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ABSTRACT

of the dissertation for the degree of Doctor of Philosophy

DIAGNOSTIC SIGNIFICANCE OF MOLECULAR MARKERS DURING CHRONIC INFLAMMATORY DISEASES OF THE INTERNAL GENİTAL ORGANS IN WOMEN DURING THE REPRODUCTIVE PERIOD

Speciality: 3215.01 – Obstetrics and gynecology

Field of science: Medicine

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Dissertation work was performed at the I Department of Obstetrics and Gynecology of Azerbaijan Medical University and at the Institute of Biophysics of ANAS.

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GENERAL DESCRIPTION OF THE WORK

Relevance of the topic. Chronic inflammatory diseases of internal genital organs (CIDIGO) in women are one of the actual problems of gynecology [1].

According to the number of applications to antenatal clinics, inflammatory processes make up 60-65% of gynecological diseases, and 30% of inpatients [2].

Persistent pelvic inflammatory disease leads to menstrual dysfunction in 50-80% of patients[3]. Approximately 20% of women with CIDIGO develop infertility and 40% have chronic pain [4]. Chronic salpingo-oophoritis is still high in the structure of women's diseases, accounting for 35% of CIDIGO[5]. Studies have shown that

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18% of women with CIDIGO between the ages of 20 and 24 experience infertility and 16,8% develop ectopic pregnancy[6].

The examination and treatment measures often do not allow complete recovery and elimination of complications [7].

The hidden forms of the disease, which are difficult to diagnose, are the main problem in solving this issue, because the delay in examinations, the incorrect assessment of the severity of the disease and the inadequate therapy carried out in this regard lead to the chronicity of the process [8].

It is required to improve the diagnosis of chronic endometritis (CE) and chronic salpingo-oophoritis (CSO) to develop safer and more effective methods [9].

The development of modern criteria for the detection and planning of the course of CIDIGO in women is of great interest.

In recent years, molecular analysis methods have been widely used to search for new markers of various diseases in blood serum[10].

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Infrared radiation was discovered in 1800 by the English scientist W. Herschel. Infrared (IR) radiation is electromagnetic radiation with wavelengths between 760 nm and 100,000 nm [11].

Fourier transform IR spectroscopy is widely used in medicine[12] and pharmaceuticals [13].

Taking into account the wide application of IR-spectrography in medical examinations, its use as a safe and effective method in women with CIDIGO, comparative analysis of the obtained results has been proposed as an urgent issue.

Object of research. The study included 100 women receiving treatment at the Educational-Surgical Clinic of Azerbaijan Medical University, on the basis of the Department of Obstetrics and Gynecology, in 2017-2021. These women were divided into two groups: I main group included 60 women with chronic endometritis, II main group included 40 women with chronic salpingo-oophoritis. 30 practically healthy women were included in the control group.

Purpose of the study: Development of new prognostic markers by applying modern molecular examination methods in the examination of chronic inflammatory diseases of internal genital organs in women of reproductive age.

The tasks of the research:

1. Comparative analysis of the course of chronic endometritis and chronic salpingo-oophoritis in women of reproductive age with

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chronic inflammatory diseases of the internal genital organs by conducting clinical and anamnestic examinations;

2. Comparative analysis of the results of microbiological, biochemical, hormonal, instrumental examinations in women of reproductive age with chronic endometritis and chronic salpingo-oophoritis;

3. Identification of potential molecular markers of the inflammatory process in blood serum during CIDIGO in women of reproductive age;

4. Comparative definition of diagnostic value and differentiation of criteria by determination of frequency of spectral peaks of blood serum content on infrared spectrophotometry in women of reproductive age with chronic endometritis and chronic salpingo-oophoritis.

5. Development of an optimized examination algorithm for differential molecular markers of chronic endometritis and chronic salpingo-oophoritis in women of reproductive age by infrared spectrophotometry.

Research methods:

- Clinical and anamnestic;
- Microscopic examination;
- Biochemical examination of blood;
- Hormonal examination;
- Ultrasound examination and dopplerometry;
- Infrared spectroscopy of blood serum;
- Mathematical-statistical analysis methods.

The main provisions of the dissertation defended:

1. Among women of reproductive age with chronic inflammatory diseases of the internal genital organs, the middle-upper reproductive age prevailed and a low level of education was determined.

2. Taking into account that after the treatment of chronic endometritis and chronic salpingo-oophoritis in women of reproductive age, clinical-laboratory and instrumental examination

parameters, as well as CRZ, which is considered a necessary inflammatory marker, returned to normal, lower or upper limits of the normal in a statistically honest manner, it was not considered appropriate to use them as markers of chronicity.

3. IR-spectrometry results of chronic endometritis and chronic salpingo-oophoritis did not change after the treatment measures taken, which allows to use it as an effective marker to show the chronicity of the process.

4. During IR-spectrophotometry in women of reproductive age with CIDIGO, it was possible to perform a differential examination as a result of distinguishing the molecular composition of blood serum and markers with high diagnostic value for similar nosologies such as chronic endometritis and chronic salpingo-oophoritis.

Scientific novelty of the research

İn modern times, with the use of clinical, anamnestic, microbiological, biochemical, hormonal and instrumental methods of examination during CIDIGO in women of reproductive age, the ideas about the clinical course, clinical characteristics and pathogenesis of the disease have been expanded. Diagnostic markers of CIDIGO were determined by the method of IR-spectrophotometry examination and scientifically substantiated by statistical methods in a comparative way. By determining the blood serum substance profile through IR-spectrophotometry, CE and CSO differential diagnostic markers have been identified and scientifically substantiated.

Practical significance of research

The expediency and relevance of studying molecular markers in blood plasma in chronic endometritis and chronic salpingo-oophoritis has been substantiated, which allows to expand the understanding of the pathogenesis of the diseases. IR-spectrophotometry determined the main inflammatory markers for CIDIGO in women. Through this examination method, it was possible to accurately differentiate between two inflammatory nosologies of the reproductive system. O-H stretching, N-H

stretching and Amide II group for chronic endometritis; CH_2-CH_3 and Amide I groups for chronic salpingo-oophoritis, have been proposed as markers with a characteristic high diagnostic value. The study of molecular characteristics during CIDIGO in women of reproductive age was recommended as an effective screening method, allowing to detect the disease at an early stage, to clarify the chronification process. The developed examination algorithm allows optimizing the examination and along with this treatment and preventive measures in women of reproductive age with sufficient medical and social significance CIDIGO.

