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Colonization resistance of vaginal secretion

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Abstract

Vaginal contents - vaginal secretion - this is a serous vascular transudate, a secret of the glands of the mucous membrane of the canine of the cervix and large glands of the vagina front, of the leukocytes and of the epithelium of the corpus luteum [1]. The main chemical composition of the vaginal secretion is water, inorganic salts, proteins (albumins, immunoglobulins), carbohydrates, fatty acids, urea, lysozyme [2, 3, 8]. Physiological maturation of epithelial cells, accumulation of glycogen in them and securing the acidity of the vaginal secretion are affected by estrogen.

To the protective mechanisms of the vagina are physiological white, the normal amount of which per day is up to 2 mg; A normal microflora of the vagina is present in the form of lactobacter bifidobacteria, which compete with the vaginal microflora for glycogen and create an acid medium. A key factor is the so-called "colonization resistance of the vaginal secretion," which includes the protective agents that enter humoral pathway here and are produced by local lymphoid tissue (IgA, IgM, IgG, lysozyme, a-lysines, complement, secretory IgA, etc.), bactericidal compounds hydrogen peroxide, lactic acid), cells of the immune system (leukocytes, macrophages, T- and B-lymphocytes) [4, 5, 6].

The purpose of the work was to study cellular and humoral indices at normal norms and at different levels of dysbiosis.

The results of the correlation analysis showed that the content of IL10 in the vaginal secretion had strong direct relations with humoral and cellular factors of colonial resistance -

IgA, sIgA, IgG_2, lysozyme, phagocytic activity of leukocytes and the index of phagocytic activity of leukocytes, C_3 and C_4, γ-INF (from r = 0.52 to r = 0.80; p <0.05 in all cases), and inverse - with all proinflammatory cytokines (from r = -0.68 to r = -0.77; p <0, 05 in all cases). Moreover, the positive association with the level of IL10 was with the level of lactobacillus (r = 0,56; p = 5,51E-26) and the main protein of myelin (r = 0,41; p = 3,15E-13), while with the indicator normobiota, the content of elective anaerobes and obligate anaerobes was negative (r = -0.49, r = -0.53 and r = -0.47, respectively, p <0.001 in all cases). This confirms the role of IL10 as a universal integrative regulator in the development of bacterial vaginosis.

Key words: colonization resistance, bacterial vaginosis, dysbiosis

Topicality. Vaginal contents - vaginal secretion - is a serous vascular transudate, a secret of the glands of the mucous membrane of the canine of the cervix and large glands of the vagina front, leukocytes and cells of the epithelium of the corpus luteum [1]. The main chemical composition of the vaginal secretion is water, inorganic salts, proteins (albumins, immunoglobulins), carbohydrates, fatty acids, urea, lysozyme [2, 3]. Physiological maturation of epithelial cells, accumulation of glycogen in them and securing the acidity of the vaginal secretion are affected by estrogen.

To the protective mechanisms of the vagina are physiological white, the normal amount of which per day is up to 2 mg; A normal microflora of the vagina is present in the form of lactobacter bifidobacteria, which compete with the vaginal microflora for glycogen and create an acid medium. A key factor is the so-called "colonization resistance of the vaginal secretion," which includes the protective agents that enter humoral pathway here and are produced by local lymphoid tissue (IgA, IgM, IgG, lysozyme, a-lysines, complement, secretory IgA, etc.), bactericidal compounds hydrogen peroxide, lactic acid), cells of the immune system (leukocytes, macrophages, T- and B-lymphocytes) [4, 5, 6].

Acidity (pH) of the vaginal secretion is normally 4.0-4.5. This is facilitated by the enzymatic cleavage of the glycogen produced by the epithelial cells in the secretory phase, into glucose, maltose and dextrose, which already due to lactobacilli are split into α-hydroxypropionic (lactic) acid [7].

The purpose of the work was to study cellular and humoral indices at normal norms and at different levels of dysbiosis.

In this study, against the background of normocenosis, the pH of vaginal secretion was from 3.76 to 4.20 (Table 1), the coefficient of variation was 3.0%.

