

The state of the cytokine profile in pregnant women with non-alcoholic fatty liver disease at the stage of non-alcoholic steatohepatitis with varying degrees of comorbid obesity under the influence of the developed complex therapy program

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of the article

Key words:

non-alcoholic steatohepatitis, cytokine profile, obesity, pregnancy complications, vitamin E, ursodeoxycholic acid.

Zaporozhye medical journal
2023; 25 (2), 136-141

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The aim of the study: to evaluate the cytokine profile state in pregnant women with non-alcoholic fatty liver disease (NAFLD) at the stage of non-alcoholic steatohepatitis (NASH) with varying degrees of obesity under the influence of the developed complex therapy program.

Material and methods. We examined 197 pregnant women with NAFLD at the stage of NASH in combination with obesity. The main group I consisted of 98 pregnant women with NAFLD at the stage of NASH with varying degrees of obesity, who were divided into 3 subgroups depending on body mass index (BMI). Among them, 26 pregnant women with BMI of 25.0–29.9 kg/m² were included in IA group, 48 pregnant women with BMI of 30.0–34.9 kg/m² were included in IB group, and 24 pregnant women with BMI of 35.0–39.9 kg/m² – in IC group. All pregnant women in the main group were prescribed complex therapy including vitamin E at a dose of 400 IU/day, ursodeoxycholic acid (UDCA) at a dose of 15 mg/kg/day, and L-carnitine at a dose of 3 g per day. The comparison group consisted of 69 women with NAFLD at the stage of NASH and abdominal obesity, who corresponded to subgroups of the main group (IIA – 23 patients, IIB – 25 women, IIC – 21 pregnant women) and received basic therapy. The control group consisted of 30 healthy women. To evaluate the cytokine profile, levels of IL-1β, IL-6, IL-10 and TNF-α were determined by ELISPOT.

Results. Analysis of the cytokine profile in women with NASH and obesity showed the presence of systemic inflammation links in the examined groups, which was manifested by increased levels of pro-inflammatory and decreased levels of anti-inflammatory interleukins in blood serum of pregnant women. A prescription of the complex treatment contributed to a decreased activity of the inflammatory response, which was manifested by an improvement in the levels of cytokine profile indicators.

Conclusions. NASH during pregnancy is accompanied by significant changes in the cytokine profile. The prescription of complex therapy in the form of vitamin E, UDCA and L-carnitine is effective in the treatment of pregnant women with NAFLD at the stage of NASH due to cumulative and potentiating effects, reducing manifestations of systemic inflammation by normalizing the level of cytokines.

Ключові слова:

неалкогольний стеатогепатит, цитокіновий профіль, ожиріння, ускладнення вагітності, вітамін Е, урсодезоксихолева кислота.

Запорізький медичний журнал.
2023. Т. 25, № 2(137).
С. 136-141

Стан цитокінового профілю у вагітних із неалкогольною жировою хворобою печінки на стадії неалкогольного стеатогепатиту на тлі ожиріння різних ступенів під впливом розробленої програми комплексної терапії

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Мета роботи – оцінити стан цитокінового профілю у вагітних із неалкогольною жировою хворобою печінки (НАЖХП) на стадії неалкогольного стеатогепатиту (НАСГ) на тлі ожиріння різних ступенів під впливом розробленої програми комплексної терапії.

Матеріали та методи. Обстежили 197 вагітних із неалкогольною жировою хворобою печінки на стадії неалкогольного стеатогепатиту в поєднанні з ожирінням. Основна (I) група – 98 вагітних із НАЖХП на стадії НАСГ на тлі ожиріння різних ступенів, яких поділили на 3 підгрупи залежно від індексу маси тіла (ІМТ). Так, до ІА підгрупи залучили 26 вагітних з ІМТ 25,0–29,9 кг/м², ІВ – 48 жінок з ІМТ 30,0–34,9 кг/м², ІС – 24 обстежених з ІМТ 35,0–39,9 кг/м². Усім вагітним основної групи призначили комплексну терапію: вітамін Е у дозі 400 МО/добу, урсодезоксихолеву кислоту в дозі 15 мг/кг/добу, L-карнітин у дозі 3 г на добу. Групу порівняння склали 69 жінок із НАЖХП на стадії НАСГ та абдомінальним ожирінням, яких за ІМТ поділили на аналогічні підгрупи (ІІА – 23 пацієнтки, ІІВ – 25 обстежених, ІІС – 21 вагітна) й отримували базисну терапію. В контрольну групу залучили 30 практично здорових жінок. Для оцінювання цитокінового профілю за допомогою ELISPOT визначали рівень ІЛ-1β, ІЛ-6, ІЛ-10 та TNF-α.

