

ANNOTATION

on the topic “CLINICAL AND IMMUNOLOGICAL ASPECTS OF CHRONIC PELVIC INFLAMMATORY DISEASE IN WOMEN OF REPRODUCTIVE AGE” of the thesis by Nuradilova Dina Maksatovna, submitted for the degree of Doctor of Philosophy (PhD) in the specialty 6D110100-Medicine

Relevance of the research topic

Pelvic inflammatory disease (PID) in women all over the world represent a huge medical, social and economic problem due to their widespread prevalence, negative impact on the reproductive function of women, the health of mother and child and, as a result, the nation as a whole.

There is still no single view on the definition of this group of diseases. The most acceptable is the definition of PID as a group of independent nosological diseases of the upper reproductive tract, manifested as an isolated disease or as any combination of endometritis, salpingitis, oophoritis, pyosalpinx, tubo-ovarian abscess and pelvic peritonitis [Brunham RC., Gottlieb SL., Paavonen JN., 2015].

In the world, about 350 million women fall ill with PID every year, most of them develop chronic inflammatory disease of the reproductive organs, which in 15% of cases lead to ectopic pregnancy, and in 40-85% of cases can lead to infertility [Catherine L Haggerty, Patricia A Totten, Gong Tang et al., 2016]. Unsuccessful attempts at in vitro fertilization in many cases are also associated with the presence of sexually transmitted infections [Dimitra Moragianni, George Dryllis, Panagiotis Andromidas et al., 2019].

The increase in the incidence of PID, which has been noted recently all over the world, including in the CIS countries, is associated with increased migration of the population, changes in moral norms and prostitution [Starodubov V.I., Sukhanova L.P., 2012].

Many researchers have noted a change in the clinical picture and outcomes of PID in modern women. So, having an indolent, low-symptomatic course, they can lead to complications that have the most severe consequences, up to the need for surgical intervention [Sweet R.L., 2012, Xin Tao, Shu-qi Ge et al., 2018, Dimitra Moragianni, George Dryllis et al., 2019].

This is primarily due to a change in the nature of the pathogenic microflora of the genital tract in modern women. If earlier E.coli, staphylococci, bacteroids, clostridium, peptostreptococci, etc. were considered the main causative agents of PID, now most researchers name the causative agents of sexually transmitted infections (STI) as the main etiological factors of the chronic inflammatory process in the reproductive organs [Eloisa Llata, Kyle T. et al., 2015, Arlene M. Butz, Ch. Gaydos, et al., 2016].

Thus, chlamydia and ureaplasma infections were noted in 70% of cases, and in the case of infertile marriage, chlamydia and/or ureaplasma are most often detected in the genital tract of the spouses [Bernice M Hoenderboom, Birgit H B van Benthem et al., 2019].

For chronic PID, polyetiology is characteristic, so the clinical picture of an isolated disease may be due to a combination of microorganisms. Conversely, one pathogen can cause inflammatory processes of various localization. The female genital tract is quite often colonized by protozoal-bacterial-fungal associations with the formation of biofilms, which is one of the reasons for the ineffectiveness of standard methods of treatment of chronic PID.

To date, there are practically no publications in Kazakhstan devoted to the problem of urogenital mixed infections in women of reproductive age with PID.

Chronic inflammation of the genital tract in women caused by sexually transmitted infections is also a risk factor for oncogynecological pathology [de Martel C., Georges D., Bray F. et al., 2018, Ferlay J., Ervic M. et al., 2020].

Currently, laboratory verification of urogenital infections presents certain difficulties. The widespread use of the polymerase chain reaction (PCR) method in practice has increased the possibilities of laboratory diagnosis of STIs. But in some cases, when the infection continues to recur, despite the ongoing antibacterial therapy, it becomes necessary to determine the sensitivity of microorganisms to antibiotics.

Violation of the immunological mechanisms of protection of a woman's genital tract can also contribute to the development of inflammatory diseases of the reproductive tract and the chronization of urogenital mixed infections, which dictates the need for adequate and rational immunocorrection.

Thus, the topicality of the problem is determined by the wide spread of chronic pelvic inflammatory disease in women, their negative impact on the reproductive health of the population and the low effectiveness of standard treatment methods carried out without taking into account the etiological factors of PID and immunological reactivity of macroorganisms.

The purpose of the research

To study the clinical and immunological aspects of chronic pelvic inflammatory disease associated with urogenital mixed infections in women of reproductive age and to develop etiopathogenetically based treatment methods.

Subject of the research

Pelvic inflammatory disease in women of reproductive age.

