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UDC – [618.3-06: 616.314.17-002]-092: 612.017.1-00864]-07-08(045)
DOI – http://dx.doi.org/10.14300/mnnc.2015.10034
ISSN – 2073-8137

SPECIFIC FEATURES OF PERIPHERAL BLOOD LYMPHOCYTES IMMUNOPHENOTYPE IN PREGNANT WOMEN WITH CHRONIC PERIODONTITIS

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ОСОБЕННОСТИ ИММУНОФЕНОТИПА ЛИМФОЦИТОВ ПЕРИФЕРИЧЕСКОЙ КРОВИ У БЕРЕМЕННЫХ ЖЕНЩИН С ХРОНИЧЕСКИМ ПАРОДОНТИТОМ

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Purpose of study was to identify the specific features of cellular immunity through immunophenotypic analysis of peripheral blood lymphocytes in pregnant women with inflammatory periodontal disease depending on the structure of the pathology as well as the severity of chronic periodontitis. 165 pregnant women with inflammatory periodontal disease; 31 pregnant women with physiological pregnancy; 32 healthy female volunteers. All the women had their immunophenotype of peripheral blood lymphocytes investigated. The immunophenotyping of the lymphocytes bearing the $CD3^+$, $CD4^+$, $CD8^+$, $CD16^+$, and $CD95^+$ markers was performed with laser flow cytometry. The study showed that along with an increasing degree of chronic periodontitis severity the pregnant women developed more pronounced lymphopenia, imbalance in T cell subsets with a decrease in the relative number of mature T-lymphocytes and T-helper cells against an increasing number of cells possessing killer and apoptotic activity. The pregnant women with chronic periodontitis revealed changes in the lym-

phocytes immunophenotype, which were basically similar to alterations in pregnancies with no dental pathologies, yet proved more prominent, which predisposed to a prolonged course of chronic inflammatory diseases.

Key words: pregnancy, chronic periodontitis, lymphocytes immunophenotype, cellular immunity

Определялись особенности клеточного иммунитета путем иммунофенотипирования лимфоцитов у беременных женщин с воспалительными заболеваниями пародонта в зависимости от структуры патологии и степени тяжести хронического пародонтита. У 165 беременных женщин с воспалительными заболеваниями пародонта, 31 здоровой беременной женщины и у 32 здоровых волонтеров изучен иммунофенотип лимфоцитов. Иммунофенотипирование лимфоцитов с маркерами $CD3^+$, $CD4^+$, $CD8^+$, $CD16^+$, $CD95^+$ проводили методом лазерной проточной цитофлюориметрии. Установлено, что у беременных женщин с хроническим пародонтитом по мере повышения степени тяжести наблюдалась более выраженная лимфопения, дисбаланс субпопуляций Т-клеток с уменьшением относительного числа зрелых Т-лимфоцитов и Т-хелперов на фоне повышения клеток с киллерной и апоптической активностью. У беременных женщин с хроническим пародонтитом изменения иммунофенотипа лимфоцитов по направленности повторяли изменения при беременности без стоматологической патологии, но были более выраженными, что предрасполагало к затяжному течению хронических воспалительных заболеваний.

Ключевые слова: беременность, хронический пародонтит, иммунофенотип лимфоцитов, клеточный иммунитет

Immunophenotypic analysis of the blood cells is the gold standard in diagnosing infectious diseases [3]. Leukocytes of each subpopulation are known to express specific surface molecules, which can serve as markers. There has been developed a systematized nomenclature of marker molecules, typically indicated with the acronym CD (Cluster of Differentiation); this helps distinguish between different subpopulations of lymphocytes, differentiate between resting cells and activated ones, and understand their interactions [2]. Cooperation of the immune cells involved into the elimination of inflammation in periodontium largely determines the progress of chronic periodontitis [1, 4, 5]. In pregnant women, the presence of chronic infection foci in the oral cavity affects the fetus, gestation period and labor. Infectious diseases of the periodontium, for instance, increase the risk of fetal growth retardation, premature labor, low birth weight, preeclampsia, and increased maternal mortality [6–9].

Investigation into the link between inflammatory processes of the periodontium and cellular immune response would help reasoning the scientific concept of systemic consequences caused by inflammatory periodontal disease in pregnant women on the whole. Given this, the purpose of this work was to identify the specific features of cellular immunity through immunophenotypic analysis of peripheral blood lymphocytes in pregnant women with inflammatory periodontal disease depending on the structure of the pathology as well as the severity of chronic periodontitis.

