ORIGINAL ARTICLE
KLİNİK ARAŞTIRMA

Diagnostic capabilities of kallistatin, IL-10 and IL-1β in patients with non-alcoholic fatty liver disease and hypertension

Alkole bağlı olmayan yağlı karaciğer hastalığı ve hipertansiyonu olan hastalarda kallistatin, IL-10 ve IL-1β'nin tanısal güçleri

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SUMMARY

Aim: Non-alcoholic fatty liver disease (NAFLD) is closely linked to hypertension (HT) and affects about 25% of the adult population. An important issue remains the search for non-invasive biomarkers for NAFLD diagnosis.

The objective of the study was to evaluate the diagnostic value of kallistatin, interleukin-10 (IL-10) and interleukin-1β (IL-1β)in diagnosis of NAFLD in combination with HT.

Materials and Methods: 115 patients with NAFLD at the stage of non-alcoholic steatohepatitis (NASH) were examined, including 63 patients with comorbidity of NASH and HT (main group) and 52 people with isolated NASH (comparison group).Clinical and laboratory parameters were evaluated; plasma kallistatin, IL-10 and IL-1β levels were measured in all patients.

Results: Kallistatin levels averaged 65.03 ng/ml, 83.42 ng/ml and 111.70 ng/ml in patients with NAFLD and HT, isolated NAFLD and control group, respectively. The IL-10 level was 12,69 ng/ml and 14,34 ng/ml in patients with comorbid and isolated NAFLD, respectively, while control results averaged 16,19 ng/ml. The IL-1β level in NAFLD and HT group was 17,55 pg/ml, and in isolated NAFLD group the indicator averaged15,72 pg/ml, which exceeded the control values (8,26 pg/ml).

Conclusions: The course of both isolated NAFLD and comorbidity NAFLD with HT was accompanied by significant changes of kallistatin, IL-10 and IL-1β levels. Higher HT stage and BP grade, increased BMI and high CRP levels are associated with significantly more pronounced deviations of these indicators in patients with NAFLD and HT. The obtained data provide the possibility to consider the kallistatin, IL-10 and IL-1β as biomarkers of NAFLD severity.

Keywords: Hypertension, IL-10, IL-1β, kallistatin, NAFLD, NASH, non-alcoholic fatty liver disease, non-alcoholic steatohepatitis

ÖZET

Giriş: Alkolsüz yağlı karaciğer hastalığı, hipertansiyonla yakından bağlantılıdır ve yetişkin nüfusun yaklaşık%25'ini etkiler. Alkolsüz yağlı karaciğer hastalığı teşhisi için invazif olmayan biyobelirteçlerin araştırılması önemli bir konu olmaya devam etmektedir.

Çalışmanın amacı, hipertansiyon ile kombinasyon halinde alkolsüz yağlı karaciğer hastalığı tanısında kallistatin, interlökin-10 (IL-10) ve interlökin-1β (IL-1β) 'nin tanısal değerini değerlendirmektir.

Materyal ve Metodlar: Alkolsüz steatohepatit evresinde alkolsüz yağlı karaciğer hastalığı olan 115 hasta incelendi; bunlara eşlik eden alkolsüz steatohepatit ve hipertansiyonu olan 63 hasta (ana grup) ve izole alkolsüz steatohepatitli 52 kişi (karşılaştırma grubu). Klinik ve laboratuvar parametreleri değerlendirildi; Tüm hastalarda plazma kallistatin, IL-10 ve IL-1β seviyeleri ölçüldü.

Bulgular: Alkolsüz yağlı karaciğer hastalığı ve hipertansiyonu olan hastalarda, izole alkolsüz yağlı karaciğer hastalığı ve kontrol grubunda ortalama kallistatin seviyeleri sırasıyla 65.03 ng/ml, 83.42 ng/ml ve 111.70 ng/ml olmuştur. IL-10 seviyesi, komorbid ve izole alkolsüz yağlı karaciğer hastalığı olan hastalarda sırasıyla 12,69 ng/ml ve 14,34 ng/ml iken, kontrol sonuçlarının ortalaması 16,19 ng/ml idi. Alkolsüz yağlı karaciğer hastalığı ve HT grubunda IL-1β seviyesi 17,55 pg/ml ve izole alkolsüz yağlı karaciğer hastalığı grubunda gösterge ortalama 15,72 pg/ml ve bu da kontroldeğerlerini (8,26 pg/ml) aştı.