Application of research results. Practical recommendations proposed in scientific work, algorithms for examining patients with chronic endometritis and salpingo-oophoritis are applied in the activities of the Educational-Surgical Clinic of AMU. The dissertation materials are used in the educational process of the I Obstetrics and Gynecology Department of AMU.

The organization in which the dissertation work was carried out. The work was performed at the Educational-Surgical Clinic of Azerbaijan Medical University, at the Department of Obstetrics and Gynecology and at the Institute of Biophysics of ANAS.

Approbation of research work. The results of the research work were reported at the following scientific conferences:

1. VIII International Scientific and Practical Conference Brain & Heart, Odessa, September 6-7, 2018;

2. The 27-th World Congress on Controversies in Obstetrics, Gynecology and Infertility (COGI) All About Women`s Health, Paris, November 21-23, 2019;

3. Oncology-XXI century: proceedings of the XXIV International Scientific Conference "Oncology-XXI Century", X Italian-Russian Scientific Conference on Oncology and Endocrine Surgery, XXIV International Scientific Conference "National Health-XXI Century" (online), Perm – Istanbul, May 6-7, 2020;

4. International Health Sciences and Innovation Congress, Antalya, Turkey, December 18-21, 2021;

5. III Russian scientific and practical conference for obstetricians and gynecologists. Scientific program. St. Petersburg, November 12-13, 2021.

6. XXVIII Russian Congress with International Participation "Outpatient care at the epicenter of women's health from menarche to menopause", Moscow, April 8, 2022;

7. Materials of scientific-practical seminar dedicated to the 100th anniversary of great leader Heydar Aliyev. Baku, 25 may, 2023;

8. XVIII International Congress of Reproductive Medicine, Moscow, January 16-19, 2024.

9. II Eurasian conference of female inventors and technological entrepreneurs. Moscow, January 20, 2025

The results of the research work were reported and discussed on may 12, 2023 at the inter-departmental meeting of AMU (Protocol № 10), on May 3, 2024 at the scientific seminar of the dissertation Council No. 2.06 ED of AMU (protocol №11).

Published works. 20 scientific works on the topic of the dissertation (8 articles, 11 theses and 1 methodological manual) were published.

Volume and structure of the dissertation. The dissertation work is written in A4 format, in Azerbaijani language, using "Times New Roman", 14 fonts and 1.5 line spacing. The dissertation consists of 184 pages of computer text (223.385 marks), table of contents (5.900 marks), introduction (9.731 marks), literature review (49.272 marks), Chapter II on research materials and methods (14.630 marks), Chapter III on the results of personal research (83.515 marks), Chapter IV on justification of the selection of the examination method and discussion of the obtained results (16.154 marks), conclusions (39.415 marks), results (3.640 marks), practical recommendations (1.128 marks). The work is illustrated with 22 graphs, 37 tables and 5 pictures. The list of literature consists of 186 sources (of which 9 domestic, 177 Russian and other foreign).

MATERIALS AND METHODS OF RESEARCH

We applied the criteria proposed by the National Center for Disease Control and Prevention (US) in 2006 to investigate the diagnosis of chronic pelvic inflammatory disease.

130 women were included in the study. 100 of these women were diagnosed with CIDIGO. 30 practically healthy women were included in the control group. The average age of women was calculated as 32.5±1.3 years.

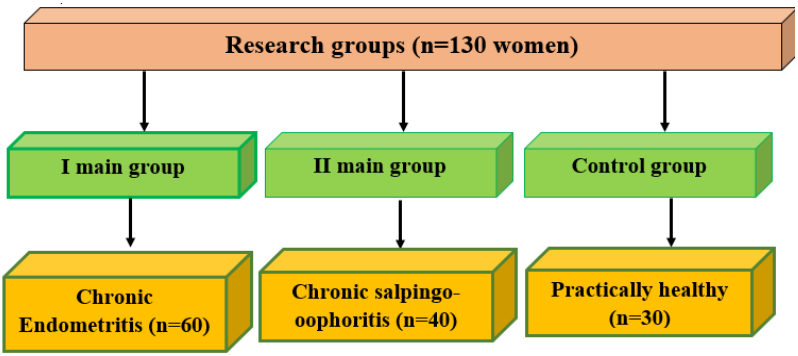


Figure 1. Research design

Inclusion criteria for the study:

1. Women aged 21-45;
2. Women who have had chronic endometritis and chronic salpingo-oophoritis;
3. Women with duration of the disease of more than 6 months;
4. Women with exacerbation of disease up to 3-4 times a year;
5. 6 months after the acute phase of the disease.

Exclusion criteria from the study:

1. Acute inflammatory diseases of female genital organs and urinary system;

2. Hormonal diseases of the female reproductive system;
3. Application of hormonal or intrauterine contraception;
4. Positive AIDS, syphilis, gonorrhea, tuberculosis tests;
5. Benign and malignant derivatives of small pelvic organs.

All patients underwent clinical, microbiological, biochemical (total protein, CRP, albumin, cholesterol, ALT, AST, total lipids, triglycerides and creatinine), hormonal (estradiol, progesterone, testosterone, follicle stimulating hormone (FSH), luteinizing hormone (LH), prolactin and cortisol) and instrumental examinations (USE, dopplerometry). IR-spectroscopy was performed as the main examination method. The obtained results were analyzed by mathematical-statistical methods.

Method of blood research in the Fourier Transform Infrared spectrometer. The measurements carried out in the study were carried out using a spectrometer of the German company Bruker (VERTEX 70VXSA). Thermostatic BioATR (ZnSe) accessories were used for measurements.

Statistical analysis of the material. The obtained data were statistically processed using Wilcoxon (W), Student (T), non-parametric test (Mann-Whitney (U), $\chi 2$-Pearson and Fisher (f) exact test. Between-group differences were assessed by one-way analysis of variance (ANOVA (A)). MS Excel, Statistics 6.0 and SPSS 24 statistical packages were used in the statistical process.

RESEARCH RESULTS AND THEIR DISCUSSION

Education, social status and previous diseases were analyzed as socio-medical characteristics of women.

