ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Subpopulations of Lymphocytes and Cytokine Status of Patients with Type 2 Diabetes and Non-Alcoholic Fatty Liver Disease.

Kondratiuk KO[1]*, Kobyliak NM[1], Gurianov VG[1], Lisyany NI[2], Mykhalchyshyn GP[1], and Bodnar PM[1].

[1]Bogomolest National Medical University, T Shevchenko Boulevard, 13, Kyiv, 01601, Ukraine.
[2]AP Romodanov Institute of Neurosurgery of NAMS of Ukraine.

ABSTRACTS

We have observed 118 patients, including 64 patients with T2DM and NAFLD (I group), 26 patients - with T2DM (II group) and 28 patients with NAFLD (III group). The control group consisted of 25 apparently healthy individuals. We have determined the quantitative content of lymphocyte subpopulations in peripheral blood, the concentration of cytokines (interleukin (IL) 1β, 2, 4, 6, 8, 10, 17, TNF-α and IFN-γ) in peripheral blood serum and in coprofiltrates, and evaluated the state of the microbiota of the intestine as a result of bacteriological examination of faeces. The studies have found that each of the studied pathologies (T2DM, NAFLD and their combination) is characterized by its own cytokine status, which reflects both the presence of inflammatory processes and focus immunopathological reactions in the body. Identified changes in the levels of cytokines and lymphocyte subpopulation content in combination T2DM with NAFLD can be used both for diagnosis and for predicting adverse development of this disease, the intensity of the proliferation of connective tissue and the degree of fibrosis formation.

Keywords: non-alcoholic fatty liver disease, type 2 diabetes, cytokines, lymphocytes.

Corresponding author

ISSN: 0975-8585

INTRODUCTION

The prevalence of diabetes mellitus (DM) grows steadily with each year. The number of diabetic patients in the world in 2013 amounted to 382 million people, and according to forecasts of the World Diabetes Federation by 2035 this number will increase to 592 million at the expense of patients with type 2 diabetes mellitus (T2DM) [1].

The leading role in the development of T2DM belongs to immunopathological mechanisms that determine the nature of the flow in the future of this pathology, the rate of propagation and severity of specific complications. The main role in the mechanisms of non-alcoholic fatty liver disease (NAFLD) development belongs to insulin resistance, activation of gluconeogenesis and induction of oxidative stress, which damages the liver cells and triggers an inflammatory process that leads to pathological changes in hepatocytes. From 10% to 75% of T2DM is associated with NAFLD. The combination of T2DM with NAFLD doubles the risk of carcinoma and liver cirrhosis [2-10]. At the same time, many questions regarding the immune mechanisms of development of T2DM and NAFLD remain understudied.

The purpose of research is to study features of cytokine status and major lymphocyte subpopulations of patients with T2DM and NAFLD.

MATERIALS AND METHODS

We have observed 118 patients, including 64 patients with T2DM and NAFLD (I group), 26 patients with T2DM (II group) and 28 patients with NAFLD (III group). The control group consisted of 25 apparently healthy individuals.

The concentration of cytokines (interleukin (IL) 1β, 2, 4, 6, 8, 10, 17, TNF-α and IFN-γ) was determined in the peripheral blood serum by enzyme immunoassay (ELISA) using kits "Vector-Best" (Russia) based on solid-phase "sandwich"- the embodiment according to the instructions of the manufacturer. The intensity of the yellow color is proportional to the concentration of the sample contained in a particular cytokine, are recorded on a spectrophotometer «Immunochem» ("High Technology, Inc.", USA) with a wavelength of 540 nm.

The quantitative composition of lymphocyte subpopulations in peripheral blood were studied by flow cytometry «FC-500» («Beckman Coulter», USA) program Cytomics CXP Software using double combinations of monoclonal antibodies production «Beckman Coulter» (USA) and antibodies of company "Sorbent", Russia. With this were evaluated in (%) the rates of T-cell population: the total number of T lymphocytes (CD3+), the number of T-helper cells (CD4+), cytotoxic T lymphocytes (CD8+), natural killer cells (NK-cells - (CD16+), B cells population (CD19 +) of lymphocytes as well as the relative content of lymphocytes which are bearing early activation marker (CD25+) and the antigen apoptosis marker (CD95+). Formulation of reactions determining subpopulations of lymphocytes with monoclonal antibodies was realized according to the instructions [11] with whole blood and followed lysis of erythrocytes.

