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The Peculiarities of Local Immunity, the Level of Cytokines and Growth Factors in Endometrioid Ovarian Cysts Depending on the Presence or Absence of Undifferentiated Dysplasia of Connective Tissues

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Abstract: In the peritoneal microenvironment level, in patients with endometrioid ovarian cysts develop immunological dysfunction, which is characterized by increased activity of macrophages and decreased activity of T-lymphocytes and natural killer cells. Certain changes are exacerbated in patients with both endometrioid ovarian cysts and undifferentiated dysplasia of connective tissue, such as a decrease in the number of peritoneal fluid lymphocyte CD4-, CD16-, CD25- lymphocytes and B lymphocytes. In patients with endometrioid ovarian cysts combined with undifferentiated dysplasia of connective tissues, have increased levels of proinflammatory cytokines which promotes a proangiogenic effect: IL1β, TNFα, IL6, IL8. In addition, when endometrioid ovarian cysts are concomitant with undifferentiated dysplasia of connective tissues the level of antitumor cytokine IL2 and IFNγ are decreased. Similar changes in the cytokine composition of the peritoneal fluid, along with reduction of the number of immunocompetent cells with antitumor cytotoxicity determine the increase of proangiogenic and decrease of antitumor activity.

Key words: Endometrioid ovarian cysts · Undifferentiated connective tissue dysplasia · Immunological dysfunction · Cytokines

INTRODUCTION

An important role in the onset and progression of endometriosis belongs to the immune system [1-4]. According to contemporary views, anti-tumoral immunity is a complex immune reactions in close co-operative interaction between the T and B - lymphocytes, natural killers and macrophages, which is regulated by cytokines [5-8]. Cytokines are cells of the immune system which mediate intercellular interactions. Cytokines form a complex and diverse, but a single system which processes intercellular cooperation, growth and differentiation of lymphoid cells, angiogenesis and neuro-immuno endocrine interactions [9].

A special interest is given to the peculiarities of immunity in areas of endometrioid foci -the ovaries and peritoneal fluid [10-13].

In the progression period of endometriosis the number of macrophages in the peritoneal fluid increases and the number of CD3, CD4 (T-helper), CD16 (natural killers) and CD25 (activated T-lymphocytes) cells reduces. The increase in the activity of peritoneal immune competent cells is accompanied by the production of factors which have not only cytotoxic effect on tumor cells, but also which intensify the processes of angiogenesis and stimulate adhesion of the endometrium cells to the peritoneum.

Similar immunity disorders are expressed in undifferentiated dysplasia of connective tissues. In consequence, with a combination of these pathologies progression of the process is a possibility. Presently, there are numerous articles, about undifferentiated dysplasia of connective tissues in the development of gynecological diseases [14-17]. But information about the peculiarities of changes in immunity as a result of both endometrioid ovarian cysts and undifferentiated dysplasia of connective tissues, are very less.

Objective: To study the peculiarities of local immunity in patients with endometrioid ovarian cysts depending on the presence or absence of undifferentiated dysplasia of connective tissues.

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MATERIALS AND METHODS

The study included 100 women aged 18 to 40 years, who were divided into several groups: We study group consisted of 35 patients with endometrioid ovarian cysts, who expressed more than 6 identified external phenotypic traits of undifferentiated dysplasia of connective tissues, II a comparison group consisted of 35 patients with endometrioid ovarian cysts who expressed 6 or less identified external phenotypic traits of undifferentiated dysplasia of connective tissues. The control group included 30 women of reproductive age, without undifferentiated dysplasia of connective tissues or concomitant gynecological pathologies, with two-phase menstrual cycles, who were admitted for surgical sterilization procedures.

To externally establish the phenotype a modified phenotypic map was used, including 63 metric units [18]. All patients went through a clinical and instrumental examination, medical and diagnostic laparoscopy. Diagnosis of endometrioid ovarian cysts were confirmed histologically.

The immunological study assesses the relative and absolute number of major subpopulations of immune competent cells in the peritoneal fluid (lymphocytes, neutrophils, mononuclear phagocytes, CD3, CD4, CD8, CD16, CD19, CD25 lymphocytes), as well as studying the level of IL1β, IL2, IL4, IL6, IL8, TNFα, IFNγ in native peritoneal fluid of patients.

