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Immunohistochemical Study of CD Markers in Tissue Samples of Fertile Age Women with Infertility with Polycystic Ovary Syndrome

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Abstract **The aim of the research** was to investigate and analyze CD markers in the tissue of ovaries in women with polycystic ovary syndrome (PCOS) and infertility. The material and methods of the study included three large groups of patients: one group with non-endocrine factors of infertility, one group with PCOS who became pregnant after treatment, and a third group of women with infertility despite having PCOS. All groups were compared with each other and were collected for comparative characterization of the behavior of molecular markers participating in the pathogenesis of PCOS. **The results and discussion.** The study revealed the behavior of such molecular patterns as receptors of the adaptive immune system, consisting of T cells and B cells, that is, lymphocytes, especially in the tissue of the ovaries. It is known that T lymphocytes participate in cell-mediated immune response, while B lymphocytes mainly participate in the humoral immune response. It is worth noting that T lymphocytes, such as CD3+, CD4+, and CD8+, play a decisive role in inhibiting inflammation by secreting pro-inflammatory cytokines in various metabolic organs and stimulating the formation of follicles, releasing specific chemokines and growth factors that promote the development of granulocytes and the selection of follicles in the ovaries, and sending cytotoxic signals. **Conclusions.** The molecular risk factors for the development of infertility in women were found to be high and moderate expression of CD3+ in the ovarian tissue in 49% and 26% of cases, respectively, high and moderate expression of CD20+ in 28.4% and 26% of cases, respectively, high and moderate expression of CD4+ in 35% and 21% of cases, respectively, and high and moderate expression of CD8+ in 23% and 18% of cases. For the second group of women: high and moderate expression of CD3+ in 16% and 15% of cases, respectively, high and moderate expression of CD20+ in 44% and 35% of cases, respectively, high and moderate expression of CD4+ in 37% and 21% of cases, respectively, and high and moderate expression of CD8+ in 34% and 49% of cases. And for the third group of women: high and moderate expression of CD3+ in 28% and 40% of cases, respectively, high and moderate expression of CD20+ in 54% and 42% of cases, respectively, high and moderate expression of CD4+ in 46% and 32% of cases, respectively, and high and moderate expression of CD8+ in 23% and 18% of cases.

Keywords Polycystic ovary syndrome, Female infertility, Endocrine disorders, CD markers, Molecular tissue markers

1. Introduction

It is already known that cells responsible for proliferation or resistance to drugs play a special role in the immunopathogenesis of various formations, including cysts, thereby causing progression or reduction of the proliferative process [1,2,3,4,8]. Recently, the identification of various CD markers in tissue has been carried out using the method of immunohistochemical analysis, which has shown that, in particular, these markers are present in ovarian tissue and can determine various functions, such as CD44+ and CD133+ cell markers in tissue [5,6,9,12]. Therefore, thanks to the development of the immunohistochemical research method, it has been possible to identify the expression of these receptors, which determine various processes.

Thus, it is considered that research in molecular biology opens new ways and opportunities for understanding genetic disorders and enables the application of the obtained information in clinical practice. For example, the study of molecular genetic disorders will allow the development of new targeted drugs, which will contribute to the further development of individualizing the therapy for each patient. Of course, the basis for making decisions about the treatment tactics will be molecular diagnostics, which, along with the clinical features of the pathological process, will accumulate more and more knowledge and experience and will contribute to early disease diagnosis and monitoring, as well

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as to improving treatment outcomes.

It should be noted that in recent years, all practical medicine, especially gynecology and endocrinology in the treatment of infertility, is based on the already known values, which play an important role in achieving the effectiveness of therapy.

According to our data and the data from the world literature, polycystic ovary syndrome (PCOS) is based on certain molecular changes [10,11,14]. We have studied several molecular markers step by step, depending on different groups. Based on the above, our aim is to study and analyze the CD markers in ovarian tissue, depending on the clinical course of polycystic ovary syndrome in women with infertility.

The aim of our research is to study and analyze CD markers in ovarian tissue depending on the clinical course of polycystic ovary syndrome in infertile women.

2. Materials and Methods

In our study, women were divided into three major groups:

Group 1 – 64 women with non-endocrine causes of infertility (tubal-peritoneal and endometriosis) with no tumorous formations or functional ovarian cysts.

Group 2 – 48 women with PCOS who became pregnant after treatment.

Group 3 – 96 women with infertility due to PCOS.

All groups were compared to each other and were collected for a comparative characteristic of the behavior of molecular markers involved in the pathogenesis of polycystic ovary syndrome. It is worth noting that women with PCOS were also obese. Below are the results of the study on the immunohistochemical examination of ovarian tissue samples in women with PCOS depending on the three groups of examined women.