Women were grouped according to secondary education, secondary vocational education and higher education. In all three groups, more women received secondary education. 26 (43,3%) women in group I, 18 (45,0%) in group II and 13 (43,3%) women in the control group received secondary vocational education. 19 (31,7%) women in group I, 15 (37,5%) women in group II and 9

(30,0%) women in the control group were satisfied with only secondary education. The number of women who completed higher education was less in all 3 groups. 15 (25,0%) women in main group I, 7 (17,5%) in group II and 8 (26,7%) women in control group graduated from higher education.There were no differences between the groups according to the level of education.

There were 12 (20%) housewives in group I, 20 (50%) in group II and 6 (20%) in the control group. There were 14 (23,3%) students in group I, 9 (22,5%) in group II and 7 (23.3%) in the control group. There were 4 (6.7%) female civil servants in group I, 3 (7,5%) in group II and 2 (6,7%) in the control group. 30 (50%) women worked in group I, 8 (20%) in group II and 15 (50%) in the control group. According to the number of working women, group I was statistically higher than group II ($\chi2$=9,168, p=0,003, f=0,003, p<0,01), the control group was statistically higher than group II ($\chi2$=6,994, p=0,009, f= 0,011, p<0,01).

Most of the women were officially married, only 3 women (5%) in main group I and 7 women (17,5%) in main group II were single.

Among somatic pathologies, gastrointestinal tract diseases were not statistically significantly higher in group I (73.3%) than in group II (70%) ($\chi2$=0.132, p=0.717, f=0.821), group I compared to the control group (76.7%) ($\chi2$=0.117, p=0.733, f=0.802) and group II compared to the control group ($\chi2$=0.385, p=0.535, f=0.596; p>0.05). There were no statistically significant differences in nose-throat-ear diseases, urinary system and cardiovascular diseases.

When considering abdominal surgical interventions, group I indicators (68,3%) were higher than group II (35,0%) ($\chi2$=10,774, p=0,002, p<0,01), as well as indicators in group I compared to the control group (16,7%) was statistically high ($\chi2$=21,366, p<0,001).

The age of menarche among women was determined. Only in the control group was 1 (3,3%) woman with menarche before the age of 12 years. There were no statistically significant differences between the groups at 12, 13, 14, 15 years of age. There were 3 (5%)

women in group I and 1 (2,5%) women in group II, whose menarche was at 16 years of age. 2 (3,3%) women with menarche at the age of 17 were from group I. Women whose menarche was later than 17 years old were not in any group.

Among genital diseases, colpitis was 58,3% in group I, 75,0% in group II and 36,7% in the control group. Colpitis in II group was statistically significantly higher than in the control group ($\chi 2$=10,381, p=0,002, f=0,002, p<0,010). Cervical disease was statistically significantly higher in group II (50,0%) than in the control group (23,3%) (p<0,05). Benign ovarian neoplasias were statistically significantly higher in group I (25%) than in the control group (6,7%) (p<0,05). Hormone-related diseases of the endometrium were statistically significantly higher in group II (25%) than in the control group (p<0,05) and in group I (33,3%) than in the control group (3,3%) (p<0,001).

A statistically high difference between I (13,3%) and control (83,3%) ($\chi 2$=32,443, p<0,001), II (15%) and control groups ($\chi 2$=42,201, p<0,001) was determined due to the absence of algodismenorrhea in women.

Study groups were analyzed according to reproductive functions. The number of nulliparous women was statistically higher in group I (21,7%) than in group II and in group II (47,5%) compared to the control group (13,3%) (p<0,01). There were no statistically significant differences between the groups according to the number of 1 and 2 births in the anamnesis. The women of group II did not have 3 births. More women without abortion were in the control group (93,3%). The number of these women in group I (38,3%) and group II (52,5%) was statistically less than the control group (p<0,001). Women with 1-2 abortions were significant more in groups I (46,7%) and II (35%) than in the control group (6,7%). Women who had 3-4 abortions were not in the control group. Miscarriages were significantly higher in groups I (35%) and II (30%) than in the control group (3,3%) (p<0,001). Ectopic pregnancy

was significantly higher in group II (30%) than both group I (8,3%) and control group (3,3%) (p<0,01).

There was no statistical difference between women in groups I (20%) and II (37.5%) in terms of the number of infertility cases. However, there is a statistical difference between the control (6.7%) and group II women ($\chi 2$=8.863, p=0.003, f=0.004, p<0.05). Primary infertility did not occur in the control group. There were no significant differences between the groups for primary and secondary infertility.

Clinical symptoms of CIDIGO were analyzed in study groups. In group I, general weakness (88,3%), pain in the lower abdomen (95%) and irritability (76,7%) were more, high temperature (18,3%), dysuria (31,7%) and dyspepsia (20%) were less. In group II, pain on bimanual examination (77.5%), irritability (90%) and growth of appendages (72.5%) were more, headache (30%), high temperature (40%) and dyspepsia (40%) were less common.

The duration of CE and CSO was studied (Table 1).

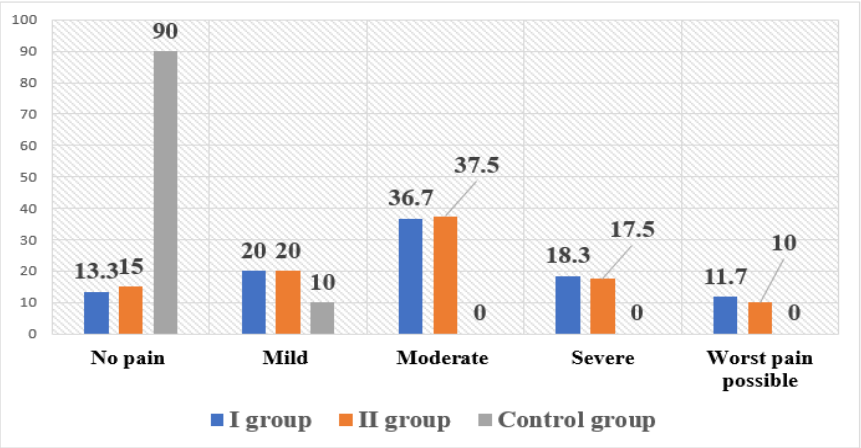
Table 1.

Distribution of patients according to the duration of the disease

Periods	I main group (n=60)		II main group (n=40)		p
	Abs.n	%	Abs.n	%	
2-3 year	12	20,0	8	20,0	P >0,05
3-5 year	14	23,3	17	42,5	P <0,05
6-10 year	26	43,3	9	22,5	P <0,05
<10 year	9	15,0	6	15,0	P >0,05

Menstruation, hypothermia, abortion, ARVI, and unknown causes were found as factors contributing to the development of CIDIGO in the study groups and there were no significant differences between the groups.