The object of the research

2360 women with pelvic inflammatory diseases, aged 18 to 45 years, selected according to the data of the appeal to the Regional Diagnostic Center. After laboratory diagnostics of 4720 samples of biomaterial obtained by scraping from the cervical canal and urethra (2 samples from each woman) for the presence of pathogens of urogenital infections, 112 women were selected, in whose samples 6 or more pathogens were identified (main group).

The control group consisted of 100 women of the same age without gynecological pathology with negative results for the presence of urogenital infections. There were no restrictions on racial, ethnic and religious affiliation in the study, women of various nationalities living in the region were represented.

The criteria for inclusion were:

- 1) Women 18-45 years old
- 2) Women suffering from PID (endometritis, salpingoophoritis, latent chronic course, previously suffered acute inflammatory process, with relapses)
- 3) Women who did not receive immunomodulators during the previous 6 months

The criteria for exclusion were:

- 1) Virgins
- 2) Pregnant women
- 3) Breast-feeding women
- 4) Women under 18 and over 45
- 5) Women with pelvioperitonitis, parametritis, tuboovarial abscess, with acute and subacute course of PID
- 6) Menopausal women
- 7) Women with immunocompromising diseases (viral hepatitis B, C, HIV, tuberculosis, diabetes mellitus, autoimmune and oncological diseases)

All 112 women of the main group gave informed consent to participate in the study in compliance with the principle of voluntariness and an explanation of the purpose of the study, the degree of risk and protection from risks.

Among women with PID, persons aged 30 to 39 years prevailed (41.8% of the total number of studied). There were 33 women aged 20 to 29 (29.5%), 29 women aged 40-45 (26.0%). The smallest group was women under the age of 20 - 3 people (2.7%). The age composition of the control group differed slightly.

The largest group (81.1%) were women of the titular nation (Kazakhs), 13.4% of the surveyed women were Russians, 2.7% were Uighurs, 1.5% were Koreans and 1.0% were Ukrainians. The distribution by social status and type of activity of women with PID showed that more than half of the surveyed women (59.8%) did not work, the number of women employed in the service sector was 19 people (17.0%), 10 workers (8.9%). The smallest group was employees (7.1%) and female students (2.7%).

The majority of the research participants were married women - 82 (73.2%), there were 21 unmarried (18.7%), 8 women were divorced (7.1%), 1 woman was in a civil marriage (1.0%).

Methods of the research

- *Anamnestic method*

To establish the complex of complaints and risk factors, all 112 women of the main group were surveyed using a specially designed questionnaire containing 29 questions and answer options.

- *General clinical methods*

The examination included a general examination, gynecological examination, laboratory methods (general clinical blood analysis, general urine analysis, biochemical studies with the determination of glucose, alanine transaminase, aspartate transaminase, creatinine, total bilirubin), ultrasound examination of the pelvic organs.

- *Special laboratory methods*

1. Microscopic examination of the separated cervical canal of the vagina and urethra

2. Vaginal ph-metry

3. Amine test

4. Bacteriological method (cultural)

The cultural test "AF-genital system" ("Liofilchem, Italy") allows to identify microorganisms most often found in the human genital tract:

- Escherichia coli
- Proteus spp.
- Pseudomonas spp.
- Gardnerella vaginalis
- Staphylococcus aureus
- Enterococcus faecalis
- Neisseria gonorrhoeae
- Streptococcus agalactiae (group B)
- Candida spp.
- Mycoplasma hominis
- Ureaplasma spp.
- Trichomonas vaginalis

In addition, the "AF-genital system" allows to identify, count semi-quantitatively and determine sensitivity to antibiotics Mycoplasma hominis and Ureaplasma spp.

1. Polymerase chain reaction method (PCR)

PCR analysis of DNA determination of Chlamydia trachomatis was performed on a 6-channel amplifier with an optical system for PCR and «real time» mode detection «Rotor – Gene 6000» («Corbett Research», Australia).

2. Flow cytometry – determination of subpopulations of lymphocytes CD3+, CD4+, CD8+, CD16+CD56+, CD19+

Immunological phenotyping was performed on a flow cytometer (BD "FACS Canto II", Becton Dickinson, the USA).

• *Methods for evaluating the effectiveness of treatment*

The effectiveness of PID treatment methods was evaluated using clinical examination, microscopic, bacteriological, immunological methods and PCR

• *Statistical analysis*

Statistical data processing was performed on a PentiumIV personal computer in the Microsofte Exel 2013 operating system using the IBM standard software package SPSS Statistics 23.0 for Windows (SPSS, Inc, Chicago, IL., USA). Parametric and nonparametric methods were used, the level of statistical significance (p) was calculated, where the critical level was assumed to be equal to or less than 0.05. At the $p < 0.05$ level, the differences were considered statistically significant.

