Despite the high content of IgG, no significant differences in the number of CD19- lymphocytes (B-cells) were detected between the groups (although a slight tendency towards a relative increase in the number of B lymphocytes was observed);

– Due to the activation of the efferent circuit, a slight decrease in the level of CICs is noted (compared to acute CI), but as before, it is 1.3 times higher than the level observed in individuals in the control group;

–increase in complement demand by 15.4% compared to the control group.

4. Changes in the efferent loop:

– 1.9-fold increase in the number of B-cells compared to the control group (cells without receptors; this group includes new cells entering the bone marrow for differentiation and senescent cells programmed for apoptosis).

We recorded persistent CI in 35 (36.8±4.9%) people. This type of infection is characterized by the retention of changes that occur in the chronic course of the infectious process and the formation of a dynamic balance between micro- and macroorganisms: the amount of IgG reaches the norm, the neutrophil/monocyte index increases sharply. Changes in the leukocytogram correspond to changes that occur during the chronic course of CI:

–preservation of relative lymphopenia;

–decrease in the lymphocyte index, increase in the leukocyte migration index;

–confirmation of impaired immune reactivity;

–increase in the leukocytic intoxication index by 1.8 times (compared to the control group);

–increase in the neutrophil/monocyte ratio index, which is an indicator of changes in the macrophage system.

Changes in the cell line during the persistent course of CI are relative: an increase in the relative amount of CD5 up to 6.0% and the percentage of CD 25 up to 10.1% (compared to the control group). This line is represented by both T- and B-cells expressing receptors for interleukin 2 (receptor for T-cell growth factor). An increase in the amount of CD25 and a decrease in the CD3/CD25 index are considered signs of activation of the T1-helper response. For the humoral line, true CD5 B-cell lymphocytosis, that is, a specific indicator of immunity in patients with persistent CI, is characteristic. In such patients, the number of CD5 B-cells is 3.5 times higher than in the control group. Constant antigenic stimulation without elimination of the agent leads to the activation of this group of cells (producing antibodies that do not have a protective function) and an increase in the amount of CICs with a 1.8-fold increase in the need for complement compared to the control group. An increase in the functional activity of professional macrophages is characteristic of the efferent loop (a 6.4- and 1.8-fold increase in spontaneous and stimulated NYT, respectively). Our examinations conducted to study the state of the immune system in patients with CI showed significant changes in both the cellular and humoral loops of immunity. For an optimal immune response, it is important to have normal levels of CD4 cells - after stimulation with chlamydia, they produce γ-interferon and other cytokines (stimulating the growth and maturation of progenitor cells that activate macrophages, cells that produce antibodies), and also accelerate the proliferation of antigen-specific cytotoxic T-lymphocytes. A decrease in the number of CD4 cells is considered an extremely unfavorable prognostic sign. When assessing the role of the macrophage-leukocyte circle in the immunopathology of CI, it is necessary to remember the structural structure and role of the macrophage system of the body. Thus, despite its seemingly weak immunogenicity, Ch. trachomatis is capable of creating a sharp imbalance by affecting practically all defense factors. Patients with CI are characterized by absolute rod-shaped neutrophilia, activation of the NST test, and an increase in the

level of circulating immune complexes. Acute CI is considered the dominant type of disease, and changes in the acute phase indicators of the general blood test are characterized by an increase in the lymphocytic index, a decrease in the lymphocyte/ESR ratio index, and an increase in the ratio of non-specific CD5 B-cells (without the involvement of the cellular immune system). The presence of receptor-free lymphocytes is characteristic of chronic CI. Activation of the T1-helper response has been detected in persistent CI. The dependence of the immune response on the nature of the course of CI necessitates the development of different approaches to the treatment of such patients.

