ANNOTATION
of the thesis
by Amirbekova Zhanna
on:
"ASSESSING THE PREVALENCE OF C.TRACHOMATIS SUSTAINABILITY TO FLUOROHINOLONS BASED ON THE STUDY OF THE TARGET GENES' MUTATION PROFILE"

in candidacy for the degree of Doctor of Philosophy (PhD)
on the specialty 6D110100 "Medicine"

The problem topicality

Chlamydia trachomatis, being an obligate intracellular bacterial pathogen, is one of the most common pathogens of sexually transmitted diseases. An important feature of this infection is that this disease is usually asymptomatic in about 75% of cases for women and 50% of cases for men [1. Sziller I., Witkin S.S., Ziegert M., 1998]. This fact ensures the prevalence of Chlamydia infection in the human population, due to people's untimely or lack of medical attention seeking in the health care organization. Moreover, without a timely and adequate diagnostic approach and chlamydial infection therapy, the process can move to the upper parts of the urogenital tract, causing new pathological processes with severe consequences, and also acquire a long chronic infectious form [2. Cohen C.R., Brunham R.C., 1999; 3. Paavonen J., Eggert-Kruse W., 1999].

The choice of drugs for treating urogenital chlamydial infection are, as a rule, macrolides, tetracyclines and fluoroquinolone series drugs [4. Ridgway G.L., 1997]. Recently, there have been reports of the "unsuccessful" anti-chlamydial antibiotic therapy, which is sometimes associated with the emergence of the C.trachomatis' resistance to antibiotics [5. Somani J., Bhullar V.B., Workowski K.A., 2000]. At the same time, an adequate assessment of the level of chlamydia pathogen resistance to antibiotics is an extremely difficult task, which is difficult to solve due to a number of objective reasons. First, the curing control for chlamydial infection is not always performed by health professionals. Second, obtaining information on an antibioticogram is extremely difficult task, since chlamydia is very difficult to cultivate. Third, it is sometimes difficult to separate the detected chlamydia after treatment (ineffective treatment, lack of cure) from reinfection, when the patient was cured really, but by the time of the subsequent examination he again became infected.

The problem of antibiotic resistance is currently relevant, and chlamydial infection is no exception. Stability mechanisms are actively being studied, and by now much is already known. At the same time, there are open questions in full understanding of the origin and prevalence of the antibiotic resistance genetic basis.

It should be understood that there are features in the life cycle of C.trachomatis - reproduction and development of chlamydia occurs inside the host cell. Finding the active part of life in the intracellular medium creates difficulties with a simple

extrapolation of understanding the prevalence of resistance genes among the usual Gram-negative and Gram-positive bacteria. Nevertheless, phenotypically resistant strains of C.trachomatis have been obtained when cultured on media in the presence of antibiotics in subinhibitory concentrations.

At the moment, data on the genetic mechanisms of the emergence of chlamydia resistance to various antibacterial drugs appear in the literature. For example, the analysis of the C.trachomatis mutant strains genes that appeared due to multiple passages in the cell culture with antibiotics has showed that mutant isolates have mutations in the gyrA and parC genes, which are associated with resistance to fluoroquinolones [6. Yokoi S., Yasuda M., Ito S., 2004; 7. Misiurina O., Shipitsina E.V., Finashutina Iu P., 2004]. This mechanism of the resistance to fluoroquinolones due to mutations in target genes, which, leading to decreased affinity of fluoroquinolones and targets, may be one of the main ones. However, at this stage, there is little enough data on the mutational profile level in the clinical C.trachomatis strains.

Thus, there is a situation when, on the one hand, there are reports of clinical unsuccessful treatment, and on the other hand, there is insufficient evidence on the molecular genetic mechanisms of resistance development in Chlamydia infection. All this has predetermined to set the aim of assessing the level of Chlamydia infection resistance to fluoroquinolones based on the study of mutations in target genes.

The research aim

To assess the level of prevalence of chlamydial infection resistance to fluoroquinolones based on the study of gyrA and parC genes mutations in the QRDR region.

The research objectives

1. Study the STIs structure and determine the proportion of the Chlamydiatrachomatis incidence in women's gynecological practice;
2. Conduct a correlation analysis to determine the STIs factors;
3. Analyze gyrA and parC genes mutations in the QRDR region of C. trachomatis clinical isolates;
4. Conduct a bioinformatic analysis of the genome-wide data of the C. trachomatis global collection for the epidemiological evaluation of resistance to fluoroquinolones.