3. Results and Discussion

The study of the main CD lymphocyte differentiation markers - CD3+ in women of the first group did not reveal the presence of mature lymphocytes with the CD3+ marker in any tissue sample, indicating that mature lymphocytes with the CD3+ marker were not identified. When studying the expression of CD20+ in ovarian tissue, high, moderate, and negative expressions were identified in the ovarian tissue samples. Thus, high expression of CD20+ was identified in 25.6% of tissue samples, moderate expression in 28.4% of cases, and negative expression in 46%.

Therefore, we can say that the studies did not reveal lymphoid infiltration based on the expression of the CD3+ marker, which is responsible for lymphoid proliferation. Against this background, a slight CD20+ lymphoid infiltration can be traced in samples of women with polycystic ovary syndrome due to infertility, although the bulk of the expression was negative and amounted to 46% of cases.

Further, it is clear that in this group of women there is high interstitial expression of CD4+ in 27% of cases, moderate in 35% of cases and negative in 38% of cases. This picture indicates the presence of proliferative activity of cells in the ovarian tissue, but to an insignificant extent. That is, we see insignificant production of proliferation mediators, which are restrained by opportunistic mediators. One of them is CD8+ expression in ovarian tissue samples, high expression of which was detected in this group of women in 49% of cases, moderate expression in 26% of cases and negative expression in 25% of cases.

It is known from the literature that interstitial expression of CD8+ is a sign of severe inflammation, this marker indicates a cytotoxic lytic function in the tissue, which is also a pro-inflammatory sign with the production of pro-inflammatory cytokines that will lead to adhesions and scarring [5,8,9,12,14].

Consequently, in the first group of women, a predominance of CD8+ expression over CD4+ expression was revealed in the ovarian tissue by 1.8 times, which is a sign of a chronic inflammatory process. Moreover, there are women with high expression of CD20+ in 25.6%, which indicates the presence of proliferative activity characteristic of this group of women.

CD4+ Th cells are central organizers of pro-inflammatory and anti-inflammatory immune responses. This inflammatory response within the ovaries leads to the accumulation of numerous follicles without ovulation and PCOS patients experience high levels of estrogen. Thus, it has been confirmed that a significant difference in the Th17/Th2 ratio with a bias towards Th17 is common among patients with PCOS [5,6,7,11,12]. Consequently, the accumulation of Th1 and Th17 cells leads to immune hyperactivity, which can often lead to the formation of autoimmune inflammation.

Below will be presented the results of women included in the second group of women with polycystic ovary syndrome. As for CD8+ cells, they are the primary effector cells of the cellular immune system. They cause cytotoxic processes aimed at destroying infected or malignantly transformed cells. As stated above, most often their increased expression leads to inflammatory diseases.

Thus, a study of the expression of the main CD - markers of lymphocyte differentiation - CD3+ in women of the second group revealed the following changes. Thus, high expression of markers of mature CD3+ lymphocytes was identified in 16% of women, moderate expression in 15% of cases and negative expression in 69% of cases.

A study of CD20+ expression in ovarian tissue revealed high expression in 44% of cases, moderate expression in 35% of cases, and negative expression in 21% of cases. Thus, high expression of CD20+ was identified in 44% of cases in tissue samples, this is a fairly high value. Moreover, the high expression of CD20+ was 2.8 times higher than the expression of CD3+, which once again indicates proliferative activity in the ovarian tissue. Therefore, we can say that the studies revealed lymphoid infiltration based on the slight expression of the CD3+ and CD20+ marker,

which is responsible for lymphoid proliferation within the ovarian tissue against the background of polycystic disease. Moreover, it should be noted that CD20+ lymphoid infiltration in samples of women with polycystic ovary syndrome against the background of infertility was high, a higher percentage than moderate and negative.

Next, interstitial expression of CD4+ was studied, which showed that high expression was detected in 37% of cases, moderate in 21% of cases and negative in 42% of cases. As can be seen, proliferation is observed, which is expressed in the production of the main pro-inflammatory proliferative cytokines.

One of the interesting and important markers of inflammation and proliferation is precisely the imbalance in the expression of CD8+ in ovarian tissue samples [1,2,4,8]. Thus, high expression of this marker was detected in the second group of women in 34% of cases, moderate expression in 49% of cases and negative expression in 17% of cases.

It is known from the literature that interstitial expression of CD8+ is a sign of severe inflammation, moreover, chronic inflammation [2,4,5,8]. Consequently, in the second group of women, a predominance of CD4+ expression over CD8+ expression was revealed in the ovarian tissue by 1.2 times. Although this is not a big difference, lymphoid proliferation is still visible.