The number of patients with uveitis of viral and chlamydial etiology has recently increased. Therefore, the determination of changes in the cytokine profile of uveitis and its recurrent forms associated with chlamydial infection will help determine the stages of the infectious process, which in turn allows for adequate immunocorrection to prevent exacerbation of the infection and direct prophylaxis against relapses of the disease. In some cases, immuno-ophthalmology, a modern medical direction, is used to explain the problem of immunodeficiency, which in turn forms ideas about immune reactivity. One of the main concepts in this direction is considered to be a secondary immunodeficiency state, which may be associated with stress, old age, infections, chronic diseases, environmental, socio-economic and other factors. In various literatures, according to different authors, the frequency of secondary immunodeficiency varies between 25-75%, which is of great interest, since immunological deficiency is considered one of the background or concomitant conditions capable of changing the course and prognosis of both infectious and non-infectious diseases. The solution of this problem is associated with the implementation of the main directions of experimental immunology: immunodiagnostics, immunoprophylaxis and immunorehabilitation. All this fully applies to immuno-phthalmology. According to the literature, eye diseases - uveitis, retinal pathologies, glaucoma, myopia and a number of other diseases are accompanied by the formation of SID, which is reflected in complex approaches to treatment using immunopharmacological

agents. The dissertation work also included a clinical and laboratory assessment of the immune status. According to the results of the collected disease anamnesis, the group as a whole had infectious immunopathological syndrome (IPS) - 58.4%, allergic syndrome - 10.5%, while in most cases - 8.1% of patients, allergy to cold was detected. According to the data of laboratory-serological diagnostics (blood IFA), the chronic form of chlamydia infection was detected in 59.8% of cases, of which signs of active inflammation (reactivation) were determined in 23.4%. Primary infection was observed in 22.6% of the registered patients, and local form (conjunctivitis) without signs of general infection of the body was observed in 18.4%. When choosing the form of observation of patients with infectious uveitis, it is important to determine the phase of the infectious process and assess the adequacy of the immune response of the macroorganism to the effects of pathogens. Clinical and laboratory examination of 95 people with uveitis was carried out. Depending on the stage of the infection under study, 3 groups of patients were formed, and during the study, the cytokine profile was studied in each group and in comparison with the control group. In the 1st group of patients, 35 people (36.9±4.3%) had an acute form of chlamydia infection, 31 people (32.6±4.8%) had a subacute course of the disease, and 29 people (30.5±4.7%) had a chronic course. We have established that in patients with uveitis, anti-inflammatory cytokines are actively produced in the acute phase of the infection, while during the exacerbation of chronic infection, there is a significant decrease in the synthesis of pro-inflammatory cytokines and an increase in anti-inflammatory cytokines. Studies have shown that in group II, the level of IL-4 was 7.1 times higher than in the control group ($p<0.001$), which was observed against the background of a simultaneous 3.2-fold increase in the amount of native IFN-γ ($p<0.001$) and a 4.9-fold decrease in the concentration of stimulated IFN-γ ($p<0.001$). Such a contrast of changes indicates, first of all, a decrease in the functional activity of IFN-γ secreting type 1 T-lymphocytes, which occurs both as a result of the direct inhibitory effect of IL-4 and as a result of the depletion of the functional reserves of its synthesis due to increased antigen loading.

