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Original Article

Features of the cytokine type in the regulation of the immune response in women with functional ovarian cysts on the background of type 2 diabetes mellitus

Olga Sergeevna Shapoval [1] *, Michael Ivanovich Sheremet [2], Madalina Piron-Dumitrascu [3], Simona Raluca Iacoban [3]

[1] Department of Gynecology, SI Zaporizhian Medical Academy of Postgraduate Education of the Ministry of Healthcare of Ukraine, Zaporizhzhia, Ukraine

[2] Department of Surgery No.1, Bukovinian State Medical University, Chernivtsi, Ukraine

[3] Department of Obstetrics and Gynecology, Polizu Clinical Hospital, Carol Davila University of Medicine and Pharmacy, Bucharest, Romania

* Correspondence to: Shapoval Olga Sergeevna, Department of Gynaecology, SI Zaporizhian Medical Academy of Postgraduate Education of the Ministry of Healthcare of Ukraine, Vinter Avenue 20, Zaporizhzhia, Ukraine. Phone: +380508195300; E-mail: shapoval_olga@ukr.net

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Abstract

The aim of the work was to reveal the features of the cytokine type in the regulation of the immune response in women with functional ovarian cysts on the background of type 2 diabetes mellitus. A total of 104 patients of reproductive age with functional ovarian cysts and type 2 diabetes mellitus were examined in the Department of Gynecology in the limited liability company "VITACENTER". A general clinical examination, a study of the immune status and a bacteriological study of the vaginal discharge were carried out. In the spectrum of microorganisms isolated from the vagina, Gram- and Gram+ bacteria, *Candida albicans* were present. In patients with infertility and nulliparity, a decrease in the functional activity of neutrophils at the digestion stage was noted. The index of the phagocytic number of neutrophils indicated incompleteness of phagocytosis against the background of the depleted functional-metabolic reserve and the parameters of the bactericidal system were reduced. In women giving birth, the functional activity of neutrophils did not change; there was a decrease in digestive capacity against the background of the preserved functional metabolic reserve. In the group of nulliparous women, a Th2/Th1 type of immune response with a predominance of Th 2 type was observed. In patients with infertility, a Th1/Th2 type of immune response was observed with a predominance of the Th1 type. In the group of women giving birth, a Th2-type of immune response was observed. The change in the state of the vaginal microbiocenosis leads to the formation of dysfunction of the immune system, particularly the cytokine type of regulation. Patients with different parity have differences in the cytokine type regulation of the immune response.

Keywords: cytokines, immune response, type 2 diabetes mellitus, functional ovarian cysts.

Introduction

The relevance of improving the treatment of tumor-like formations of the ovaries is determined by the consistently high frequency of this pathology and the tendency to increase in recent years [1, 2]. Numerous studies indicate the development of cysts against the background of dysbiosis of the vaginal flora. The state of this microsystem, along with others, determines the immunological balance of the organism. Complex relationships of immunocompetent cells in the occurrence and development of pathological processes are mediated by universal molecules of intercellular interaction – cytokines [3]. Their induction is a direct response to the presence of microorganisms [4]. The choice of the immune system method of protection (Th1-, Th2-type)

depends on the type of antigen and its resistance to phagocytosis [4, 5].

A detailed study of the cytokine status in functional ovarian cysts against the background of type 2 diabetes mellitus was not made, which greatly complicated the understanding of this pathology's cytokine regulation features.

The article is aimed to study the features of the cytokine type of regulation of the immune response in women with functional ovarian cysts on the background of type 2 diabetes mellitus.

Material and methods

A total of 104 patients of reproductive age with functional ovarian cysts and type 2 diabetes mellitus were examined in the Department of Gynecology in the Limited liability company "VITACENTER" for the period 2019–2022.

Patients were divided into the following groups:

- Group 1 (control) – 50 healthy non-pregnant women of reproductive age who were consulted about contraception;
- Group 2 – patients with functional ovarian cysts with type 2 diabetes mellitus, who were divided into subgroups:
  - Subgroup 2 a – 25 women who did not give birth for various reasons;
  - Subgroup 2 b – 28 women with infertility;
  - Subgroup 2 c – 51 women who had previously given birth.

According to existing standards, all patients underwent general clinical and gynecological examinations. Ultrasound examination of the pelvic organs showed the presence of tumor-like formation of the ovaries size from 3 to 5 cm.