The severity of pain between groups in patients was determined according to the D. Barlow scale (Graph 1).



Graph 1. Dynamic description of pain sensation by severity in study groups

It has been established that the secretions are curd-like, mucous, purulent. Mucous and purulent secretions were absent in the control group. There were no significant differences in secretions between the main groups.

According to regular use of barrier contraceptives, no statistical difference was found between groups I and II ($\chi 2=0,554$, $p=0,457$, $f=0,492$; $p>0,05$), statistical difference was recorded between I and control, II and control groups (respectively, $\chi 2=15,977$, $p<0,001$; $\chi 2=9,287$, $p=0,003$, $f=0,003$, $p<0,01$).

The number of women with ≥2 sexual partners was small in the groups (13,3%, 15%, 3,3%, respectively) and did not differ significantly. There were no women in the control group who had not had sex in the last 6 months and there were no differences between groups I (5%) and II (4%). Sexual life started at the average age of

21 years in group I, 20 years in group II and 22 years in the control group.

Microscopic examination of vaginal smears did not reveal normocenosis in group II. In the control group (70%) was more significant than in the I group (1,7%) ($p_{I-c}<0.001$).
Mesosinosis was slight in all 3 groups (3,3%, 5%, 10%), no significant differences were found. Aerobic vaginitis was significantly higher in groups I (26,7%) and II (20%) than in the control group (3,3%) ($p<0,05$). There were no significant differences between the main groups for bacterial and candidal vaginitis. The coexistence of candidiasis and aerobic vaginitis was significantly higher in groups I (23,3%) and II (47,5%) ($p<0,001$) than in the control group (3,3%) ($p<0.05$).

In the study groups, rod-shaped flora, mixed flora and coccal flora were found. The rod-shaped flora was found in 8,3% in the I main group, 7,5% in the II main group and 53,3% in the control group. Mixed flora was found in 50% of the main group I, in 42,5% of the main group II and in 10% of the control group. Mixed flora is more common in main group I. Coccal flora was identified in 41,6% of the main group I and in 50,0% of the main group II. This type of flora was not detected in the control group. The number of women with a large amount of bacteria in the smear was 76,7% in group I and 57,5% in group II.

We grouped the number of leukocytes in the smear as 0-10, 10-20, 20-40 and more than 40 in the visual field. The number of leukocytes in the field of vision was less than 10 in 25% of the CE group, 42.5% in the CSO group and 6.7% in the control group. According to this indicator, the number of women in the main groups was statistically significantly higher than that of healthy women ($p<0.001$, $p<0.05$). In the smear of healthy women, the number of 10-20, 20-40 and more than 40 leukocytes in the field of vision were not detected. There were no honest differences between women with CE and CSO due to the number of leukocyte counts of 10-20, 20-40 and more than 40 in the field of vision.

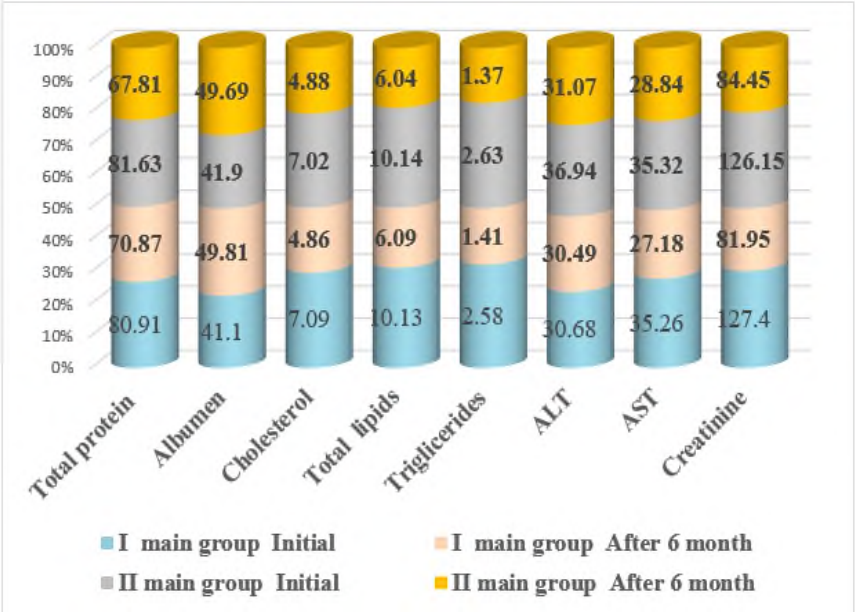
In group I sterile uterine cavity aspirate sample was recorded only in 1 (1.7%) woman. There was no sterile aspirate sample in group II. Lactobacillus spp. monocultures were detected in 20.0% of the CE group and 22.5% of the CSO group. Lactobacillus spp. along with Bifidobacteria spp. 20.0% were identified in the CE group and 12.5% in the CSO group.

In the microbiological examination of the aspirate taken from the uterine cavity, facultative anaerobic flora prevailed over conditionally pathogenic microflora. Low titer of Enterobacteriaceae and obligate anaerobes were statistically significantly higher in CE and Gardnerella vaginalis in CSO ($p<0.01$). Enterobacteriaceae and obligate anaerobes with high titer were found in CSO, while Gardnerella vaginalis had a statistically significantly higher in CE ($p<0.01$). Candida albicans was found in both groups with a low titer.

A comparative analysis of laboratory and instrumental parameters of patients with chronic endometritis and salpingo-oophoritis at the initial visit and 6 months after treatment was performed.

The necessary biochemical parameters were studied in the research groups (Graph 2). During biochemical examinations, the level of total protein, albumin, cholesterol, total lipid, triglyceride, ALT, AST, creatinine parameters was determined and their significant normalization was observed. However, in the I main group, only the level of ALT did not differ statistically significantly 6 months after treatment.

The biochemical marker CRP, which is of particular importance as an inflammatory marker, we also studied at the initial visit and after 6 months. Between the groups, CRP was statistically significantly higher according to Mann-Whitney at the initial examination ($U_{I/II}=1952,0$, $U_{I/c}=1800,0$, $U_{II/c}=1200,0$ $p<0,001$).



