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FEATURES OF THE EXPRESSION OF MOLECULAR BIOMARKERS OF POLYCYSTIC OVARY SYNDROME IN WOMEN WITH INFERTILITY

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SUMMARY

The aim of the research was to study the state of molecular markers depending on the clinical course of polycystic ovary syndrome in women with infertility. **Material and Research Methods.** The material is presented by three groups of women: Group 1 – 64 women with non-endocrine factors of infertility (tubal-peritoneal and endometriosis) with the absence of tumor-like formations or functional ovarian cysts; Group 2 – 48 women with polycystic ovary syndrome who became pregnant after treatment and group 3 – 96 women with infertility due to polycystic ovary syndrome. The studies were carried out using immunohistochemical analysis on histological material of the ovaries of women with infertility using test systems produced by Novocastra. **The results obtained and their Discussion.** When comparing clinical, laboratory and molecular data, a proliferation pattern is visible, which is characterized by three main types of expression. **Conclusions:** Women in group 1 are characterized by a predominance of moderate expression of Ki-67 in 68% of cases over high and moderate expression of p53 and Ki-67, which indicates slight hyperplasia and proliferation. Women in group 3 were characterized by a predominance of high and moderate expression of Ki-67 in 49% and 45% of cases over high and moderate expression of p53, which indicates more pronounced hyperplasia and proliferation in the ovarian tissue. And women of group 2 are characterized by a predominance of moderate expression of Ki-67 in 51% of cases over high and moderate expression of p53, which indicates significantly pronounced hyperplasia and proliferation in the ovarian tissue.

KEYWORDS: polycystic ovary syndrome, infertility in women, endocrine disorders, molecular markers of proliferation and ovarian hyperplasia.

INTRODUCTION

Despite the active search for new universal diagnostic, therapeutic and/or prognostic molecular biological markers for polycystic ovary syndrome, the problem of full diagnosis and tactics for choosing targeted therapy has still not been solved.[2,3,6,17,18,20,21]

Molecular biology research opens up new ways to understand more subtle molecular biological disorders and makes it possible to apply the information obtained in clinical practice. Thus, the study of molecular genetic disorders will allow the development of new targeted drugs, which will contribute to the further development of individualization of therapy for each patient.[3,7,8,10,12]

It is known that p53 protein, which is a product of the TP53 gene, is involved in the regulation of basic cell functions, such as cell movement through the cell cycle, cell death, differentiation, DNA repair, and the formation of blood vessels. For its many important functions, it has been called the "guardian of the genome." P53 activates the P21 TS gene, which prevents the transition from phase 01 to phase S of the cell cycle. There are many reports of TP53 mutations in various types of tumors.[6,12,16,18]

Genetic instability due to disruption of P53-mediated DNA repair contributes to the occurrence of genetic abnormalities leading to chronicity of the pathological process and its consequences. TP53 mutation profiles differ depending on the histological picture of the pathological process, including polycystic ovary syndrome.[10,13,14,15,17]

The aim of the study was to conduct methods and analysis to study the state of molecular markers depending on the clinical course of polycystic ovary syndrome in women with infertility. This article is devoted to the analysis of the state of molecular markers of polycystic ovary syndrome in women with infertility, depending on the clinical course of polycystic ovary syndrome with consequences.

MATERIAL AND RESEARCH METHODS

The women in our study were divided into 3 large groups: Group 1 – 64 women with non-endocrine factors of infertility (tubal-peritoneal and endometriosis) with the absence of tumor-like formations or functional ovarian cysts; Group 2 – 48 women with PCOS who became pregnant after treatment and Group 3 – 96 women with infertility due to polycystic ovary syndrome. All groups became comparison groups with each other and were collected to comparatively characterize the behavior of molecular markers involved in the pathogenesis of polycystic ovary syndrome. Moreover, women with polycystic disease had concomitant obesity. It is important to note that polycystic ovary syndrome (PCOS), according to world literature, is based on certain molecular changes.[9,18,19,21] It was shown above in the chapter that proliferative changes in the reproductive organs are of great importance. We examined several molecular markers in stages depending on the different groups. As shown above, first we present data on the study of the expression of the proliferative activity of the Ki-67 antigen, normal and mutant p53. The studies were carried out using immunohistochemical analysis on histological material of the ovaries of women with infertility using test systems produced by Novocastra.