Sonuç: Hem izole alkolik olmayan yağlı karaciğer hastalığı hem de alkolsüz yağlı karaciğer hastalığı ile birlikte hipertansiyonun seyrine kallistatin, IL-10 ve IL-1βseviyelerinde önemli değişiklikler eşlik etti. Alkolsüz yağlı karaciğer hastalığı ve hipertansiyonu olan hastalarda daha yüksek hipertansiyon evresi ve kan basıncı derecesi, artan vücut kitle indeksi ve yüksek C-reaktif protein seviyeleri, bu göstergelerin önemli ölçüde daha belirgin sapmalarıyla ilişkilidir. Elde edilen veriler, kallistatin, IL-10 ve IL-1β'nın alkolik olmayan yağlı karaciğer hastalığı ciddiyetinin biyolojik belirteçleri olarak dikkate alınmasını sağlar.

Anahtar Kelimeler: Alkolden bağımsız karaciğer hastalığı, alkolden bağımsız karaciğer yağlanması, IL-1β, IL-10, kallistatin, yüksek tansiyon

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is the one of the most common chronic liver diseases. The prevalence of pathology in the world averages 25.2% worldwide, and the prevalence of non-alcoholic steatohepatitis (NASH), as a more severe type of NAFLD, averages 4% in the general population (1,2).

Non-alcoholic fatty liver disease is closely associated with metabolic disorders (3). Considerable attention is paid to the relationship between NAFLD and arterial hypertension (HT) that affects about one third of the world's adult population. The prevalence of HT is significantly higher in individuals with NAFLD than in the general population, and near 50% of patients with hypertension have NAFLD (4).

NAFLD can contribute to the development of hypertension, as well as occur on the background of increased blood pressure (5). It is also known that cardiovascular diseases significantly complicate the course of NAFLD (6). Therefore, diagnosis of fatty changes in the liver is especially important in the case of a comorbid combination of NAFLD and HT.

The liver biopsy is the "gold standard" for the NAFLD diagnosis. But this expensive invasive procedure is associated with a high risk of complications and false negative results in patients with NASH (7). It is the reason why a lot of studies are connected with the determine level of biomarkers to create models for steatosis and fibrosis diagnosing and predicting the course of NAFLD (8).

Cytokines play an important role in the pathogenesis of NAFLD, as NAFLD is associated with inflammatory processes in the liver (9). The variety of effects of cytokines determines their active role in the development and progression of NAFLD by stimulating hepatic inflammation, necrosis, apoptosis, and fibrosis (10).

The importance of IL-1β in the development of NAFLD has been actively studied, it is known that its activity increases in patients with NASH and correlates with the severity of fatty liver infiltration (10,11). At the same time, there is evidence that interleukin-10 (IL-10) acts as an anti-inflammatory cytokine, therefore, on the contrary, limits organ damage and prevents the progression of hepatic steatosis (12,13). Only a few studies have been performed to investigate the role of endogenous IL-10 in the progression of NAFLD (14).

Changes in cytokine balance are also observed in patients with persistently elevated blood pressure. Hypertension is thought to be associated with increased production of proinflammatory cytokines, including IL-1 (15). However, IL-10 shows protective activity under conditions of high pressure, as it counteracts the activity of the angiotensin-mediated system, as well as prevents the development of vascular dysfunction (16).

Kallistatin (kallikrein-binding protein) belongs to the family of serine protease inhibitors, which are synthesized in the liver and distributed between the tissues of the heart and blood vessels (17).

This protein maintains the level of blood pressure (BP) and exhibits anti-inflammatory, antioxidant, antiangiogenesis and antitumor effects (18,19). Various studies confirm that kallistatin may be an effective biomarker for detection of liver fibrosis in various liver diseases (20,21).

Different studies suggest that patients with NAFLD have an imbalance of pro-inflammatory and anti-inflammatory cytokines (22). Therefore, the determination of the activity of biomarkers can play a significant role in diagnosing the impact of concomitant hypertension on the course of NAFLD and determining the optimal management of this category of patients.

The aim of the study to determine the role of kallistatin, IL-1β and IL-10 as diagnostic biomarkers of NAFLD severity in patients with concomitant HT.

MATERIALS AND METHODS

The study was approved by the Ethics and Bioethics Commission of institution in which the study was conducted. The experimental part of this study respects the ethical standards in the Helsinki Declaration of 1975, as well as the principles of Good Clinical Practice (GCP) and the national law. All patients voluntarily decided to participate in the study and signed the patient's informed consent.