Graph 2. Graphic representation of average indicators of biochemical examination in study groups

After 6 months, in group I was statistically significantly higher than in group II ($U_{I/II}$=1917,5, p<0,001), as well as in group I than in the control group ($U_{I/c}$=1387,0, p<0,001). According to the Student (T) criterion, a statistical difference was recorded between CRP indicators in group I ($T_{initial/6months}$=10,034, p<0,001) and in group II ($T_{initial/6months}$=23.553, p<0,001) between initial visit and 6 months later.

Hormonal tests were performed between the study groups. It was found that estradiol levels were statistically significantly lower in women with CSO than in women with CE and healthy women, as well as in women with CE than in healthy women (p<0.001). No statistically significant differences were found between groups in progesterone levels (p>0.05). Testosterone and prolactin levels were

statistically significantly higher in women with CE and CSO than in healthy women ($p<0.001$). The levels of FSH ($p<0.001$) and LH ($p<0.05$) were statistically significantly higher in women with CE than in healthy women. Cortisol levels were statistically significantly higher in women with CE and CSO than in healthy women ($p<0.001$). Thus, although there were some statistically significant differences in hormone levels between the groups, the indicators varied within the normal range.

USE was performed in the groups and it was determined that the parameters studied at the initial visit were higher than the indicators after 6 months. Thus, it is known from the results conducted with the Wilcoxon parametric criterion that there are significant differences in the characteristic indicators of each ovary.

Indicators of right ovaries (length, width, thickness) in group I are W_L = 2811,0, W_w = 2586,5, W_T = 2831,5; in group II – W_L=1295,0, W_W = 1352,5, W_T = 1525,5.

Corresponding indicators (length, width, thickness) for left ovaries in group I are W_L = 3479,0, W_W = 3420,0 and W_T =3600,0; in the II main group – W_L = 1095,0 ($p=0,004$), W_W = 1146,5, W_T = 706,5, ($p=0,356$, there is no difference for the W_T indicator) ($p<0,001$). During the comparative assessment of the indicators of the right and left ovaries between the groups at the initial visit and 6 months later, it was revealed that there was a significant statistical difference ($p<0,05$).

According to the Student's criterion, there were significant differences in the measurements of the uterus (length, width, front-back measurement) at the initial examination and after 6 months: group I - T_L=48,21, T_W= 32,44, T_{FBM} = 38,11 ($p<0,001$); II group – T_L = 23,22, T_W = 17,75, T_{FBM} = 7,32 ($p<0,001$).

Thus, in patients with chronic endometritis and chronic salpingo-oophoritis, there were significant differences in USE parameters compared to healthy women.

Within the scope of the study carried out by us, the Pulse Index (PI), Resistance Index (RI) and systolic-diastolic ratio in the left and

right uterine arteries and ovarian artery in follicular (phase I) and lutein phases (phase II) of the menstrual cycle in endometritis, salpingo-oophoritis and practically healthy women were determined.

In the initial examination, the differences of PI of the left uterine artery in phase I by groups ($U_{I/II}$ = 1987,5; $U_{I/c}$ = 1577,5, $U_{II/c}$ = 987,0, p<0,001), RI ($U_{I/II}$=1938,0, $U_{I/c}$=1430,0, p<0,001), SDR ($U_{I/II}$=2400,0, $U_{I/c}$=1800,0, p<0,001) were significant. In the initial examination, differences in PI ($U_{I/II}$ = 2258,0, $U_{I/c}$ = 1696,0, p<0,001), RI ($U_{I/II}$ = 2218,0, $U_{I/c}$ = 1689,0, p<0,001) and SDR ($U_{I/II}$ = 2273,0, $U_{I/c}$ = 1800,0, $U_{II/c}$ = 204,0, p<0,001) parameters of the left uterine artery in phase II were significant.

In phase I, there was a statistical difference between the groups on the parameters of the right uterine artery: PI ($U_{I/II}$ =20,5, $U_{I/c}$ = 289,0, $U_{II/c}$ = 36,5, p<0,001), RI ($U_{I/II}$ = 1655,0, $U_{I/c}$ = 1728,500, $U_{II/c}$ = 0,5, p<0,001), SDR ($U_{I/II}$ = 2371,5, $U_{I/c}$ = 1792,0, $U_{II/c}$ = 316,5, p<0,001). In the II phase, a statistical difference was recorded between the groups on PI ($U_{I/II}$ = 1909,5, $U_{I/c}$ = 1573,0, $U_{II/c}$ = 214,5, p<0,001), RI ($U_{I/II}$ = 2327,5, $U_{I/c}$ = 1759,5, $U_{II/c}$ = 317,0, p<0,001) and SDR ($U_{I/II}$ = 2400,0, $U_{I/c}$ = 1800,0, $U_{II/c}$ = 417,0, p<0,001) parameters.

After 6 months, there was an significant difference between PI ($U_{I/c}$ = 1525,0, $U_{II/c}$ = 987,0 p<0,001), RI ($U_{I/II}$ = 1911,5, $U_{I/c}$ = 1385,5, p<0,001), SDR ($U_{I/II}$ = 2400,0, $U_{I/c}$ = 1800,0, p<0,001) parameters in the left uterine artery in phase I.

After 6 months, in the second phase of the cycle, a statistical difference was determined between PI ($U_{I/II}$ = 2016,0, $U_{I/c}$ = 1440,0, p<0,001) and RI ($U_{I/c}$ =1225,0, p=0,005, p<0,010; $U_{II/c}$ = 769,0 p=0,043, p<0,050) indicators, and SDR ($U_{I/II}$ = 465,0; $U_{II/c}$ = 996,0, p<0,001) in the left uterine artery.

After 6 months, in phase I of the cycle, a statistical difference was recorded for PI ($U_{I/II}$ = 738,0, p=0,001, p<0,001; $U_{II/c}$ = 843,5, p=0,004, p<0,010) and SDR ($U_{I/II}$ = 1511,5, p=0,028, p<0,050) in the right uterine artery. After 6 months, in the II phase of the cycle, there were significant differences in the indicators of PI ($U_{I/II}$ = 1969,0, $U_{I/c}$

= 1489,5, p<0,001), RI ($U_{I/II}$ = 1818,5, p<0,001; $U_{II/c}$ = 374,0, p=0,007, p<0,010) and SDR ($U_{I/II}$ = 1928,0, $U_{II/c}$ = 239,0, p<0,001) of the right uterine artery.