Composition of the peritoneal fluid is determined by flow cytometry. The first stage was the processing of cells. Solutions of antibodies conjugated with fluorescein isothiocyanateîì (FITC) and phycoerythrin (PE) [(«Caltag Laboratories, USA) using flow cytometer Bio Rad («Brute-HS », USA)] was added in to eight samples of peritoneal fluid with a volume of 100 mkl each.

The concentration of IL1β, IL2, IL4, IL6, IL8, TNFα, IFNγ in the peritoneal fluid was determined by the method of enzyme-linked immune sorbent assay (ELISA), on the tablet photometer «Labsystems iEMS Reader MF» (Finland) using toolkits «Vectorbest» (Russia) according to standard methods.

Statistical data processing was performed on a computer running PENTIUM IV with the use of the program «Statistica 6.0» [19]. In accordance with the purposes and objectives of the study, as well as taking into account the specifics of the analyzed variables the elementary statistics were solved with (mean values (M), medium errors (m), calculation of shares (%), standard error of the proportion (N)); comparison of the qualitative parameters in the groups studied with the help of non-parametrical methods x^2, Fisher's adjusted Yeats; comparison of quantitative indicators using the nonparametric criterion Mann-Whitney. The criterion for statistical validity of the obtained conclusions was $p<0.05$, as considered in conventional medicine.

RESULTS AND DISCUSSION

When analyzing the data, it was estimated that in the peritoneal fluid of the main group compared with patients without undifferentiated dysplasia of connective tissues and healthy women, there was a significantly greater relative (62,35±1,59 %, 56,58±1,43 % and 37,82±of 2.25 % respectively) and absolute (1,62±0,13 x109/l, 1,54±1,43 x109/l and 0.61±0,10x109/l, respectively) number of macrophages, while the percentage of lymphocytes (20,95±1,39 %, 24,62±1,10 % and 43,40±of 1.92 %, respectively) decreased. It should be noted that the decrease in the relative number of lymphocytes in the peritoneal fluid of patients with endometrioid ovarian cysts in the primary group and the comparison group, was not accompanied by a decrease in the absolute number. Which is associated with the increased total «cellularity» in the peritoneal fluid (Table 1).

When studying the composition of monoclonal cells in the peritoneal fluid, statistically there was a significant decrease in the absolute number of CD4-lymphocytes (0,22±0,02 x109/l, 0,28±0,02 x109/l and 0.37±0,02x109/l, respectively), as well as relative (12,41±0,95 %, 15,69±0,91 % and 24,00±2,00 %, respectively) and absolute (0,06±0,01 x109/l and 0.09±0,01 x109/l and 0.17±0,01x109/l, respectively) number CD16 and relative (17,92±1,34 %, 22,55±1,43 % and 32,10±3,70 %, respectively) and absolute (0,10±0,01x109/l to 0.15±0,01x109/l and 0.22±0,03x109/l, respectively) number of CD25 lymphocytes in the peritoneal fluid of patients endometrioid ovarian cysts and undifferentiated dysplasia of connective tissues compared to the other two groups (Table 2).

The relative and absolute number of CD8- and CD19-lymphocytes in patients with prostatic endometrioid ovarian cysts did not undergo significant changes, regardless of whether they had undifferentiated dysplasia of connective tissues or not (Table 2).

Table 1: Composition of immune-competent cells in the peritoneal fluid of patients with endometrioid ovarian cysts depending on the presence or absence of undifferentiated dysplasia of connective tissues

Index	Healthy women (n=30)	Patients with endometrioid ovarian cysts: With undifferentiated dysplasia of connective tissues (n=35)	Patients with endometrioid ovarian cysts: Without undifferentiated dysplasia of connective tissues (n=35)
Group No.	1	2	3
		M±m*	
Leukocytes x 10^9/l	1,60±0,12	2,55±0,15	2,60±0,16
Reliability of differences**	P1-2=0,001		
	P1-3=0,001	P2-3=0,820	
Neutrophils, %	14,60±0,75	13,24±0,36	13,42±0,26
Reliability of differences	P1-2=0,107		
	P1-3=0,142	P2-3=0,686	
Neutrophils, x10^9/l	0,23±0,01	0,29±0,03	0,30±0,04
Reliability of differences	P1-2=0,062		
	P1-3=0,094	P2-3=0,842	
Macrophages, %	37,82±2,25	62,35±1,59	56,58±1,43
Reliability of differences	P1-2=0,001		
P1-3=0,001	P2-3=0,009		
Macrophages, x10^9/l	0,61±0,10	1,62±0,13	1,54±0,11
Reliability of differences	P1-2=0,001		
P1-3=0,001	P2-3=0,001		
lymphocytes, %	43,40±1,92	20,95±1,39	24,62±1,10
Reliability of differences	P1-2=0,001		
	P1-3=0,001	P2-3=0,042	
lymphocytes, x10^9/l	0,69±0,12	0,54±0,04	0,63±0,05
Reliability of differences	P1-2=0,240		
	P1-3=0,646	P2-3=0,164	