For obtaining copra filtrates (supernatants) 1 g of homogenized feces and then suspended in isotonic sodium chloride solution at a ratio of 1:10. After centrifugation at 2 000 revs/min during 30 min supernatant (copra filtrate) were collected in a separate container and subjected to further research. The state of intestine microbiota was evaluated based on the results of bacteriological examination of faeces.

Data were analyzed using MedCalc v.14.12 (MedCalc Software Inc, Broekstraat, Belgium). Descriptive statistics were used to analyze and report the data. For presentation of normally distributed data, arithmetical average ($\overline{X}$) and standard error (±m) were calculated. For non-normally distributed data the median (Me), first and third quartiles (Q_I – Q_{III}) were calculated. ANOVA and post-hoc Scheffe's test (normally distributed data) or Kruskal-Wallis test and post-hoc Dunn's test (non-normally distributed data) were conducted for multiple comparisons. The degree of association between the variables was calculated using Spearman's rank correlation coefficient. The significance threshold was set at $p<0,05$.

ISSN: 0975-8585

RESULTS AND DISCUSSION

During the study the content of the main lymphocyte subpopulations of patients with T2DM, NAFLD and with their combination was determined the multidirectional dynamics. So NAFLD patients (group III) or with combination of T2DM with NAFLD (I group) was observed a decrease of level CD3+ lymphocytes (T cell), and a significant increase in CD20 lymphocytes (B cell) in I and II Group (p□0,01). The level in blood of T-helper lymphocytes (CD4) rose maximum with T2DM, whereas the level of CD8-cytotoxic lymphocytes decreased significantly in all three groups of patients, which resulted in an increase in immunoregulatory index (p□0,01). Therefore there is a decrease in blood level of cytotoxic T lymphocytes and an increase in the level of B lymphocytes, while maintaining a level of total T lymphocytes and T helper cell subpopulations. The content of subpopulation of NK (CD16) was significantly (p□0,01) reduced in patients with III and I group (table 1).

Comparing the relative and absolute content of individual subpopulations of lymphocytes in the blood of patients in all treatment groups was observed unidirectional changes in immune parameters, which were showing on the one hand the reduction of cytotoxic level, and on the other hand the stimulation of B cell component of immune system.

The important indicator of the state of the immune system is studying of activating processes and apoptosis in cells of the immune system. The study of the level CD25+ lymphocytes, which reflect the early processes of lymphocyte activation by acting on receptors of IL-2 showed a slight increase of their reference values at T2DM or NAFLD. Thus the T2DM patients with NAFLD were reveal statistically insignificant decrease in blood levels of CD25+ positive cell, that indicate to the normal not impaired activation processes of immune cell, particularly, CD4 lymphocytes which provide the development of immunopathological reactions in this pathology. At the same time, the readiness of apoptotic cells detected by the level of CD95+ cells in all three groups was increased by 1,8-2,0 times (p<0,001).

