

Diagnostic Value Determination of Antibodies to Antigens of Micro-Organisms in Women with Inflammatory Diseases of the Pelvic Organs

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Abstract To study and determine the diagnostic value of determining the titers of antibodies to antigens of certain etiological PID agents in women of childbearing age. To achieve the goal, 304 women of reproductive age with inflammatory diseases of the pelvic organs were examined. ELISA settings were performed using diagnostic test systems for determining serum antibodies to antigens Chlamydia spp, Toxoplasma gondi, Mycoplasma spp, Cytomegalovirus, herpes simplex viruses type 1 and 2 - HSV 1,2 (test systems from XEMA, RF) Ureoplasmaurealyticum (test system of the company "Vector-Best", Russia). Antibodies to Mycoplasma spp were detected in the examined women; they were detected in 120 cases ($39.5 \pm 2.8\%$). It turned out that the monoculture of microorganisms is detected 4.2 times more often than associations, in 7.6% of cases negative samples are detected. Most antibodies were detected against antigens of other species Mycoplasma spp, Chlamydia spp, Cytomegalovirus and Herpes simplex virus types 1 and 2.

Keywords Antibodies, Antigens, Enzyme immunoassay, Women, Pelvic inflammatory diseases

1. Introduction

According to statistics all over the world in recent years there has been an increase in the frequency of inflammatory diseases and the reproductive system of women. In Uzbekistan, over the past 5 years, the incidence of PID has increased by 30% and continues to grow. At the same time, more than half of the cases of modern PID have erased clinical symptoms, and each of the generally accepted diagnostic and laboratory indicators has deviations from the norm in less than half of the cases. [3,9].

Inflammatory diseases of the pelvic organs pelvic inflammatory disease (PID) characterized by different symptoms depending on the level of destruction and the force of the inflammatory response. The disease develops as a result of the penetration of the pathogen in sexual ways and in the presence of favorable conditions for its reproduction. Such conditions are created in the post-partum and post-abortion period, during menstruation, for different intrauterine manipulations [1,5].

The decisive role in causing inflammation play: the state of the microorganism, the massiveness of infection,

virulence of the etiological agent [2].

It was found that opportunistic pathogen bacterium (OPB) does not only cause of inflammation of the pelvic organs and cause of fetal and neonatal infections [1].

In this regard, microbiological tests have a high diagnostic value in the diagnostic of PID. Along with bacteriological diagnostics methods in recent years, other methods are widely used, in particular immunoassay - ELISA [4].

ELISA is an immunological laboratory method of qualitative determination and quantitative measurement of antigens. ELISA is based on the principle of interaction immunosorbent - pathogen antigen with antibodies in conjunction antigen-antibody complex with immunoglobulins containing enzyme label [3].

2. The Aim of the Study

Study and definition of the diagnostic value of detection of antibody titers to the antigens of some etiologic agents of PID in women of childbearing age.

3. Materials and Methods

To achieve the goal were studied 304 women of reproductive age with pelvic inflammatory disease. All patients were treated in the Khorezm branch of the

Republican Scientific Center for Emergency Medical Care Ministry of Health of Uzbekistan. They were distributed by age as follows: 18-21 years - 12 patients ($3,9 \pm 1,1\%$), 22-29 years - 136 patients ($44,7 \pm 2,9\%$), 30-35 years - 56 patients ($18,5 \pm 2,2\%$), 36-49 years - 100 patients ($32,9 \pm 2,7\%$).

The main number of women were married ($97,7 \pm 0,9\%$), rural residents accounted for $69,4 \pm 2,6\%$, and the urban $30,6 \pm 2,6\%$. Among all women surveyed took a major amount of housewives and non-working women ($84,5 \pm 2,1\%$).

Patients often revealed acute salpingitis, oophoritis, piosalpinx ($98,4 \pm 0,7\%$). The diagnosis was verified by means of clinical, clinical, instrumental and laboratory studies on the proposals of the National Center for Disease Control and Prevention (CDC, USA, 2006).

For setting up ELISA were used diagnostic test systems for the determination of serum antibodies to the antigens of *Chlamydia* spp, *Toxoplasma gondi*, *Mycoplasma* spp, Cytomegalovirus, Herpes simplex virus type 1 and 2 - HSV 1,2 («XEMA» company's test systems, RF) *Ureoplasmaurealyticum* (test-system of the company "Vector-Best", Russia). The principle of the method lies in the qualitative detection of antibodies to the above mentioned antigens by indirect ELISA test on polystyrene. The results were obtained by spectrophotometric method at a wavelength of 492 nm.

During carry out this research were observed all the ethical principles for medical research involving human subjects, the Helsinki Declaration adopted by the World Medical Association in 1964 (the latest addition in Seoul on the 59th General Assembly of the World Medical Association in 2008).

Statistical analysis of the material performed by traditional methods of variation statistics, all the results and conclusions based on the principles of evidence-based medicine.

The results of research and discussion. The obtaining results showed that antibodies are not detected by the above pathogens always. Most of the time the women surveyed revealed antibodies to *Mycoplasma* spp, they were detected in 120 cases ($39,5 \pm 2,8\%$). It is known that the genus *Mycoplasma* in practical medicine the main place is occupied kinds *M.hominus*, *M.genitalium* and *M.pneumoniae*. All these types are common antigenic determinants and ELISA method to determine them separately is not possible. If we consider that *Mycoplasma* spp causes acute and chronic inflammatory diseases of the urinary tract, they may be etiologic agents of PID in women [7].

