Proinflammatory interleukins 2, 6 and tumor necrosis factor alpha in patients with hypertension and diabetes mellitus depending on the presence of metabolic-associated liver steatosis

Abstract. Background. Lack of information about proinflammatory interleukins (IL) and tumor necrosis factor alpha (TNFα) levels in case of metabolic-associated liver steatosis (MALS) and their roles in its progression to steatohepatitis are key reasons for the relevance and actuality of our study. The purpose: to evaluate proinflammatory interleukins 2, 6, and TNFα levels in concomitant liver steatosis. Materials and methods. Thirty-five patients with hypertension stage II–III, type 2 diabetes mellitus were examined. All of them were treated on an outpatient basis according to the guidelines of the Ministry of Health of Ukraine and the Declaration of Helsinki. Participants were divided into the main group with MALS (n = 24, males 45.8 %, females 54.2 %; average age 55.83 ± 0.89 years) and the control group without steatosis (n = 11, males 54.5 %, females 45.5 %; average age 53.00 ± 1.55 years). In addition to standard parameters, levels of IL6, IL2, TNFα, selectin, resistin, insulin, C-peptide, glycated hemoglobin, non-esterified fatty acids were evaluated, and some indexes were calculated, including triglyceride-glucose index and Castelli indexes I and II. Results were processed statistically, with significance level of p < 0.05. Results. Although MALS is not followed by qualitative differences in proinflammatory IL2, IL6 and TNFα compared to no steatosis, the risk of TNFα elevation was 5 times higher in patients with MALS (odds ratio 5.08; 95% confidence interval 1.02–25.17). An increase in IL2 and TNFα is unfavorable for patients with MALS, it can be considered as a marker of steatosis progression to steatohepatitis, as it is associated with transaminase activation, endogenous intoxication, lipid distress and glucose intolerance. IL6 was rather lower in patients with MALS compared to those without steatosis, but its growth was exponential and proceeded simultaneously to IL2 and TNFα. Conclusions. MALS was not associated with significant changes in IL2, IL6 and TNFα compared to no steatosis, but their elevation can be criteria for transformation into steatohepatitis due to the activation of transaminases, inflammation, endogenous intoxication, lipid distress, glucose intolerance.

Keywords: diabetes mellitus; interleukin-2; interleukin-6; tumor necrosis factor alpha; metabolic-associated liver steatosis

Introduction
Metabolic dysfunction-associated fatty liver disease (MAFLD), which gradually progresses from metabolic-associated liver steatosis (MALS) to steatohepatitis and liver cirrhosis, is a worldwide pandemic. It affects one third of adult population in the developed countries [1] and causes a decrease in the effectiveness of treatment for other diseases due to the high metabolic activity of the liver. It was recently discovered that liver itself is an endocrine-paracrine organ, which secretes regulatory substances hepatokines, including those with marked pro-inflammatory activity, particularly interleukins, tumor necrosis factor alpha (TNFα), and more commonly known orosomucoids, C-reactive protein, fibrinogen.
Interleukin 2 (IL2) is produced by CD4+ and CD8+ T-lymphocytes, some B-lymphocytes, and dendritic antigen-presenting cells, located in liver tissue, providing immunological response to multiple blood antigens. It adjusts leukocyte activity and immune system function (prevents...
autoimmune reactions, controls T-lymphocyte sub-
populations ratio, increases activity of natural killer cells and
and cytotoxic T-lymphocytes), but its precise role in MAFLD
origin and progression remains unknown. It was outlined that
in patients with non-alcoholic steatohepatosis, the quantity
of IL2 receptors on cell surface rises simultaneously with
tissue fibrosis development. Thuswise, level of IL2 receptors
can be used as a MAFLD marker [2] or as a marker of ad-
vanced cirrhosis (contrary to mild or no cirrhosis) in patients
with MAFLD [3]. In a trial, IL2 induced minor increase in
lactate production in isolated hepatocyte suspension [4].

IL6 is produced by macrophages, including those located
in liver parenchyma; however, its role in MAFLD patho-
genesis is also unclear. In experiment, IL6 affected isolated
hepatocyte suspension in a similar way as IL2, inducing less
noticeable lactate increase, which is a marker of gluneneo-
genesis activation [4], specifically IL6 instead of TNFα en-
hanced lipogenesis in culture of rat hepatocytes [5].