We examined 115 patients with NAFLD at the stage of non-alcoholic steatohepatitis (NASH). Participants included 57 men and 58 women aged 38 to 59 years (Mean=48,4). The patients were divided into two groups depending on the presence of concomitant HT. The main group (n=63) consisted of 32 men and 31 women aged 38 to 59 years (Mean=48,4) with NAFLD and HT, and the comparison group (n=52) included 25 men and 27 women aged 39 to 59 years (Mean=48,3) with an isolated course of NAFLD.
NAFLD and HT I stage were observed in 21 patients (12 men and 9 women; aged 39 to 57 years (Mean=46,9), and NAFLD and HT II stage were in 42 patients (20 men and 22 women; aged 38 to 59 (Mean=46,9)).

The control group (n=20) consisted of practically healthy 12 women and 8 men aged 38 to 56 years (Mean=47,1), randomized by age and sex.

NAFLD was diagnosed in the previous stages of managing patients according to the EASL-EASD-EASO Clinical Practice Guidelines, 2016. Hypertension was diagnosed in the

previous stages of managing patients according to the criteria of European (ESH / ESC) clinical guidelines for arterial hypertension, 2018.

The study of anthropometric parameters included measuring height in centimeters using a height meter, as well as body weight in kilograms using an electronic weigh scale. Body mass index (BMI) was calculated according to generally accepted formula: weight (in kilograms) divided by height (in meters) squared.Biochemical parameters of liver functional activity were determined by spectrophotometric and colometric methods (including alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyl transpeptidase (GGT)).

Plasma kallistatin, interleukin-1β and interleukin-10 levels were measured using the Human SERPINA4 (Kallistatin) ELISA Kit (Elabscience, USA), Human IL-1β (Interleukin 1 Beta) ELISA Kit (Elabscience, USA) and the Human IL-10 (Interleukin 10) ELISA Kit (Elabscience, USA), respectively. The level of C-reactive protein (CRP) was determined using the hs-CRP ELISA Kit (Biomerica USA).

All statistical processing of obtained results was performed using computer software packages "Excel 2019" (Microsoft), "Statistica 8.0. for Windows" (StatSoft Inc.). Continuous variables are presented as mean (M) or median (Me) depending on the correspondence with the normal distribution and confidence intervals with established reliability γ=0,95 (95% CI) and coefficient of variation (CV) expressed as a percentage. With CV≤10%, the sample was considered weakly variable, with CV ranging from 10% to 20%, the sample was considered moderate variable, with CV≥20%, the sample was considered highly variable.

Determination of the statistical significance of differences in relative indicators was performed using Pearson's chi-squared test. The Mann-Whitney U-test was used to determine the difference between the two independent samples by the trait level. Spearman's rank correlation coefficient was determined and Evans's scale was used to determine the strength of the relationship. The maximum allowable probability of committing a type I error (p-value) was established as the value of the level of statistical significance less or equal than 0,05.

RESULTS

There were no significant differences between anthropometric parameters of patients with NAFLD and HT comorbidity and isolated NAFLD. At the same time, significant differences were found in comparison with the control group in almost all indicators of both the main and the comparison group. It was expected that blood pressure values differed significantly between groups (Table 1).

Among the indicators of the functional state of the liver, the levels of hepatic transaminases had exceeded normal values and were significantly higher in patients with NAFLD and HT compared with the group of isolated NAFLD. Alkaline phosphatase and gamma-glutamyl transpeptidase were predominantly within the normal range, but in the group with comorbid NAFLD its levels were statistically significantly higher than in the group with isolated NAFLD and in the control group (Table 1).

Determination of C-reactive protein levels as a non-specific marker of inflammatory processes also revealed a significant difference in its content among the groups. The highest CRP value was found in the NAFLD and HT group (7,90 mg/l (95% CI 7,96-8,75; CV=24,70%)) versus 6,55 mg/l (95% CI 6,47-7,57; CV=50,25%) and 2,07 mg/l (95% CI 1,83-2,85;CV=20,50%) in the isolated NAFLD and control group, respectively (Table. 1).