Table 1: Contents of the main lymphocyte subpopulations in peripheral blood

Parameters		Group, $\overline{X}$ ±m				p-value
		I(n=64)	II(n=26)	III(n=28)	Control (n=25)	
leukocytes, 10^9/l		5,8±0,1^{d}	6,3±0,2	6,9±0,2^{ab}	5,7±0,1^{d}	<0,001
lymphocy-tes	%	36,1±0,4^{cd}	40,8±0,9^{abd}	31,1±0,7^{bc}	33,8±0,5^{c}	<0,001
CD 3, 10^9/l	%	54,6±0,3^{acd}	60,0±1,3^{bd}	51,5±0,5^{abd}	61,4±0,9^{bd}	<0,001
	abs,	1139±27^{c}	1546±72^{acd}	1074±22^{ac}	1179±35^{cd}	<0,001
CD 4, 10^9/l	%	32,1±0,4^{ac}	36,9±0,6^{ad}	31,2±0,5^{abc}	34,3±0,6^{bcd}	<0,001
	abs,	671±18^{c}	944±36^{abd}	651±16^{c}	658±20^{c}	<0,001
CD 8, 10^9/l	%	19,7±0,3^{a}	19,8±0,5^{a}	19,5±0,4^{a}	24,4±0,5^{bcd}	<0,001
	abs,	413±12^{ac}	503±18^{bd}	407±11^{c}	469±14^{b}	<0,001
immunoregulatory index		1,64±0,02^{ac}	1,89±0,05^{abd}	1,61±0,03^{ac}	1,4±0,03^{bcd}	<0,001
CD 20,10^9/l	%	12,3±0,2^{ad}	13,1±0,4^{ad}	9,0±0,2^{bc}	9,5±0,2^{bc}	<0,001
	abs,	256±6^{acd}	333±14^{abd}	188±6^{bc}	182±6^{bc}	<0,001
CD 16,10^9/l	%	11,8±0,3^{a}	12,0±0,3^{a}	11,5±0,3^{a}	16,6±0,4^{bcd}	<0,001
	abs,	247±9^{ac}	306±10^{bd}	241±8^{ac}	320±12^{bd}	<0,001
CD 25, 10^9/l	%	7,9±0,1^{cd}	8,7±0,2^{bd}	9,4±0,2^{abc}	8,5±0,2^{d}	<0,001
	abs,	166±4^{cd}	222±10^{ab}	196±6^{ab}	163±6^{cd}	<0,001
CD 95, 10^9/l	%	3,7±0,1^{a}	4,0±0,1^{a}	4,0±0,1^{a}	2,3±0,4^{bcd}	<0,001
	abs,	76±2^{ac}	90±3^{ab}	83±3^{a}	43±6^{bcd}	<0,001

Notes: p – difference between all study groups calculated using one-way ANOVA (normally distributed data) or Kruskall-Wallis test (non-normally distributed data); ^{abcd} – for pairwise comparison of subgroups used post-hoc Scheffe's test (normally distributed data) or Dunn's test (non-normally distributed data);^{a} – difference from the control group statistically significant, p<0,01 ; ^{b} – difference from the 1 group statistically significant, p<0,01; ^{c} – difference from the 2 group statistically significant, p<0,01; ^{d} – difference from the 3 group statistically significant, p<0,01

Consequently, the change in the immune status of patients with T2DM with NAFLD is accompanied with expression on lymphocytes the apoptosis marker antigen CD95, which expressed on CD4+ and CD8+ of subpopulations on human lymphocytes regulated by IL-2 and IFN-γ. Probably, such changes are the cause of imbalance in the composition of lymphocytes, especially CD8, and CD16 cells marked in this group of patients.

The decrease of levels of NK cells in blood secreting Th2-type cytokines, including IL-4 and IL-13 may contribute to the development of Th1 pro-inflammatory condition that leads to excessive production of TNF-α and IFN-γ. Increase of pro-inflammatory cytokines contributes to the formation of oxidative stress in the liver, the development of inflammatory process and hepatocyte necrotic changes [12].

The decrease of cytotoxic lymphocytes and NK can be explained by their migration from blood into the affected organs (liver and pancreas). At the same time, there was not found a significant activation of lymphocytes that evaluated by the receptor level for IL-2 (CD25+ cells). This can be explained by a large predominance in these processes of B cell-humoral link.

According to the results of our studies we revealed the presence of imbalance in subpopulation compound of lymphocytes that are conformed with the results of other authors, who noted the increase of immunoregulatory index, apoptotic preparedness of lymphocytes and decrease of cytotoxic T lymphocytes level of patients with T2DM [3,10]. In the studies of mentioned authors also was not found significant early activation of lymphocytes, detectable by the level of CD25 cells. At the same time, our findings do not coincide with the data [13], been reported in patients with non-alcoholic steatohepatitis (NASH) the decrease of CD4 lymphocytes level in the blood with maintaining CD8 and CD16 cytotoxic cells, that can be explained with the different stage of severity on examined patients and methods of examination, it is the difference between the immunomorphological methods and cytometric determination of these cells in the blood.