All the patients in the main group had type 2 diabetes mellitus. The disease was at the stage of compensation. The average level of HbA1c was (5.8±0.21)% due to taking Metformin hydrochloride 1500 mg daily.

Microbiological examination of vaginal discharge and cervix was carried out by an obstetrician-gynecologist by ingesting the material from the vagina with a sterile cotton swab. In the laboratory, the material was sifted into 5% blood agar and the Endo by the Gold method in order to count the growing colonies). Crops were placed in a thermostat at 37°C for 24–72 hours. When colonies were found on nutrient mediums, their counting and sifting into sectors of nutrient media and simple, nutritious agar for the identification of crops was carried out [the indicator of microbial seeding was determined – colonizing units/ml (CU/ml)].

In patients of all groups, indicators of the phagocytic link of the immune system were studied. The phagocytic activity of blood neutrophils has been determined according to the method of determining their absorption and digestion ability in relation to the microbial test culture after joint preincubation (Friemel N., 1984); the state of the oxygen-dependent metabolism of neutrophils (NST-test) (Vixman M.E., 1979); activity of myeloperoxidase (MPO) of neutrophils (Narcissov R.P., 1964); the content of cationic proteins (CP) in neutrophils (Shubich M.G., 1974).

Cytokine status was studied in the first phase of the menstrual cycle using the corresponding monoclonal antibodies from the sets of test systems "VECTOR-BEST" by enzyme immunoassay. The content of pro-inflammatory and anti-inflammatory cytokines – IL-4, IL-6, IL-10, IL-12, TNF-α, IF-γ – has been determined.

Statistical processing of the obtained data was performed using computer programs of the STATISTICA package (StatSoftStatistica v.6.0). An assessment of the nature of the distribution of the analyzed indicators according to the Kolmogorov-Smirnov consensus criterion determined that most of them do not obey the normal law. The statistical significance of the compared indicators was established using the Wald-Wolfowitz series criterion at the level of significance $P<0.05$. The analyzed data are presented as the median (Me) and the interquartile span (RQ), which is the difference between the values of the 75^{th} and 25^{th} percentiles (RQ=75% UQ-25% LQ), where UQ is the top quartile; LQ is the lower quartile.

Results

The results of a microbiological study of vaginal discharge showed that in the spectrum of microorganisms isolated from the samples, there were both Gram-positive and Gram-negative bacteria as well as *Candida albicans*.

In patients with functional ovarian cysts with type 2 diabetes mellitus, regardless of the previously implemented generative function, the sown flora was presented by *Str. pyogenes*, *S. epidermidis*, *S. aureus*, *E. coli*, *C. albicans*. *Str. pyogenes* was determined in concentration more than 10^6 CU/ ml in every third patient and in moderate concentration (10^{4}–10^6 CU/ml) in every seventh woman. *S. epidermidis* in moderate concentration (10^{4}–10^6 CU/ml) was determined in every

third patient. S. *aureus* and Ent. *faecalis* in moderate concentration (10^4–10^6 CU/ml) were determined in every fourth patient. In 25.96% of patients, the concentration of E. coli was more than 10^6 CU/ml (Table 1). The study of the microbial biotope of the genital tract found the presence of C. *albicans* in low and medium concentrations in 32.69% of patients. In 34% of cases, associations of E. coli – C. *albicans* were diagnosed in vaginal discharge in the group of patients with infertility and ovarian cysts and 25% – associations of S. *epidermidis* – C. *albicans*.

Based on the study of the functional and metabolic status of neutrophils in patients of subgroup 2 a, compared to the data of the control group, a decrease of 26% in the functional activity (index) of neutrophils (FIN) by 30 minutes and its significant decrease by 120 minutes by 34% was established.

Clinically significant in subgroup 2 a, there was an increase in the absorption capacity of neutrophils (phagocytic neutrophil number (PNN) by 30 min) by 19% and the digestive ability of neutrophils (PNN by 120 min) by 17% relative to the data of the control group. The NST test did not differ from the data of the control group, but the indicators of the stimulated NST test were reduced by 21%. A significant decrease in the activity rate of MPO by 30% relative to the data of healthy women was established. The content of CP was reduced by 9% as well.

In subgroup 2 b, a complete reduction in FIN was established both by 30 minutes – by 42%, and by 120 minutes – by 44%, respectively, of the control group data. PNN for 30 minutes and PNN for 120 minutes for infertility were significantly reduced by 45% and by 60% relative to healthy women (reliably). The NST test was reduced by 7%, and the stimulated NST test by 62% (reliably), respectively. The activity of MPO was reduced by 57%, and the CP – by 64% (reliably) relative to the results of the control group.