Результати. Аналіз цитокінового профілю в жінок із НАСГ та ожирінням показав наявність ланок системного запалення в групах обстеження. Це виявляли за підвищенням рівня прозапальних і зниженням протизапальних інтерлейкінів у сироватці крові вагітних. Призначення комплексного лікування сприяло зниженню активності запальної відповіді; це підтверджено покращенням показників цитокінової ланки.

Висновки. НАСГ під час вагітності супроводжується суттєвими змінами цитокінового профілю. Призначення комплексної терапії: вітаміну Е, урсодезоксихолевої кислоти та L-карнітину – є ефективним у лікуванні вагітних із неалкогольною жировою хворобою печінки на стадії неалкогольного стеатогепатиту завдяки кумулювальному та потенціувальному ефектам, зменшує прояви системного запалення шляхом нормалізації рівня цитокінів.

Nonalcoholic fatty liver disease (NAFLD) is one of the most common diseases of the hepatobiliary system and represents a wide spectrum of pathological conditions from simple steatosis with accumulation of lipids in the liver parenchyma with a fat liver content starting at 5 % to non-alcoholic steatohepatitis (NASH), characterized by chronic inflammation and fibrosis, progressing to cirrhosis and hepatocellular carcinoma [1,4,6]. According to the EASL-EASD-EASO recommendations, the frequency of NAFLD among the adult population ranges from 17 % to 46 % [1]. In Ukraine, according to the STEPS study (2019), this disease is found in women of reproductive age in 24.8 % of the population, which corresponds to the prevalence of such diseases as obesity, metabolic syndrome, hypertension, the presence of which significantly complicates the course of pregnancy and increases the risk of developing obstetric and perinatal complications [2]. Individual scientists have proven the relationship of pathogenetic mechanisms in the development and progression of NAFLD at the stage of NASH during pregnancy with obesity, since the presence of lipid metabolism disorders increases the inflammatory reaction in the body and significantly complicates the course of gestation at the stages of steatosis and steatohepatitis [3–5,8].

A defining aspect in the pathogenesis of nonalcoholic steatohepatitis is systemic inflammation [6,9]. An overload of toxic lipids, mainly free fatty acids, causes oxidative stress and induces specific signals that cause hepatocyte apoptosis as the predominant mechanism of cell death in NASH, correlating with the degree of liver inflammation and fibrosis [6,7,10]. Systemic inflammation is also an important pathogenetic trigger of endothelial dysfunction, which is associated with the activation of IL-6 and TNF- α cytokines in obese patients [13,15]. A large amount of placental TNF- α is released during pregnancy into the maternal bloodstream (94 %) and less into the fetal bloodstream (6 %) [11,15,21]. There is a positive correlation between the level of TNF- α and placental dysfunction, depending on its severity [21]. Research conducted by Lao (2020) confirmed the effect of TNF- α on the coagulant system, which can cause the development of DIC, the occurrence of microthrombosis with the subsequent development of retroplacental hematomas, hemangiomas, and placental abruption. IL-1 induces the synthesis of cytokines IL-6, TNF- α , acute phase proteins in the liver, insulin, and also promotes the production of progesterone and estrogens by placental cells [10].