Table 2: Contents of cytokines in blood serum (pg/ml)

Parameters	Groups, Me ($Q_I - Q_{III}$)				p-value
	I (n=64)	II (n=26)	III (n=28)	control (n=25)	
IL-1β	$6,85^{acd}$ (6,6–7,2)	$2,8^{b}$ (2,6–3,1)	$4,15^{ab}$ (3,85–4,6)	$1,6^{bd}$ (1,5–1,72)	<0,001
IL-17	$6,4^{acd}$ (6,3–6,8)	$2,3^{b}$ (2–2,4)	$5,55^{ab}$ (5,2–5,85)	$1,6^{bd}$ (1,5–1,77)	<0,001
IL-2	$1,7^{ac}$ (1,5–2)	$0,9^{bd}$ (0,8–1,1)	$1,7^{ac}$ (1,5–1,95)	$1,1^{bd}$ (1–1,2)	<0,001
IFN-γ	$4,8^{acd}$ (4,5–5,1)	2^{bd} (1,7–2,2)	$4,5^{abc}$ (4,2–4,9)	$0,9^{bd}$ (0,8–1)	<0,001
IL-4	$0,4^{ac}$ (0,3–0,5)	$0,7^{bd}$ (0,6–0,8)	$0,5^{ac}$ (0,4–0,6)	$0,9^{bd}$ (0,7–0,9)	<0,001
IL-10	$12,55^{acd}$ (11,5–13,75)	$8,25^{abd}$ (7,8–8,4)	$10,25^{abd}$ (9,6–10,65)	$3,2^{bcd}$ (3,1–3,3)	<0,001
IFN-γ	$4,8^{acd}$ (4,5–5,1)	2^{bd} (1,7–2,2)	$4,5^{abc}$ (4,2–4,9)	$0,9^{bd}$ (0,8–1)	<0,001
TNF-α	$4,15^{acd}$ (3,7–4,85)	$1,25^{b}$ (0,9–2)	$2,15^{ab}$ (1,6–2,75)	$0,6^{bd}$ (0,5–0,6)	<0,001
IL-6	$4,9^{acd}$ (4,4–5,55)	$2,7^{bd}$ (2,4–3,2)	$3,9^{abc}$ (3,7–4,2)	2^{bd} (1,9–2,1)	<0,001
IL-8	$4,5^{acd}$ (4,15–5,1)	$2,4^{bd}$ (2,2–2,9)	$3,65^{abc}$ (3,4–3,9)	$1,9^{bd}$ (1,8–2)	<0,001

Notes: p – difference between all study groups calculated using Kruskall-Wallis test; ^{abcd} – for pairwise comparison of subgroups used post-hoc Dunn's test; ^{a} – difference from the control group statistically significant, p<0,01 ; ^{b} – difference from the 1 group statistically significant, p<0,01; ^{c} – difference from the 2 group statistically significant, p<0,01; ^{d} – difference from the 3 group statistically significant, p<0,01

The study of cytokine blood status in patients with T2DM, NAFLD and its combination has showed that T2DM (group II) the level of one of the major pro-inflammatory cytokines IL-1β in blood serum was above (p<0,01) the reference value in 1,7 times (table 1). The patients with NAFLD (group III) were found to increase

in the level of IL-1β in 2,6 times (p□0,01), indicating that there is an evidence of systemic inflammatory reaction. The concentration of IL-1β in the blood of patients with T2DM with NAFLD (I group) as compared with other groups of examined patients was the highest (p<0,001). This indicates an increase of the inflammatory reaction in the body, particularly in the liver with this combined pathology and confirmed to data in the scientific literature [14].

In our studies was established a statistically significant (p□0,001) elevation of the level of pro-inflammatory cytokine IFN-γ. Thus the patients with T2DM were fixed more than double increase of IFN-γ as compared with appropriate indicators of control. Considerable variation of it in blood serum was found in patients with NAFLD (p□0,01), and in patients with T2DM with NAFLD was found more then 5-fold increase IFN-γ content in comparison with control values and doubly exceeded the corresponding parameters of patients only with T2DM (p□0,01). The increase in blood serum concentration of IFN-γ in combination with T2DM and NAFLD indicates the significant activity in participation of Th-1 link immune responses for liver injury in this pathology. The IFN-γ in vitro disturbs insulin signaling in adipocytes – is an effect which can bind the inflammation mediated by T-cells with insulin resistance [15].