Table 1

PH indicator in vaginal secretion (M ± m)

Groups	Subgroups	pH
1st group (normocenosis), n = 53	–	3,98±0,02
2nd group (1st degree dysbiosis), n = 128.	1st, n=23	4,07±0,04[1]
	2nd, n=83	4,16±0,02[1,2]
	3rd, n=22	4,28±0,03[1-3]
3rd group (II degree dysbiosis), n = 117	1st, n=34	4,66±0,03[1-4]
	2nd, n=83	5,32±0,03[1-5]
Statistical procedure for comparing results		
p(MW)[1]		0,078
p(MW)[2]		1,02E-07
p(MW)[3]		1,39E-10
p(MW)[4]		4,76E-15
p(MW)[5]		1,00E-22
F		388,440
p		0,00E-01

Notes. The probability of discrepancies using the Mann-Whitney U-criterion between the corresponding indices in group 1 and: p (MW) 1 - in the 1st subgroup of the 2nd group, p (MW) 2 - in the 2nd subgroup of the 2nd group, p (MW) 3 - in the 3rd subgroup of the 2nd group, p (MW) 4 - in the 1st subgroup of the 3rd group, p (MW) 5 - in the 2nd subgroup of the 3rd group; F - result and p - probability of dispersion analysis of the estimation of differences between the corresponding indices between subgroups

Such small differences in data indicate that there is a rigid mechanism for maintaining the pH of the vaginal secretion. In the case of dysbiosis of the 1st degree, the pH value was increased, and it increased as the amount of lactobacilli decreased and, accordingly, an increase in the norm of normobiote.

In the second stage of dysbiosis, with increasing degradation of vaginal biota, the pH value was significantly increased ($p < 0.001$).

A discriminant analysis, which determines which variables play a leading role in group distribution, has shown that for pH this criterion is $F = 5.55$ ($p = 0.004$).

Immune factors of colonization resistance of the vagina (content of immunoglobulins and lysozyme) are given in Table 2.

Table 2.

Indicators of the content of immunoglobulins and lysozyme in vaginal secretion (M ± m)

Group, subgroup		IgA, mkg/ml	IgM, mkg/ml	IgG, mkg/ml	IgG_2, mkg/ml	sIgA, mkg/ml	Lysozyme, mkg/ml
1st (normocenosis), n=53		89,7± 1,4	9,41± 0,19	112,5± 2,2	38,3± 0,7	132,5± 2,8	10,41± 0,18
2nd (dysbiosis of the 1st degree), n=128	1-a, n=23	84,8± 2,5	9,44± 0,24	111,6± 5,2	34,8± 1,5[1]	155,4± 1,0[1]	19,96± 0,85[1]
	2-a, n=83	85,2± 1,4[1]	14,08± 0,25[1,2]	113,7± 1,9	33,9± 0,6[1]	131,0± 2,3[2]	15,43± 0,26[1,2]
	3-я, n=22	72,6± 3,4[1-3]	21,15± 0,86[1-3]	81,4± 3,0[1-3]	21,2± 1,0[1-3]	80,4± 3,4[1-3]	8,26± 0,20[1-3]
3rd (II degree dysbiosis, n=117	1-a, n=34	67,1± 1,6[1-3]	24,09± 0,68[1-4]	82,2± 2,2[1-3]	25,3± 0,8[1-4]	37,6± 0,6[1-4]	4,07± 0,12[1-4]
	2-a, n=83	40,6± 0,7[1-5]	32,97± 0,64[1-5]	111,1± 1,9[4,5]	29,4± 0,7[1-5]	19,7± 0,4[1-5]	1,48± 0,03[1-5]
Statistical procedure for comparing results							
p(MW)[1]		0,084	0,743	0,717	0,050	1,55E-07	5,89E-11
p(MW)[2]		0,026	4,50E-19	0,869	4,21E-06	0,761	1,70E-19
p(MW)[3]		5,53E-05	1,22E-11	1,72E-08	2,93E-11	5,93E-11	1,97E-08
p(MW)[4]		1,68E-12	4,76E-15	2,60E-11	3,21E-13	4,76E-15	4,76E-15
p(MW)[5]		1,00E-22	1,00E-22	0,532	2,43E-14	1,00E-22	1,00E-22
F		207,027	359,215	28,808	50,931	756,724	724,307
p		0,00E-01	0,00E-01	9,79E-24	0,00E-01	0,00E-01	0,00E-01

Notes. The probability of discrepancies using the Mann-Whitney U-criterion between the corresponding indices in group 1 and: p (MW) 1 - in the 1st subgroup of the 2nd group, p (MW) 2 - in the 2nd subgroup of the 2nd group, p (MW) 3 - in the 3rd subgroup of the 2nd group, p (MW) 4 - in the 1st subgroup of the 3rd group, p (MW) 5 - in the 2nd subgroup of the 3rd group; F - result and p - probability of dispersion analysis of the estimation of differences between the corresponding indices between subgroups

With the deepening of dysbiosis, a progressive decrease in the level of vaginal IgA content was detected - up to 45-75% with dysbiosis of the II degree.