TNFα is produced by macrophages (including liver paren-
chyma macrophages) and by a variety of other cells (lympho-
cytes, cardiomyocytes, adipocytes, hepatocytes, fibroblasts,
and neurons) in response to bacteria lipopolysaccharide and
IL1 elevation. It stimulates insulin resistance, maintains im-
une response, induces apoptosis, causes fever and cachexia,
regulates anti-inflammatory response alongside with IL6,
activates pro-inflammatory hepatokine synthesis in liver (oro-
somucoids, C-reactive protein, fibrinogen) and in excessive
amount it can trigger cytokine storm and shock [6]. Lack of
information about ILs and TNFα levels in case of MALS and
their role in its progression to steatohepatitis are key reasons
for the relevance and actuality of our study.

The purpose of study was to evaluate proinflammatory in-
terleukins 2, 6, and TNFα levels in concomitant liver steatosis.

Materials and methods

Thirty-five patients with hypertension II–III stage, grade
2–3, compensated heart failure I–II functional class ac-
cording to NYHA classification, type 2 diabetes mellitus
were examined. All of them were treated on an outpatient
basis according to the guidelines of the Ministry of Health
of Ukraine and the Declaration of Helsinki.

Participants were divided into two groups: the main group
with MALS (n = 24, males 11/45.8 %, females 13/54.2 %; av-
average age 55.83 ± 0.89 years) and the control group without ste-
atosis (n = 11, males 6/54.5 %, females 5/45.5 %; average age
53.00 ± 1.55 years). Groups were identical in gender, age, and
hypertension duration (9.83 ± 2.09 and 10.10 ± 3.25 years).
At the same time, it turned out that in 19 (79.2 %) patients
with MALS, hypertension was associated with chronic forms
of coronary artery disease (CAD) with duration of 1.76 ± 0.43
years, whereas in control group, no patients had CAD
(p < 0.05). Duration of type 2 diabetes was 3.37 ± 0.55 years
in main group; in controls, it was newly diagnosed (p < 0.05).

Although according to body mass index, patients with
MALS were obese and controls were overweight (33.47 ± 1.00
and 28.72 ± 1.09 kg/m²; p < 0.05), both groups were identical
in nature of obesity and waist-to-hip ratio (1.03 ± 0.05 and
0.98 ± 0.02; p > 0.05), which allows us to evaluate adipose
tissue development as analogical. In addition to standard li-
ver function parameters, endogenous intoxication parame-
ters (creatinine, urea), lipid and carbohydrate metabolism,
insulin, C-peptide, glycated hemoglobin (HbA1c) and oral
glucose tolerance test, non-esterified fatty acids, IL6, IL2,
TNFα, selectin, resistin levels were evaluated and variety of
complex indexes was calculated, including De Ritis ratio,
triglyceride-glucose index (Tg/G = TG (mmol/l) × fasting
glucose (mmol/l)) / 2 [7] and two Castelli risk indexes (1 —
total cholesterol (TC)/high-density lipoproteins (HDL); II —
low-density lipoproteins (LDL)/HDL). Results were pro-
cessed statistically, with admitted significance level of p < 0.05.

Results

In the examined patients, MALS presence did not interfere
with IL2 level, which ranged within 0.56—
15.1 pg/ml and was 6.52 ± 0.67 pg/ml in patients with MALS
and 5.67 ± 0.54 pg/ml in those without steatosis (p > 0.05).
Level of IL2 in MALS correlated directly with adipokytone
resistin (r = 0.62; p < 0.01) and cytokine TNFα levels (r = 0.54; p < 0.05).
It also had negative correlation with TyG (r = –0.46; p < 0.05) —
criteria of insulin resistance [8], pre-clinical athe-
rosclerosis [9], atherosclerotic plaques stability in carotid and

In patients with concomitant MALS, IL2 level above ave-
rage (9.57 ± 0.58 pg/ml) was associated with significant dif-
fences: shorter CAD duration (0.78 ± 0.17 vs 2.46 ± 0.68 years),
but higher levels of both transaminases (aspartate amio-
transferase: 46.46 ± 6.79 vs 31.84 ± 1.85 mmol/h/l; alanine
aminotransferase: 50.82 ± 7.39 vs 37.61 ± 1.95 mmol/h/l), less
pronounced endogenous intoxication syndrome (creatinine:
81.37 ± 3.56 vs 94.56 ± 5.02 μmol/l), p < 0.05 for all findings,
respectively. results of TNFα levels in case of MALS and
its role in their progression to steatohepatitis are key reasons
for the relevance and actuality of our study.