It is known that IFN-γ is an inductor of macrophage activation, which plays a significant role in T2DM and NAFLD immunogenesis. It stimulates macrophage production of pro-inflammatory cytokines such as IL-1, IL-6 and TNF-α [16], which in their turn activate the proteolysis activity of these cells. The unbalanced increase in the intensity of proteolysis leads to progressive destruction in membranes of hepatocytes, accelerating of their apoptotic death, aggressive degradation in key components of the extracellular matrix of liver tissue [17]. The study of content of IL-2 has revealed a significant increase in its level (p<0,01) in the blood of patients with NAFLD (group III) or a combination of T2DM with NAFLD (I group) compared with the control, but at the same time in patients with T2DM only we have not recorded the increased production of this interleukin.

The average level of IL-17 in I and III groups of patients was significantly reliable (p<0,01) exceeded the control values. The increase of its level (3,5-4 times) was found only in patients with NAFLD or in patients with a combination of T2DM and NAFLD, which can indicate the presence of a significant inflammatory reaction in this group of patients. It is known that IL-17 participates in the development of insulin resistance: disturbs the insulin mediated glucose transport, inhibits the genes expression involved in lipid metabolism and also aggravates obesity [18].

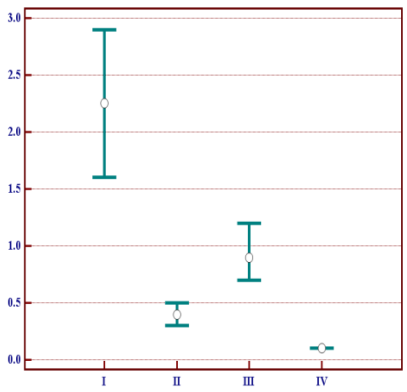
The study of pro-inflammatory cytokines in the blood of studied patients showed the multidirectional nature of their changes. The level of IL-4 in patients with NAFLD was lower value than the control group (p<0,01) and did not change significantly with T2DM (p>0,05). In patients with T2DM and NAFLD was recorded statistically significant decrease in IL-4 as compared with control values and with appropriate indicators of patients with T2DM only (p<0,001).

The level of IL-10 was significantly increased in the blood of patients of all the groups studied (p□0,001). The increase in IL-10 levels with T2DM and NAFLD is a marker of change of the liver tissue to connective tissue, and is treated as a kind of "protective" reaction in the liver on inflammatory and destructive processes which occurred in it [19]. The detected changes in cytokine status in examined patients indicate predominance in the body and in the liver, in particular, inflammatory and immunopathological processes.

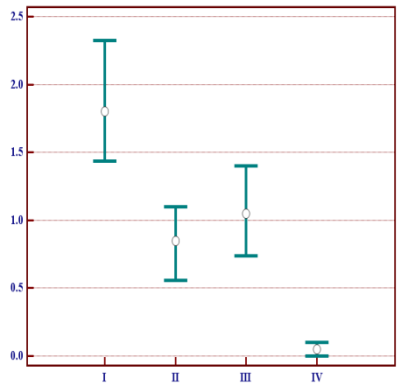
Most patients with T2DM in combination with NAFLD (I group) registered profound qualitative and quantitative changes of intestinal microflora: disbacteriosis of III degree was detected in 46 (71,9%) patients, disbacteriosis of II degree - in 18 (28,1%) examined patients. The frequency of registration of disbacteriosis of III and II degree in patients with NAFLD (III group) composed 12 (42,9%) and 16 (57,1%) cases accordingly.

During the study of the level of pro-inflammatory cytokines in the blood serum and coprofiltrates of patients with T2DM, NAFLD and combined T2DM with NAFLD has revealed the high concentrations of TNF-α, IL-6 and IL-8 (Table 2, Figure 1-3).

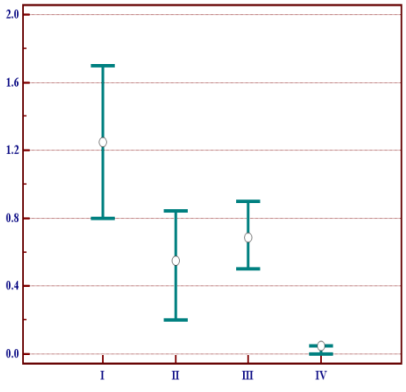
ISSN: 0975-8585



Figue 1. Indicators of TNF-α (pg/ml) in coprofiltrates of examined patients: I - T2DM + NAFLD, II – T2DM, III -NAFLD, IV - control.
(Me and 95% CI). Differences are statistically significant (Kruskall-Wallis test, p<0,001).