Scientific novelty

Behavioral factors associated with chlamydial infection based on the multifactor analysis have been studied, showing the role of the early debut of sexual life with acquiring STIs.

For the first time, the prevalence of the level of fluoroquinolones resistance to C.trachomatis clinical isolates, isolated in the gynecological practice of Karagandy city, was assessed based on the analysis of topoisomerase genes mutations in the QRDR region. The results of this study indicate a low percentage of resistant strains in

the Karaganda region, which makes it possible to select fluoroquinolones as a priori therapy for the chlamydial infection.

A complex in silico analysis of the genomic sequences of the global collection was conducted for the first time to identify mutations in target genes for fluoroquinolones using a complex of bioinformatic programs. The results of analysis of the chlamydia global collection indicate an extremely low resistance of C.trachomatis to fluoroquinolones. The results obtained from the clinical strains of C.trachomatis and the analysis of genomic data from the international collection of C.trachomatis are consistent, indicating a low level of chlamydia resistance to fluoroquinolones at the current stage.

Practical significance

- Low spread of C.trachomatis resistance to fluoroquinolones, studied on the basis of genetic mechanisms of resistance, allows using fluoroquinolones as empirical antibacterial chemotherapy at the present stage.
- Recommend the methodology for determining mutant strains on the QRDR region of genes-topoisomerases based on PCR, developed in Scientific Research Institute of Antimicrobial Chemotherapy (Smolensk), followed by the analysis of melting curves temperature (HRM analysis) in diagnostic practice, in order to detect genetic mechanisms of the chlamydia resistance to fluoroquinolones and confirm the resistance in cases of "clinically" unsuccessful treatment.
- To continue and expand the study on the "classical" genetic mechanisms of chlamydia resistance to fluoroquinolones, in order to clarify the epidemiological assessment of the resistance prevalence in the territory of the Republic of Kazakhstan.

Introduction of results to practice

The research results obtained during the preparation of this thesis work, as well as on the basis of the analysis of literature data, national STI (sexually transmitted diseases) programs and WHO recommendations, are introduced to clinical practice and the work of the Department of Obstetrics and Gynecology. At present, the principle of a laboratory test for the detection of chlamydia resistance to fluoroquinolones has been introduced, and a form of a questionnaire for collecting information on the sexual behavior of people has been developed to assess the risk of STI infection (Appendixes A and B).

The main provisions of the thesis work placed for defense

1. The proportion of chlamydial infection in modern gynecological practice in Karaganda, estimated on the basis of PCR-based diagnostics, was 11% (95% PS: 8.57-14.02).
2. The absence of clinical symptoms in sexual infections, as well as earlier initiation of sexual activity, is risk factors for STI infection.
3. The low prevalence of fluoroquinolone resistance in chlamydia, assessed by the determination of the mutational profile in the target genes of topoisomerases,

allows using drugs of this group as an empirical antibacterial therapy at the present stage.

4. In silico research using bioinformatic and statistical approaches based on the full genomic sequences of pathogens from the STI group, including C.trachomatis, is an important informative tool that provides data not only on the presence / absence of mutations associated with resistance, but also on resistance genes, as well as data on pathogenicity and intraspecific clustering factors.

Link of the thesis work with other research papers

This thesis work was carried out within the framework of international cooperation between the Karaganda State Medical University (Karaganda, Kazakhstan) and the State Budget Educational Institution Smolensk State Medical Academy of the Ministry of Health of the Russian Federation (Smolensk, Russia).

Thesis work approbation

The main provisions and results of the work were reported at:

The 26th European Congress of Clinical Microbiology and Infectious Diseases - ECCMID, Amsterdam (Netherlands), 9-12 April 2016;

XVIII IACMAC International Congress “Antimicrobial Therapy”, Moscow (Russia), May 25-27, 2016

International Conference of Young Scientists "The World of Science and Youth: New Ways of Development", Karaganda (Kazakhstan), April 12, 2016.