Note

* M - mean, m - mean deviation

** - the differences between groups are significant at $p<0.05$, the Mann-Whitney criterion

Table 2: Composition of monoclonal cells in the peritoneal fluid of patients with endometrioid ovarian cysts depending on the presence or absence of undifferentiated dysplasia of connective tissues.

Index	Healthy women (n=30)	Patients with endometrioid ovarian cysts: With undifferentiated dysplasia of connective tissues (n=35)	Patients with endometrioid ovarian cysts: Without undifferentiated dysplasia of connective tissues (n=35)
Group No.	1	2	3
CD3, %	72,50±5,50	57,03±1,38	57,16±1,43
Reliability of differences **	P1-2=0,008		
P1-3=0,009	P2-3=0,948		
CD3, x 10^9/l	0,50±0,07	0,31±0,02	0,35±0,02
Reliability of differences	P1-2=0,011		
P1-3=0,043	P2-3=0,162		
CD4, %	53,00±1,00	41,34±1,00	42,12±1,11
Reliability of differences	P1-2=0,001		
	P1-3=0,001	P2-3=0,603	
CD4, x 10^9/l	0,37±0,02	0,22±0,02	0,28±0,02
Reliability of differences	P1-2=0,001		
	P1-3=0,001	P2-3=0,038	
CD8, %	25,50±1,50	26,84±1,19	27,79±1,19
Reliability of differences	P1-2=0,486		
	P1-3=0,236	P2-3=0,574	
CD8, x 10^9/l	0,18±0,02	0,14±0,01	0,18±0,01
Reliability of differences	P1-2=0,078		
	P1-3=1,000	P2-3=0,078	
CD16, %	24,00±2,00	12,41±0,95	15,69±0,91
Reliability of differences	P1-2=0,001		
	P1-3=0,001	P2-3=0,015	

Table 2: Continued

Index	Healthy women (n=30)	Patients with endometrioid ovarian cysts: With undifferentiated dysplasia of connective tissues (n=35)	Patients with endometrioid ovarian cysts: Without undifferentiated dysplasia of connective tissues (n=35)
Group No.	1	2	3
CD16, x 10^9/l	0,17±0,01	0,06±0,01	0,09±0,01
Reliability of differences	P1-2=0,001		
	P1-3=0,001	P2-3=0,038	
CD19, %	23,00±2,00	21,09±0,86	20,25±0,78
Reliability of differences	P1-2=0,383		
	P1-3=0,205	P2-3=0,472	
CD19, x 10^9/l	0,16±0,04	0,12±0,01	0,13±0,01
Reliability of differences	P1-2=0,335		
	P1-3=0,469	P2-3=0,482	
CD25, %	32,10±3,70	17,92±1,34	22,55±1,43
Reliability of differences	P1-2=0,001		
	P1-3=0,019	P2-3=0,021	
CD25, x 10^9/l	0,22±0,03	0,10±0,01	0,15±0,01
Reliability of differences	P1-2=0,001		
	P1-3=0,030	P2-3=0,001	

Note

* M - mean, m - mean deviation

** - the differences between groups are significant at $p<0.05$, the Mann-Whitney criterion

Table 3: The levels of cytokines in the peritoneal fluid of patients with endometrioid ovarian cysts depending on the presence or absence of undifferentiated dysplasia of connective tissues