One of the methods to correct the pathogenetic links of NAFLD is the use of antioxidant therapy, polyunsaturated fatty acids, which reduce hepatic steatosis, manifestations of lobular inflammation and hepatocellular ballooning [13]. A complex use of vitamin E, ursodeoxycholic acid (UDCA) and L-carnitine deserves special attention, since potentiates the anti-steatosis effect and improves the course of pregnancy [16,18,20,28,29]. Patients with NASH have hyperglycemia, an excess of intrahepatic iron, that contribute to and increase lipid peroxidation [20]. Lipid-soluble vitamin E (α -tocopherol) is a powerful free radical scavenging agent which interrupts the processes of polyunsaturated fatty acid peroxidation [16,17,21,29]. On the other hand, UDCA has numerous hepatoprotective properties, including replacement of toxic bile acids, stabilization of cell membranes, and immunomodulatory effects [16,18,19]. UDCA inhibits

apoptosis of hepatocytes, can modulate the mitochondrial membrane potential [22]. The administration of L-carnitine can positively affect the β -oxidation of fatty acids in mitochondria, thus promoting lipid metabolism by increasing the absorption of fatty acids and reducing the accumulation of lipids in hepatocytes [28]. Studies conducted by Jean-Francois Dufour (2006, 2011, 2022) have confirmed a low level of vitamin E, which is well known as a lipophilic antioxidant, in the blood serum of patients with NAFLD and obesity [16,17,29]. At the same time, Vlad Ratziu (2022) points to the immunomodulatory functions and direct anti-apoptotic properties of UDCA that block the progression of NAFLD/NASH by reducing serum levels of TNF- α and restoring the defective activity of natural killer cells, thus suppressing fibrosis in patients with NASH [18]. The above data became the basis for the possibility of correcting liver steatosis in pregnant women with NASH by improving the processes of lipotoxicity, endothelial dysfunction and systemic inflammation to prevent the development of clinically significant obstetric and perinatal complications.

Aim

The aim of the study was to evaluate the cytokine profile state in pregnant women with NAFLD at the stage of NASH with varying degrees of obesity under the influence of the developed complex therapy program.

Material and methods

In the study, we examined 197 pregnant women with NAFLD at the stage of NASH in combination with obesity, who were brought to Ternopil Regional Clinical Perinatal Center "Mother and Child" to undergo inpatient treatment and formed the main and control groups. The main group I – 98 pregnant women with NAFLD at the stage of NASH with varying degrees of obesity were divided into 3 subgroups depending on body mass index (BMI). Among them, 26 pregnant women with BMI of 25.0–29.9 kg/m² were included in IA group, 48 pregnant women with BMI of 30.0–34.9 kg/m² were included in IB group, and 24 pregnant women with BMI of 35.0–39.9 kg/m² comprised IC group. All pregnant women were prescribed complex therapy including vitamin E at a dose of 400 IU/day, ursodeoxycholic acid at a dose of 15 mg/kg/day and L-carnitine at a dose of 3 g per day in a continuous mode in accordance with the pharmacodynamic properties of the drugs used when the diagnosis of NASH was confirmed. The comparison group was made up of women with NAFLD at the stage of NASH and abdominal obesity, representative of the pregnancy course and age, who corresponded to the subgroups of the main group (IIA – 23 patients, IIB – 25 women, IIC – 21 pregnant women) and received a basic therapy. The basic therapy of pregnant women with NASH was performed in accordance with the Order of the Ministry of Health of Ukraine No. 826 dated November 6, 2014 "Non-alcoholic steatohepatitis" and the 2016 EASL-EASO practical recommendations [2]. Accordingly, such treatment included taking alpha-tocopherol acetate in a daily dose of 200–400 mg (25–36 drops), Omega-3 in the form of 300 mg eicosapentaenoic acid, 200 mg docosahexaenoic acid, 498 mg other fatty acids, 2 mg D- α -Tocopherol (1 capsule) twice a day within 15 minutes

Table 1. Age distribution of pregnant women in all clinical groups

Clinical groups	Age range	Median age (M ± m)
Main group (n = 98)	21–37	27.2 ± 2.5
Comparison group (n = 69)	19–38	28.4 ± 3.4
Control group (n = 30)	18–39	25.1 ± 2.3

Differences between the median age are not significant ($P > 0.07$).

