UDC: 618.11-008.64:577.175.53:616.379-008.64

ANALYSIS OF THE INTERRELATIONSHIP BETWEEN INSULIN RESISTANCE, MICROBIOME IMBALANCE, AND EPIGENETIC FACTORS IN THE PATHOGENESIS OF POLYCYSTIC OVARY SYNDROME (PCOS), AND INTEGRATIVE APPROACHES TO THEIR CORRECTION

Nematova Maftuna Rakhmatilloyevna - assistant of the department obstetrics and gynecology No. 3,
Bukhara state medical institute,
Uzbekistan.

ABSTRACT. In this study, the relationship between insulin resistance, microbiome imbalance, and epigenetic factors was studied in 24 women of reproductive age with polycystic ovary syndrome (PTTS). The results of the study showed that PTTS has a multifactorial pathogenesis and there is a close relationship between changes in the intestinal microbiota composition, impaired levels of DNA methylation, and insulin resistance. When using a complex integrative approach (diet therapy + probiotic + metformin), positive changes in metabolic and hormonal indicators were noted.

Keywords: PTTS, insulin resistance, microbiome, epigenetics, hyperandrogenism, integrative therapy

АНАЛИЗ ВЗАИМОСВЯЗИ МЕЖДУ ИНСУЛИНОРЕЗИСТЕНТНОСТЬЮ, ДИСБАЛАНСОМ МИКРОБИОМА И ЭПИГЕНЕТИЧЕСКИМИ ФАКТОРАМИ В ПАТОГЕНЕЗЕ СИНДРОМА ПОЛИКИСТОЗНЫХ ЯИЧНИКОВ (СПКЯ) И ИНТЕГРАТИВНЫЕ ПОДХОДЫ К ИХ КОРРЕКЦИИ

Нематова Мафтуна Раҳматиллоевна - ассистент кафедры акушерства и гинекологии № 3
Бухарского государственного медицинского института.
Узбекистан.

АННОТАЦИЯ. В данном исследовании была изучена взаимосвязь между инсулинорезистентностью, дисбалансом микробиома и эпигенетическими факторами у 24 женщин репродуктивного возраста с синдромом поликистозных яичников (ПСЯЯ). Результаты исследования показали, что ПТТС имеет многофакторный патогенез и существует тесная связь между изменениями состава микробиоты кишечника, нарушением уровня метилирования ДНК и инсулинорезистентностью. При использовании комплексного интегративного подхода (диетотерапия + пробиотик + метформин) отмечены положительные изменения метаболических и гормональных показателей.

Ключевые слова: PTTS, инсулинорезистентность, микробиома, эпигенетика, гиперандрогения, интегративная терапия

Introduction. Polycystic Ovarian Syndrome (PTTS) is the most common endocrine metabolic disorder in women, characterised by hormonal imbalance,

ovulation dysfunction, and insulin resistance. Studies conducted in recent years have shown that the imbalance of the intestinal microbiome and epigenetic changes play an important role in the development of PCOS.

Insulin resistance is the central pathogenetic link of PCOS: this process leads to hyperinsulinemia, which in turn leads to hyperandrogenism and ovulation disorders. At the same time, the microbiota-intestinal-ovarian axis is closely related to hormonal regulation. Epigenetic mechanisms, including DNA methylation and miRNA expression, alter the activity of genes associated with PTTS.

The purpose of the study is to determine the interaction of these three factors (insulin resistance, microbiome imbalance, epigenetic changes) in the pathogenesis of PCOS and to evaluate the effectiveness of the integrative treatment approach in their correction.

Over the past decade, views on the etiopathogenesis of PCOS have changed dramatically. While classical theories mainly explained the syndrome as hyperandrogenemia and ovulatory dysfunction, current scientific literature assesses PTTS as a multisystem (multisystem) metabolic disorder (Lizneva D. et al., 2020).

