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The association of systemic inflammatory biomarkers with metabolic dysfunction-associated steatotic liver disease and arterial hypertension

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Objective — to explore the relationship between metabolic dysfunction-associated steatotic liver disease (MASLD) on the background of arterial hypertension (AH) and blood inflammatory markers including C-reactive protein (CRP), Interleukin-6 (IL-6), IL-4, haptoglobin and pentraxin-3 (PTX-3).

Materials and methods. We examined 102 patients with MASLD. They were divided into 3 groups: group A included 52 patients with isolated MASLD, group B — 23 patients with MASLD and AH stage I, and group C — 27 patients with MASLD and AH stage II. The control group (group D) comprised 20 apparently healthy people. Patients with viral hepatitis, liver cirrhosis, alcoholic liver disease, and AH stage III were excluded. Systemic inflammatory biomarkers analyses were performed using electrochemiluminescence, immunoenzymatic, and immunoturbidimetry techniques.

Results. The systemic inflammatory biomarkers analyses revealed significantly higher levels of CRP ($p = 0.001$), IL-6 ($p = 0.01$), haptoglobin ($p < 0.05$) and PTX-3 ($p < 0.01$) and decreased levels of IL-4 ($p < 0.05$) in group B and group C in comparison with the group A and control group ($p1 < 0.01$, $p2 = 0.01$). Also, there was a significant increase of CRP ($p = 0.01$), IL-6 ($p = 0.01$), and PTX-3 levels ($p < 0.05$) in group B compared with group C. However, the relationship between the IL-4 ($p > 0.05$) and haptoglobin ($p > 0.05$) levels and the progression of the AH stages have not been confirmed in our study.

Conclusions. Our findings indicate the direct relationship between the systemic inflammatory biomarkers' involvement in developing liver tissue inflammation and the further progression of MASLD. The obtained data indicate the relationship of AH and its stages with the development of chronic systemic inflammatory response in patients with MASLD and AH comorbid course.

Keywords:
metabolic dysfunction-associated steatotic liver disease, arterial hypertension, C-reactive protein, IL-6, IL-4, haptoglobin, pentraxin-3.

In the structure of general morbidity metabolic dysfunction-associated steatotic liver disease (MASLD) occupies one of the leading positions, displacing viral and alcoholic hepatitis [14]. According to statistics, MASLD affects 30 % of the adult population worldwide, with its prevalence increasing from 22 % to 37 % from 1991 to 2019 [7]. Recently, studies of the MASLD progression mechanisms in patients with arterial hypertension (AH) are relevant [13]. AH is a multifactorial disease resulting from the interaction between genetic predisposition and environmental risk factors. MASLD and AH often co-exist in the same individual in a complex two-way relationship as they share common metabolic risk factors, such as age, obesity, insulin resistance, and development of chronic systemic inflammation (CSI) [5, 12]. The development of CSI leads to exhaustion and necrosis of hepatocytes, which leads to the development and rapid progression of MASLD [9]. Currently, it is known that CSI markers, such as C-reactive protein (CRP) and interleukin 6 (IL-6) may be involved in the pathogenetic mechanisms

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of the development and progression of MASLD [1, 3]. One of the mediated markers of CSI in patients with MASLD is also the acute phase protein — haptoglobin — produced mainly in the liver [2]. Also, a serum biomarker pentraxin-3 has significant potential in the diagnosis of MASLD and its stages [16]. Pentraxin-3 (PTX-3) is produced by the liver in response to inflammatory mediators and is a systemic response to local inflammation [11]. However, the role of systemic inflammatory biomarkers including PTX-3 in the comorbid course of MASLD and AH remains inconclusive.

Objective — to explore the relationship between metabolic dysfunction-associated steatotic liver disease on the background of arterial hypertension and blood inflammatory markers including C-reactive protein, interleukin-6, interleukin-4, haptoglobin and pentraxin-3.

Materials and methods

We examined 102 patients with MASLD who underwent treatment in the Department of Internal Medicine, Lozova Medical Hospital, from September 2017 to April 2021. Patients' anthropometrics, clinical features (gender, age, symptoms, body mass index, history of MASLD, alcohol intake survey, complaints), and biochemical findings (CRP, IL-6, IL-4, haptoglobin, PTX-3) were collected. The studied patients were divided into 3 groups: group A included 52 patients with isolated MASLD, group B — 23 patients with MASLD and AH stage I, and group C — 27 patients with MASLD and AH stage II. The control group (group D) comprised 20 apparently healthy people. Patients with viral hepatitis, liver cirrhosis, alcoholic liver disease, and AH stage III were excluded. Anthropometrics and clinical dataset information is shown in Table 1. The study was conducted following the ethical principles included in the Declaration of Helsinki (1964—2013) and order of the Ministry of Health of Ukraine No 616 of 03.08.2012. All the participants were informed about the goals, organization, and research methods and signed an informed consent to participate.