The results obtained and discussion. Below we will present an analysis of the state of molecular markers in women with polycystic ovary syndrome depending on the clinical course of the disease. Thus, analysis of the expression of the proliferative activity of Ki-67 and p53 in samples of ovarian tissue from women of fertile age in three groups of women showed that in all groups proliferative activity is observed in polycystic ovary syndrome, which affects the course, prognosis and effectiveness of therapy. Comparison of immunohistochemical analysis data with clinical course data depending on groups of women revealed the following patterns. We know that to assess the proliferative activity (PA) of the tissue, the number of Ki-67-positive cells in each group of material was counted. PA values were assessed based on the most commonly used assessment method: 0-10% - low PA, 10-50% - moderate, above 50% - high. Consequently, the following results were obtained, characteristic of the first group of material, that is, material from women with non-endocrine factors of infertility (tubal-peritoneal and endometriosis) with the absence of tumor-like formations or ovarian cysts.

Immunohistochemical studies have shown that in this course of the pathological process there is a moderate expression of p53 proliferative activity in 32% of women, negative in 67% of cases, and high in no cases. Thus, moderate expression of Ki-67 is observed in 68% of women, high in 18% of women, and negative in 21% of cases. This marker is a marker of proliferation and is expressed in almost all phases of the mitotic cycle, and reflects the size of the proliferative pool, which proves the predominance of proliferative processes in polycystic ovary syndrome. As for the expression of the apoptosis marker, moderate and negative expression of mtp 53 was most often detected. Based on the above, it becomes obvious that the attention of clinicians should be paid not only to the restoration of reproductive function, but also to the treatment of endometrial hyperplastic processes and metabolic changes. Despite the fact that the oncological aspects of polycystic ovary syndrome have been known for a long time[4,5,8,10], the problem of prevention and differentiated therapy, taking into account modern ideas about endocrine and metabolic disorders, remains relevant not only in the medical, but also in the social aspect and requires further research.

Women in this group had a regular ovular menstrual cycle against the background of normative levels of gonadotropins and sex steroids. It should be noted the age of the women in this group, which was 26.7±0.4 years. Moreover, the duration of infertility in these women was 2.2±0.3 years. It is also clear from clinical and anamnestic data that infertility was primary in almost 70% of women in this group. As for the causes, we have identified the following causes: tubo-peritoneal factor and endometriosis. It should be said that until the time of this study, almost half of the women were constantly examined and treated.

An important aspect and great significance in infertility were women with obesity, or rather with metabolic syndrome. It should be noted that in this group of women, obesity was observed in 14% of cases. It is known that metabolic syndrome is one of the factors in the unfavorable course of the disease. Thus, the average BMI values in the first group described were 25.1±1.3 kg/m2. Also, insulin resistance occurred in 7.8% of cases, hypothyroidism in 1 case. As for obese women, which occurred in 14% of cases, we see that these women were characterized by slightly different hyperplastic activity of the ovaries. Thus, molecular studies have shown that high p53 expression was detected in 3% of cases, moderate expression in 44% of cases, and negative expression in 53% of cases. This shows moderate activity of the hyperplastic reaction in obese women. At the same time, the expression of Ki-67 was detected as moderate expression, which once again confirms a slight hyperplastic reaction; high expression was not detected in this group. Moreover, the 1st group of women was characterized by concomitant diseases, such as cardiovascular system - 8%, gastrointestinal - 16%, anemia - 11%, urinary system - 9%, respiratory diseases - 6%.

As for gynecological diseases, in this group, against the background of unexpressed hyperplasia, the following diseases occur, such as polyps - 8%, pathology of the fallopian tubes - 33%, adhesions in the pelvic area - 3%. With regard to ovarian reserve, the first group had the lowest levels of LH, FSH, estradiol, testosterone,

prolactin and progesterone when compared with the data of the other two groups.