Table 2. NASH-FibroTest indicators in pregnant women with NAFLD at the stage of NASH depending on the degree of obesity (M ± m)

Parameter, units of measurement	Control group (n = 30)	NASH (n = 167)		
		Overweight IA + IIA group (n = 49)	Class I obesity IB + IIB group (n = 73)	Class II obesity IC + IIC group (n = 45)
Steato-test, cu.	0.17 ± 0.01	0.52 ± 0.01*	0.64 ± 0.01***	0.72 ± 0.01****
Ash-test, cu.	0.05 ± 0.01	0.07 ± 0.01	0.12 ± 0.01	0.13 ± 0.01
NASH-test, cu.	0.17 ± 0.01	0.50 ± 0.01*	0.69 ± 0.01***	0.77 ± 0.01****

cu.: conditional unit. *: the difference is significant in comparison with the parameter of the control group ($P < 0.05$); **: the difference is significant in comparison with the parameter of IA + IIA subgroups ($P < 0.05$); ***: the difference is significant in comparison with the parameter of IB + IIB subgroups ($P < 0.05$).

before a meal. The control group consisted of 30 practically healthy women with a mean BMI of 23.53 ± 0.38 kg/m².

The study was conducted in the period from 2019 to 2021 on the basis of the Communal Institution of the Ternopil Regional Council "Ternopil Regional Clinical Perinatal Center "Mother and Child", Ukraine. The Ethic Committee of the Ivan Horbachevsky Ternopil National Medical University of the Ministry of Health of Ukraine approved the study on 29 October 2019, protocol of the session No. 14. The study was conducted in accordance with the ethical standards of the 1975 Helsinki Declaration, 2008(5) revision, as well as the national law. In all cases, informed consent was obtained from patients.

The diagnosis of NASH and obesity was established in accordance with the 2013 global practical recommendations of the World Gastroenterology Organization (WGO) global guidelines on obesity, standardized protocols for the diagnosis and treatment of digestive system diseases based on the order of the Ministry of Health of Ukraine No. 826 dated November 6, 2014, the unified clinical protocol of primary, secondary (specialized) medical care "Non-alcoholic steatohepatitis, ICD-10", on the grounds of anamnesis data, clinical, instrumental examinations and biochemical markers by standard methods following the EASL-EASD-EASO Clinical practice guidelines for the management of NAFLD [1,2].

Pregnant women involved in the study did not have a history of alcohol abuse, they did not have serum markers of viral hepatitis B or C, autoimmune or hereditary liver diseases. BMI was determined by the Quetelet index:

$$\text{BMI} = \text{weight (kg)} / \text{height}^2 \text{ (m)}$$

BMI results were evaluated according to the WHO classification: 18.5–24.9 kg/m² – normal body weight; 25.0–29.9 kg/m² – excess body weight; 30.0–34.9 kg/m² – class I obesity; 35.0–39.9 kg/m² – class II obesity; 40 and more kg/m² – class III obesity.

Table 1 shows the age distribution of pregnant women in all groups. The given values indicate the age homogeneity of women in all groups ($P > 0.07$).

The criteria for inclusion in the study were: the presence of a desired singleton pregnancy without abnormalities of fetal development, the absence of severe extragenital pathology in the stage of decompensation, the absence

of abnormalities of uterine development, the presence of excess body weight or obesity, voluntarily provided written informed consent for additional examinations, namely the NashTest, elastography and steatometry of the liver, as well as adherence to the recommended treatment regimen.

Exclusion criteria comprised viral hepatitis B, C, D, autoimmune hepatitis, toxic liver damage, genetic liver pathology, liver cirrhosis, the presence of extragenital pathology in the stage of decompensation, type 1 and 2 diabetes mellitus, congenital anomalies of the uterus, multiple pregnancy.

After an explanatory interview, all patients signed an informed consent to participate in the study.