It is known from the literature that the presence of high and moderate expression of CD4+ indicates the proliferative activity of cells, i.e. in the production of proliferation mediators, which explains the increased proliferation activity against the background of polycystic ovary syndrome [9,10,11,14]. According to our data, there is a predominance of high CD4+ expression over CD8+ expression, which indicates a proliferative process. Further, the results obtained from studying the expression of CD - markers of lymphocyte differentiation in the third group of women will be described below. Thus, the analysis showed that high expression of CD3+ in women of the third group was detected in 28% of cases, moderate expression in 40% of cases and negative expression in 32% of cases. Thus, it was shown that the high expression of this marker was insignificant, but turned out to be functional enough to enhance proliferative activity.

A study of high expression of CD20+ in ovarian tissue showed that it was found in 54% of cases, moderate in 42% of cases and negative in 4% of cases. Thus, the high expression of CD20+ identified in ovarian tissue turned out to be quite significant for increased proliferative activity in this group of women. From the data obtained it is clear that high CD20+ expression prevailed over high expression in frequency of occurrence among women in this group by 2 times higher than the frequency of occurrence of high CD3+ expression. Consequently, high expression of CD20+ occurred 2 times more often in this group, which indicates lymphoid proliferation within the ovarian tissue against the background of polycystic disease and the production of pro-inflammatory cytokines, which reflect evident proliferation.

Further, the intratissular expression of CD4+ was studied, which showed that high expression was detected in 46% of cases, moderate expression in 32% of cases, and negative expression in 14% of cases. It is evident that there is proliferation, which is manifested in the production of major proinflammatory proliferative cytokines due to the frequent occurrence among women of this group with high CD4+ expression.

The study of the inflammation marker CD8+ in ovarian tissue samples showed that high CD8+ expression was found in 23% of cases, moderate expression in 18% of cases, and negative expression in 59% of cases. It is known from the literature that intratissular CD8+ expression is a sign of pronounced inflammation, specifically, chronic inflammation. From our data, it follows that the presence of more negative CD8+ expression indicates more of a proliferative process than an inflammatory one. Therefore, in the third group among women, a predominance of high CD4+ expression over CD8+ expression by 2 times was detected in ovarian tissue, indicating a pronounced high proliferation based on the expression of CD20+ and CD3+ in ovarian tissue.

Above, we were able to analyze the behavior of such molecular patterns as cell receptors of the adaptive immune system, which consists of T-cells and B-cells, that is, lymphocytes, especially in the ovarian tissue. It is known that T-lymphocytes are involved in cell-mediated immune response, while B-lymphocytes mainly mediate the humoral immune response. We know that the balance of these cells is an important mechanism for maintaining ovarian tissue homeostasis [3,4,5,8]. It is also important to note that T-lymphocytes, such as CD3+, CD4+ and CD8+, play a crucial role in mediating inflammation by secreting pro-inflammatory cytokines in various metabolic organs and stimulating the formation of follicles, releasing specific chemokines and growth factors that contribute to the development of granulosa cells and the selection of ovarian follicles, and also sending cytotoxic signals.

The results we have obtained from studying the behavior of individual populations and subpopulations in ovarian tissue can serve as a diagnostic and prognostic criterion in the diagnosis and understanding of the pathogenesis of proliferative processes, particularly in the case of developing ovarian polycystic ovary syndrome. It is also known that IGH markers reflect the functional state of proliferative intra-tissue cells, which is important in assessing the features of the course and outcome of the pathological process. It should be noted that CD20+ and CD4+ have been the most prominent proliferative markers, which are responsible for cell proliferation in the tissue, as well as for the production of proliferative mediators of inflammation that support the active proliferative process. Furthermore, concomitant obesity is one of the leading factors determining the frequency and nature of various forms of ovarian proliferative processes. Visceral obesity is important. Therefore, the diagnosis and treatment of women with polycystic ovary syndrome should be comprehensive and combine components of hormonal and metabolic therapy.

4. Conclusions

The molecular risk factors for infertility in women with PCOS were as follows: for group 1 women, the absence of CD3+ expression in ovarian tissue was accompanied by high and moderate expression of CD20+ in 26% and 28.4% of cases, high and moderate expression of CD4+ in 27% and 35% of cases, as well as high and moderate expression of CD8+ in 49% and 26% of cases. For group 2 women, high and moderate expression of CD3+ was observed in 16% and 15% of cases, high and moderate expression of CD20+ in 44% and 35% of cases, high and moderate expression of CD4+ in 37% and 21% of cases, as well as high and moderate expression of CD8+ in 34% and 49% of cases. For group 3 women, high and moderate expression of CD3+ was observed in 28% and 40% of cases, high and moderate expression of CD20+ in 54% and 42% of cases, high and moderate expression of CD4+ in 46% and 32% of cases, as well as low expression of CD8+ in 23% and 18% of cases.