The functional capacity of IFN-γ to synthesize adequate antigenic load is also reduced by up to 25% in group III compared to the control group. At the same time, the level of stimulated IFN-γ in individuals in group I was 1.6 times higher than in the control group ($p< 0.001$), which indicates the activation of IFN-γ–producing cells in response to the introduction of an infectious agent and is accompanied by the development of a type 1 T helper (Th1) response, which mainly prevents the highly damaging effect of the pathogenic agent on the macroorganism. An increase in the amount of IL-2 was noticeable in all analyzed groups: its level was 2.6 times higher in group I ($p<0.001$) and 1.4 times higher in group III ($p<0.01$) compared to the control group. This increase in this indicator reflects the degree of activation of T-helper cells, the main producers of IL-2, by antigens of the infectious agent. The targets of IL-2 are Th1, cytotoxic T-cells, B-cells, natural killers (NK) and macrophages, which play an important role in the development of non-sterile immunity at any stage of the course of infection. In group I, IL-1β and TNF-α were 4.5 times ($p<0.001$) and 9.2 times ($p<0.001$) higher than in the control group, respectively. Considering that these cytokines participate in the activation of local and systemic inflammatory reactions, activate T-, B- NK-cells, polymorphonuclear leukocytes, and also play an important role in the coordination of the inflammatory response and the activation of the cytokine cascade, and, being an inducer for the synthesis of other cytokines, cause hyperproduction of IL-1β and TNFα in the acute phase of infection, in our opinion, they have a positive significance by directing the reaction of all links of immunity towards the elimination of the pathogen. Thus, considering the differences in the immune system in the cytokine profile indicators in patients with chlamydial uveitis, the following conclusions can be drawn.

In the acute phase of infection, the synthesis of pro-inflammatory cytokines increases significantly, which is associated with the direct cytotoxic effect of antigens on the tissues of the macroorganism, including the organ of vision. In the recurrent form of infection, the depletion of the synthesis of pro-inflammatory cytokines and an increase in anti-inflammatory cytokines are mainly observed, which

can lead to the development of an autoimmune inflammatory process of the organ of vision in the future, and the antigens of the causative agent in this case can participate as trigger factors.

A comprehensive examination of patients with chlamydia has revealed the fact that in the chronic form of chlamydial infection, the majority of patients have background immune changes. The immunopathogenesis of chlamydial infection and background immunodeficiency mainly cause the activation of the Th-2-conditioned humoral response (with hyperproduction of serum immunoglobulins) and an increase in circulating immune complexes. At this time, a significant increase in activated B-lymphocytes and, in connection with this, increased production of IgE is considered normal. These symptoms, together with eosinophilic infiltration of the conjunctival mucosa in acute and subacute forms of chlamydial conjunctivitis, as well as the frequency of development of allergy to cold, which occurs concomitantly against the background of chlamydiosis, indicate the possibility of an allergic component in the immunopathogenesis of chlamydial infection, including ophthal-mochlamydia.

We evaluated the effectiveness of laboratory and immunological methods for the diagnosis of hematogenous forms of ophthalmic chlamydiosis and immunomodulatory treatment. Under observation, 95 patients were diagnosed with hematogenous forms of chlamydial eye damage - chlamydial episcleritis and scleritis, uveitis: anterior, posterior and pan-uveitis, as well as uveitis with the spread of the inflammatory process to the cornea (keratouveitis), retina, uveitis of the optic nerve (uveoretinoneuritis), etc. Laboratory and immunological examination included: examination of scrapings taken from the conjunctiva and urethra, as well as lacrimal fluid by immunofluorescence, polymerase chain reaction and the “gold standard” culture method; examination of blood serum to determine specific antibodies to C. trachomatis and their titers; immunological examination of the patient's serum and leukocytes in humoral and cellular immune reactions to identify immunodeficiency states, conduct immunocorrective therapy and carry out immunological control of the effectiveness of treatment.

When the source of urogenital infection was determined in patients and specific antibodies were detected in the blood confirming chlamydial infection as an etiological factor of eye disease, we used a specific method - hypersensitivity reaction in the diagnosis of ophthalmic chlamydia.