In subgroup 2 c, a significant decrease in the indicators of FIN was determined. In relation to the data of the control group, FIN was reduced by 33% by 30 minutes and by 120 minutes – 34%, respectively. PNN was

Table 1: Indicators of the microflora of the genital tract in women of reproductive age with functional ovarian cysts and diabetes mellitus.

Type of microorganism	Concentration, CU/ml	Control group (n=50)		Patients with ovarian cysts and diabetes mellitus (n=104)	
		N	%	n	%
	10^2–10^4	4	8	4	3.85
Str. Pyogenes	10^4–10^6	1	2	15	14.42
	$>10^6$	0	0	31	29.81
	10^2–10^4	2	4	10	9.62
S. epidermidis	10^4–10^6	1	2	33	31.73
	$>10^6$	-	-	40	3.85
	10^2–10^4	5	10	4	3.85
S. aureus	10^4–10^6	2	4	26	25
	$>10^6$	-	-	12	11.54
	10^2–10^4	1	2	12	11.54
Ent. Faecalis	10^4–10^6	-	-	27	25.96
	$>10^6$	-	-	2	1.92
	10^2–10^4	4	8	5	4.81
E. coli	10^4–10^6	-	-	13	12.5
	$>10^6$	-	-	27	25.96
	10^2–10^4	3	6	22	21.15
C. albicans	10^4–10^6	2	4	12	11.54
	$>10^6$	-	-	-	-

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reduced by 19% by 30 minutes and the PNN by 120 minutes – by 27%, respectively. A decrease in indicators from the spontaneous and stimulated NST test was determined by 23% and 38% relative to the indicators of the control group. The activity of MPO was reduced by 32%, and the content of CP by 22% (reliably).

The data obtained on the state of the functional and metabolic status of neutrophils coincides with the results of other authors [4–6] and confirm the opinion that bacteria have developed various mechanisms of protection against phagocytosis, as evidenced by the identified violations of the functional activity of phagocytes – their digesting ability.

In the study of the state of cytokine status in women of subgroup 2 a, a significant increase in the concentration of TNF-α by 166% and IL-12 by 227% was found. Concentration L-4 was also significantly increased – by 193%, respectively, to the indicators of the control group.

At the same time, there was a significant decrease in the concentration of IL-6 by 47% and IL-10 by 64% compared with the results of healthy women. The concentration of IF-γ was also significantly reduced by 39%. In accordance with the results obtained indicators of pro- and anti-inflammatory cytokine fractions, in patients with ovarian cysts and type 2 diabetes mellitus who did not give birth, there was a Th1/Th2 type of immune response with a predominance of Th1-type. This indicates the state of immune mechanisms aimed at realizing the antimicrobial effect and implementing the mechanism of antibody-dependent cytotoxicity (Table 2) [7].

In subgroup 2 b, there was a significant decrease in the level of IL-6 by 87% and IL-10 by 56%. A significant increase in the concentration of IL-12 was established by 585% (reliably) compared to the control group and IL-4 by 361%. The TNF-α and IF-γ levels also significantly increased by 96% and 79% compared to the indicators of the control group. Thus, patients with ovarian cysts and infertility experienced a Th1-type immune response with an increase in the proportion of cellular reactions regulated by Th1-type [4].

In subgroup 2 c, there was a 64% reduction in IL-6 levels. Concentrations of IL-10 and IF-γ were also reduced by 66% (significantly) and 30% relative to the results of healthy women. The level of IL-12 and IL-4 was significantly increased – by 146% (reliably) and 139%, respectively. The concentration of TNF-α exceeded the values of the control group by 59%. Thus, in patients with ovarian cysts and type 2 diabetes mellitus with realized reproductive potential, a Th1/Th2-type immune response with a predominance of Th1-type was observed (Table 3) [6].

Thus, the data obtained on the existing features of the cytokine type of regulation of the immune response in patients with functional ovarian cysts and type 2 diabetes mellitus make it possible to identify the cause of the low effectiveness of therapeutic measures in this group of patients. Thus, nulliparous patients have Th1/Th2-type immune response with a predominance of Th1-type; in infertility – Th1-type with an increase in the proportion of cellular reactions regulated by Th1-type; in patients who gave birth – Th1/Th2-type immune response with a predominance of Th1-type.