The content of IgM, on the contrary, was gradually increased - in 2,6-3,5 times (p <0,001). Such "switching" may be due to the growth of anaerobic flora, since IgM binds endotoxins of gram-negative bacteria (lipopolysaccharides).

IgG content was significantly reduced only in patients of the 3rd subgroup of group I and subgroup 1 of group II (up to 72-73% of the level of normocenosis). A flattening of IgG

level with the deepening of dysbiosis reflects the general tendency to develop an immunodeficiency under conditions of bacterial vaginosis. In view of this, it is interesting to raise the level of IgG in the 2nd subgroup of Group II, where, in fact, there was bacterial vaginosis. Perhaps this can be seen in the secondary activation of humoral immunity against the background of bacterial vaginosis, since the synthesis of IgG is activated in the presence of bacterial infections caused by predominantly gram-positive microorganisms.

This assumption was consistent with elevation against the background of second grade dysbiosis after a preliminary decrease in the content of IgG-IgG2 subfraction, the most resistant to the action of proteolytic enzymes and the one that strongly binds the antigen. Reduced levels of IgG2 are often accompanied by a deficiency of IgG4 and IgA. The latter was also noted in our studies.

Discriminant analysis revealed a significant role in the group distribution of IgM and IgG2 ($F = 3.85$; $p = 0.022$ and $F = 5.76$; $p = 0.004$, respectively).

The content of these local factors of colonial resistance, such as secretory IgA and lysozyme, was systematically reduced and reached a 6-7-fold decrease in dysbiosis of grade II.

At the same time, there was a significant increase in the level of both factors in the 1st subgroup with dysbiosis of the I degree (by 17% and 92%, respectively, $p < 0.001$ in both cases). Obviously, against the background of dysbiosis, there is a progressive depletion of the reserves of the synthesis of secretory IgA and lysozyme with an increase in the proportion of the conditionally pathogenic microflora.

Both of these factors played a significant role in the group distribution according to the results of the discriminatory analysis: for secretory IgA $F = 7.41$ ($p = 0.001$), for lysozyme $F = 104.1$ ($p < 0.001$).

Indicators of nonspecific resistance - the level of phagocytic activity of leukocytes and the index of phagocytic activity of leukocytes are given in Table 3.

The index of phagocytic activity of leukocytes, which in percent reflects the proportion of cells that entered phagocytosis, from their total, varied from 14% to 45% in normocenosis, with most of the indicators (68%) being in the range of 27% to 36%. That is, about one third of leukocytes found in vaginal secretions were in a state of phagocytosis.

Phagocytic activity of leukocytes in the vaginal secretion at normocenosis varied in rather wide limits - from 2.9 s.u. to 6.4 s.u., with most indicators (74%) were in the range of 2.9-4.3 s.u.. In other words, on average, each phagocytic leukocyte had from 3 to 5 absorbed latex particles.

According to the data, both indicators were increased with dysbiosis of the I degree in the 1 st subgroup, while the statistical confirmation of this increase was obtained only for phagocytic activity of leukocytes - by 26.5% ($p < 0.001$). Subsequently, both indicators decreased systematically and simultaneously, and in the second grade dysbiosis in the 2nd subgroup they accounted for 18% of the corresponding indicators of normocenosis. The distribution of the index of phagocytic activity of leukocytes was from 3% to 0%, that is, phagocytic cells were observed very little, and they contained at most one latex lobe. Based on these data, it can be concluded that essential inhibition of phagocytic activity of leukocytes in the vaginal secretion as the degree of dysbiosis increases and, accordingly, the development of a local non-specific cellular immune deficiency.