Table 1. Clinical and laboratory characteristics of the examined patients

Indicator	NAFLD + HT (n = 63)	NAFLD (n = 52)	Control group (n = 20)	Reliability between groups
BMI, kg/m²	26.9(95% CI 24.45-29.34;CV=9.96%)	25.1(95% CI 25.38-26.56;CV=11.06%)	22.7(95% CI 22.41-23.46;CV=9.01%)	$p_{1-2} = 0.477$ $p_{1-3} < 0.001$ $p_{2-3} < 0.001$
SBP, mm Hg	140 (95% CI 137.86-140.55;CV=3.83%)	120 (95% CI 120.83-122.24;CV=2.08%)	123 (95% CI 121.94-126.56;CV=3.98%)	$p_{1-2} < 0.001$ $p_{1-3} < 0.001$ $p_{2-3} = 0.059$
DBP, mm Hg	85 (95% CI 82.72-86.17;CV=8.11%)	70 (95% CI 70.54-73.30;CV=6.90%)	75 (95% CI 73.75-79.25;CV=7.68%)	$p_{1-2} < 0.001$ $p_{1-3} < 0.001$ $p_{2-3} = 0.004$
ALT, IU/l	79.00(95% CI 80.00-86.98;CV=16.60%)	69.00(95% CI 65.29-70.79;CV=14.53%)	20.00(95% CI 18.77-23.92;CV=25.76%)	$p_{1-2} < 0.001$ $p_{1-3} < 0.001$ $p_{2-3} < 0.001$
AST, IU/l	75.05(95% CI 68.13-75.17;CV=19.46%)	54.00(95% CI53.16-56.99;CV=12.49%)	16.50(95% CI 15.36-20.04;CV=28.22%)	$p_{1-2} < 0.001$ $p_{1-3} < 0.001$ $p_{2-3} < 0.001$
ALP, IU/l	1840.00 (95% CI 1764.83-1872.79;CV=11.79%)	1150.00 (95% CI 1059.91-1213.17;CV=24.22%)	1160.00 (95% CI1032.76-1222.24;CV=17.95%)	$p_{1-2} < 0.001$ $p_{1-3} < 0.001$ $p_{2-3} = 0.826$
GGTP, IU/l	64.00(95% CI 63.31-70.53;CV=21.44%)	57.83(95% CI 55.08-60.57;CV=17.07%)	28.15(95% CI23.84-32.46;CV=32.77%)	$p_{1-2} < 0.001$ $p_{1-3} < 0.001$ $p_{2-3} < 0.001$
CRP, mg/l	7.90 (95% CI 7.96-8.75;CV=24.70%)	6.55 (95% CI 6.47-7.57;CV=50.25%)	2.07 (95% CI 1.83-2.85;CV=20.50%)	$p_{1-2} < 0,001$ $p_{1-3} < 0,001$ $p_{2-3} < 0,001$

Note: *p < 0,05 – the difference is statistically significant between groups;*
p1-2 – the difference between the NAFLD + HT group and the isolated NAFLD group;
p1-3 – the difference between the NAFLD + HT group and the control group;
p2-3– the difference between the isolated NAFLD group and the control group.

Maltepe Tıp Dergisi Cilt: 13 Sayı: 2/ 2021
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The kallistatin level in patients with NAFLD and HT was 65,98 ng/ml (95% CI 62,85-69,12;CV=22,28%). It was respectively in 1,3 (p<0.001) and 1,7 times (p<0.001) less, than in isolated NAFLD (83,42 ng/ml (95% CI 81,89-84,94; CV=6,56%)) and control group (111,70 ng/ml (95% CI 106,14-113,22;CV=6,90%)) (Fig. 1).

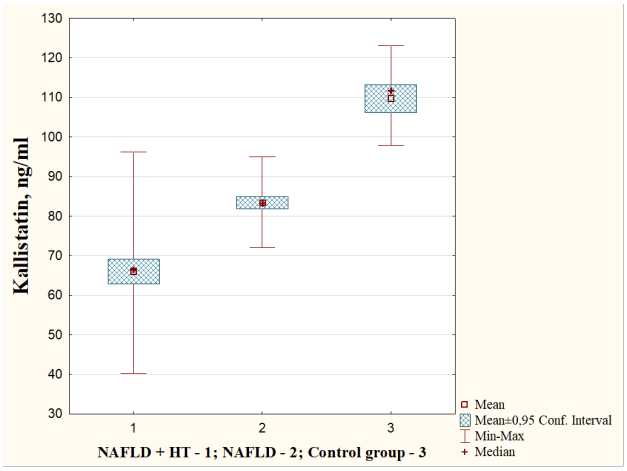

Figure 1. Kallistatin levels in the examined patients

The IL-10 level in the group of patients with NAFLD and HT averaged 12,69 ng/ml (95% CI 11,93-12,95; CV=15,88%), and in the group with isolated NAFLD it was 14,34 ng/ml (95% CI 13,27-14,34; CV=16,34%). It was 1,27 (p<0,001) and 1,1 (p<0,001) times less than in the control group (16,19 ng/ml (95% CI 15,15-17,74; CV=16,82%)), respectively (Fig. 2).