During the dopplerometric examination of the ovaries, the main parameters were determined only in women with CSO. At the first visit, in phase I, PI ($U_{II/c}$ = 1200,0, p<0,001), RI ($U_{II/c}$ = 1200,0, p<0,001) indicators of the left ovarian artery were statistically significantly higher than in the control group.

In the initial examination, there were significant differences in RI ($U_{II/c}$ =1200,0, p<0,001) and PI (p<0,001) in phase II of the left ovary.

In phase I, there were significant differences in the indicators of PI ($U_{II/c}$ = 1200,0, p<0,001), RI ($U_{II/c}$ = 1200,0, p<0,001) of the right ovarian artery.

In the initial examination, there were significant differences in PI ($U_{II/c}$ = 1200,0, p<0,001), RI ($U_{II/c}$ =1200,0, p<0,001) of the right ovarian artery in phase II.

After 6 months, no statistically significant differences were found in the parameters of PI ($U_{II/c}$ = 536,0, p=0,445, p>0,050), RI ($U_{II/c}$ = 580,5, p=0,815, p>0,05), SDR ($U_{II/c}$ = 757,0, p=0,061, p>0,050) in the left ovarian artery in phase I. In phase II, although the difference in the PI ($U_{II/c}$ = 430,5, p=0,044, p<0,050) indicator was found, statistically significant differences in the RI ($U_{II/c}$ = 492,0, p=0,197, p>0,050) and SDR ($U_{II/c}$ =549,0, p=0,543, p>0,050) parameters were not determined.

After 6 months, no statistically significant difference was detected in the PI parameters ($U_{II/c}$ = 575,5, p=0,770, p>0,050) of the right ovarian artery in phase I, but a statistically significant difference was detected in terms of RI ($U_{II/c}$ = 367,0, p=0,005, p<0,010) and SDR indicators ($U_{II/c}$ =192,5, p<0,001). In phase II, no statistical difference was observed between the PI ($U_{II/c}$ = 454,0, p=0,080, p>0,050) and RI ($U_{II/c}$ = 487,0, p=0,176, p>0,050) indicators between the groups, but a difference was recorded in SDR ($U_{II/c}$ = 968,0 p<0,001).

Thus, in the right and left arteries of the ovaries, significant differences were noted in parameters of PI, RI and SDR.

We determined the diagnostic and prognostic value of molecular markers in blood serum in the diagnosis of chronic endometritis and salpingo-oophoritis. During the examination, initially, the spectrogram was conditionally divided into 5 rows and the spectral peaks were recorded for them (Table 2).

Table 2.
Quantitative indicators of peaks and absorption band parameters during IR-spectroscopy

Indicators	Chronic endometritis (n=60)		Chronic salpingo-oophoritis (n=40)	
	Spectral peak (cm^{-1})	Absorption band	Spectral peak (cm^{-1})	Absorption band
Row 1	1570,2±24,7	0,16±0,04	1529,3±46,4	0,14±0,02
Row 2	1634,2±12,8	0,16±0,02	1691,6±24,4	0,18±0,03
Row 3	2808,0±103,7	0,05±0,01	2948,0±120,7	0,06±0,02
Row 4	3340,0±107,8	0,09±0,05	3076,0±247,7	0,06±0,02
Row 5	3524,0±66,6	0,07±0,02	3210,5±246,9	0,06±0,02

Note: $p<0,001$ statistically significant; $p>0,05$ statistically non-significant.

Based on the calculations, significant changes in spectral analysis of spectral peaks were found in patients with endometritis and salpingo-oophoritis. The nature of the obtained values is significantly different from the normative data.

The marker Amide II group obtained on row 1, according to the spectral peak ($U_{I/II} = 492,5$, $p<0,001$) and absorption band ($U_{I/II} = 412,5$, $p=0.024$, $p<0.050$) between groups I and II was a statistically significant difference. A statistically significant difference was observed in spectral peak ($U_{I/c} = 405,0$, $p<0.001$) and absorption band ($U_{I/c} = 384,5$, $p<0,001$) between I and control groups. No statistical difference was observed between the II and control groups

on spectral peak ($U_{II/c}$ = 139,5, p=0,723) and absorption band ($U_{II/c}$ =195,5, p=0,123, p>0,050).

The marker obtained on row 2 corresponded to marker Amide I group and the spectral peak ($U_{I/II}$ = 181,0, p=0,018) and absorption band ($U_{I/II}$ =187,5, p=0,024, p<0,050) indicators between groups I and II differed statistically significant. A statistical difference was recorded between the I and control groups according to the spectral peak ($U_{I/c}$ =312,0, p=0,036, p<0,050), no statistical difference was recorded between the groups according to the absorption band ($U_{I/c}$ = 269,5, p=0,274, p>0,050). A statistically significant difference was recorded between II and control groups according to spectral peak ($U_{II/c}$ = 247,0, p<0,01) and absorption band ($U_{II/c}$ = 225,0, p=0,011, p<0,050).

The marker obtained on row 3 corresponded to marker CH_2-CH_3 group marker and a statistically significant difference was recorded between groups I and II according to the spectral peak ($U_{I/II}$ = 92,5, p<0,001) and absorption band ($U_{I/II}$ = 174,5, p=0,008, p<0,010). A statistical difference was recorded between the I and control groups according to the spectral peak ($U_{I/c}$ = 307,0, p=0,043, p<0,050) and absorption band ($U_{I/c}$ =450,0, p<0,001). A statistically significant difference on spectral peak ($U_{II/c}$ = 249,5, p=0,001, p<0,010) and absorption band ($U_{II/c}$ =300,0, p<0,001) was recorded between II and control groups.

The marker obtained on row 4 corresponded to the marker N-H stretching and a statistically significant difference between groups I and II was determined according to the spectral peak ($U_{I/II}$ = 477,0, p<0,001) and absorption band ($U_{I/II}$ = 408,0, p=0,031, p<0,050). A statistically significant difference between the I and the control groups according to spectral peak ($U_{I/n}$ = 450,0, p<0,001) and absorption band ($U_{I/c}$ =422,0, p<0,001) indicators was determined. A statistically significant difference between II and control groups was determined according to the spectral peak ($U_{II/n}$=212,0, p=0,036, p<0,050) and absorption band ($U_{II/n}$=269,0, p<0,001).