Figue 2. Indicators of IL-6 (pg/ml) in coprofiltrates of examined patients: I - T2DM + NAFLD, II – T2DM, III -NAFLD, IV - control.
(Me and 95% CI). Differences are statistically significant (Kruskall-Wallis test, p<0,001).



Figue 3. Indicators of IL-8 (pg/ml) in coprofiltrates of examined patients: I - T2DM + NAFLD, II – T2DM, III -NAFLD, IV - control.
(Me and 95% CI). Differences are statistically significant (Kruskall-Wallis test, p<0,001).

As shown data in Table 2 the patients with type 2 diabetes (II group) revealed a two-fold increase in TNF-α and one and half times increased levels of IL-6 and IL-8 in blood serum. In coprofiltrates higher content of all three cytokines at the same time (TNF-α, IL-6 and IL-8) was found in type 2 diabetes with disbacteriosis of III degree.

The relationships (Spearman's rank correlation coefficient, r≠0 at p<0,05) were detected between the dysbiosis disoder rate and cytokine production in coprofiltrates of examined patients from group II: for TNF-α – r= 0,778; for IL-6 - r= 0,740; for IL-8 - r= 0,500. The absence or low level (0,2 pg/ml) in the biotope simultaneously of two cytokines (TNF-α and IL-6) were detected in 7,7% of cases, which was combined with sub-compesatory form of disbacteriosis in patients with T2DM (Table 3).

Table 3: The correlations between the level of production of pro-inflammatory cytokines and dysbiosis disoder rate.

Group of patients	Parameters	Spearman's rank correlation coefficient, r
I	TNF-α serum / TNF-α coprofiltrate	0,649 [a]
	IL-6 serum / Il-6coprofiltrate	0,522 [a]
	IL-8serum / Il-8coprofiltrate	0,419 [a]
	TNF-α coprofiltrate / dysbiosis disoder rate	0,538 [a]
	IL-6 coprofiltrate / dysbiosis disoder rate	0,528 [a]
	IL-8coprofiltate/ dysbiosis disoder rate	0,586 [a]
II	TNF-α serum / TNF-α coprofiltrate	0,778 [a]
	IL-6 serum / IL-6coprofiltrate	0,740 [a]
	IL-8 serum / IL-8 coprofiltrate	0,500 [a]
	TNF-α coprofiltrate / dysbiosis disoder rate	0,683 [a]
	IL-6 coprofiltrate / dysbiosis disoder rate	0,616 [a]
	IL-8 coprofiltrate / dysbiosis disoder rate	0,760 [a]
III	TNF-α serum / TNF-α coprofiltrate	0,785 [a]
	IL-6 serum / IL-6 coprofiltrate	0,514 [a]
	IL-8 serum / Il-8 coprofiltrate	0,334 [a]
	TNF-α coprofiltrate / dysbiosis disoder rate	0,444 [a]
	IL-6 coprofiltrate/ dysbiosis disoder rate	0,666 [a]
	IL-8 coprofiltrate / dysbiosis disoder rate	0,554 [a]

Note: If the value of the Spearman's rank correlation coefficient (r) <0,3 - weak link, from 0,31 to 0,5 – moderate, > 0,5 – significant. [a] – Spearman's rank correlation coefficients differ from zero at significance level p<0,05.

In patients of I group (T2DM with NAFLD) was found the greatest increase in the level of pro-inflammatory cytokines. In 65,6% of cases we have found a significant (7-fold) increase in the level of TNF-α in blood serum as compared with rates of control group ($p<0,01$). Thus, was found 2,5-fold increase of IL-8 content and 2-fold increase of IL-6 in blood serum of the same patients ($p<0,01$).

Thus, as a result of studies we found that each of the studied pathologies (T2DM, NAFLD and their combination) is characterized by its own cytokine status, which reflects both the presence of inflammatory processes and focus of immunopathological reactions in the body. Identified changes in the levels of cytokines and lymphocyte subpopulation content in the combination with T2DM and NAFLD can be used both for diagnosis and for predicting adverse development of this disease, the intensity of the proliferation of connective tissue and the degree of fibrosis formation.