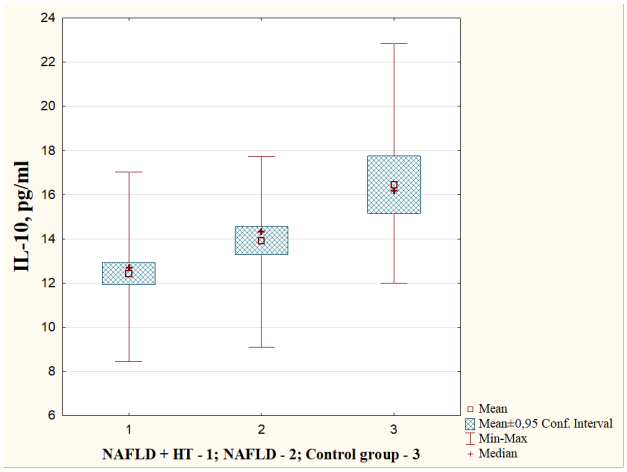

Figure 2. Interleukin-10 levels in the examined patients

At the same time, the level of IL-1β significantly exceeded the control values in both groups of patients with NAFLD. In the group with comorbidity of NAFLD and HT, the cytokine level was 17,55 pg/ml (95% CI 17,06-19,73; CV=28,81%), and in the group with isolated NAFLD the indicator averaged 15,72 pg/ml (95% CI 15,25-17,44; CV=23,97%), which exceeded the control values (8,26 pg/ml (95% CI 7,79-8,46; CV=8,77%) by 2,1 and 1,9 times, respectively (Fig. 3).

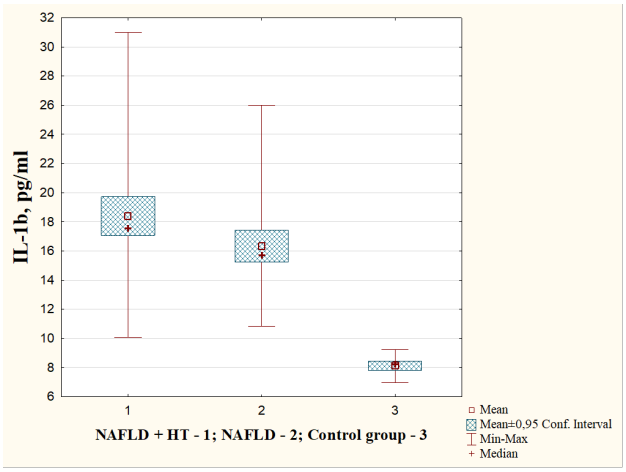

Figure 3. Interleukin-1β levels in the examined patients

The level of kallistatin in patients with NAFLD and HT I was 71,82 ng/ml (95% CI 70,16-79,51; CV=13,73%), which was significantly higher than in patients with HT II (58,62 ng/ml (95% CI 55,81-64,45; CV=23,06%; p<0.001). There were also differences between the levels of IL-1β, the activity of which in patients with NAFLD and HT II (19,47 pg/ml (95% CI 17,93-21,37; CV=28,14%)) significantly exceeded similar indicators in patients with NAFLD and HT I pg/ml (14,65 (95% CI 14,16-17,63; CV=23,97%), p=0,010) (Fig. 4).

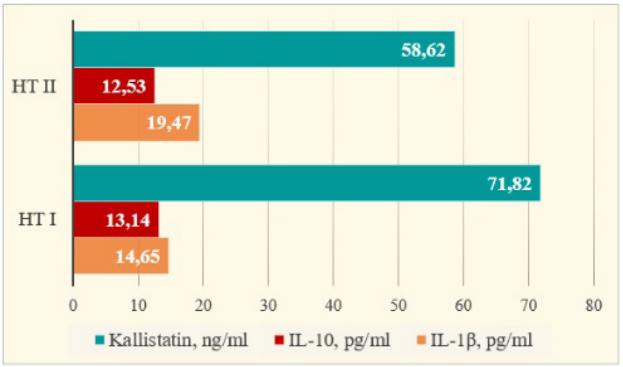

Figure 4. Biomarker levels in patients with NAFLD on the background of HT depending on HT stage and BP grade

At the same time, a significant difference was found only between plasma kallistatin levels in patients with HT II, BP grade I and HT II, BP grade II (p=0,011) (Table 2).