The marker obtained on row 5 corresponded to marker O-H stretching and a statistically significant difference was observed between groups I and II according to spectral peak ($U_{I/II}$=492,0, p<0,001) and absorption band ($U_{I/II}$ = 424,0, p=0,013, p<0,050). A statistically significant difference was observed between the I and control groups according to the spectral peak ($U_{I/c}$ = 440,0, p<0,001) and absorption band ($U_{I/c}$ = 390,0, p<0,001). A difference was observed between II and control groups according to the spectral peak ($U_{II/c}$ = 210,0, p=0,045) and absorption band ($U_{II/c}$ = 223,0, p=0,015, p<0,050).

It can be concluded that the application of IR-spectrophotometry, along with complex clinical and laboratory examination is of particular importance in determining systemic inflammation in patients with CIDIGO and studying the pathogenetic mechanism of the development of infectious-inflammatory diseases.

Determining the differential diagnostic value of markers obtained by IR-spectrophotometry.

As is known, IR-spectrophotometric parameters have varied in terms of diagnostic value for chronic endometritis in wide ranges. During CE, diagnostic sensitivity for Amide II was 79,31%, diagnostic specificity was 56,25% and diagnostic efficiency was 71,11%. Positive results of this molecular marker were determined in 46 (76,66%) women, negative results in 14 (23,33%) women.

The diagnostic sensitivity of group Amide I marker for chronic endometritis was 61,53%, the diagnostic specificity was 31,25% and the diagnostic efficiency was 40,0%. Positive results of this marker were determined in 16 (26,66%) women, negative results in 44 (73,33%) women.

Different results were also obtained for CE on the group CH_2-CH_3 marker. For this marker, positive results were achieved in 4 (6,67%) patients and negative results in 56 (93,33%) patients. Diagnostic sensitivity was 33,33% diagnostic specificity was 28,2% and efficiency was 28,88%.

We also analyzed N-H stretching during chronic endometritis. It was found that positive results were found in 60 (100%) women, there were no negative results. The fact that the positive results are at such a high level is also reflected in the diagnostic values. Diagnostic sensitivity for this marker was 81,1%, specificity was 100% and efficiency was 84,44%.

In chronic endometritis high values were determined for O-H stretching as well as for N-H stretching. Thus, 60 (100%) women had a positive result for these markers and there were no negative results. The superiority of the positive results of the marker has also shown itself in the diagnostic values. Thus, diagnostic sensitivity was 85,7%, specificity was 100% and efficiency was 88,88%.

From the conducted analysis, it is known that Amide II group, N-H stretching and O-H stretching had a high efficiency value for chronic endometritis. This shows the accuracy of the spectrophotometric value of these molecular markers for that inflammatory nosology.

The diagnostic sensitivity of Amide II for CSO was 40,0%, the diagnostic specificity was 36,0% and the diagnostic efficiency was 37,14%. Positive results of this marker were determined in 8 (20%) women, negative results in 32 (80%) women.

Diagnostic sensitivity of Amide I group for CSO was 70,58%, diagnostic specificity was 55,55%, diagnostic efficiency was 62,85%. Positive results of this marker were determined in 24 (60%) women, negative results in 16 (40%) women.

For CSO, different results were obtained for the marker CH_2-CH_3 group. According to this marker, positive results were achieved in 32 (80%) patients and negative results in 8 (20%) patients. Diagnostic sensitivity was 80%, diagnostic specificity was 73,33% and efficiency was 77,14%.

N-H stretching was also analyzed during CSO. It was found that positive results were recorded in 14 (35%) women and negative results in 26 (65%) patients. The low level of positive results is also reflected in the diagnostic values. Diagnostic sensitivity for N-H

stretching was 50%, specificity was 38,9% and efficiency was 42,85%.

Low values were determined during CSO for O-H stretching as well as for N-H stretching. Thus, a positive result for these markers was determined in only 4 (10%) and a negative result was determined in 36 (90%) patients. Thus, diagnostic sensitivity was 28,57%, specificity was 35,71% and efficiency was 34,28%.

From the conducted studies, it was found that the values of Amide I and CH_2-CH_3 group markers were very high in terms of higher diagnostic specificity, sensitivity and efficiency for chronic salpingo-oophoritis.

Optimized algorithm of examination during CIDIGO by IR-spectrophotometry. When our proposed markers are detected in certain ranges, it is possible to diagnose CIDIGO in women (Figure 2).

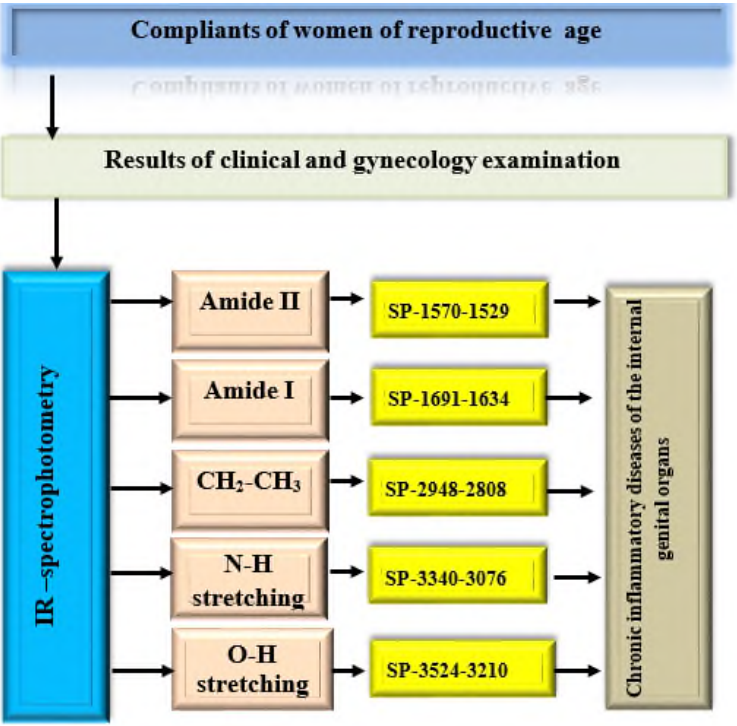


Figure 2. Optimal algorithm for detection of CIDIGO in women of reproductive age

Then we developed a schematic representation of markers for the differentiation of CE and CSO.