The following presents the data from the analysis of women from group 3; these were women with infertility due to polycystic ovary syndrome. Molecular studies have shown that immunohistochemically, during this course of the proliferative process, there is high expression of p53 in 25% of cases, moderate expression of proliferative activity of p53 in 42% of cases, and negative expression in 33% of cases. As can be seen, with this proliferative process there is a high frequency of proliferation activity compared to moderate and negative expression, which explains the pronounced hyperplastic process in women with clinically significant polycystic ovary syndrome.

Thus, moderate expression of Ki-67 in the 3rd group of women is observed in 45% of women, high in 49% of women, and negative in 6% of cases. This marker is a marker of proliferation and is expressed in almost all phases of the mitotic cycle, and reflects the size of the proliferative pool, which proves a more pronounced predominance of proliferative processes in polycystic ovary syndrome in this group of women.

As for the second group of patients, which included 48 women with PCOS and who became pregnant after treatment, in this group of women there is still not such pronounced proliferation as in the third group. Clinical studies showed that in the third group of women, compared to the second group, a significantly increased frequency of insulin resistance was revealed (19.8%) compared to 16.7% in the second group, and diabetes mellitus as a diagnosis was identified in women in the third group, almost 3%. cases, further analysis showed that in the third group hyperprolactinemia was detected in 4% of cases, hypothyroidism in 4.2% of cases. And they also have cardiovascular diseases, which occur in 51% of cases, while in the second group - in 35% of cases, diseases of the gastrointestinal tract in the third group occur in 28% of cases, anemia - in 24%, diseases of the urinary system - in 24 % of cases, respiratory diseases - in 21% of cases.

As can be seen from the data presented, the third group of women with polycystic ovary syndrome turned out to be the most complex with concomitant diseases, with a pronounced picture of proliferation and hyperplasia. The analysis showed significant differences between the groups, especially between the second and third groups of women.

From the above data, characteristic pronounced clinical and molecular signs of polycystic ovary syndrome have been established, which turned out to be characteristic of women with this pathology. These signs also include the characteristics of the ovarian reserve, where a significant increase in LH levels was observed in women of groups 2 and 3 ($p < 0.05$). In women of the third group, a significant increase in LH values was recorded in relation to the second group ($p < 0.05$). The level of total testosterone in women of the third group was significantly increased when compared with the first and second groups of women ($p < 0.05$).

In the third group of women with PCOS, the average volume of the ovaries and the number of antral follicles turned out to be significantly higher compared to the data of women. All this is explained by pronounced proliferation, that is, ovarian hyperplasia, which can be substantiated by the identified elevated proliferation markers. Consequently, statistically significant differences were found between the studied groups in such indicators as ovarian volume and the number of antral follicles. From our data it follows that the higher the proliferation and pronounced values of hyperplasia, the higher the likelihood of developing infertility in patients with PCOS. It was in the third group of women that the highest BMI indicators were observed, which adversely affected the course of the disease in 48% of cases ($p < 0.05$-0.01). It should be noted that when surveyed, women in the third group did not report positive results when trying to reduce their weight. Immunohistochemical studies showed that pronounced hyperplastic activity of the ovaries was detected, which showed the following picture: high p53 expression was detected in 16% of cases, moderate in 57% of cases, and negative in 27% of cases. Thus, high activity of p53+ expression was more common than in groups 1 and 2 of women. At the same time, Ki-67 expression was also more common in this group of women than in the first and second groups and amounted to a high expression of 24% of occurrence, which confirms a pronounced hyperplastic reaction.

The second group was characterized by the following immunohistochemical picture: high p53 expression in 14% of cases, moderate expression of p53 proliferative activity in 36% of cases, and negative expression in 50% of cases. Moderate expression of Ki-67 in the third group of women is observed in 51% of women, high in 31% of women, and negative in 18% of cases.