Table 2. Levels of biomarkers in patients with NAFLD on the background of HT depending on the HT stage and BP grade

Biomarker	HT I (n = 21)		Reliability between groups	HT II (n = 42)		Reliability between groups
	BP grade I (n = 16)	BP grade II (n = 5)		BP grade I (n = 3)	BP grade II (n = 39)	
Kallistatin, ng/ml	72.25 (95% CI 69.15-79.93; CV=13.56%)	71.82 (95% CI 60.93-90.61; CV=15.77%)	p=0,240	78.25 (95% CI 69.50-88.49; CV=4.84%)	58.12 (95% CI 54.37-62.99; CV=22.64%)	p=0,009
IL-10, pg/ml	13.57 (95% CI 12.17-14.05; CV=13.43%)	12.56 (95% CI 11.15-13.61; CV=8.02%)	p=0,240	14.00 (95% CI 11.79-16.53; CV=6.74%)	12.41 (95% CI 11.34-12.70; CV=17.39%)	p=0,05
IL-1β, pg/ml	14.35 (95% CI 13.40-16.39; CV=18.81%)	19.57 (95% CI 12.68-25.47; CV=26.98%)	p=0,867	13.51 (95% CI 3.46-28.99; CV=31.67%)	19.75 (95% CI 18.12-21.71; CV=27.77%)	p=0,810

Note: *p<0.05 – the difference is statistically significant between groups.*

Analysis of kallistatin and interleukin levels in the distribution of patients in both groups by BMI (Fig. 5) revealed significant changes in all biomarkers with weight gain in the group of patients with NAFLD on the background of HT. But in patients with normal weight and overweight patients in the group with isolated NAFLD a significant difference was found only between plasma kallistatin levels (p = 0.002) (Table 3).

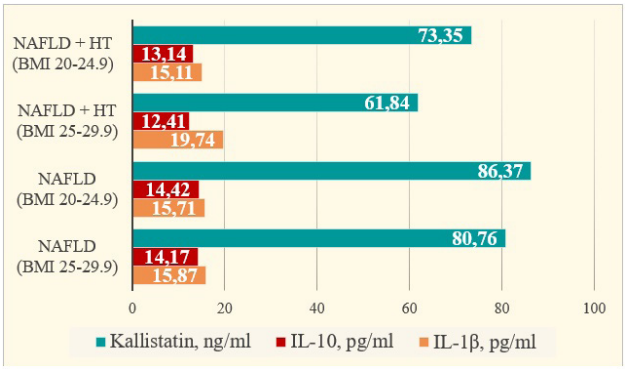

Figure 5. Biomarker levels in the examined patients depending on BMI

Table 3. Biomarker levels in the examined patients depending on BMI

Biomarker	NAFLD + HT (n = 63)		Reliability between groups	NAFLD (n = 52)		Reliability between groups
	BMI 20-24.9 (n = 22)	BMI 25-29.9 (n = 41)		BMI 20-24.9 (n = 26)	BMI 25-29.9 (n = 26)	
Kallistatin, ng/ml	71.06 (95% CI 67.57-78.16; CV=16.38%)	59.21 (95% CI 56.38-65.28; CV=23.18%)	p<0,001	86.37 (95% CI 83.35-88.03; CV=6.77%)	80.76 (95% CI 79.49-82.80; CV=5.04%)	p=0,002
IL-10, pg/ml	13.14 (95% CI 12.42-13.82; CV=12.10%)	12.41 (11.40-12.71; CV=17.21%)	p=0,037	14.42 (95% CI 13.36-14.97; CV=14.06%)	14.17 (95% CI 12.63-14.68; CV=18.59%)	p=0,647
IL-1β, pg/ml	15.11 (95% CI 14.31-17.57; CV=23.09%)	19.75 (95% CI 17.95-21.48; CV=28.39%)	p=0,012	15.71 (95% CI 14.30-17.25; CV=23.09%)	15.87 (95% CI 15.23-18.60; CV=24.64%)	p=0,400

Note: *p<0.05 – the difference is statistically significant between groups*

In the group with NAFLD and HT, the level of kallistain negatively correlated with the HT stage and BP grade (r=-0,52), and both relationships were defined as moderate. At the same time, with an increase in BP grade in patients with NAFLD and HT, the level of IL-10 significantly decreased (r=-0,32). The relationship between the levels of the proinflammatory cytokine IL-1β and the HT stage in the group with NAFLD and HTwas regarded as a direct weak (r=0,33), but the relationship with BP grade was moderate (r=0,42).