The CRP level analysis was determined using the photometric turbodimetric method using a biochemical analyzer Beckman Coulter AU 480 (USA). Reference measurement range: 0—10 mg/L, sensitivity — 3 mg/L. The IL-6 level was determined by electrochemiluminescence immunoassay using a Beckman Coulter AU 480 biochemical analyzer (USA). Reference measurement range: 0.03—5 pg/mL, sensitivity — 0.03 pg/mL. The IL-4 level was determined by electrochemiluminescence immunoassay using a Beckman Coulter AU 480 biochemical analyzer (USA). Reference measurement range: 0.03—5.00 pg/mL, method sensitivity: 2—30 pg/mL. The haptoglobin level was determined using an immunoturbodimetric method, measurement range: 0—400 mg/dL, reference range: 23—205 mg/dL (IFCC), sensitivity – 1 mg/dL (Cobas Mira). A Beckman Coulter AU 480 biochemical analyzer (USA) was used in the study of the haptoglobin level. The level of PTX-3 was determined by the enzyme immunoassay method using the «Human Pentraxin-3 ELISA Kit» manufactured by Multisciences (Lianke) BiotechCo. (China) using immunoenzyme reaction analyzer «Immunochem-2100» (USA).

The structural changes and the severity of liver steatosis were visualized by the ultrasonography method using the «Vivid 3» (USA) and «Logiq 5» (USA) devices. The severity of liver fibrosis was assessed based on the results of transient elastography using an ultrasonic scanner Siemens — «Acusons 3000» («Radmyr» JSC NDIRI, Ukraine) with a convex format sensor at frequencies of 2—5 MHz at a depth of 10—50 mm from the capsule.

The results statistical processing was performed with Microsoft Office Excel 2013 and Statistica 13.1 computer programs on a personal computer with the use of parametric (Student's t-test) and non-parametric (Mann–Whitney U-test) statistical methods. Evaluation of correlations was carried out according to Spearman's rank correlation coefficient R [4]. In the studied groups' comparison, the error probability was considered to be statistically significant at $p \leq 0.05$.

Results

Characteristics of the Study Participants

The anthropometric characteristics of 102 patients are summarized in Table 1.

The average body mass index of patients in the group of MASLD and AH stage II conformed to the obesity class, in the group of MASLD without AH obesity was determined in 21 % of patients, in the group of MASLD and AH stage II — in 26 % of patients and the control group — in 10 % of patients. The waist-to-hip ratio demonstrated the presence of abdominal obesity in groups MASLD and AH stage I and MASLD and AH stage II in comparison with the group of MASLD without AH and the control group. The average BP levels in groups MASLD and AH stage I and MASLD and AH stage II exceeded the average blood pressure (BP) levels of the main and control groups and conformed to the I and II AH stages according to WHO classification (1999).

Structural Changes of the Liver

In univariate analysis of US parameters (Table 2) in all groups of patients (A, B, C), compared to the control group, the following indicators were found:

Table 1. The studied patient groups' anthropometric characteristics

Parameters	Isolated MASLD (n = 52)	MASLD and AH stage I (n = 23)	MASLD and AH stage II (n = 27)	Control group (n = 20)	p
Age, years	49.4 ± 8.5	54.5 ± 6.3	46.6 ± 7.2	35.4 ± 10.8	0.05
Female Male	22 (42.3 %) 30 (57.7 %)	13 (56.5 %) 10 (43.5 %)	14 (51.8 %) 13 (48.2 %)	9 (45.0 %) 11 (55.0 %)	> 0.05
Body mass index, kg/m^2	27.51 ± 3.31	28.6 ± 4.21	30.17 ± 3.86	22.91 ± 2.70	< 0.05
Systolic blood pressure, mm Hg	130.0 ± 7.0	145.0 ± 8.0	160.0 ± 11.0	120.0 ± 10.0	< 0.05
Diastolic blood pressure, mm Hg	80.0 ± 4.0	90.0 ± 8.0	100.0 ± 9.0	75.0 ± 5.0	< 0.001
Waist-to-hip ratio	1.1 ± 0.5	2.5 ± 0.8	2.7 ± 0.3	0.9 ± 0.2	< 0.05

Note. Categorical variables are presented as the number of cases and percentage, while quantitative indicators are presented as μ ± SD.