Index	Healthy women (n=30)	Patients with endometrioid ovarian cysts: With undifferentiated dysplasia of connective tissues (n=35)	Patients with endometrioid ovarian cysts: Without undifferentiated dysplasia of connective tissues (n=35)
Group No.	1	2	3
		M±m*	
IL1β, pg/ml	131,60±7,18	519,40±16,96	475,20±13,30
Reliability of differences **	P1-2=0,001		
P1-3=0,001	P2-3=0,043		
TNFα, pg/ml	8,09±1,32	58,29±4,38	46,44±3,61
Reliability of differences	P1-2=0,001		
P1-3=0,001	P2-3=0,041		
IFNγ, pg/ml	121,30±10,80	50,18±4,43	68,60±5,41
Reliability of differences	P1-2=0,001		
P1-3=0,001	P2-3=0,010		
IL2, pg/ml	29,70±1,60	18,57±1,16	22,28±1,26
Reliability of differences	P1-2=0,001		
P1-3=0,001	P2-3=0,034		
IL4, pg/ml	60,10±5,10	55,94±3,79	65,90±4,15
Reliability of differences	P1-2=0,515		
P1-3=0,381	P2-3=0,081		
IL6, pg/ml	44,52±2,75	98,09±6,07	80,33±6,06
Reliability of differences	P1-2=0,001		
P1-3=0,001	P2-3=0,042		
IL8, pg/ml	23,30±2,70	274,30±11,82	227,20±19,88
Reliability of differences	P1-2=0,001		
P1-3=0,001	P2-3=0,046		

Note

* M - mean, m - mean deviation

** - the differences between groups are significant at $p<0.05$, the Mann-Whitney criterion

In the peritoneal fluid of patients with both endometrioid ovarian cysts and undifferentiated dysplasia of connective tissues compared with those of the comparison group and healthy women, had an increased level of IL1β (519,40 ± 16,96 pg /ml, 475,20 ± 13,30 pg /ml and 131,60 ± 7,18 pg / ml, respectively), TNFα (58,29 ± 4,38 pg/ml, 46,44 ± 3,61 pg /ml and 8,09 ± 1,32 pg /ml respectively), IL6 (98,09 ± 6.07 pg/ml, 80,33 ± 6,06 pg /ml and 44,52 ± 2,75 pg /ml, respectively) and IL8 (274,30 ± 11,82 pg /ml, 227,20 ± 19, 88 pg / ml and 23,30 ± 2,70 pg /ml respectively) and a reduced level of IL2 (18,57 ± 1,16 pg / ml, 22,28 ± 1,26 pg /ml, 29,70 ± 1.60 pg /ml, respectively) and IFNγ (50,18 ± 4,43 pg /ml, 68,60 ± 5,41 pg /ml and 121,30 ± 10,80 pg/ml, respectively), and the level of IL4 has no changes (Table 3).

CONCLUSION

Summarizing the data obtained, it can be argued that the level of peritoneal microenvironment in patients with endometrioid ovarian cysts develop immunological dysfunctions, such as the increase in activity of macrophages and the decrease in the activity of T-lymphocytes and natural killer cells. These changes are compounded in patients endometrioid ovarian cysts combined with undifferentiated dysplasia of connective tissues, as indicated by the decrease in the number of CD4-lymphocytes 1.3 times compared with patients without undifferentiated dysplasia of connective tissues and 1.7 times compared with healthy women, CD16-lymphocytes - 1.5 and 2.8 times, respectively and CD25 lymphocytes - 1.5 and 2.2 times, respectively and increase in the number of macrophages is 2.7 times compared to healthy women.

The changes in the composition of monoclonal cells in the peritoneal fluid of patients with endometrioid ovarian cysts combined with undifferentiated dysplasia of connective tissues, compared to patients with endometrioid ovarian cysts without undifferentiated dysplasia of connective tissues and healthy women, are naturally accompanied by an increase in the level of proinflammatory cytokines with proangiogenic action: the content of IL1β increases in 1.1 and 3.9 times, respectively, TNF-a - 1.3 and 7.25 times, respectively, IL6 - 1.2 and 2.2 times, respectively, IL8 - 1.2 and 11.7 times, respectively. In addition, when endometrioid ovarian cysts are combined with undifferentiated dysplasia of connective tissues, reduction of anticancer cytokines in native peritoneal fluid is IL2 1.2 times and IFNγ 1,36 times compared with patients without undifferentiated dysplasia of connective tissues. Similar changes in cytokine composition of the peritoneal fluid, along with a reduction in the number of immune-competent cells, with antitumor cytotoxic effect determines the increase proangiogenic and reduction of antitumor activity, which may be one of the preconditions for the increase of the frequency of relapses of patients with endometrioid ovarian cysts combined with undifferentiated dysplasia of connective tissues. The obtained data require further study.

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