Thus, when comparing clinical, laboratory and molecular data, we emerge a picture of proliferation, which is characterized by three main types of expression, which we can characterize and present in the form of a logically complex picture. Thus, it turned out that high and moderate expression was most characteristic of the 3rd group of women with a severe clinical picture. And for the 1st group of women, a pronounced moderate pattern of proliferation with negative expression of p53 was most characteristic.

As shown by immunohistochemical studies of the material in combination with clinical and laboratory data, all this allows us to obtain a morphological description of the process, an assessment of the level of proliferation, an assessment of the level of expression of various

receptors, which provides an accurate assessment of the further development and prognosis of the pathological process. It should also be noted that IHC studies significantly increase the diagnostic capabilities of morphological studies and are used to identify the exact histogenesis, which makes it possible to objectify the diagnosis, differential diagnosis of neoplasms and hyperplastic and proliferative processes that have morphological similarities.

Thus, according to the literature and our data, a promising marker of proliferation is the Ki-67 antigen, which is expressed in almost all phases of the mitotic cycle and, accordingly, reflects the size of the proliferative pool. Thus, we conducted an IHC study aimed at determining the activity of ovarian hyperplasia. During the IHC study, specially synthesized labeled antibodies were added to ovarian tissue samples containing cells with Ki-67 antigens. During the reaction, "antigen-antibody" complexes were formed, which demonstrate the number of cells in the active phase of division, then the index of proliferative activity was calculated, which was indicated as a percentage. It is known that Ki-67 is present in cell nuclei throughout the cell life cycle, except for G0 and G1. The presence of this gene in cells in all phases of the mitotic cycle, the absence during the transition to the resting period and during DNA repair, allows us to consider Ki-67 an ideal marker of proliferation, especially when assessing the activity of the proliferative process, the growth of a tumor or pathological tissue, which has an important role for characteristics of the oncological essence of the tumor and its aggressiveness, in which this indicator can be selected as one of the decisive prognosis factors.[3,4,7,9,13,15] A set of signs is often used that allows the properties of the hyperplastic process under study to be clarified as much as possible.

CONCLUSIONS

Immunohistochemical studies showed that women in group 1 were characterized by: moderate expression of p53 proliferative activity in 32% of cases, negative expression in 67% of cases, high expression in no cases. Moderate expression of Ki-67 is observed in 68% of female cases, high in 18% of female cases, and negative in 21%. This marker is a marker of proliferation and is expressed in almost all phases of the mitotic cycle, and reflects the size of the proliferative pool, which proves the predominance of proliferative processes in polycystic ovary syndrome, all this proves the presence of endometrial hyperplastic processes and metabolic changes. Consequently, there is a predominance of moderate expression of Ki-67 in 68% of cases over high and moderate expression of p53 and Ki-67, indicating little significant hyperplasia and proliferation.

In all groups, women with obesity, or rather with metabolic syndrome, were identified. Molecular studies have shown that high p53 expression was detected in 3% of cases, moderate expression in 44% of cases, and negative expression in 53%. This shows moderate activity of the hyperplastic reaction in obese women. At the same time, the expression of Ki-67 was detected as moderate expression, which once again confirms a slight hyperplastic reaction; high expression was not detected in this group.

Women in group 3 were characterized by: high expression of p53 in 25% of cases, moderate expression of proliferative activity of p53 in 42% of cases, and negative expression in 33% of cases. Moderate expression of Ki-67 in the third group of women is observed in 45% of women, high in 49% of women, and negative in 6% of cases. Consequently, there is a predominance of high and moderate expression of Ki-67 in 49% and 45% of cases over high and moderate expression of p53, indicating more pronounced hyperplasia and proliferation in the ovarian tissue.

Women in group 2 were characterized by: high expression of p53 in 14% of cases, moderate expression of proliferative activity of p53 in 36% of cases, and negative expression in 50% of cases. Moderate expression of Ki-67 in the third group of women is observed in 51% of women, high in 31% of women, and negative in 18% of cases. Consequently, there is a predominance of moderate expression of Ki-67 in 51% of cases over high and moderate expression of p53, which indicates significantly pronounced hyperplasia and proliferation in the ovarian tissue.

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