Indicator of IR in the 1st and 2nd subgroups for the dysbiosis of the I degree compared with such for the normocenosis did not change significantly. In the 3rd subgroup for dysbiosis of the 1st degree and in the 1st subgroup for the second stage dysbiosis, this indicator was significantly higher (1.4-1.5 times; $p < 0.001$), while in the 2nd group for the dysbiosis II the degree of the content of IR was twice lower than the norm of normocenosis ($p < 0,001$).

Consequently, the initial stages of dysbiosis (IUPP from -3 lg GE / sample and PNB up to 1.0 lg GE / sample) were not accompanied by elevated IR formation. Further growth of conditionally pathogenic microflora led to an increase in the formation of IR in vaginal secretion, which corresponded to the dynamics of increasing the level of IgM (Table 2).

Moreover, on the background of bacterial vaginosis (in the 2nd subgroup for the second stage dysbiosis) the content in the vaginal secretion of IgM continued to increase significantly, but the formation of the IC significantly decreased.

It is probable that the humoral link of nonspecific resistance to a different degree of dysbiosis was in a different functional state - activated in the process of transition to second grade dysbiosis and was suppressed (despite a rather high level of IgM) against bacterial vaginosis. In the latter case, the extremely low levels of secretory IgA and lysozyme supplemented the picture of deep local non-specific immunodeficiency and, along with the decrease in the indicators of phagocytic activity of leukocytes, allowed to state the presence of a combination of localized immunodeficiency against the background of bacterial vaginosis.

As you know, the complement of the IR is included. The content of components of the complement in the vaginal secretion (Table 3) corresponded to changes in the level of inflammation - was elevated compared to the norm for the normocenosis for the dysbiosis of the 1st degree (2^{nd} subgroup - 1.3 times; $p < 0.05$), decreased with the transition to dysbiosis of the second degree (up to 50-66% of the level of normocenosis) and sharply decreased on the

background of BV (up to 20% for C_3 and up to 17% for C_4; p <0,05).

Table 3

Indices of phagocytic activity of leukocytes and its index, content of immune complexes and components of the complement of C_3 and C_4 in vaginal secretion (M±m)

Group, subgroup		PHAL, s.u.	Index PHAL, %	IK, u/ex.	C_3, mkg/ml	C_4, mkg/ml
1st group (normocenosis), n = 53		3,24± 0,13	32,0± 1,0	4,89± 0,16	15,6± 0,43	3,53± 0,07
2nd group (1st degree dysbiosis), n = 128	1st, n=23	4,10± 0,12[1]	35,4± 1,5	5,03± 0,29	17,4± 0,8	3,64± 0,11
	2nd, n=83	2,03± 0,04[1,2]	22,3± 0,4[1,2]	5,13± 0,09	20,5± 0,3[1,2]	4,62± 0,08[1,2]
	3rd, n=22	1,20± 0,05[1-3]	11,9± 0,4[1-3]	7,18± 0,21[1-3]	12,5± 0,4[1-3]	2,28± 0,07[1-3]
3rd group (II degree dysbiosis), n = 117	1st, n=34	1,02± 0,03[1-4]	8,1± 0,2[1-4]	6,86± 0,19[1-3]	7,8± 0,2[1-4]	1,19± 0,03[1-4]
	2nd, n=83	0,60± 0,01[1-5]	5,9± 0,1[1-5]	2,46± 0,04[1-5]	3,1± 0,1[1-5]	0,60± 0,01[1-5]
Statistical procedure for comparing results						
p(MW)[1]		2,60E-05	0,094	0,751	0,065	0,541
p(MW)[2]		4,36E-15	7,95E-15	0,153	1,43E-13	4,83E-14
p(MW)[3]		1,82E-11	1,82E-11	6,16E-09	5,01E-05	5,49E-11
p(MW)[4]		5,10E-15	4,76E-15	6,39E-10	4,76E-15	4,76E-15
p(MW)[5]		1,00E-22	1,00E-22	1,56E-21	1,00E-22	1,00E-22
F		349,545	380,932	174,747	555,406	654,759
p		0,00E-01	0,00E-01	0,00E-01	0,00E-01	0,00E-01

Notes. The probability of discrepancies using the Mann-Whitney U-criterion between the corresponding indices in group 1 and: p (MW) 1 - in the 1st subgroup of the 2nd group, p (MW) 2 - in the 2nd subgroup of the 2nd group, p (MW) 3 - in the 3rd subgroup of the 2nd group, p (MW) 4 - in the 1st subgroup of the 3rd group, p (MW) 5 - in the 2nd subgroup of the 3rd group; F - result and p - probability of dispersion analysis of the estimation of differences between the corresponding indices between subgroups

Perhaps it was the reduction in the level of the complement that led to a decrease in the formation of inflammation in the background of bacterial vaginosis, when the level of IgM was quite high.