Intradermal injection of chlamydial antigen was performed, followed by a study of the focal reaction in the eye. Taking into account indications and contraindications, a test was performed on 21 eyes of 15 patients aged 14-60 years with suspected chlamydial etiology of damage to the choroid, retina, optic nerve, sclera and cornea. The control group consisted of 10 patients (13 eyes) with similar diseases of non-chlamydial etiology. Chlamydia antigen was titrated in dilutions of 1:100, 1:101, 1:102, 1:103, 1:104, 1:105, 1:106 and injected intradermally in 0.1 ml. The dilution was carried out depending on the activity, severity, spread and localization of the inflammatory process: in cases of damage to the anterior segment of the eye, the introduction of chlamydial antigen began with a dilution of 1:103-1:104: in cases of damage to the retina and choroid, with a dilution of 1:106 - 1:104. In total, from one to three samples were taken with an interval of 48 hours. In the absence of any reaction or if the result of the first injection is doubtful, a higher concentration of antigen is re-introduced after 48 hours (but not more than twice). The focal reaction occurring in the tissues of the eye was recorded using traditional ophthalmological, complex functional and immunological methods before and after 24-48 hours. A positive focal reaction in the eye in response to the introduction of chlamydial antigen was expressed in the form of an increase in the inflammatory reaction, which was manifested by an increase in fluid exudation in the anterior chamber and vitreous body, an increase in corneal infiltrates, the appearance of fresh deposits, increased iris edema and the formation of new synechiae, its borders, an expansion of the caliber of retinal vessels, the appearance of small hemorrhages. A positive result of the test was considered an increase in visual acuity by 0.1 or more: changes in the area of opacity by at least 1/3, the boundaries of the visual fields - at least 10-20°, the amplitude of the total (a-, b-waves) biopotential of the retina - at least 20 μV, the threshold of

electrical sensitivity - not less than 10 μA, and electrical conductivity - not less than 5 Hz. Thus, the dynamics of two or three indicators after the introduction of the antigen was considered a positive focal reaction. In 95 patients (124 clinical samples) using the method of direct immunofluorescence microscopy, chlamydial antigens were detected in 80% of conjunctival and 92% of urethral specimens. Polymerase chain reaction was used to examine samples (lacrimal fluid and conjunctival scrapings) taken from 95 patients. Chlamydial DNA was detected in 92±2.37% of conjunctival scrapings, and in 87±2.15% of lacrimal fluid (31 samples).

52% of patients with suspected atypical and persistent forms of infection showed the presence of antibodies to chlamydia in titers from 1:64 to 1:256 and higher, which is characteristic of an acute inflammatory process or severe uveitis, panuveitis, and spread to other structures.

High titers of specific IgG indicate a severe and widespread inflammatory process. In 48% of patients with chronic chlamydial infection with frequent relapses of the disease, IgG titers against the background of previous antibiotic therapy, regardless of sensitivity to the chlamydial pathogen, did not exceed 1:64, which is associated with the development of combined immunodeficiency in the patient's body. Analysis of immunological defense factors in this category of patients in the study of serum and leukocytes shows a weakening of all components of the immune response.

A decrease in the total number of T and B lymphocytes was detected. Significant variability was found in the T-cell circle of the immune system: in half of the patients, the ratio of immunoregulatory subpopulations of T-lymphocytes (CD4/CD8) was disturbed, in a third of the patients this was due to a decrease in the number of T-helpers/inducers (CD4+). In case of immunodeficiency, we included in the complex therapy new synthetic broad-spectrum immunomodulatory drugs and interferon inducers, which restore the status of interferon due to the synthesis of endogenous interferons and have a stimulating effect on various mechanisms of the immune response. In 2/3 of the patients with suspected chlamydial etiology, a positive result of the focus test was

obtained after intradermal injection of chlamydial antigen. It was found that the positive focus reaction (73.4%) was significantly higher in the observed patients than in the control group (8.3%). In a quarter of the patients, a therapeutic or adverse reaction was detected in the eye, which was expressed in a decrease in the symptoms of inflammation. Each of the immunodiagnostic methods has its own advantages and disadvantages, therefore, it is more expedient to use a combination of several examination methods.

RESULTS

1. In our study, 64.21±2.34% of the patients included in the main group had uveitis, 25.26±1.47% had scleritis, and 10.52±1.47% had episcleritis.