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The purpose of study was to evaluate proinflammatory in-
terleukins 2, 6, and TNFα levels in concomitant liver steatosis.
TNFα concentration, in addition to IL2, had direct correlation with resistin levels in the examined patients (r = 0.50; p < 0.05). Nevertheless, in MALS, the risk of TNFα elevation was 5 times higher (OR 5.08; 95% CI 1.02–25.17). According to absolute value of determined parameters, patients with higher (11.24 ± 0.96 pg/ml) and lower (3.87 ± 0.38 pg/ml) than average levels of TNFα did not differ from each other.

Correlational analysis has shown that among patients with above average TNFα level, its further growth would be associated with endogenous intoxication syndrome (urea: r = 0.78; p < 0.05). It also correlates directly with resistin levels (creatinine-resistin: r = 0.71; p < 0.05) that can be considered as a marker of MALS progression to steatohepatitis. Whereas increased resistin level leads to elevation of C-peptide and TC (r = 0.63; p = 0.07 and r = 0.77; p < 0.05), markers of carbohydrate and lipid metabolism impairment. It means that MALS is characterized by normal TNFα level, but 5 times higher probability of its elevation, which in turn would be associated with development of endogenous intoxication, lipid distress, glucose intolerance and progression of steatosis into steatohepatitis.

In the examined patients, presence of steatosis did not significantly influence IL6 level (3.22 ± 0.67 and 3.23 ± 0.84 pg/ml; p > 0.05), its concentration in patients with MALS was characterized by a great amplitude (0.47–13.7 pg/ml) and did not correlate with other parameters. Interestingly, in most cases (79.2 %), level of this cytokine was below average, whilst elevated in 20.8 % (p < 0.05), but in that case, it was 2.4–4.2 times above limit. One-fifth of patients with increased IL6 (8.60 ± 1.67 pg/ml) was characterized by shorter hypertension duration (4.40 ± 1.50 vs 11.26 ± 2.52 years; p < 0.05), less intense course of endogenous intoxication syndrome (creatinine: 76.10 ± 2.10 vs 93.09 ± 4.14 μmol/l; p < 0.01; urea: 4.16 ± 0.33 vs 5.27 ± 0.39 mmol/l; p < 0.05) and better lipid profile (LDL: 2.30 ± 0.33 vs 3.91 ± 0.53 mmol/l; TC: 4.67 ± 0.31 vs 5.98 ± 0.48 mmol/l; p < 0.05 for both; Castelli index II: 1.44 ± 0.26 vs 3.16 ± 0.49; p < 0.01).

In our opinion, low IL6 level is unfavorable, as it is observed in case of long-term hypertension and is followed by distress of lipid metabolism. In patients with MALS and IL6 concentrations above average, among all measured cytokines, only TNFα correlated significantly with creatinine (r = 0.94; p < 0.05) and non-esterified fatty acids (r = −0.97; p < 0.01).

Amidst patients with IL6 level below average, its concentration was significantly associated with elevated production of other cytokines, IL2 (r = 0.67; p < 0.01), TNFα (r = 0.66; p < 0.05) and resistin (r = 0.63; p < 0.05), which were also connected significantly (IL2-resistin: r = 0.60; IL2-TNFα: r = 0.60; resistin-TNFα: r = 0.51), on the background of better carbohydrate profile (TyG: r = −0.68; p < 0.05; C-peptide: r = 0.94); all p < 0.05.

MAFLD is a growing global health burden among the population with high susceptibility to obesity and insulin resistance [17]. The most effective prevention strategy for MAFLD is lifestyle modification [18]. Considering the prevalence and its impact, several novel methods, including therapeutic and surgical approaches, are currently under investigation [19]. Pharmacological interventions, such as treatment with antidiabetic medications, and anti-obesity drugs, can be considered for MAFLD patients but should be chosen wisely on a case-to-case basis. In addition, bariatric surgery aimed to obtain durable weight loss is an option for MAFLD patients who either fail medical management or have associated comorbidities. Novel approaches in drug delivery would be ideal for managing MAFLD in the future [20].