On the next place on detectability were antibodies to *Chlamydia* spp - $27,0 \pm 2,5\%$ ($n = 82$). The main representative of this kind, which may be the causative agent of TORCH-infections is *C.trachomatis*, other (*C.psittaci* and *C.pneumoniae*) rarely cause disease in humans [8]. Detection of specific serum IgG-antibodies activity reflects the extent of reproduction *Chlamydia* spp. Determination of IgG antibodies may be used for establishing and monitoring of disease recurrence of the infection.

A distinctive feature of our research was that most

frequently detected antibodies to such antigens - Cytomegalovirus (CMV) and Herpes simplex virus type 1 and 2 (HSV 1,2) respectively in $20,4 \pm 2,3\%$ ($n = 62$) and $12,5 \pm 1,9\%$ ($n = 38$) cases. If we consider that CMV is found in 60-90% of the adult population, the CMV problem in women, especially pregnant women, it is very serious. [9] Although IgG-antibodies do not protect against reactivation of latent virus, but can serve as an indirect indicator of CMV activity in the body of women. HSV 1 & 2 (herpes simplex virus type 1 and 2) are also seen very often, hit about 90% of the population [6]. Women particularly common HSV 2 (genital herpes) and identification of IgG-antibodies indicates a remission or recurrence of herpes simplex disease.

Relatively few have been identified antibodies to *Toxoplasma gondi* ($5,9 \pm 1,4\%$, $n = 18$) and *Ureoplasmaurealyticum* ($3,3 \pm 1,0\%$, $n = 10$). It is known that the IgG antibodies to *Toxoplasma gondi* have a protective function, and provide a stable immunity against re-infection [10], so the definition of IgG antibodies to *Toxoplasma gondi* is used for the purpose of state immunity anti-toxoplasmonic of women. The same diagnostic objective pursued determining IgG antibodies to *Ureoplasmaurealyticum* [8].

There were revealed total 330 cases of detection of positive samples from 304 women, but it is necessary to take into account that $7,6 \pm 1,5\%$ ($n = 23$) of the women surveyed were found negative samples. Consequently, the 330 positive samples were found in the surveyed 281 women with PID. The percentage of detection of antibodies in healthy women who have not had PID was low and significantly different from women with pelvic inflammatory disease ($P < 0.001$).

Given the fact that the identification of positive samples was observed in the form of monocultures and microbial associations, we were interested to study the relationship between detection of each another.

The results show that most often encountered in associations and CMV antigens from different *Chlamydia* spp microorganisms (for $n = 20$) and *Mycoplasma* spp ($n = 14$).

In most cases the association was observed with the following organisms: CMV and HCV 1,2 to $3,3 \pm 1,0\%$ ($n = 10$); And *Mycoplasma* spp CMV in $2,6 \pm 0,9\%$ ($n = 8$); *Chlamydia* spp and *Mycoplasma* spp in $2,6 \pm 0,9\%$ ($n = 8$); CMV, *Chlamydia* spp and HSV 1,2 to $2,0 \pm 0,8\%$ ($n = 6$) patients. Other associations met from $0,7 \pm 0,5\%$ ($n = 2$) to $1,3 \pm 0,6\%$ ($n = 4$) cases.

The detected associations were observed between 2 and 5 microorganisms. They were as follows: 2 in association microorganism was $13,8 \pm 2,0\%$ ($n = 42$), 3 in the microorganism $5,3 \pm 1,3\%$ ($n = 16$) 4 microorganisms $1,3 \pm 0,6\%$ ($n = 4$) and to 5 microorganisms $0,7 \pm 0,5\%$ ($n = 2$) samples.

It is interesting to note that the monoculture of microorganisms identified ($n = 266$) occurred 4.2 times more frequently than the association of these microorganisms ($n =$

64). The presence of a certain number of negative samples ($7,6 \pm 1,5\%$, $n = 23$) antigen above microorganisms in these studies points to the discovery of other etiologic agents (gram-negative bacteria, gram-positive cocci, anaerobes), pelvic inflammatory disease in women of childbearing age who are found bacteriological methods.

The findings indicate that in addition to bacteriological methods of qualitative determination of antibodies in the serum of women against antigens of various microorganisms using the ELISA method has a certain diagnostic value and along with other methods can be used for the diagnosis of PID.

4. Conclusions

1. In the serum examined women with PID most commonly detected IgG antibodies to antigens of *Mycoplasma* spp, *Chlamydia* spp, Cytomegalovirus, and Herpes simplex virus type 1 and 2, the least frequently detected IgG antibodies to antigens of *Toxoplasma gondi*, *Ureoplasma urealyticum*.

2. Total 330 positive samples were found, which samples with 64 monocultures and 266 microorganisms association. Monoculture occurred in 4.2 times more frequently than the association of microorganisms, $7,6 \pm 1,5\%$ cases were found negative samples.

3. Women who were surveyed most often in association met Cytomegalovirus and *Chlamydia* spp 20 times, *Mycoplasma* spp 14 times with other microorganisms and each other.

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