Many studies show insulin resistance as the main mechanism of PCOS. When the sensitivity of insulin receptors decreases, compensatory hyperinsulinemia develops. This stimulates the secretion of androgens in the granulosa cells of the ovaries, disrupting the ovulation cycle (Escobar-Morreale, 2018).

In the study of Wojciechowska A. (2022), it was found that epigenetic modification of the IRS-1, INSR genes in the insulin signaling pathways plays an important role in the development of PCOS.

Torres P.J. et al. (2021) found that in women with PCOS, the diversity of the intestinal microbiota sharply decreases, and an increase in the genera Bacteroides and Prevotella increases insulin resistance.

The modification of the intestinal microbiota is directly related to hormonal balance and lipid metabolism, which justifies the "gut-ovary axis" theory. Clinical trials based on probiotics and prebiotics showed positive changes in the levels of hormones and glucose (Qi X. et al., 2022).

Epigenetic changes - DNA methylation, histone modification, and microRNAs - control the expression of genes associated with PTTS.

It has been established that miRNA-21, miRNA-93, and miRNA-222 are closely related to hyperandrogenism and insulin resistance (Jansen E., 2021).

Also, in patients with increased methylation of the CYP17A1 and INSR genes, hormonal balance and the process of folliculogenesis are disrupted.

Traditional treatment (metformin, combined contraceptives) controls the symptoms, but does not completely eliminate the cause. Therefore, complex approaches based on microbiota modification + diet therapy + metformin + epigenetic correction are considered a new prospect (Li L. et al., 2023).

Such approaches are effective in improving metabolic indicators, balancing hormones, and restoring ovulation.

Analysis of the literature indicates that the study of PTTS is relevant not only as a hormonal disorder, but also as a complex syndrome characterized by the interaction of metabolic, microbiological, and epigenetic mechanisms. This approach indicates the need to introduce the principles of personalized medicine in the diagnosis and treatment of PCOS.

Materials and Methods. The study was conducted among 24 women (age range: 20-38 years) under outpatient observation at the clinical base of the Bukhara region. All participants were confirmed with a diagnosis of polycystic ovary syndrome (PTTS) in accordance with the Rotterdam criteria (ESHRE/ASRM, 2003) based on clinical, laboratory, and ultrasound parameters. All patients who participated in the study expressed their informed consent in writing.

The study participants were divided into two groups:

- Main group (n=12) - treatment based on an integrative approach (metformin, probiotic complex, diet therapy, psychological support);
- Control group (n=12) - standard therapy for PTTS (only metformin 1500 mg/day).

The duration of the study was 12 weeks. All patients were assessed at the initial (week 0) and final (week 12) stages.

- Research criteria and evaluation parameters. Metabolic metrics:
- Blood plasma glucose level (mmol/L);
- Insulin level (μU/mL);
- The level of insulin resistance was assessed using the HOMA-IR index (glucose × insulin / 22.5).

Hormonal indicators:

- The levels of luteinizing hormone (LH), follicle-stimulating hormone (FSH), testosterone, and antimyuller hormone (AMH) were measured by enzyme-linked immunosorbent assay (ELISA).
- The LH/FSH ratio was also calculated, which was considered an important indicator of ovulation dysfunction.

Microbiome analysis:

- The composition of the intestinal microbiota was assessed using the 16S rRNA gene sequencing method from fecal samples.
- The ratio of rocks of the main taxonomic groups - Bacteroides, Lactobacillus, and Prevotella - was analyzed.
- Microbiome diversity was assessed according to the Shannon index.

Epigenetic analyses:

- The degree of methylation of the CYP17A1 and INSR genes in the DNA of blood samples was measured by methyl-specific PCR.
- The expression levels of miRNA-21 and miRNA-222 were analyzed using qPCR (real-time PCR).
- These markers were selected as the main genes involved in the mechanisms of insulin signaling and hyperandrogenism associated with PTTS.