25th European Congress of Obstetrics and Gynecology in conjunction with the 15th Congress of the Turkish Association of Gynecologists and Obstetricians - EBCOG, Antalya (Turkey), May 17-21, 2017

XIX IACMAC / ESCMID International Congress “Antimicrobial Therapy”, Moscow (Russia), May 17-19, 2017

International Conference of Young Scientists "The World of Science and Youth: New Ways of Development", Karaganda (Kazakhstan), April 12, 2017.

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The pre-defense was held at a meeting of the Scientific-Expert Commission of the KSMU, Protocol No. 9 of June 29, 2017.

Publications

On the basis of the thesis materials 11 works were published. In the publications recommended by the Committee for Control in the Sphere of Education and Science, 7 papers were published. In the publications included in the Scopus database, 1 article was published.

Structure and volume of the thesis work

The thesis work includes 99 pages, consists of the following parts: introduction, main part, conclusion and list of cited references, including 259 references to sources. The work contains 11 tables, 18 figures and 3 appendixes.

Materials and methods

The primary stage of the current research included reception, registration and survey of patients who applied to the City Hospital No 1. After inspection of the presence of clinically significant inflammation symptoms, the results of which were made in questionnaire and register of patients, the sampling of clinical material - urogenital scraping was performed. Subsequently, the samples were directed to the clinical diagnostic laboratory for the diagnosis of a wide range of STI pathogens, including chlamydia, ureaplasma, mycoplasma, gardnerella, trichomonas, candida, cytomegalovirus and herpes simplex virus type 1/2, based on PCR studies.

According to the laboratory study results, as well as compliance with the inclusion / exclusion criteria, DNA samples with positive PCR results for C. trachomatis DNA were sent to State Budget Educational Institution Smolensk State Medical Academy of the Ministry of Health of the Russian Federation (Smolensk, Russia). Together with a group of researchers, headed by Eidelstein Inna Alexandrovna, a PCR analysis for the presence of mutations in the DNA-gyrase and topoisomerase IV genes in the QRDR region by high-resolution melting was conducted.

At the next stage, it was decided to carry out a bioinformatic analysis of the genomic data of C.trachomatis on the presence of single nucleotide substitutions in the region of the connection of fluoroquinolones with target genes. As a result of qualitative assembly of DNA sequences into longer contiguous and scaffolds,903 full genomic sequence was obtained in which the internal fragments of gyrA and parCen were detected by virtual PCR. The multiple alignment method made it possible to detect SNP in the desired genes and determine the type of mutations.

In the final stage, the test of cure of both PCR diagnosis of C.trachomatis and the method of examining patients for signs of inflammation was performed.

Conslusions:

1. The structure of the STI obtained on the basis of PCR diagnostics is shown in the following form: U.parvum (34.5% (95% CI: 30.54-38.68)), Candidaalbicans 28.5% (95% CI: 24 , 73-32.46). Herpes simplex virus type I / II 24.2% (95% CI: 20.73-28.09). G.vaginalis 21.5% (95% CI: 18.1-25.2). U.urealyticum 20.4% (95% CI: 17.1-24). CMV 19.5% (95% CI: 16.3-23.1). M.hominis 15.9% (95% CI: 12.88-19.18). M.genitalium 8.8% (95% CI: 6.63-11.59). T. vaginalis 2.75% (95% CI: 1.61-4.6). Chlamydiatrachomatis accounted for about 11% (95% CI: 8.57-14.02).
2. In all cases, a statistically significant difference between the presence of symptomatic signs and the STI etiologic agent ($p > 0.05$) was not detected, except in the case of M.hominis ($p = 0.0237$). This fact shows that the absence of symptoms occurs in the same way as the presence of clinical symptoms, which, in turn, is a factor that increases the risk of STI acquiring.

3. Early sexual debut is a statistically significant factor that increases the risk of STI acquiring. It was found that an earlier sexual activity for every 1-4 years increases the risk of infection by 1.16 times (p = 0.0480).

4. Analysis of mutations in the QRDR region of gyrA and parC genes associated with resistance to fluoroquinolones, collection of clinical isolates of C. trachomatis (n = 55) showed that chlamydia resistance to fluoroquinolones is observed in 0% (95% CI: 0-6.5%).

5. In silico studies on the genome sequences of the international collection of Chlamydiatrachomatis (n = 913) revealed chlamydia resistance to fluoroquinolones at 0.1% (95% CI: 0.002-0.6%).

Bibliography:

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