To assess the presence and severity of steatosis, liver elastography with steatometry (IU22×MATRIX ultrasound (US) diagnostic system, Phillips Ultrasound, USA) and NASH-FibroTest non-invasive diagnostic test (BioPredictive laboratory, France) were performed, and a comprehensive assessment of morphological changes in hepatocytes was determined. Blood was taken in the morning on an empty stomach from the elbow vein to determine the following indicators: alpha2 macroglobulin, haptoglobin, apolipoprotein A1, total bilirubin, gamma-glutamyltransferase, ALAT, ASAT, glucose level, total cholesterol, triglycerides taking into account BMI and the age of a woman. The test results were graphically evaluated on 3 scales: the presence of fatty hepatosis (SteatoTest scale) and alcoholic or non-alcoholic steatohepatitis (AshTest and NashTest scales).

To evaluate the cytokine profile, levels of IL-1β, IL-6, IL-10, and TNF-α were determined by enzyme-linked immunosorbent assays (ELISPOT), BD Biosciences (USA). Examination of pregnant women for the cytokine profile evaluation was carried out before the start of treatment and no earlier than 1.5 months after receiving the therapy.

For statistical data analysis, the license program "IBM SPSS Statistics" was used for Windows software version 21.0. Quantitative indicators were given as arithmetic mean and standard error of the mean (M ± m). The paired Student's t-test and the one-way ANOVA were used to compare mean indicators in all subgroups, and the non-parametric Mann-Whitney test was used in case of non-normal distribution. Adjusted odds ratios (OR) and 95 % confidence intervals (CI) were calculated. Differences between the values were considered significant at a value of $P < 0.05$.

Results

According to the blood test results in pregnant women with NAFLD and obesity it was found that patients of all groups had a significant grade of steatosis (Table 2) based on the integral Steato-test, Ash and NASH tests. On the basis of the Ash-test results, we concluded that steatosis in pregnant women was exclusively of non-alcoholic etiology. According to the Steato-test results, the highest rate was observed in women of IC and IIC subgroups, which was 1.38 and 1.2 times higher than the results of the examined pregnant women of IA + IIA as well as IB + IIB subgroups ($P < 0.05$). Similar results were obtained after the NASH-test: the rate in pregnant women with NASH and II degree obesity exceeded that among women of IA + IIA subgroups by 1.54 times and among patients of IB + IIB subgroups by 1.11 times ($P < 0.05$), significantly indicating a direct relationship with an increase in BMI.

Determination of the liver steatosis grade in pregnant women based on the results of US steatometry was performed according to the scale of US wave attenuation proposed by M. Sasso et al. and the NAS (NAFLD activity score) validated by the morphological scale of fatty infiltration: S0 – no signs of steatosis were detected (less than 5 % of hepatocytes with fatty infiltration): from 1.0 dB/cm to 2.19 dB/cm; S1 – the minimum grade of steatosis (the proportion of hepatocytes with fatty infiltration was 6–33 %): from 2.20 dB/cm to 2.29 dB/cm; S2 – moderate steatosis (the proportion of hepatocytes with fatty infiltration was 34–66 %): from 2.30 dB/cm to 2.90 dB/cm; S3 – pronounced steatosis (the proportion of hepatocytes with fatty infiltration was >66 %): >2.90 dB/cm [23].

No grade of steatosis (S0) was detected at liver elastography with steatometry among the examined women of IB + IIB and IC + IIC groups. S1 grade of steatosis was recorded 3.6 times and 5.5 times more often among pregnant women of IA + IIA group compared to indicators of IB + IIB and IC + IIC groups of pregnant women, respectively, ($P < 0.05$). The frequency of S2 in pregnant women with NASH and class I obesity was observed 14 times higher than that in the group of patients with NASH and overweight, and 3.2 times higher in comparison with IC + IIC group of pregnant women ($P < 0.05$). S3 grade of steatosis was found in 71.1 % of women in IC + IIC group, which significantly, 3 times ($P < 0.05$), exceeded the rate of pregnant women in IB + IIB group. Therefore, in pregnant women with NASH, the steatosis grade increased with the higher degree of obesity, producing unfavorable conditions for the pregnancy course and childbirth in the future.

Analyses of the cytokine profile in women with NASH and varying degrees of obesity showed the presence of systemic inflammation links in the examined groups, which was manifested by an increase in the level of pro-inflammatory (IL-1 β , IL-6, TNF- α) and a decrease in anti-inflammatory (IL-10) interleukins in blood serum of pregnant women, thus helping conclude about the negative effect of NASH during pregnancy on the regulation of the systemic immune response.