The following complex integrative treatment program was applied to the main group for 12 weeks:

- Metformin - 1500 mg/day (3 times, after meals);
- Probiotic complex - capsules containing Lactobacillus acidophilus and Bifidobacterium bifidum strains (1 time a day);
- Diet therapy - a diet with a low glucose index, rich in fiber, rich in omega-3 fatty acids and antioxidants (vegetables, fruits, whole grains, fish);
- Psychological support - weekly sessions that include elements of stress reduction, sleep hygiene, and motivational counseling.

The control group was treated only with metformin according to the standard treatment protocol.

Statistical analysis. The obtained data were analyzed in the SPSS 26.0 program. The average values were represented as M ± SD. Student's t-test (parametric) or Mann-Whitney's test (nonparametric) was used to assess the differences between the groups.

The relationship between microbiome ratios and epigenetic markers was analyzed using Pearson or Spearman correlation coefficients. The value $P < 0.05$ was considered statistically significant.

1. Changes in metabolic indicators. According to the study results, significant positive dynamics in metabolic indicators were observed in the main group after 12 weeks of integrative treatment.

At the initial stage, insulin resistance (HOMA-IR > 2.5) was noted in all patients. After integrative therapy, the HOMA-IR index in the main group decreased from 3.8±0.4 to 2.1±0.3 ($p<0.01$), while in the control group the change was from 4.0±0.5 to 3.5±0.4, with no statistically significant difference ($p>0.05$).

These results indicate that the combination of metformin and the probiotic complex has a synergistic effect on the restoration of glucose-homeostasis. Probiotics enhance the synthesis of short-chain fatty acids (SCFA) in the intestine, improve insulin sensitivity, and normalize liver lipid metabolism.

Table 1. Changes in metabolic indicators in PTTS patients before and after treatment

Indicator	Main group (n=12) before treatment	Main group after treatment	Control group (n=12) before treatment	Control group after treatment	P (main group)
Glucose (mmol/L)	6.2 ± 0.5	5.3 ± 0.4	6.1 ± 0.6	5.9 ± 0.5	<0.05
Insulin (μU/mL)	18.5 ± 3.1	12.2 ± 2.8	17.9 ± 3.4	16.8 ± 3.1	<0.01
HOMA-IR index	3.8 ± 0.4	2.1 ± 0.3	4.0 ± 0.5	3.5 ± 0.4	<0.01

2. Improvement of the hormonal profile. In patients who underwent integrative therapy, the restoration of hormonal balance was observed. At the end of 12 weeks in the main group:

- The LH/FSH ratio decreased from 2.6 ± 0.5 to 1.4 ± 0.3 ($p < 0.01$);

- The level of testosterone decreased from 2.8 ± 0.4 nmol/L to 1.9 ± 0.3 nmol/L ($p < 0.05$);
- The level of AMH (antimuller hormone) showed a tendency to decrease from 7.2±0.6 to 5.1±0.5 ng/ml.

These results confirm that the normalization of the ovulation cycle and the reduction of androgen secretion occur faster through an integrative approach. In the control group, no significant changes were observed in the ratio of testosterone and LH/FSH.

Table 2. Dynamics of hormonal parameters after 12 weeks of treatment

Hormonal indicator	Main group (n=12) before treatment	Main group after treatment	Control group (n=12) before treatment	Control group after treatment	P (main group)
LH (mIU/mL)	12.8 ± 2.1	8.5 ± 1.8	12.4 ± 2.0	11.2 ± 2.3	<0.05
FSH (mIU/mL)	4.8 ± 0.9	5.9 ± 1.0	4.6 ± 0.8	4.9 ± 1.0	<0.05
LH/FSH ratio	2.6 ± 0.5	1.4 ± 0.3	2.7 ± 0.6	2.3 ± 0.5	<0.01
Testosterone (nmol/L)	2.8 ± 0.4	1.9 ± 0.3	2.7 ± 0.5	2.5 ± 0.4	<0.05
AMH (ng/mL)	7.2 ± 0.6	5.1 ± 0.5	7.0 ± 0.7	6.8 ± 0.6	<0.05

3. Correction of microbiome imbalance. The results of 16C rRNA analysis of the intestinal microbiome showed a violation of the Bacteroides/Prevotella ratio in patients with PTTS. In the initial stage, Bacteroides dominated, and the Lactobacillus population decreased.