In the group distribution, according to the discriminant analysis, the complement component C_3 played a significant role (F = 13.1; p <0.001).

The content of pro- and anti-inflammatory cytokines is presented in Tables 4 and 5. Of all the proinflammatory cytokines studied in group 1 of group 2 (dysbiosis of the I degree), only TNFα, whose content was higher than that for normocenosis in 1.4 times ($p < 0.05$). The content of the remaining cytokines in this group has not changed.

Tables 4

The content of proinflammatory cytokines in the vaginal secretion (M±m)

Group, subgroup		IL1β, пг/ml	IL2, пг/ml	IL6, пг/ml	IL8, пг/ml	TNFα, пг/ml
1st group (normocenosis), n = 53		18,4±0,6	19,2±0,8	12,4±0,5	39,0±1,2	19,5±0,4
2nd group (1st degree dysbiosis), n = 128	1-a, n=23	20,1±0,06	18,5±1,4	12,6±0,6	40,3±2,1	$28,3±0,9^{1}$
	2-a, n=83	$26,3±0,4^{1,2}$	21,3±0,4	$25,0±0,3^{1,2}$	$69,5±1,3^{1,2}$	$35,0±0,7^{1,2}$
	3-я, n=22	$37,6±1,2^{1-3}$	$39,3±1,3^{1-3}$	$28,3±1,1^{1-3}$	$78,1±3,0^{1-3}$	$37,5±1,6^{1,2}$
3rd group (II degree dysbiosis), n = 117	1-a, n=34	$41,4±1,0^{1-4}$	$40,5±1,1^{1-3}$	$40,8±1,0^{1-4}$	$87,0±2,9^{1-4}$	$36,8±1,2^{1,2}$
	2-a, n=83	$51,1±0,9^{1-5}$	$40,5±0,8^{1-3}$	$42,4±0,9^{1-4}$	$94,0±1,7^{1-5}$	$58,4±1,1^{1-5}$
Statistical procedure for comparing results						
$p(MW)^1$		0,111	0,519	0,512	0,449	1,36E-09
$p(MW)^2$		4,39E-17	0,025	4,46E-22	4,95E-21	9,73E-22
$p(MW)^3$		1,22E-11	2,31E-11	1,97E-11	1,97E-11	1,32E-11
$p(MW)^4$		4,76E-15	4,76E-15	4,76E-15	1,01E-14	6,26E-14
$p(MW)^5$		1,00E-22	3,43E-22	1,43E-22	1,14E-22	1,00E-22
F		290,399	180,844	298,438	150,455	213,452
p		0,00E-01	0,00E-01	0,00E-01	0,00E-01	0,00E-01

Notes. The probability of discrepancies using the Mann-Whitney U-criterion between the corresponding indices in group 1 and: p (MW) 1 - in the 1st subgroup of the 2nd group, p (MW) 2 - in the 2nd subgroup of the 2nd group, p (MW) 3 - in the 3rd subgroup of the 2nd group, p (MW) 4 - in the 1st subgroup of the 3rd group, p (MW) 5 - in the 2nd subgroup of the 3rd group; F - result and p - probability of dispersion analysis of the estimation of differences between the corresponding indices between subgroups

As the dysbiosis deepened, the content of proinflammatory cytokines increased synchronously and systematically. Thus, the content of IL1β in the 2nd group of dysbiosis II degree (for BV) exceeded the norm of normocenosis by 2.8 times, IL2 - by 2.1 times, IL6 -

3.4 times, IL8 - 2.4 times, and TNFα - 3.0 times (all differences were statistically

significant - p <0.001).

The degree of increase in the level studied interleukins were distributed as follows: IL6> TNFα> IL1β> IL8> IL2. It can be noted that TNFα first responded to dysbiosis, IL6 demonstrated the maximum increase, and IL2 was the most inert reaction.