Abnormal liver function with T2DM leads to an imbalance of intestinal microbiocenosis by increasing number of potentially pathogenic Gram-negative bacterias, a significant accumulation of endotoxins which stimulate the systemic and local overproduction of pro-inflammatory cytokines. The increasing of the concentration of pro-inflammatory cytokines in peripheral blood is a reflection of systemic reaction to local enteropathy, which is necessary to consider when developing pathogenically based treatment of T2DM complicated with NAFLD.

CONCLUSION

- In patients with T2DM, NAFLD or combination were marked changes in cytokine status in blood serum, reflecting the intensity of the inflammatory immune reactions, their direction and possible forecast of fibrosis formation in the liver.
- Revealed imbalance in the composition of lymphocyte subpopulations, observed by decreased blood levels of CD8+ and CD16+ cells at a stable level of T helper (CD4+) cells and increased content of CD20+ cells.
- In patients T2DM with NAFLD was registered an increased readiness of apoptotic lymphocytes, which revealed an increase in the content of CD95+ cells in the blood.
- The comparative analysis of cytokines content, depending on the intestine dysbiosis disoder rate showed that in patients with T2DM, NAFLD or in combination have marked significant increase in the concentration of pro-inflammatory cytokines in blood serum and coprofiltrates, indicating that there are a significant inflammatory and immunopathological reactions.
- The indicators of cytokines level and lymphocyte subpopulation content are informative for the diagnosis and assessment of severity of disorders in T2DM and NAFLD.

REFERENCES

[1] IDF Diabetes Atlas Sixth edition. IDF, 2013;155
[2] HvorostInka VM, Lavrinenko OV, Zhuravlova LV. Modern Gastroenterol 2009;3:91-97
[3] Tihonova TM. Int Med Mag 2011; 4: 82-86.
[4] Mammaev SN, Bagomedova NV, Bogomolov PO. The Russian J Gastroenterol Hepatol Coloproctol 2007;4:35-39.
[5] Bodnar PM, Mykhalchyshyn GP, Kobyliak NM. Endocrinol 2012; 17: 94-101.
[6] Klyaritska IL, Stilidi OI. Arch Clin Exp Med 2010; 19:167-192.
[7] Diehl AM. Am J Physiol Gastrointest Liver Physiol 2002; 282: 105-115.
[8] Sulskaya YuV, Beloglazov VA. Immunol Allergol 2009; 4: 55-61.
[9] Belyakova NA, Rudenko EV, Mihaylova DG, Egorova EN, Nekrasova EG, Gogina ED. Questions of Pathogenesis. 2011; 2: 9-11
[10] Trushina EN, Mustafina OK, Soto SH, Sentsova TB, Plotnikova OA, Sharafetdinov HH, Semenchenko IYu, Kuznetsov VD. Nutr 2012; 81: 60-65.
[11] Pinegin BV., Yarilin AA., Simonova AV., Klimova SV., Mazurov DV., Dambaeva SV., Bahus GO. Manual for doctors, technicians. State scientific center, Russia. Institute of Immunology, Ministry of Public Health, Russia. 2001: 53.
[12] MI Kremer MI, Hines IN. World J Gastroenterol 2008; Vol. 14: 487–488.
[13] Zak KP, Tronko MV, Popova VV, Butenko AK. Kyiv: Knyga - plus, 2014: 500.

[14] Korniychuk IYu. Bukovynskiy Med Bull 2011; 15: 214-217.
[15] McGillicuddy FC, Chiquoine EH, Hinkle CC. et al. J Biol Chem 2009; 284: 31936-31944.
[16] Ketlinskiy SA, Kalinina NM. Immunol 1995; 3: 30-44.
[17] Sivkova AA, Lareva NV, Luzina EV, Tomina EA. Zabaikalskii Medi Bull 2011; 2: 79-85.
[18] Zuniga LA, Shen WJ, Joyce-Shaikh B. et al. Immunol 2010; 185: 6947-6959.
[19] Zahran WE, Salah El-Dien KA, Kamel PG, El-Sawaby AS. Indian J Clin Biochem 2013; 28: 141-146.