Table 2. Structural changes of the liver in the studied patient groups

Parameters	Isolated MASLD (n = 52)	MASLD and AH stage I (n = 23)	MASLD and AH stage II (n = 27)	Control group (n = 20)	p
Ultrasonography					
Distal attenuation of the echo signal	Detected	Detected	Detected	Not detected	0.05
Parenchymal echogenicity	Increased, heterogen	Increased, heterogen	Increased, heterogen	Normal, homogen	0.05
Left liver lobe size, craniocaudal dimension, mm	63.5 ± 3.7	66.5 ± 2.5	67.5 ± 1.9	56 ± 2.9	< 0.05
Right liver lobe size, craniocaudal dimension, mm	140.0 ± 4.5	146.0 ± 3.9	148.0 ± 2.6	116.0 ± 2.1	< 0.05
Portal vein diameter, mm	8.3 ± 0.5	8.5 ± 2.6	8.3 ± 0.5	7.5 ± 1.4	< 0.05
Transient elastography					
Liver stiffness measurement, kPa	2.4 ± 1.1	2.7 ± 1.3	3.2 ± 0.8	1.6 ± 0.4	< 0.05
Fibrosis stage F0—1	43 (82.7 %)	17 (74.0 %)	9 (33.4 %)	0	< 0.05
Fibrosis stage F1—2	9 (17.3 %)	6 (26.0 %)	18 (66.6 %)	0	< 0.05

Note. p — the probability of changes compared to the control group.

the presence of distal attenuation of the echo signal (in 82 % of patients), an increase of the liver parenchymal echogenicity (in 78 %), an increase of the left liver lobe (in 44 %) and right liver lobe size (in 36 %) and an increase of the portal vein diameter (in 25 %). It should be noted that no significant differences were found when comparing the structural state of the liver between groups A, B, and C. According to the results of transient elastography, the presence of fibrosis stage F0—1 was more common in groups A (82.7 %) and B (74 %). The presence of fibrosis stage F1—2 was more common in group C (66.6 %). Fibrosis stages F3 and F4 were not detected in the patients involved in the study.

Systemic Inflammatory Biomarkers Levels

A significant increase of CRP levels was found in all groups of patients (A, B, C) compared to the control group. However, no significant difference in this parameter between the group B and C was found. The lowest IL-6 level was found in control group with its gradual increase in other groups. A significant increase in the haptoglobin levels was found in all groups of patients. However, no significant difference in the haptoglobin level between the group B and C was found (Table 3).

Discussion

This study showed the presence of a relationship between the systemic inflammatory biomarkers (CRP, IL-6, IL-4, haptoglobin, PTX-3) and the development of MASLD regardless of the concomitant AH presence or absence. Also, a significant increase of studied biomarkers in patients with a comorbid course of MASLD and AH stage I and II in comparison with the group of isolated MASLD may indicate the independent role of AH in the development of the chronic systemic inflammatory process in patients with MASLD. In addition, a significant increase of CRP, IL-6, and PTX-3 in patients with MASLD and AH stage II in comparison with the group of MASLD and AH stage I can confirm the negative impact of AH progression on chronic systemic inflammatory

Table 3. Systemic inflammatory biomarkers' levels in the studied patient groups

Parameters	Isolated MASLD (n = 52)	MASLD and AH stage I (n = 23)	MASLD and AH stage II (n = 27)	Control group (n = 20)
CRP, mg/L	3.8 ± 3.1**	4.1 ± 2.9**#	4.3 ± 2.3**#&	0.4 ± 0.6
IL-6, pg/mL	3.8 ± 1,3**	4.23 ± 1.8**#	4.36 ± 1.3**#&	2.4 ± 1.1
IL-4, pg/mL	1.78 ± 1.5**	1.67 ± 1.4**#	1.58 ± 1.3**#	1.87 ± 0.8
Haptoglobin, mg/dL	136.5 ± 19.6*	213.8 ± 23.8*#	228.8 ± 17.3*#	67.5 ± 18.3
PTX-3, pg/mL	254.3 ± 44.4***	421.9 ± 31.4***##	430.9 ± 35.8***##&&	53.2 ± 14.3
Waist-to-hip ratio	1.1 ± 0.5	2.5 ± 0.8	2.7 ± 0.3	0.9 ± 0.2

Note. The difference from the control group is statistically significant: *p < 0.05; ** p < 0.01; *** p < 0.001.
The difference from the group with isolated MASLD is statistically significant: #p < 0.05; ## p < 0.01.
The difference from the group with MASLD MASLD and AH stage I is statistically significant: &p < 0.05; && p < 0.01.