Material and Methods. The 165 pregnant women that entered the clinical group and had gingivitis, chronic periodontitis of mild to moderate severity underwent immunophenotyping of peripheral blood lymphocytes through determination of the absolute and relative amounts of lymphocytes with markers $CD3^+$ (total T-lymphocytes), $CD4^+$ (T-helper cells), $CD8^+$ (cytotoxic cells), $CD16^+$ (NK-cells), $CD95^+$ (FAS/APO-1 antigen inducing factor of apoptosis), and the total count of lymphocytes per 1 ml of blood. The absolute number of leukocytes was calculated as $\times 10^9$/l. The two control groups enrolled 31 pregnant women with physiological pregnancy and no dental diseases diagnosed, and 32 healthy female volunteers.

In the clinical group the population's age varied from 18 to 39, while the healthy pregnant women were aged 18–34, and the age of the healthy donors was 17 through 40. The mean age of the pregnant women in the clinical group was 28.1±1.7; among the healthy pregnant women – 25.8±1.9, and in the healthy donors' group – 26.3±2.0.

Mononuclear blood cells were isolated by centrifugation employing density gradient ficoll-verografin (density 1.077 g/cm^3). The immunophenotyping of lymphocytes was performed through laser flow cytometry (Cytomics FC500, BeckmanCoulter, USA) using mouse monoclonal antibodies LT3, LT4, LT8, LNK16, LT95, FITC-labeled (JSC SORBENT, Moscow, Russia) to the following antigens: $CD3^+$ (total T-lymphocytes), $CD4^+$ (T-helper cells), $CD8^+$ (cytotoxic cells), $CD16^+$ (NK-cells), $CD95^+$ (FAS/APO-1 antigen inducing factor of apoptosis).

The statistical analysis of the data obtained was performed with the STATISTICA 10.0 software (StatSoft Inc., USA). The investigated values were presented as sample mean and standard error of the mean value. The significance of the mean value differences of independent samples was evaluated using the parametric t-test for a normal distribution and the Mann-Whitney non-parametric test for the non-normal distribution. The decision on distribution of the variables in the sample was developed using the test for normality and the Shapiro-Wilk test. Each criteria value had its significance level of p calculated, whereas the critical level of significance was p=0.05.

Results and Discussion. Among periodontal diseases the frequency of chronic catarrhal gingivitis reached 14.5% (n=24), hypertrophic gingivitis – 22.4% (n=37), chronic ulcerative gingivitis – 6.7% (n=11), and chronic periodontitis of mild to moderate severity – 56.4% (n=93). This means that in the clinical group gingivitis was detected in 72 (43.6%) women while mild to moderate chronic periodontitis – in 93 (56.4%).

In the clinical group, localized chronic periodontitis was observed in 32 (34.4%) women, while another 61 (65.6%) patients with chronic periodontitis had this ailment in its generalized form. The subgroup of pregnant women with chronic periodontitis joined 51 (54.8%) and 42 (45.2%) patients with mild and moderate severity of the disease, respectively.

Table 1 shows the number of lymphocyte subpopulations in general in the clinical group compared with the control groups. Unlike the healthy donors, the healthy women with normal pregnancy demonstrated a 10% decrease in the total number of lymphocytes as well as in the percentage of mature T-lymphocytes ($CD3^+$), T-helper cells ($CD4^+$), by 8.9% ($p<0.05$) and 7.4% ($p<0.05$), respectively, against an increase in the T-cytotoxic ($CD8^+$) cells by 19.5% ($p<0.05$). The change in the numbers of helper and killer lymphocytes resulted in a reduced immunoregulatory index CD4/CD8 (by 17.4%; $p<0.05$). The absolute and relative number of lymphocytes with natural killer activity CD16+ went down by 19.6%, while the number of lymphocytes bearing the cell death programming receptor was elevated by 26.2% ($p<0.05$). The registered change in lymphocyte immunophenotyping in the pregnant women with physiological pregnancy was evidence of slight immunosuppression.