- After 12 weeks of integrative therapy in the main group:
- The ratio of Lactobacillus increased from 1.2 to 3.4 times ($p < 0.01$);
- The proportion of Bacteroides decreased from 60% to 38%;
- The Shannon index of the microbiome increased from 3.1 to 3.9, which indicates the restoration of microbial diversity.

No significant changes were noted in the control group. These findings indicate that the balance of hormonal and metabolic signaling is restored through the microbiota-intestinal-ovarian axis (gut-ovary axis).

Table 3. Microbiome composition and diversity indicators (based on 16S rRNA analysis)

Taxonomic group / indicator	Main group before treatment (%)	Main group after treatment (%)	Control group before treatment (%)	Control group after treatment (%)	P (main group)
Bacteroides	60 ± 8	38 ± 6	58 ± 7	55 ± 6	<0.01
Lactobacillus	15 ± 3	34 ± 5	16 ± 4	18 ± 3	<0.01
Prevotella	25 ± 4	28 ± 5	26 ± 4	27 ± 5	>0.05

4. Analysis of epigenetic markers. According to the results of epigenetic analysis, a state of hypometylation in the promoter regions of the CYP17A1 and INSR genes was observed in patients with PCOS, which is considered a contributing factor to hyperandrogenism and insulin resistance.

Against the background of integrative treatment:

- The degree of methylation of the CYP17A1 gene increased from 42% to 58% ($p<0.05$);

- In the INSR gene, a tendency towards an increase in methylation from 39% to 53% was noted;
- At the same time, the expression of miRNA-21 and miRNA-222 decreased by 1.8 times, which may be associated with the normalization of insulin signaling.

An inverse correlation ($r = -0.61$; $p < 0.01$) was found between epigenetic markers and HOMA-IR, which confirms the relationship between genetic regulation and metabolic adaptation.

Table 4. Changes in epigenetic markers (DNA methylation and miRNA expression analysis)

Epigenetic marker	Main group (before)	Main group (after)	Control group (before)	Control group (after)	P (main group)
CYP17A1 methylation (%)	42 ± 5	58 ± 6	43 ± 4	46 ± 5	<0.05
INSR methylation (%)	39 ± 4	53 ± 5	40 ± 5	43 ± 5	<0.05
miRNA-21 expression (relative)	1.00	0.58 ± 0.1	1.00	0.88 ± 0.1	<0.01
miRNA-222 expression (relative)	1.00	0.62 ± 0.1	1.00	0.91 ± 0.1	<0.01

5. Clinical effect of the integrative approach. The integrative approach improved not only the metabolic and hormonal status, but also the general psycho-emotional state of patients. Depressive symptoms (according to the Beck scale) decreased from 18.2±3.1 to 10.4±2.7 ($p<0.05$).

In 67% of patients, restoration of the ovulation cycle was observed, while in the control group this indicator was 33%.

The obtained results show that in the pathogenesis of PCOS, insulin resistance, microbiome imbalance, and epigenetic changes constitute an interconnected multi-component system.

An integrative approach (metformin + probiotic + diet therapy + psychological support) affects all the main links of this system and normalizes metabolic, hormonal, and genetic regulation as a whole.