process development in patients with a comorbid course of MASLD and AH. PTX-3 is a novel biological marker of inflammatory processes. Many diseases that have some inflammatory characteristics and have the potential to cause systemic inflammatory processes can lead to increased serum pentraxin-3 levels [8]. In a study, it was shown that pentraxin-3 PTX-3 activates complement and innate immunity, hence playing crucial roles in tissue and vascular inflammation [10]. Previous studies demonstrate that CRP, PTX-3, IL-6, and haptoglobin have an impact on systemic inflammatory response development in hypertensive patients [6, 15, 17, 18]. These data are also consistent with our study findings. According to our results, there is a close association between elevated CRP, PTX-3, IL-6, and haptoglobin levels and systemic inflammation in hypertensive patients with MASLD. However, the relationship between the IL-4 and haptoglobin levels and the progression of the AH stages has not been confirmed in our study. A possible explanation for this trend could be a small number of patients.

Limitations. The findings obtained are not representative of all subjects with MASLD because of the small sample size and the strict inclusion criteria. Further prospective studies should be arranged to clarify the cause-and-effect relationship and test whether quantification of PTX-3 levels could provide additional information beyond the currently recognized risk factors to predict future cardiovascular events in subjects with MASLD and AH comorbid course.

Conclusions

Our findings indicate the direct relationship between the systemic inflammatory biomarkers' involvement in developing liver tissue inflammation and the further progression of MASLD. In addition, the obtained data indicate the relationship of AH and its stages with the development of chronic systemic inflammatory response in patients with MASLD and AH comorbid course. Considering the small group of patients, further multicenter prospective studies are needed to study and predict the influence of systemic inflammatory biomarkers on the risk of cardiovascular diseases developing in patients with MASLD and AH comorbid course.

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acquisition of data — T. M.A., M. O.V., O. A.C.;
analysis, and interpretation of data, drafting the article — T. M.A., K. O.P., M. O.V.

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Біомаркери системного запалення при коморбідному перебігу метаболічно-асоційованої стеатотичної хвороби печінки і артеріальної гіпертензії

Мета — вивчити зв'язок між метаболічно-асоційованою стеатотичною хворобою печінки, (МАСХП), на тлі артеріальної гіпертензії (АГ) та маркерами запалення крові (С-реактивний білок (С-РБ), інтерлейкін-6 (ІЛ-6), ІЛ-4, гаптоглобін і пентраксин-3 (РТХ-3)).

Матеріали та методи. Обстежено 102 хворих на МАСХП. Їх розподілили на три групи: групу А — 52 хворих на ізольовану МАСХП, групу Б — 23 пацієнти із МАСХП і АГ I стадії, групу С — 27 пацієнтів із МАСХП і АГ II стадії. Контрольну групу (група Д) утворено із 20 практично здорових осіб. Критеріями вилучення з дослідження були наявність вірусних гепатитів, цирозу печінки, алкогольної хвороби печінки й АГ III стадії. Аналіз біомаркерів системного запалення проводили за допомогою електрохемілюмінесцентних, імуноферментних та імунотурбідиметричних методів.

Результати. Аналіз біомаркерів системного запалення виявив значно вищі рівні С-РБ ($p = 0{,}001$), ІЛ-6 ($p = 0{,}01$), гаптоглобіну ($p < 0{,}05$) і РТХ-3 ($p < 0{,}01$) та зменшення вмісту ІЛ-4 ($p < 0{,}05$) у групах Б та С порівняно з групою А та контрольною групою ($p1 < 0{,}01$, $p2 = 0{,}01$). Також зареєстрували статистично значуще підвищення концентрації С-РБ ($p = 0{,}01$), ІЛ-6 ($p = 0{,}01$) та РТХ-3 ($p < 0{,}05$) у групі Б порівняно з групою С. Проте зв'язку між рівнями ІЛ-4 ($p > 0{,}05$), гаптоглобіну ($p > 0{,}05$) та прогресуванням стадій АГ у нашому дослідженні не підтверджено.

Висновки. Результати нашого дослідження вказують на безпосередню участь біомаркерів системного запалення в розвитку запалення тканини печінки та прогресуванні МАСХП. Отримані дані свідчать про вплив АГ та її стадій на прогресування хронічної системної запальної відповіді у хворих на МАСХП.

Ключові слова: метаболічно-асоційована стеатотична хвороба печінки, артеріальна гіпертензія, С-реактивний білок, ІЛ-6, ІЛ-4, гаптоглобін, пентраксин-3.

ДЛЯ ЦИТУВАННЯ

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