Table 2: The state of the functional-metabolic status of neutrophils in women with functional ovarian cysts.

	Me (75%Q–25%Q=RQ)							
Indicator, units of measurement	**FIN by 30 min, %**	**PNN by 30 min**	**FIN by 120 min, %**	**PNN by 120 min, %**	**NST sp, c.u.**	**NST st, c.u.**	**CP, c.u.**	**MPO, c.u.**
1 group n=50	67.5 (74.2-49.1= 25.1)	3.1 (6.5-1.3= 5.2)	58.4 (68.3-39.8= 28.5)	5.7 (6.1-4.0= 2.1)	1.2 (1.3-1.0= 0.3)	1.3 (2.2-0.8= 1.4)	2.2 (3.0-1.4= 1.6)	2.3 (3.4-0.7= 2.7)
Subgroup 2a n=25	50.2 (62.1-35.3= 26.8)	3.7 (6.2-1.9= 4.3)	45.1* (58.3-28.8= 29.5)	6.7 (7.8-2.9= 4.9)	1.2 (1.7-0.8= 0.9)	0.9 (1.7-0.2= 1.5)	2.0 (2.9-0.8= 2.1)	1.4* (2.2-0.7= 1.5)
Subgroup 2b n=28	39.4* (54.3-31.3= 23.0)	1.7* (3.2-1.1= 2.1)	32.8* (40.1-25.6= 14.5)	2.3* (3.9-1.8= 2.1)	1.0 (1.8-0.7= 1.1)	0.8* (1.4-0.4= 1.0)	0.9* (2.0-0.5= 1.5)	1.0 (2.0-0.6= 1.4)
Subgroup 2c n=51	39.4* (54.3-31.3= 23.0)	1.7* (3.2-1.1= 2.1)	32.8* (40.1-25.6= 14.5)	2.3* (3.9- 1.8= 2.1)	1.0 (1.8-0.7= 1.1)	0.5 (1.3-0.2= 1.1)	0.8* (1.4-0.4= 1.0)	1.0 (2.0-0.6= 1.4)

Note: * – statistically significant differences ($p<0.05$) relative to the control group.

Table 3: Condition of cytokine status in women with functional ovarian cysts and type 2 diabetes mellitus.

Indicator, pg/ml	Me (75%Q–25%Q=RQ)					
	Nulliparous women		Patients with infertility		Women who gave birth	
	Control	Subgroup 2 a	Control	Subgroup 2 b	Control	Subgroup 2 c
IL-6	0.79 (1.4-0.71= 0.69)	0.42* (0.69-0.23= 0.46)	4.44 (5.79-2.31= 3.48)	0.59* (0.62-0.43= 0.19	1.55 (1.7 – 1.4= 0.3)	0.56 (0.87-0.35= 0.52)
Il-10	4.90 (8.19-4.53= 3.66)	1.77 * (8.1-1.4= 6.7)	4.18 (5.12-3.64= 1.48)	1.85* (10.6-1.77= 8.83)	6.65 (8.86 – 5.11= 3.75)	2.31* (3.67-1.02= 2.65)
IL-12	1.92 (2.28-1.81= 0.47)	6.29* (7.75-5.50= 2.25)	1.13 (1.25-1.01= 0.24)	7.75* (11.7-5.3= 6.4)	1.50 (2.03 – 1.34= 0.69)	3.7* (5.21-2.78= 2.43)
IL-4	0.43 (0.5-0.33= 0.17)	1.26 (1.32-1.22= 1.0)	0.34 (0.47-0.23= 0.24)	1.57 (2.16-0.87= 1.29)	0.56 (0.88 – 0.46= 0.42)	1.34 (1.45-1.17= 0.28)
TNF-α	1.20 (1.41-1.17= 0.24)	3.19* (12.5-1.56= 10.94)	1.32 (1.78-0.91= 0.87)	2.6* (3.87-1.41= 2.46)	1.46 (1.75 – 1.27= 0.48)	2.33 (7.21-1.13= 6.08)
IF-γ	5.70 (6.17-5.07= 1.10)	3.51* (3.83-2.88= 0.95)	1.96 (3.84-1.04= 2.8)	3.51* (3.67-2.89= 0.78)	3.67 (6.01 – 3.2= 2.81)	2.57 (3.20-1.56= 1.64)

Note: * – statistically significant differences ($p<0.05$) relative to the control group.