Kallistatin levels were decreased in patients with increasing body mass index (BMI) both in the group with NAFLD and HT (r=-0,58; p<0,001) and in the group with isolated NAFLD (r=-0,54; p=0,002).With an increase in body weight, the activity of IL-1β also increased (r=0,32) and the strength of the anti-inflammatory protection of IL-10 (r=-0,27) decreased in the group with comorbidity of NAFLD and HT.

The activity of kallistatin decreased with the progression of HT in patients with NAFLD (p<0,001). The correlations between kallistatin and CRP were very strong (r=-0,89) and strong (r=-0,61) in groups with comorbid and isolated NAFLD, respectively. At the same time, an inverse relationship between the level of CRP and IL-10 was observed in both groups of patients with NAFLD, but in cases with concomitant HT, the strength of the relationship was significantly higher (r=-0,69 and r=-0,30, respectively). In addition, in patients with NAFLD and HT with higher CRP levels, the activity of the proinflammatory cytokine IL-1β increased (r=0,61).

We also obtained data on the inverse relationship between kallistatin and hepatic transaminases in both groups of patients with NAFLD, while in cases with concomitant HT, the correlations were stronger. Also, an inverse relationship between the level of kallistatin and ALPwas found in all patients with NAFLD, but inverse correlations between the kallistatin level and GGTP (r=-0,43) was found only in the group with NAFLD and HT (Table 4).

Maltepe Tıp Dergisi Cilt: 13 Sayı: 2/ 2021
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Table 4. Correlation coefficients between biomarkers and clinical and laboratory parameters in the examined patients, r (p value)

	Groups of patients					
	NAFLD + HT (n = 63)			NAFLD (n = 52)		
	Kallistatin	IL-10	IL-1β	Kallistatin	IL-10	IL-1β
HT stage	-0,52 (p < 0.05)	-0,19 (p > 0.05)	0,33 (p < 0.05)	-	-	-
BP grade	-0,52 (p < 0.05)	-0,32 (p < 0.05)	0,42 (p < 0.05)	-	-	-
SBP	-0,30 (p < 0.05)	-0,17 (p > 0.05)	0,15 (p > 0.05)	-0,09 (p > 0.05)	0,10 (p > 0.05)	0,04 (p > 0.05)
BMI	-0,58 (p < 0.05)	-0,27 (p < 0.05)	0,32 (p < 0.05)	-0,54 (p < 0.05)	-0,09 (p > 0.05)	0,11 (p > 0.05)
CRP	-0,84 (p < 0.05)	-0,69 (p < 0.05)	0,61 (p < 0.05)	-0,28 (p < 0.05)	-0,30 (p < 0.05)	0,21 (p > 0.05)
ALT	-0,67 (p < 0.05)	-0,01 (p > 0.05)	0,07 (p > 0.05)	-0,52 (p < 0.05)	-0,23 (p > 0.05)	0,17 (p > 0.05)
AST	-0,55 (p < 0.05)	0,00 (p > 0.05)	-0,02 (p > 0.05)	-0,47 (p < 0.05)	-0,28 (p < 0.05)	0,15 (p > 0.05)
ALP	-0,47 (p < 0.05)	-0,01 (p > 0.05)	0,01 (p > 0.05)	-0,29 (p < 0.05)	0,20 (p > 0.05)	-0,18 (p > 0.05)
GGTP	-0,43 (p < 0.05)	-0,17 (p > 0.05)	0,17 (p > 0.05)	-0,37 (p > 0.05)	0,01 (p > 0.05)	0,07 (p > 0.05)

Note: *p<0.05 – the correlation is statistically significant*

DISCUSSION

The effect of concomitant hypertension on the clinical manifestation of NAFLD has been determined in various studies. Ampuero J. et al. showed that HT was independently linked to significant fibrosis in patients with NAFLD (23). The possibility of the two-way relationship between these diseases remains under study. Prospective cohort study of Liu P. et.al. also showed bidirectional association between NAFLD and hypertension was found (24).

The study of Ma J. showed that NAFLD is associated with increased odds of incident HT and patients with hypertension at baseline had higher odds of NAFLD development (25). According to Aneni E. C. et al. study results more prevalent NAFLD may occur early in the development of HT, even in conditions of the absence of other metabolic risk factors. Authors concluded that controlling blood pressure levels among even non-obese hypertensive patients may be important in preventing or limiting NAFLD (26).