As can be seen, the following molecular features were characteristic for women with infertility against the background of PCOS: in group 1 women, inflammatory changes were at the forefront, accompanied by slight expression of CD20+ and CD4+ against the background of pronounced expression of CD8+ in 49% and 26% of cases, which further confirms our assumption about chronic inflammatory process. For group 2 women with PCOS and infertility, slight expression of CD3+ was often observed, with frequent expression of CD20+ and CD4+ against the background of moderate expression of CD8+. For group 3 women with PCOS and infertility, low expression of CD8+ was observed against the background of pronounced expression of CD3+ in 28% and 40% of cases, significant expression of CD20+ in 54% and 42% of cases, and CD4+ in 46% and 32% of cases.

REFERENCES

[1] Bannert N., Kurth R. (2006). The evolutionary dynamics of human endogenous retroviral families. Annu. Rev. Genomics Hum. Genet. 7 149–173. 10.1146/annurev.genom. 7.080505.115700.

[2] Cordaux R., Batzer M. A. (2009). The impact of retrotransposons on human genome evolution. Nat. Rev. Genet. 10 691–703. 10.1038/nrg2640.

[3] Corn C. M., Hauser-Kronberger C., Moser M., Tews G., Ebner T. (2005). Predictive value of cumulus cell apoptosis with regard to blastocyst development of corresponding gametes. Fertil. Steril. 84 627–633. 10.1016/j.

[4] Gershon E., Plaks V., Dekel N. (2008). Gap junctions in the ovary: expression, localization and function. Mol. Cell Endocrinol. 282 18–25. 10.1016/j.mce.2007.11.001.

[5] Kazazian H. H., Jr. (2004). Mobile elements: drivers of genome evolution. Science 303 1626–1632. 10.1126/science.1089670

[6] Labarta E., de Los Santos M. J., Escriba M. J., Pellicer A., Herraiz S. (2019). Mitochondria as a tool for oocyte rejuvenation. Fertil. Steril. 111 219–226. 10.1016/j.fertnstert.2018.10.036.

[7] Li T., Mo H., Chen W., Li L., Xiao Y., Zhang J., et al. (2017). Role of the PI3K-Akt signaling pathway in the pathogenesis of polycystic ovary syndrome. Reprod. Sci. 24 646–655. 10.1177/1933719116667606.

[8] Liu Q., Li Y., Feng Y., Liu C., Ma J., Li Y., et al. (2016). Single-cell analysis of differences in transcriptomic profiles of oocytes and cumulus cells at GV, MI, MII stages from PCOS patients. Sci. Rep. 6:39638. 10.1038/srep39638.

[9] Magoffin D. A. (2006). Ovarian enzyme activities in women with polycystic ovary syndrome. Fertil. Steril. 86(Suppl. 1) S9–S11. 10.1016/j.fertnstert.2006.03.015 [PubMed] [CrossRef] [Google Scholar].

[10] Malki S., van der Heijden G. W., O'Donnell K. A., Martin S. L., Bortvin A. (2014). A role for retrotransposon LINE-1 in fetal oocyte attrition in mice. Dev. Cell 29 521–533. 10.1016/j.devcel.2014.04.027.

[11] Ollinger R., Childs A. J., Burgess H. M., Speed R. M., Lundegaard P. R., Reynolds N., et al. (2008). Deletion of the pluripotency-associated Tex19.1 gene causes activation of endogenous retroviruses and defective spermatogenesis in mice. PLoS Genet 4: e1000199. 10.1371/journal.pgen.1000199.

[12] Rotterdam E. A.-S. P. C. W. G. (2004a). Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. Fertil. Steril. 81 19–25. 10.1016/j.fertnstert.2003.10.004.

[13] Yuan P., Zheng L., Liang H., Li Y., Zhao H., Li R., et al. (2018). A novel mutation in the TUBB8 gene is associated with complete cleavage failure in fertilized eggs. J. Assist. Reprod. Genet. 35 1349–1356. 10.1007/s10815-018-1188-.

[14] Zhang J., Bao Y., Zhou X., Zheng L. (2019). Polycystic ovary syndrome and mitochondrial dysfunction. Reprod. Biol. Endocrinol. 17: 67. 10.1186/s12958-019-0509-4.