Table 1

Absolute and relative numbers of lymphocytes and their subpopulations in clinical and control groups

Index	Clinical group (n=165)	Healthy donors (n=32)	Healthy pregnant women (n=31)
Lymphocytes, %	24.6±0.6*°	30.1±1.2	27.1±0.8*
Lymphocytes, $x10^9$/l	1.6±0.2	1.9±0.4	1.7±0.3
CD3+,%	60.8±1.2*°	70.5±2.2	64.2±1.1*
CD3+,$\times10^9$	0.97±0.1	1.1±0.2	1.0±0.1
CD4+,%	38.2±0.4*°	43.3±0.6	40.1±0.5*
CD4+,$\times10^9$	0.61±0.08	0.73±0.09	0.62±0.06
CD8+,%	28.9±0.5*°	21.5±1.3	25.7±0.7*
CD8+,$\times10^9$	0.47±0.05	0.42±0.03	0.48±0.02*
CD4/CD8 ratio	1.32±0.1*°	1.9±0.3	1.57±0.2
CD16+,%	23.5±0.7*°	20.9±1.0	16.8±0.6*
CD16+,$\times10^9$	0.37±0.04°	0.40±0.03	0.28±0.02*
CD95+,%	9.9±0.6*°	6.5±0.9	8.2±0.4*
CD95+,$\times10^9$	0.16±0.01*	0.10±0.02	0.13±0.01

Note: * – significant difference in comparison to the healthy donors at $p<0.05$, ° – significant difference in comparison to the healthy pregnant women.

In the pregnant women with inflammatory periodontal disease, the decrease in the total number of lymphocytes, mature T-lymphocytes ($CD3^+$) and lymphocytes with helper and inductor properties ($CD4^+$) occurred by 18.3% ($p<0.05$), 13.8% ($p<0.05$) and 11.8% ($p<0.05$), respectively, if compared to healthy donors. At the same time there was a significant increase in the number of cytotoxic cells as well as lymphocytes possessing natural killer activity. Due to this, the immunoregulatory index in the pregnant women occurring along with inflammatory periodontal disease went down significantly by 30.5% ($p<0.05$) compared to the healthy volunteers. The pregnant women of the clinical group revealed an increase by 52.3% ($p<0.05$) in the relative amount of lymphocytes characterized by apoptotic activity, which bore a predisposition to cytokine imbalance.

Thus, if compared to the healthy pregnant women, the pregnant women with inflammatory periodontal disease had their immunosuppression expressed to a greater extent, which implied a predisposition to a prolonged course of chronic inflammatory diseases.

The change in the cell-mediated immune responses depended on the type of periodontal disease (Table 2). The pregnant women with mild to moderate chronic periodontitis had (compared to the patients with gingivitis) a more pronounced decrease in the total number of lymphocytes (23.3% vs. 8.3%), mature T-lymphocytes (16.7% vs. 9.9%), T-helper cells (1.4% vs. 8.3%). T-lymphocytes with cytotoxic activity ($CD8^+$) in the pregnant women with mild to moderate chronic periodontitis went 38.6% above the same index in the healthy donors, which exceeded the increase observed in comparison to the patients suffering from gingivitis (by 21.4%). The number of lymphocytes expressing $CD16^+$ went up significantly, compared with the healthy volunteers, only in the patients with chronic periodontitis of mild to moderate severity (by 18.7%). In the patients with gingivitis there were no significant changes in $CD16^+$ lymphocytes observed if compared to the control numbers. When compared to the healthy donors, the immunoregulatory index CD4/CD8 had a more significant drop down in the pregnant women with mild to moderate chronic periodontitis (by 36.8%) rather than in those with gingivitis (by 18.4%). The relative number of lymphocytes bearing marker of apoptotic activity was significantly increased in the patients with chronic periodontitis (by 4.6%).

Therefore, the pregnant women with mild to moderate chronic periodontitis revealed a pronounced elevation in the cytotoxic potential of T-lymphocytes compared to the patients with gingivitis. In the patients with chronic periodontitis an increase in the T-cells' killer activity as more prominent than the helper-and-inductor components, resulting in decreased immunoregulatory index.