**Conclusions**

Although MALS is not followed by qualitative differences in proinflammatory IL2, IL6 and TNFα compared to no steatosis, the risk of TNFα elevation was 5 times higher in patients with MALS (OR 5.08; 95% CI 1.02–25.17). An increase in IL2 and TNFα is unfavorable for patients with MALS, it can be considered as a marker of steatosis progression to steatohepatitis, as it is associated with transaminase activation, endogenous intoxication, lipid distress and glucose intolerance. IL6 was rather lower in patients with MALS compared to those without steatosis, but its growth was exponential and proceeded simultaneously to IL2 and TNFα.

Further studies perspectives: to study levels of these cytokines in other metabolic-associated liver diseases.

**References**


Висновки

Наявність МАСП не викликала істотних змін ІЛ-2, ІЛ-6, ФНП-α, проте зростання їхнього рівня можна вважати маркером переходу стеатозу в стеатогепатит, оскільки це асоціюється з активацією трансаміназ, системним запаленням, ендогенною інтоксикацією, ліпідним дисром та непереносимістю глюкози. Вміст ІЛ-6 у пацієнтів із МАСП було дещо нижчим, ніж в осіб без стеатозу, однак його зростання відповідає ризику переходу в стеатогепатит, оскільки воно було пов’язано з активацією трансаміназ, оскільки воно було пов’язано з активацією трансаміназ, ендогенною інтоксикацією, ліпідним дисром та непереносимістю глюкози.

Ключові слова: цукровий діабет; інтерлейкін-2; інтерлейкін-6; фактор некрозу пухлин альфа; метаболічно-асоційований стеатоз печінки.

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Прозапальні інтерлейкін-2, -6 і фактор некрозу пухлин альфа в пацієнтів з артеріальною гіпертензією та цукровим діабетом залежно від наявності метаболічно-асоційованого стеатозу печінки

Резюме. Актуальність. Недостатня кількість інформації про вміст прозапальних інтерлейкінів (ІЛ) і фактора некрозу пухлин альфа (ФНП-α) за умов метаболічно-асоційованого стеатозу печінки (МАСП) та її ролі в процесі переходу в стеатогепатит зумовлені доцільності та актуальність нашого дослідження. Метеорожка. Оцинити вміст прозапальних ІЛ-2, ІЛ-6 та ФНП-α за умов супутнього МАСП. Матеріали та методи. Обстежено 35 пацієнтів з артеріальною гіпертензією та цукровим діабетом 2-го типу, яких лікували амбулаторно відповідно до стандартів МОЗ України та Гелінської декларації. 

Вміст прозапальних ІЛ-2, ІЛ-6, ФНП-α в пацієнтів з метаболічно-асоційованим стеатозом печінки (МАСП) нарахований методом імплюсованого амплендування з використанням стандартних зразків. Реактиви та методиця: звичайні прозапальних ІЛ-2, ІЛ-6, ФНП-α, селективні, ресінуїнд, інсуліну, С-пептидіт, глюкозного гемоглобіну, вільних жирних кислот, розраховували тригліцерид-глюкозний індекс та індекс Castelli 1, II. Результати оприлюднено статистично. Результати. Хоча при МАСП не спостерігалося відмінностей у рівнях прозапальних ІЛ-2, ІЛ-6 та ФНП-α порівняно з пацієнтами без стеатозу, особливо з МАСП мали в 5 разів вищу ймовірність зростання ФНП-α (відносний ризик 5,08; 95% довірчий інтервал 1,02–25,17). Несприсятивним для пацієнтів із МАСП було збільшення ІЛ-2 і ФНП-α, яке можна вважати маркером переходу стеатозу в стеатогепатит, оскільки це асоціюється з активацією трансаміназ, ендогенною інтоксикацією, ліпідним дисром та непереносимістю глюкози. Вміст ІЛ-6 у пацієнтів із МАСП був дещо нижчим, ніж в осіб без стеатозу, однак його зростання було експоненційним і відбувалося паралельно ІЛ-2 та ФНП-α. Висновки. Наявність МАСП не викликала істотних змін ІЛ-2, ІЛ-6, ФНП-α, проте зростання його рівня можна вважати несприятливим чинником переходу в стеатогепатит, оскільки воно було пов’язано з активацією трансаміназ, системним запаленням, ендогенною інтоксикацією, ліпідним дисром та непереносимістю глюкози. Ключові слова: цукровий діабет; інтерлейкін-2; інтерлейкін-6; фактор некрозу пухлин альфа; метаболічно-асоційований стеатоз печінки.