In different studies high hs-CRP values was found in hypertensive patients and it was considered as an independent risk factor for HT. Authors concluded that this biomarker can aggravate hypertension by participating in local and systemic inflammatory responses (27). Significant differences in the CRP level obtained during the examination of patients with comorbid and isolated NAFLD course also confirm this statement.Summary from 19 prospective studies by Lonardo A. at al. suggest that HT can lead to differences in NAFLD course and condition the rapid deterioration of fatty liver patients and combination of this pathologies could be a precursor of the metabolic syndrome (28).

Various studies suggest that kallistatin concentrations vary in different chronic liver diseases, which may be associated with decreased hepatic protein secretion activity (4). Cheng Z. et al. found a significantly lower content of kallistatin in patients with liver fibrosis (29). Halla M. et al. proved that even a single determination of the biomarker level allows to detect patients in the initial liver fibrosis stages with a sensitivity of 96,7% and a specificity of 50% (7).

Frühbeck, G. et al. proved that kallistatin has a potential protective role in the obesity development in NAFLD (30). In our study, significant changes in kallistatin levels with weight gain in NAFLD patients with and without HT were detected. This may indicate the importance of the protein in protecting against metabolic changes in adipose tissue in NAFLD, but these findings require further investigation.

Another important issue is the kallistatin role in the cardiovascular diseases. The hypertension development is associated with activity of the kallikrein-kinin system decrease. Studies show that endogenous kallistatin is a protective agent against vascular oxidative stress, inflammation and fibrosis in animal hypertension models (31). The results of our study showed that kallistatin levels are significantly lower in patients with NAFLD and HT than in isolated NAFLD patients (p<0,001).

However, literature data indicate a relatively low sensitivity and specificity of kallistatin as an isolated indicator of liver parenchyma changes (21). Thus, it is necessary to consider the possibility of combining the kallistatin determination with other non-invasive NAFLD tests.

Our study showed that the level of anti-inflammatory cytokines (interleukin-10) in patients with isolated NAFLD was significantly higher than in patients with a combination of NAFLD and HT, and in the control group the anti-inflammatory activity was significantly higher than in patients with NAFLD. Meanwhile, the level of IL-1β in patients with concomitant pathology of NAFLD on the background of HT significantly exceeded the corresponding value in the group with isolated NAFLD, and in general the level of inflammatory marker in patients of the main group and the comparison group exceeded control values.

These results confirm the findings of Nelson J. and other authors that proinflammatory cytokines, such as IL-1β, are elevated in nonalcoholic steatohepatitis (32). Statistically significant differences were found between kallistatin and

IL-1β levels in patients with NAFLD on the background of HT depending on the stage of hypertension. We also obtained data indicating the effect of the progression of hypertension on the proinflammatory activity of IL-1β, as well as a significant inverse dependence of the anti-inflammatory activity of calistatin on blood pressure. These results indicate a deepening of pro-inflammatory and anti-inflammatory deviations in patients with comorbid NAFLD in the transition to more severe stages of HT and with an increase in BP grade. Also noteworthy was the fact that a significant decrease in the anti-inflammatory activity of kallistatin with increasing BMI was observed in all patients with NAFLD, and an increase in the effect of interleukins on the course of NAFLD with increased BMI was determined only under the additional influence of HT.

The lack of a clear significant relationships between IL-10 and clinical and laboratory parameters of the examined patients partially confirms the opinion of the authors that this cytokine has different effects. Thus, Saraiva M. and co-authors claim that its antifibrotic activity is detected only at the beginning of the disease, and with the progression of NAFLD the cytokine may even stimulate irreversible changes in the liver parenchyma (33).
Therefore, it is important to measure the levels of these biomarkers in combination to diagnose the severity of NAFLD more accurately and to determine the effect of hypertension on the course of NAFLD.

CONCLUSIONS

The course of both isolated NAFLD and comorbidity NAFLD with HT was accompanied by significant changes of kallistatin, IL-10 and IL-1β levels, which confirmed the pathophysiological role of chronic low-grade systemic inflammation in NAFLD. The combination of NAFLD and HT led to a significant increase in the severity of deviations in these indicators, which makes it possible to consider HT as a trigger factor of NAFLD progression.

Higher HT stage and BP grade, increased BMI and high CRP levels are associated with significantly more pronounced deviations of these indicators and should be considered as additional factors of NAFLD progression in patients with HT.

The obtained data provide the possibility to consider the kallistatin, IL-10 and IL-1β as biomarkers of NAFLD severity

Ethics Committee Approval: All the patients signed an informed consent. The study was approved by the Ethics and Bioethics Committee of the Kharkiv National Medical University
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