Although it should be noted that this distribution can be considered conditional, since all proinflammatory interleukins have demonstrated a friendly increase in the level and a pronounced reaction to the activation of opportunistic microflora, confirming the position of the "chain cascade" of the interleukin reaction against the background of inflammation. The peculiarity of such a reaction in the vaginal secretion, in our opinion, can be considered a friendly and parallel increase in their content with an increase in the degree of dysbiosis.

According to discriminant analysis in the distribution, the most significant role was played by TNFα (F = 13.3; p <0.001) and IL6 (F = 8.9; p <0.001). For anti-inflammatory cytokines, the opposite trend was noted: their content decreased significantly with the increase of dysbiosis (Table 5).

The content of γ-INF did not actually change in the 1st subgroup of dysbiosis of the 1st degree, but reached the maximum in the 2nd subgroup (increased by 1.3 times; p <0.001). As the dysbiosis deepened, the content of γ-INF decreased and at BV was 21.9% of the normocytosis. Similarly, the level of component of complement C3 (Table 3) also changed.

The content of IL4 and IL10, like most other interleukins, did not actually change in the 1st grade dysbiosis subgroup. With an increase in the degree of dysbiosis, it was systematically reduced and made up by 22% for IL4 and 25% for IL10 from the level of normocenosis (p <0.001 for both indicators) by bacterial vaginosis.

Both cytokines played a significant role in the group distribution of patients: IL4 - F = 3.1; p = 0.047; IL10 -F = 3.1; p = 0.047.

The level of TGF-1β in vaginal secretion in the 1st degree of dysbiosis of the 1st degree also did not actually change. With the deepening of dysbiosis, a double step increase in the content of this cytokine was recorded in comparison with the norm of normocenosis - in 1,2-1,3 times in the 2nd and 3rd subgroups of the dysbiosis of the I degree and in 2,4-2,5 times in the second grade dysbiosis . Obviously, this can explain the significance of this factor in the group distribution (F = 57,4; p <0,001).

Table 5

The content of regulatory cytokines in vaginal secretions (M±m)

Group, subgroup		γ-INF, пг/ml	IL4, пг/ml	IL10, пг/ml	TGF-1β, пг/ml
1st group (normocenosis), n = 53		1,87±0,06	26,8±0,9	11,44±0,40	37,8±1,1
2nd group (1st degree dysbiosis), n = 128	1-a, n=23	1,93±0,05	29,1±0,7	7,85±0,29	35,5±2,2
	2-a, n=83	2,47±0,04[1,2]	18,9±0,3[1,2]	6,28±0,11[1,2]	45,5±0,9[1,2]
	3-я, n=22	0,84±0,03[1-3]	12,5±0,4[1-3]	3,16±0,10[1-3]	48,4±1,6[1,2]
3rd group (II degree dysbiosis), n = 117	1-a, n=34	0,71±0,02[1-4]	12,4±0,3[1-3]	3,00±0,08[1-3]	92,3±2,4[1-4]
	2-a, n=83	0,41±0,01[1-5]	5,9±0,1[1-5]	2,81±0,05[1-5]	94,7±1,0[1-4]
Statistical procedure for comparing results					
p(MW)[1]		0,415	0,032	1,01E-07	0,371
p(MW)[2]		2,38E-12	1,91E-14	1,56E-18	4,19E-07
p(MW)[3]		1,43E-11	1,43E-11	2,13E-11	4,18E-06
p(MW)[4]		4,76E-15	7,20E-15	8,85E-15	4,76E-15
p(MW)[5]		1,00E-22	1,00E-22	1,63E-22	1,00E-22
F		550,794	379,834	315,207	454,711
p		0,00E-01	0,00E-01	0,00E-01	0,00E-01

Notes. The probability of discrepancies using the Mann-Whitney U-criterion between the corresponding indices in group 1 and: p (MW) 1 - in the 1st subgroup of the 2nd group, p (MW) 2 - in the 2nd subgroup of the 2nd group, p (MW) 3 - in the 3rd subgroup of the 2nd group, p (MW) 4 - in the 1st subgroup of the 3rd group, p (MW) 5 - in the 2nd subgroup of the 3rd group; F - result and p - probability of dispersion analysis of the estimation of differences between the corresponding indices between subgroups

The results of discriminant analysis are summarized in Table 6.