According to our findings (Tables 4, 5, 6), a significant increase in the levels of IL-1 β , IL-6, and TNF- α ($P < 0.001$) was found in all groups of pregnant women with NASH, which were positively correlated with BMI. So, before starting the treatment, the level of IL-1 β in IA group was significantly higher by 1.42 times, in IB group – by 2.63 times, and in IC group – by 4.12 times ($P < 0.001$) compared to the control one. During pregnancy, the level of IL-6, in contrast to the control group, was also higher in all groups of examined women. Therefore, in IA group, the found significant difference was higher by 1.52 times, in IB group – by 2.62 times, in IC group – by 3.29 times ($P < 0.001$).

Evaluating the cytokine system in pregnant women of all groups, a statistically significant decrease in the level of IL-10 was found in comparison with the control group of women: in IA group – by 1.53 times, in IB group – by 1.93 times, in IC group – by 2.35 times ($P < 0.001$).

The concentration of TNF- α showed similar results, as in women of the three main examined groups it was 1.68, 2.88 and 3.58 times higher, respectively, than that of the control group ($P < 0.001$).

The complex treatment prescription in the form of vitamin E, UDCA and L-carnitine contributed to a decrease in

Table 3. The severity of hepatic steatosis in pregnant women with NASH depending on the degree of obesity

Grade of steatosis	NASH (167)					
	Overweight IA + IIB group (n = 49)		Class I obesity IB + IIB group (n = 73)		Class II obesity IC + IIC group (n = 45)	
	n	%	n	%	n	%
S0	12	24.4	0	0	0	0
S1	35	71.5	14	19.1	5	11.1
S2	2	4.1	42	57.6	8	17.8
S3	0	0	17	23.3	32	71.1

Table 4. The state of cytokine profile indicators in overweight pregnant women with NASH before and after the complex drug therapy ($M \pm m$)

Parameter, units of measurement	Control group (n = 30)	NASH + Overweight			
		IA (n = 26)		IIA (n = 23)	
		Before treatment	After treatment	Before treatment	After treatment
IL-1 β , pg/ml	2.42 \pm 0.09	3.45 \pm 0.11*	2.96 \pm 0.08**	3.44 \pm 0.12*	3.17 \pm 0.09*
IL-6, pg/ml	3.27 \pm 0.16	4.98 \pm 0.29*	3.51 \pm 0.15***	4.97 \pm 0.27*	4.43 \pm 0.18*
IL-10, pg/ml	9.69 \pm 0.21	8.71 \pm 0.44*	9.83 \pm 0.32***	8.68 \pm 0.41*	8.96 \pm 0.28
TNF- α , pg/ml	4.51 \pm 0.28	7.59 \pm 0.39*	5.12 \pm 0.23***	7.58 \pm 0.37*	7.17 \pm 0.37*

*: significance of differences between parameters in relation to the control group ($P < 0.001$); **: significance of differences between parameters in relation to baseline ones (before treatment) ($P < 0.001$); *: significance of differences between parameters in relation to those of the comparison group (after treatment).

Table 5. The state of cytokine profile indicators in pregnant women with NASH and class I obesity before and after the complex drug therapy ($M \pm m$)

Parameter, units of measurement	Control group (n = 30)	NASH + Class I obesity			
		IB (n = 48)		IIB (n = 25)	
		Before treatment	After treatment	Before treatment	After treatment
IL-1 β , pg/ml	2.42 \pm 0.09	6.38 \pm 0.16*	3.35 \pm 0.12***	6.37 \pm 0.12*	5.88 \pm 0.14*
IL-6, pg/ml	3.27 \pm 0.16	8.57 \pm 0.38*	4.56 \pm 0.18***	8.58 \pm 0.39*	8.03 \pm 0.21*
IL-10, pg/ml	9.69 \pm 0.21	7.46 \pm 0.37*	8.91 \pm 0.24***	7.45 \pm 0.36*	7.61 \pm 0.33*
TNF- α , pg/ml	4.51 \pm 0.28	13.03 \pm 0.36*	8.14 \pm 0.19***	13.05 \pm 0.34*	11.29 \pm 0.43*

*: significance of differences between parameters in relation to the control group ($P < 0.001$); **: significance of differences between parameters in relation to baseline ones (before treatment) ($P < 0.001$); *: significance of differences between parameters in relation to those of the comparison group (after treatment).