2. While the incidence of uveitis in the main group of patients with chlamydia infection was 64.21%, it was 43.33% in the control group. The odds ratio (Odd Index) was calculated to be 2.34. This indicates that the incidence of uveitis among patients with ocular Chlamydia trachomatis infections is 2.34 times higher than that of eye lesions of other etiologies. Although the incidence of uveitis among children in the main group was 36.36±2.13%, it was 28.57±1.73% in the control group. However, the odds ratio (Odd Index) of uveitis between groups was 1.27, which is equal to 1 if we round it. The obtained result shows that uveitis cannot be considered a specific symptom (pathology) among children with chlamydia.

3. As a result of the examinations conducted in the main and control groups with the IFA method, it was found that the sensitivity of the method was 87.36±3.04%, and the specificity was 93.33±4.55%. When we look at the results of the PCR examinations, we see that the sensitivity of the method was 93.68±2.94%, and the specificity was 96.66±3.28%. This also indicates that the PCR method is considered a more sensitive method for Chlamydia trachomatis than the IFA method. However, the specificity of both methods is considered high, which allows the IFA method to differentiate chlamydia infection from other clinically similar infections in the laboratory.

4. Significant changes were noted in the T-cell circle of the immune system of patients with ophthalmic chlamydia: in half of the patients the ratio of immunoregulatory subpopulations of T-lymphocytes (CD4/CD8) changed, in 1/3 of the patients this was associated with a decrease in the number of T-helper/inducers (CD4+). In chronic forms of chlamydial infection, the majority of patients had background immune changes. The immunopathogenesis of chlamydial infection and background immunodeficiency mainly lead to the activation of the Th-2-conditioned humoral response (with hyperproduction of serum immunoglobulins) and an increase in CICs. In this case, a significant increase in activated B lymphocytes (CD23+) and, in connection with this, increased production of IgE is considered normal.

5. In the acute phase of ocular Chlamydia trachomatis infections, the synthesis of pro-inflammatory cytokines is significantly increased, which is associated with the direct cytotoxic effect of antigens on the tissues of the macroorganism, including the organ of vision. In the recurrent form of infection, the depletion of the synthesis of pro-inflammatory cytokines and an increase in anti-inflammatory cytokines are mainly observed, which can lead to the development of an autoimmune inflammatory process of the organ of vision in the future, and the antigens of the causative agent in this case can participate as trigger factors.

PRACTICAL RECOMMENDATIONS

1. When infectious-inflammatory eye problems occur in patients, the correct assessment of the clinical form helps to make a timely diagnosis. This is especially important when examining patients with uveitis for chlamydia.

2. In all clinical forms of chlamydia, it is advisable to use PCR and IFA test systems, which have high sensitivity and specificity, are inexpensive, and are used in seroepidemiological diagnostics. In chronic diseases, the simultaneous use of these diagnostic methods or one of them 2 times with an interval of 10-15 days is highly effective.

3. In the diagnosis of chlamydiosis, observation of changes in the T-cell circuit is very effective in differentiating the disease. Detection of changes in pro-inflammatory and anti-inflammatory cytokines can help prevent the development of autoimmune inflammatory processes in the organ of vision.

4. It is considered appropriate to use the obtained data in the curriculum of medical educational institutions and in the practical work of ophthalmology departments of our republic.

USED ABBREVIATIONS

1. CICs - circulating immune complexes
2. CI – chlamydia infection
3. DNA - Deoxyribonucleic Acid
4. ELISA - enzyme-linked immunosorbent assay
5. ESR - erythrocyte sedimentation rate
6. IFA – Immunofluorescence assay
7. PCR - polymerase chain reaction
8. SAFE - surgery for trachomatous trichiasis; antibiotics for C.trachomatis; facial hygiene; and environmental improvement
9. SID - secondary immunodeficiency

List of published articles related to the dissertation

1. Aghayeva G., Aghayev İ. / Oftalmoxlamidioz zamanı kompleks laborator-immunoloji müayinə metodları / Tezis / «Təbabətin aktual problemləri» / Bakı- 2018 / İşarələrin sayı:2070 / səh.148
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