Table 2

Absolute and relative numbers of lymphocytes and their subpopulations in pregnant women with gingivitis and periodontitis, and healthy donors

Index	Gingivitis (n=72)	Mild to moderate chronic periodontitis (n=93)	Healthy donors (n=32)
Lymphocytes, %	27.6±0.5*	23.1±0.4*°	30.1±1.2
Lymphocytes, $x10^9$/l	1.73±0.1	1.57±0.2	1.9±0.4
CD3+,%	63.5±1.3*	58.7±1.1*°	70.5±2.2
CD3+,$\times10^9$	1.09±0.09	0.92±0.1	1.1±0.2
CD4+,%	39.7±0.5*	36.2±0.4*°	43.3±0.6
CD4+,$\times10^9$	0.69±0.07	0.57±0.06	0.73±0.09
CD8+,%	26.1±0.4*	29.8±0.8*°	21.5±1.3
CD8+,$\times10^9$	0.47±0.06	0.47±0.05	0.42±0.03
CD4/CD8 ratio	1.55±0.2	1.20±0.1*°	1.9±0.3
CD16+,%	21.6±0.5	24.8±0.3*°	20.9±1.0
CD16+,$\times10^9$	0.37±0.06	0.39±0.04	0.40±0.03
CD95+,%	8.2±0.4*	10.7±0.3*°	6.5±0.9
CD95+,$\times10^9$	0.14±0.02	0.17±0.01*°	0.10±0.02

Note: * – significant difference in comparison to the healthy donors at $p<0.05$, ° – significant difference in comparison to the healthy pregnant women.

Table 3 offers a view on the absolute and relative numbers of lymphocytes and their subpopulations in the pregnant women with chronic periodontitis of various degrees of severity taken in comparison to the healthy donors. Icreasing severity of periodontal disease came alongside a decrease in the total number of lymphocytes, mature T-lymphocytes, T-helpers, and an increase in the number of T-lymphocytes with cytotoxic and killer, apoptotic activity. In the cases of chronic periodontitis of moderate severity, unlike the cases of the healthy donors, the relative number of lymphocytes went down by 22.6%, mature T-lymphocytes – by 16.5%, T-helper cells – by 15.9% given a general background of an increased relative number of T-lymphocytes with marker $CD8^+$ by 37.7%, CD16+ by 19.1%, and $CD95^+$ by 67.7% ($p<0.05$).

Table 3

Absolute and relative numbers of lymphocytes and their subpopulations in pregnant women with mild to moderate chronic periodontitis and healthy donors

Index	Chronic periodontitis, mild (n=51)	Chronic periodontitis, moderate (n=42)	Healthy donors (n=32)
Lymphocytes, %	24.8±0.6*	23.3±0.7*°	30.1±1.2
Lymphocytes, $x10^9$/l	1.62±0.3	1.58±0.2	1.9±0.4
CD3+,%	61.2±0.9*	58.9±1.1*°	70.5±2.2
CD3+,$\times10^9$	0.97±0.1	0.92±0.05	1.1±0.2
CD4+,%	37.8±0.4*	36.4±0.3*°	43.3±0.6
CD4+,$\times10^9$	0.61±0.08	0.57±0.06*	0.73±0.09
CD8+,%	27.4±0.5*	29.6±0.7*°	21.5±1.3
CD8+,$\times10^9$	0.46±0.05	0.47±0.03	0.42±0.03
CD4/CD8 ratio	1.32±0.1*	1.21±0.06*	1.9±0.3
CD16+,%	22.7±0.7	24.9±0.8*°	20.9±1.0
CD16+,$\times10^9$	0.37±0.04	0.39±0.06	0.40±0.03
CD95+,%	8.7±0.6*	10.9±0.4*°	6.5±0.9
CD95+,$\times10^9$	0.16±0.01	0.17±0.03	0.10±0.02

Note: * – significant difference in comparison to the healthy donors at $p<0.05$, ° – significant difference in comparison to the healthy pregnant women.

Conclusions. Inflammatory periodontal diseases in pregnant women are associated with disturbances in the cellular immunity. Unlike the patients with gingivitis, and along with an increasing severity of periodontal disease, the pregnant women with mild to moderate chronic periodontitis demonstrated more of lymphopenia, imbalance in T cell subsets with a decrease in the relative number of mature T-lymphocytes and T-helper cells against an increasing number of cells with killer and apoptotic activity. The peculiarities of peripheral blood lymphocytes immunophenotype mostly followed the changes in the pregnant women free from dental pathology yet were more prominent. More pronounced immunosuppression in the pregnant women with inflammatory periodontal disease was predisposing to a prolonged course of chronic inflammatory diseases.

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