Table 6. The state of cytokine profile indicators in pregnant women with NASH against the background of obesity II degree before and after complex drug therapy ($M \pm m$)

Parameter, units of measurement	Control group (n = 30)	NASH + Class II obesity			
		IC (n = 24)		IIC (n = 21)	
		Before treatment	After treatment	Before treatment	After treatment
IL-1 β , pg/ml	2.42 \pm 0.09	9.99 \pm 0.53*	3.71 \pm 0.15***	9.98 \pm 0.51*	8.63 \pm 0.37**
IL-6, pg/ml	3.27 \pm 0.16	10.77 \pm 0.48*	5.13 \pm 0.26***	10.79 \pm 0.45*	9.45 \pm 0.37**
IL-10, pg/ml	9.69 \pm 0.21	5.38 \pm 0.49*	8.22 \pm 0.36***	5.38 \pm 0.44*	6.12 \pm 0.28*
TNF- α , pg/ml	4.51 \pm 0.28	16.17 \pm 0.42*	10.54 \pm 0.27***	16.18 \pm 0.39*	14.73 \pm 0.39*

*: significance of differences between parameters in relation to the control group ($P < 0.001$); **: significance of differences between parameters in relation to baseline ones (before treatment) ($P < 0.001$); *: significance of differences between parameters in relation to those of the comparison group (after treatment).

the inflammatory response activity, which was manifested by an improvement in the levels of cytokine indicators. Hence, the level of IL-1 β in group IA was not significantly different from the control until the end of pregnancy and decreased by 1.16 times, in group IB – by 1.90 times, in group IC – by 2.69 times ($P < 0.001$). With additional complex therapy, the level of IL-6 was decreased by 1.41 times in group IA being not significantly higher than in healthy pregnant women, in group IB – by 1.87 times, while IL-6 level was 2.09 times

decreased only in group IC and did not reach the control indicators ($P < 0.001$).

After the course of treatment, the serum level of IL-10 was increased by 1.12 times in patients of IA group, by 1.19 times – in IB group, by 1.52 times – in IC group ($P < 0.001$).

Similarly, TNF- α levels in all main groups were decreased by 1.48, 1.60, and 1.53 times, respectively, compared to the baseline levels ($P < 0.001$).

Evaluating the given results, we could regard the prescribed complex treatment with vitamin E, UDCA and L-carnitine as effective in reducing the levels of both pro-(IL-1 β , IL-6, TNF- α) and anti-inflammatory cytokines (IL-10), suppressing signs of the systemic inflammation.

Discussion

NAFLD is the most common cause of liver disease worldwide and it is the hepatic manifestation of metabolic syndrome, which is often accompanied by obesity, dyslipidemia, systemic inflammation, and endothelial dysfunction. Of particular concern are reports of an increase in the number of NAFLD cases in the stage of non-alcoholic steatohepatitis with excess body weight and obesity in adolescence, which contributes to the development of end-stage liver disease much earlier in adulthood than classical suggestions describe. Compounding the problem is the fact that most adults worldwide are overweight or obese, but women of reproductive age are leading the trend, gaining weight faster than men and older women [24,25].

Our analysis of the cytokine profile indicators in pregnant women with NAFLD at the stage of NASH with varying degrees of obesity has shown the significant increase in the levels of pro-inflammatory (IL-1 β , IL-6, TNF- α) and the decrease in anti-inflammatory (IL-10) interleukins, which were correlated with the increase in BMI, and could serve as a trigger for the development of obstetric and perinatal complications.