Discussion

Treatment of functional cysts of the ovaries often begins with surgery performed in an unreasonably large volume without rehabilitation. It may cause the relapse of cysts [3, 4].

Benign tumor-like formations of the ovaries are diagnosed in 19–25% of patients with ovarian tumors [7, 8]. This problem is especially important in patients of reproductive age with comorbid pathology. Waiting for tactics, chaotic administration of hormonal drugs, the development of chronization of the process, conjugation and infertility contribute to an increase in the frequency of surgical aggression in patients with ovarian pathology. It decreases ovarian reserve and reproductive potential [3–6, 9].

That is why the work aimed to study the features of the cytokine type in the regulation of the immune response in women with functional ovarian cysts on the background of type 2 diabetes mellitus.

The results of microbiological examination of the microflora of the genital tract in patients with functional ovarian cysts and type 2 diabetes mellitus indicate the development of a pathological process against the background of vaginal dysbiosis in the presence of pathogenic gram-positive and gram-negative microflora.

Factors of pathogenicity of pathogens, including conditionally pathogenic ones, in various ways, affect the evolutionarily debugged mechanisms of regulation of the immune defense of the macroorganism [6, 9, 10]. It plays a key role in ensuring its homeostasis and minimizing the consequences of almost any pathological process, the outcome of which largely depends on the adequate functioning of various parts of immunity [4].

It was revealed that in the spectrum of microorganisms isolated from the samples, there were both Gram-positive and Gram-negative bacteria as well as *Candida albicans*.

In patients with functional ovarian cysts with type 2 diabetes mellitus, the sown flora was presented by *Str. pyogenes*, *S. epidermidis*, *S. aureus*, *E. coli*, *C. albicans*. *Str. pyogenes* in high and moderate concentration.

In 34% of cases in the group of patients with infertility and ovarian cysts association, *E. coli* – *C. albicans* were diagnosed in vaginal discharge and 25% – association of *S. epidermidis* – C. albicans. This fact leads to a reduction of nonspecific reactivity and maintenance of the existence of both diabetes mellitus and inflammatory process and can be counted as one of the reasons for infertility.

In patients of all groups, indicators of the phagocytic link of the immune system were studied.

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In patients of subgroup 2 a, a decrease in the functional activity of neutrophils and their digesting ability was diagnosed. It indicates incomplete phagocytosis against the background of a reduced functional and metabolic cell reserve.

In the patients of subgroup 2 b, a sharp decrease in the functional activity of neutrophils and their absorbing and digesting ability was established. It indicates an unauthorized phagocytosis against the background of depletion of cells' functional and metabolic reserves.

In patients of subgroup 2 c, the functional activity of neutrophils did not change; there was a decrease in their digesting ability against the background of a preserved functional and metabolic reserve.

Based on the results of the microbiological study of the biomaterial from the vagina, it seems likely that there is a direct effect of dysbiotic changes in the vaginal microbiocenosis on the state of the immune system.

In the group of nulliparous women, a Th2/Th1 type of immune response with a predominance of Th 2 type was observed. In patients with infertility, a Th1/Th2 type of immune response was observed with a predominance of the Th1 type. In the group of women giving birth, a Th2-type of immune response was observed. Thus, the change in the state of the vaginal microbiocenosis leads to dysfunction of the immune system, particularly the cytokine type of regulation. Patients with different parity have differences in the cytokine type regulation of the immune response.

Conclusions

A change in the state of the vaginal microbiocenosis can affect the normal functioning of the phagocytic link of the immune system, which leads to the formation of dysfunction of the immune system, particularly the cytokine type of regulation. An increase in the level of cytokines and a violation of their imbalance in the blood indicates the tension of the immune response and plays a significant role in the development of chronicity and progression of the disease. The obtained results, based on the various reproductive potential of women with functional ovarian cysts and type 2 diabetes mellitus, made it possible to identify differences in the functioning of both the phagocytic link of the immune system and the features of the formation of the cytokine type of regulation of the immune response. The data obtained indicate the need for an individual approach to treating these patients and the appropriate use of immunotropic drugs in complex therapy.

Conflict of interest

The authors declare no conflict of interest.

Ethics approval

The approval for this study was obtained from the Ethics Committee of the SI Zaporizhian Medical Academy of Postgraduate Education (approval ID: 04-11.02.2021).

Consent to participate

Written informed consent was obtained from the participants.

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