Conclusion. Polycystic Ovarian Syndrome (PTTS) is a multifactorial disease in which insulin resistance, microbiome imbalance, and epigenetic changes are interrelated. According to the results of the conducted research, the integrative approach (methformin, probiotics, diet therapy, and psychological support) significantly improved metabolic, hormonal, and epigenetic indicators in patients. Restoration of the microbiota plays an important role in increasing insulin sensitivity and normalizing ovulation. In the future, broader clinical studies will contribute to a more in-depth study of the long-term effectiveness of this approach.

REFERENCES

1. Azziz, R., Carmina, E., Chen, Z., Dunaif, A., Laven, J. S. E., Legro, R. S., & Lizneva, D. (2016). Polycystic ovary syndrome. Nature Reviews Disease Primers, 2, 16057. https://doi.org/10.1038/nrdp.2016.57

2. Qi, X., Yun, C., Sun, L., Xia, J., Wu, Q., Wang, Y., & Liang, X. (2019). Gut microbiota–bile acid–interleukin-22 axis orchestrates polycystic ovary

syndrome. Nature Medicine, 25(8), 1225–1233. https://doi.org/10.1038/s41591-019-0509-0

3. Escobar-Morreale, H. F. (2018). Polycystic ovary syndrome: Definition, aetiology, diagnosis and treatment. Nature Reviews Endocrinology, 14(5), 270–284. https://doi.org/10.1038/nrendo.2018.24

4. Zhao, X., Jiang, Y., Xi, H., Chen, L., & Feng, X. (2020). Exploration of the relationship between gut microbiota and polycystic ovary syndrome (PCOS): A review. Reproductive Biology and Endocrinology, 18(1), 3–12. https://doi.org/10.1186/s12958-019-0567-0

5. Louwers, Y. V., & Laven, J. S. E. (2020). The genetics of polycystic ovary syndrome: A review. Reproductive BioMedicine Online, 40(3), 389–400. https://doi.org/10.1016/j.rbmo.2019.12.006

6. Jiang, Y., Zhang, Z., & Zhang, J. (2022). Epigenetic mechanisms and therapeutic perspectives in polycystic ovary syndrome. Frontiers in Endocrinology, 13, 861020. https://doi.org/10.3389/fendo.2022.861020

ИСПОЛЬЗОВАННАЯ ЛИТЕРАТУРА:

1. Аззиз, Р., Кармина, Э., Чен, З., Дунайф, А., Лавен, Дж. С. Э., Легро, Р. С., & Лизнева, Д. (2016). Синдром поликистозных яичников. Nature Reviews Disease Primers, 2, 16057. https://doi.org/10.1038/nrdp.2016.57

2. Qi, X., Yun, C., Sun, L., Xia, J., Wu, Q., Wang, Y., & Liang, X. (2019). Микробиота кишечника-жёлчная кислота-интерлейкин-22 организует синдром поликистозных яичников. Природная медицина, 25 (8), 1225-1233. https://doi.org/10.1038/s41591-019-0509-0

3. Эскобар-Морреаль, Х. Ф. (2018). Синдром поликистозных яичников: определение, этиология, диагностика и лечение. Nature Reviews Endocrinology, 14 (5), 270-284. https://doi.org/10.1038/nrendo.2018.24

4. Чжао, Х., Цзян, Ю., Си, Х., Чен, Л., и Фэн, Х. (2020). Исследование взаимосвязи между микробиотой кишечника и синдромом поликистозных яичников (СПКЯ): Обзор. Репродуктивная биология и эндокринология, 18 (1), 3-12. https://doi.org/10.1186/s12958-019-0567-0

5. Louwers, Y. V., & Laven, J. S. E. (2020). Генетика синдрома поликистозных яичников: Обзор. Репродуктивная биомедицина онлайн, 40 (3), 389-400. https://doi.org/10.1016/j.rbmo.2019.12.006

6. Jiang, Y., Zhang, Z., & Zhang, J. (2022). Эпигенетические механизмы и терапевтические перспективы при синдроме поликистозных яичников. Границы в эндокринологии, 13, 861020. https://doi.org/10.3389/fendo.2022.861020