Dyslipidemia and endothelial dysfunction are not only associated risk factors for NASH, but also the main pathogenetic links that trigger mechanisms of systemic inflammation associated with obesity during pregnancy. Scientific research by Lee (2016) and Qian et al. (2022) indicate that an obesity-induced increase in the concentration of pro-inflammatory cytokines serves as the basis for the development of preeclampsia, premature rupture of the amniotic membrane and postpartum bleeding [9,26]. Dyah et al. (2021) highlights a direct correlation between increased interleukin levels and high frequency of placental dysfunction with a violation of the utero-placental and placental-fetal circulation, which is directly proportional to an increase in BMI in pregnant women [25].

Our study has shown a direct relationship between NASH and manifestations of systemic inflammation detected during pregnancy, which ultimately led to a pathological course of pregnancy in the form of a high risk to develop preeclampsia, placental dysfunction, fetal growth retardation, premature rupture of the amniotic membranes compared to healthy pregnancies. The given results are comparable to scientific works by M. Sarkar (2020), V. Ratziu (2022), N. Li (2021) J. F. Dufour (2022), which confirm increased levels of IL-1 β , IL-6 and TNF- α and decreased levels of IL-10 with increasing BMI in NASH patients [12,18,27,28].

The elimination of these pathophysiological links is a key principle in the regression of liver steatosis and preventing the development of serious obstetric and perinatal pathology. The prescription of complex treatment including vitamin E, ursodeoxycholic acid and L-carnitine is appropriate in achieving anti-steatotic and hepatoprotective effects, improving metabolic processes in the liver and placental microcirculation [27]. Vitamin E inhibits lipid peroxidation processes due to immunomodulatory and radioprotective properties. On the other hand, mitochondrial dysfunction and apoptosis of hepatocytes are characteristic features of NASH associated with overweight [7,17,28]. UDCA has hepatoprotective properties and immunomodulating effects, and by virtue of that, suppresses these phenomena by modeling the mitochondrial membrane potential, as well as suppressing the expression of some HLA-1 histocompatibility antigens on the hepatocyte membrane, as a result of which a decrease in pro-inflammatory interleukins is observed [14,17,18,19,21]. L-carnitine inhibits the inflammatory reaction through the transfer of β -oxidized long-chain fatty acids into mitochondria and the removal of toxic substances in the process of fatty acid metabolism, contributing to the reduction of oxidative stress and systemic inflammatory response [27].

Thus, it is the combination of pathogenetically justified complex of UDCA, vitamin E and L-carnitine that helps to reduce the activation of systemic inflammation in pregnant women with NASH and may prevent the development of certain obstetric and perinatal complications.

Conclusions

1. Nonalcoholic fatty liver disease at the stage of non-alcoholic steatohepatitis during pregnancy is accompanied by significant changes in the cytokine profile, which is manifested by an increase in the levels of pro- (IL-1 β , IL-6, TNF- α) and anti-inflammatory interleukins (IL-10). An increase in the body mass index directly contributes to the increase in these indicators in pregnant women with nonalcoholic steatohepatitis.

2. The complex therapy prescription including vitamin E, ursodeoxycholic acid and L-carnitine is effective in the treatment of pregnant women with non-alcoholic fatty liver disease at the stage of non-alcoholic steatohepatitis due to cumulative and potentiating effects, reducing manifestations of systemic inflammation by normalizing the level of cytokines.

Prospects for further scientific research. The obtained data justify the need to examine long-term outcomes in pregnant women with NASH and varying degrees of obesity.

Funding

This work is a fragment of the comprehensive research works of Ivan Horbachevsky Ternopil National Medical University of the Ministry of Health of Ukraine "Development and implementation of effective methods and means of diagnosis, treatment and prevention of disorders of reproductive health of women and perinatal protection of the fetus" (state registration number 0110U001941, 2010–2012) and "Comprehensive approach to symptom control, direct and distant prognosis in conditions of comorbid pathology in the clinic of internal diseases and the practice of a family doctor" (state registration number 0118U000361, 2018–2022).

Conflicts of interest: authors have no conflict of interest to declare.
Конфлікт інтересів: відсутній.

Надійшла до редакції / Received: 05.12.2022
Після доопрацювання / Revised: 20.01.2023
Прийнято до друку / Accepted: 30.01.2023

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