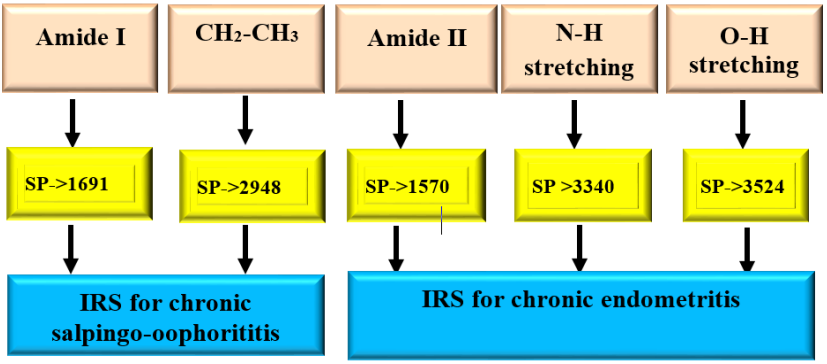


Figure 3. Schematic representation of the optimal differential algorithm for the İR-examination of CE and CSO

These images allowed the rapid and effective management of patients during differential examination of CE and CSO and the initiation of treatment measures at the maximum early stage. We consider it expedient to use Fourier transform IR-spectrophotometry as a sufficient and financially viable method of its application as a screening method, thereby early detection of a group of patients with CE and CSO (Figures 3).

Thus, our results not only have a positive effect on the protection of the health of women of reproductive age, but also contribute to the solution of a problem of medical and social importance, such as infertility.

RESULTS

1. In the prospective analysis of chronic inflammatory diseases of the internal genital organs, CE occurred mostly in the upper

reproductive age (50%), CSO occurred in the middle reproductive age (42,5%). Among somatic pathologies, diseases of the gastrointestinal tract prevailed (CE – 73,3%, CSO – 70,0%), from gynecological diseases - colpitis (CE – 58,3%, CSO - 75%). Intermenstrual bleeding was more prevalent in women with XE and XSO, 70% and 75%, respectively ($p<0.001$). 1-2 cases of abortion were recorded 55% in CE and 40,8% in CSO, infertility 20% in CE and 37,5% in CSO [3,5,7,15].

2. During microscopic examination, normosenosis was 70,0% in healthy women, but it was not present in women with CSO. Aerobic vaginitis (26,7%) and candidiasis (25%) prevailed separately in women with CE and these changes were recorded in 47,5% of women with CSO. In the examination of the aspirate taken from the uterine cavity, more conditionally pathogenic microorganisms were dominant. In the upper titer Gardnerella vaginalis in CE, Enterobacteriaceae and obligate anaerobs in CSO were statistically significantly higher ($p<0.01$). The level of C-reactive protein was – 13.02 in CE and 10.65 mg/l in CSO ($p<0.001$). Among biochemical indicators, total protein, cholesterol, triglycerides, AST and creatinine increased, while albumin decreased ($p<0.001$). Compared to healthy women, the differences on estradiol was significantly lower, testosterone, prolactin, cortisol and FSH statistically significantly higher in women with CE and CSO and differences in progesterone and LH were insignificant. There were also statistically significant differences ($p<0.001$) in the NI, RI parameters of the uterine artery during dopplerometry, which is an indicator of blood circulation disorders in the pelvic organs [7,8,12,14,18].

3. Markers corresponding to conventional 5 rows of spectral peaks obtained during İRS were determined: row 1 Amide II group (CE – 1570 cm^{-1}, CSO – 1529 cm^{-1}), row 2 Amide I group (CE – 1634 cm^{-1}, CSO – 1691 cm^{-1}), row 3 CH_2-CH_3 group (CE – 2808 cm^{-1}, CSO – 2948 cm^{-1}), row 4 N-H stretching (CE – 3340 cm^{-1}, CSO – 3076 cm^{-1}), row 5 O-H stretching (CE – 3524 cm^{-1}, CSO – 3210 cm^{-1}) [12,13,14,16,20].

4. Amid II group with high diagnostic sensitivity, diagnostic specificity, diagnostic efficiency (79.31%, 56.25%, 71.11%, respectively), O-H stretching (85.71%, 100.0%, 88.88%, respectively), N-H stretching (81.1%, 100.0%, 84.44%, respectively) in chronic endometritis and in chronic salpingo-oophoritis Amid I (respectively, 70.58%, 55.55%, 62.85%), CH_2-CH_3 group (respectively, 80.0%, 73.33%, 77.14%) groups were evaluated as high diagnostic markers [17].

5. Based on the results of detailed clinical and gynecological examinations for the maximal simplification of diagnostic measures, an algorithm was designed according to the levels of spectral peaks of markers of infrared spectrophotometry for chronic inflammatory diseases of the internal genital organs in general and chronic endometritis and chronic salpingo-oophoritis separately [19].

PRACTICAL RECOMMENDATIONS

1. Inflammatory diseases of the internal genital organs in women of reproductive age have a serious impact on the reproductive function and lead to infertility, so its timely and correct examination is of great importance. In this regard, timely and correct examination during chronic endometritis and chronic salpingo-oophoritis is of great importance.

2.Timely examination, treatment and rehabilitation of inflammatory diseases of the internal genital organs in women of reproductive age, prevention of recurrence is a necessary issue.

3. Application of infrared spectrophotometric analysis of blood plasma to determine molecular markers can be considered an effective diagnostic method for inflammatory diseases of internal genital organs in women of reproductive age. So, as a method of choice, it does not require expensive reagents and has high diagnostic efficiency.

4. It is considered appropriate to use an algorithm based on blood plasma spectrophotometric markers for rapid and differential diagnosis of chronic endometritis and chronic salpingo-oophoritis. Based on this algorithm, it is possible to approach patients with CE and CSO individually, choose the optimal treatment tactics and give the correct prognosis.

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LIST OF CONVENTIONAL ABBREVIATIONS

ALT	–	alanine aminotransferase
AST	–	aspartate aminotransferase
ARVI	–	acute respirator viral infection
CE	—	chronic endometritis
CIDIGO	–	chronic inflammatory diseases of internal genital organs
CSO	–	chronic salpingo-oophoritis
FBM	–	front-back measurement
FSH	–	follicle stimulating hormone
L	–	length of ovaries (uterus)
LH	–	luteinizing hormone
İR	–	infrared
İRS	–	infrared spectrophotometry
Pİ	–	pulse index
Rİ	–	resistance index
SDR	–	systolic-diastolic ratio
TO	–	thickness of ovaries
USE	–	ultrasound examination
W		the width of the ovaries (uterus)

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