Table 6

Results of discriminatory analysis on the indices of factors of local colonial resistance (located as the F was killed)

Indicator	Wilks'-Lambda	F	p
Lysozyme	0,016	104,065	0,00E-01
TGF-1β	0,013	57,356	1,52E-21
IL10	0,013	53,630	2,16E-20
TNFα	0,010	13,259	3,18E-06
C_3	0,010	13,076	3,76E-06
IL6	0,010	8,893	1,81E-04
sIgA	0,010	7,414	7,31E-04
IgG_2	0,010	5,764	0,004
pH	0,010	5,546	0,004
IgM	0,010	3,850	0,022
ФАЛ	0,010	3,061	0,048
IL4	0,010	3,090	0,047

Determining role in subgroup distribution, depending on the degree of dysbiosis, was played by lysozyme, TGF-1β and IL10. This fact agrees with the data on the leading role of lysozyme as the main protective factor of colonial resistance. High values of the F factor for TGF-1β and IL10 were less expected.

TGF-1β is one of the most studied representatives of the TGF supermassage. Its source is a lot of cells, primarily activated leukocytes and regulatory T-lymphocytes, and the function is the autocrine effect, i.e., suppression of secretion and responses to IL1, IL2, γ-INF, TNFα and other proinflammatory cytokines. As the correlation analysis showed, the TGF-1β content in the vaginal secretion had strong direct interactions with the content of all proinflammatory interleukins and IgM (the coefficient of correlation r ranged from 0.71 to 0.82).

Moreover, the positive correlation with the TGF-1β content was with the index of opportunistic microflora ($r = 0.75$; $p < 0.001$) and normobiotic index ($r = 0.51$; $p = 1.01E-20$), while with the general the bacterial mass and the amount of lactobacilli was negative (r = -

0.54 and r = -0.63, respectively, p <0.05 in both cases).

Based on these results, it can be assumed that TGF-1β served as a factor suppressing colonial resistance to vaginal secretion and, having immunoregulatory activity, suppressed the immune response against the background of vaginal dysbiosis. This assumption is confirmed by the presence of a negative correlation of TGF-1β with IgA, secretory IgA, lysozyme, phagocytic activity of leukocytes, complement components of C_3 and C_4, γ-INF, IL4 and IL10 (r value from -0.69 to -0, 81; p <0.05 in all cases).

The most influential factors for immunosuppression were (Table 6) lysozyme, IL10, and the complement of C_3, the content of which by bacterial vaginosis decreased by 7.0, 4.1 and 5.0 times, respectively. Together with TGF-1β, immunosuppression may be considered to be an increase in the vaginal secretion of levels of TNFα and IL6, which was 3.0 and 3.4 times, respectively.

Among the conditionally pathogenic microflora, TGF-1β levels were associated with the content of obligate anaerobes (r values ranged from 0.31 to 0.40, p <0.05 in all cases).

Nearly the same as in TGF-1β, criterion F was at IL10 (F = 53.6; p <0.001). This anti-inflammatory cytokine is produced by activated monocytes, macrophages, T-helper cells and is a very potent inhibitor for these cells. Thus, it is known that IL10 inhibits the formation of γ-INF, proinflammatory cytokines, expression of TNFα and IL12 receptors. The activity of IL10 in inhibiting the formation of TNFα, IL1 and IL6 is due to its ability to inhibit the formation of IL12.

As the results of the correlation analysis showed, the content of IL10 in the vaginal secretion had strong direct relationships with the humoral and cellular factors of colonial resistance - IgA, sIgA, IgG_2, lysozyme, phagocytic leukocyte activity, and the index of phagocytic activity of leukocytes, C_3 and C_4, γ-INF from r = 0.52 to r = 0.80; p <0.05 in all cases), and inverse - with all proinflammatory cytokines (from r = -0.68 to r = -0.77; p <0, 05 in all cases). Moreover, the positive relationship between the content of IL10 and the level of lactobacilli (r = 0,56; p = 5,51E-26) and obligate bacteria (r = 0,41; p = 3,15E-13), while with the norm of normobiotic , the content of facultative anaerobes and obligate anaerobes were negative (r = -0.49, r = -0.53 and r = -0.47 respectively, p <0.001 in all cases). This confirms the role of IL10 as a universal integrative regulator in the development of bacterial vaginosis.

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