Objectives of the research

1. To study the structure, features of the clinical course of PID and concomitant gynecological pathology of women of reproductive age.

2. To study the prevalence of urogenital infections and antibiotic sensitivity of ureaplasmas and mycoplasmas in women with PID of reproductive age.

3. To study the state of immunological reactivity of women with PID associated with urogenital mixed infections.
4. To develop etiopathogenetically based methods of treatment of PID in women of reproductive age.

Scientific novelty

1. Clinical and immunological aspects of PID associated with urogenital mixed infections (6 or more pathogens) were studied for the first time in the Republic of Kazakhstan.
2. A comprehensive approach was used to verify the pathogens of urogenital infections using modern methods of laboratory diagnostics – PCR in the "real time" mode and a cultural method for identifying 12 pathogens of pathogenic urogenital infections with the determination of the sensitivity of microorganisms to antibiotics.
3. For the first time in the Republic of Kazakhstan, new etiopathogenetically based approaches to the treatment of women with PID have been proposed, taking into account the antibiotic sensitivity of pathogens of urogenital infections with elements of immunocorrection.
4. It has been proven that full-fledged eradication of pathogens of urogenital infections leads not only to the disappearance of clinical symptoms of PID, but also to a complete clinical recovery of women, and in some cases to the restoration of their reproductive function.

The main provisions submitted for defense

- 1 The leading factor in the pathogenesis of inflammatory diseases of the pelvic organs in women are urogenital mixed infections, among which chlamydia, myco-ureaplasmosis, bacterial vaginosis, candidiasis and staphylococcal infection were most often identified.
- 2 The current course of PID is characterized by a variety of pathogenic flora with a predominance of STI pathogens, multifocal inflammation of the female genital tract and the transformation of the clinical picture in 38.3% of cases towards indolent low-symptom forms.
- 3 Women with chronic PID were characterized by a significant decrease in the subpopulation of T-helper cells and natural killer cells, which play an important role in the timely elimination of intracellular bacteria.
- 4 The developed scheme of eradication of urogenital infections is an etiopathogenetically justified and effective method of treatment of inflammatory diseases of the pelvic organs in women of reproductive age. Simultaneous use of an immunomodulator in the eradication scheme significantly increases its effectiveness and tolerability of drugs.

Practical significance of the obtained results

- 1 The most informative and reliable methods of laboratory diagnosis of urogenital infections are proposed - a unique instrumentless, highly sensitive and specific cultural express test for cultivation and identification of 12 pathogenic pathogens of urogenital infections with the determination of the sensitivity of mycoplasmas and

ureaplasmas to 9 antibiotics, which can be implemented even at the stage of primary medical and social assistance and real-time PCR.

2 To improve the efficiency of diagnosis and treatment, the concept of the leading role of pathogenic urogenital infections in the recurrence, chronicity and occurrence of complications of inflammatory diseases of the pelvic organs in women of reproductive age is proposed.

3 For implementation in practical healthcare, a scheme for the eradication of causative agents of urogenital mixed infections has been proposed, taking into account their antibiotic sensitivity.

Conclusions

- 1 Among the clinical variants of chronic PID in the examined women, salpingoophoritis (75.0%), chronic endometritis (16.1%) prevailed, a combination of endometritis and salpingoophoritis (8.9%) was less common. The most common concomitant gynecological pathology in women with PID was endocervicitis (33.0%). 21.4% of women had a combination of PID with recurrent urethritis, 17.0% with ectopia of the cervix, 12.5% with menstrual irregularity, 13.4% with reproductive dysfunction, 8% with Bartholinitis, 8.0% - with ovarian cysts, 6.2% - with endometriosis and 5.3% - with uterine fibroids.
- 2 *Staphylococcus aureus* (67.3%), *Gardnerella vaginalis* (37.4%), *Candida* spp. (26.4%), *Ureaplasma* spp. (23.0%), *Mycoplasma hominis* (7.6%) were shown the most frequently from the genital tract of women with PID using the cultural method. *Chlamydia trachomatis* was identified by quantitative PCR in 8.3% of women.
- 3 The study of antibiotic sensitivity showed that most strains of ureaplasmas and mycoplasmas are highly sensitive to doxycycline (99.5% of ureaplasmas and 98.3% of mycoplasmas). The use of josamycin is impractical, because only 63.3% of ureaplasmas and 15.6% of mycoplasmas showed sensitivity to it.
- 4 For women with inflammatory diseases of the pelvic organs associated with urogenital mixed infections, the same type of changes in immunological parameters were characteristic: depression of cell-mediated immunity reactions – a decrease in the specific weight of cells with helper phenotype ($p < 0,05$) and a decrease in the content of NK cells ($p < 0,05$).
- 5 The use of an optimized etiotropic therapy scheme for PID contributed to a decrease in the proportion of women with vaginal discharge from 85.5% to 21% ($p < 0,001$), with pain in the lower abdomen from 83.9% to 21.1% ($p < 0,001$), with genital itching from 16.1% of women to 3.2% ($p < 0,001$). After treatment, dyspareunia disappeared in all women with PID.
- 6 Inclusion of the interferon inducer tiloron in the optimized treatment scheme for PID was accompanied by a faster and more pronounced clinical effect – the proportion of women with vaginal discharge decreased from 88.0% to 4.8% ($p < 0,001$), with pelvic pain from 87.9% to 6.0% ($p < 0,001$). Genital itching, dyspareunia and dysuria disappeared in all women with PID who received combination therapy (etiotropic treatment + immunomodulator).

- 7 Our proposed scheme of etiopathogenetically based therapy of chronic inflammatory process of the genital tract of women contributed not only to the disappearance of clinical symptoms of PID, but also led to complete eradication of pathogens of urogenital mixed infections. The use of an optimized eradication scheme with the inclusion of an immunomodulator contributed to the positive dynamics of immunological reactivity indicators – an increase in the number of mature T-lymphocytes (p0.05), natural killer cells (p0.05) and allowed for faster remission, as well as improved the tolerability of antimicrobial drugs.

Personal contribution of the doctoral student

The doctoral candidate personally carried out planning, determination of goals and objectives, carried out scientific and methodological substantiation of the thesis research.

The author personally carried out the design of a questionnaire to determine risk factors and subjective symptoms, questionnaires, general clinical and gynecological examination, immunological examination of women of the main and control groups. The candidate personally developed schemes for the eradication of pathogens of urogenital mixed infections using an immunomodulator with subsequent evaluation of their effectiveness.

Statistical data processing and interpretation of all the obtained results was carried out, as well as the thesis was written and designed personally by the author.

Approbation of the results of the thesis

The main provisions of the thesis are reported on:

- extended meeting of the Department of Obstetrics and Gynecology of KazNMU named after. S.D. Asfendiyarov No. 10 dated May 12, 2022
- meeting of the Scientific Commission on topic of "Surgical diseases" KazNMU named after. S.D. Asfendiyarov No. 7 dated 7.06.2022
- The Third International Emergency Medicine Symposium, Almaty, Kazakhstan at 29.03.2015
- II International Forum of the Republic of Kazakhstan and the Russian Federation on the problems of modern clinical laboratory diagnostics, Kazakhstan, Almaty, April 1-2, 2015.
- IInd International Scientific and Practical Conference "Science and Education - Our Future". November 22-23, 2015, Ajman, UAE.
- VI Congress of Obstetricians and Gynecologists of the Republic of Tajikistan, Dushanbe, the Republic of Tajikistan, December 2, 2016.
- IInd International Scientific and Practical Conference «International Trends in Science and Technology» March 16, 2018, Warsaw, Poland.
- VI International Congress of the Kazakhstan Association of Medical Laboratory "Modern interdisciplinary and integrated technologies in laboratory medicine", Republic of Kazakhstan, Almaty, April 19-20, 2018.
- II Regional Conference "Modern problems of reproductive medicine", Republic of Kazakhstan, Aktau, June 14-15, 2018.

Publications on the topic of the thesis

Based on the materials of the dissertation, 11 scientific papers were published, including 2 articles in journals indexed in the Scopus database, 4 articles in journals recommended by the Committee for Control in the field of Education and Science of the Ministry of Education and Science of the Republic of Kazakhstan, 7 publications in the materials of International and Republican conferences. 6 acts of implementation and 4 copyright certificates were received.

The structure and volume of the thesis

The dissertation is made in the amount of 142 pages and consists of the following chapters: Introduction; Modern ideas about the epidemiology, Clinical diagnostic criteria and treatment of inflammatory diseases of the pelvic organs in women (literature review); Material and research methods; Chapters of the results of their own research (“Prevalence of urogenital infections in women of reproductive age with PID”, “Clinical and immunological characteristics of PID in women of reproductive age”, “Results of a study of the effectiveness of pathogenetically substantiated methods of treating PID associated with urogenital mixed infections”); Discussion of the obtained results; Conclusions; Practical recommendations; List of used sources; Appendices.

The work is illustrated with 19 tables, 27 figures, 3 appendices. The bibliographic index includes 243 sources in Russian and foreign languages.