PROTO-HORMONES LEVELS OF ADIPOSE TISSUE, INTERLEUKIN-6 AND INDICATORS OF DETOXIFYING FUNCTION OF THE LIVER IN PATIENTS WITH HYPERTENSION AND OBESITY COMBINED WITH NON-ALCOHOLIC FATTY LIVER DISEASE

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Abstract: The research estimates the state of liver detoxifying function and analyzes the changes in the levels of leptin, adiponectin and interleukin-6 in patients with arterial hypertension in combination with obesity and non-alcoholic fatty liver disease.

Aim. The aim of the study is to evaluate levels of proto-hormones adipose tissue, interleukin-6 and indicators of detoxifying function of the liver in patients with hypertension and obesity combined with non-alcoholic fatty liver disease (NAFLD).

Materials and methods. The study involved patients with arterial hypertension combined with obesity and NAFLD. All patients underwent anthropometric, general clinical, laboratory (blood lipid profile) and instrumental diagnostics (electrocardiography, echocardiography, ultrasonography, 13C-metacetin breath test, ELISA (adiponectin, leptin, IL-6). The patients' height and weight were measured, the body mass index was calculated according to standard formulas.

Results. Patients with arterial hypertension combined with obesity and NAFLD at the stage of steatohepatitis showed an increase in the levels of leptin and IL-6 and a decrease in the level of adiponectin. This group also revealed a moderate decrease in liver detoxifying function, as indicated by the results of 13C-MBT due to a 46.7 % decrease in the metabolic rate and a decrease in cumulative doses of CUM40 by 40 % and CUM120 by 46.8 %, respectively.

Conclusions. The elevated levels of leptin and IL-6 and lowered adiponectin levels can be used to determine the degree of activity of non-alcoholic steatohepatitis and predict the course of NAFLD in combination with hypertension and obesity.

An increased level of leptin and IL-6 and a low level of adiponectin in patients with such a comorbid pathology lead to an increase in the left ventricular myocardial mass index and aggravate the course of arterial hypertension.

Keywords: arterial hypertension, obesity, non-alcoholic steatohepatitis, leptin, adiponectin, IL-6, 13C-metacetin breath test.

1. Introduction

Proto-hormones of adipose tissue, such as leptin, adiponectin, as well as proinflammatory cytokine IL-6, play an important role in the progression of arterial hypertension in obesity.

Adiponectin is a protein hormone, which is mainly synthesized in the adipose tissue; its expression is higher in subcutaneous than in visceral fat [1]. It affects carbohydrate and lipid metabolism, participates in the regulation of blood pressure, prevents inflammatory processes in the endothelium of blood vessels, reduces the damage due to ischemia and reperfusion of the cardiac muscle and also reduces liver fibrosis [2, 3]. The level of adiponectin is significantly reduced in case of obesity, insulin resistance, type 2 diabetes, hyperglycemia and many cardiovascular diseases (myocardial infarction, acute coronary syndrome, ischemic heart disease) [4].

Leptin is a product of the expression of the obesity gene, a protein-producing hormone, synthesized by cells of white and brown fatty tissue, in a lesser extent – in the skeletal muscle, stomach and placenta [5]. According to literature, leptin has antisteatogenic effects, regulates and controls intracellular homeostasis of glucose and free fatty acids, prevents the development of glucotoxicity and lipotoxicosis [6]. Other authors established the profibrogenic effect of leptin [7]. Hyperleptinemia stimulates the development of NAFLD, in particular, fibrosis [8].

According to many studies, IL-6 is associated with the development of metabolic disorders [9, 10]. It is produced by fibroblasts, endothelial cells, monocytes and adipocytes, it is also characterized by multifactoriality and a wide range of biological effects.

The aim of the study was to assess the status of the detoxifying function of liver and analyze changes in the level of leptin, adiponectin and IL-6 in patients with arterial hypertension in combination with obesity and non-alcoholic fatty liver disease in accordance with the degree of activity of non-alcoholic steatohepatitis and myocardial mass index.

2. Materials and methods

The study involved 50 patients with stage II–III AH who were selectively hospitalized in Lviv City Clinical Emergency Hospital (Lviv, Ukraine) for the period from 2018–2019. The patients, who underwent clinical and diagnostic examination, 28 (56 %) were women of a mean age of 58.2±0.97. All patients underwent anthropometric, general clinical, laboratory (blood lipid profile) and instrumental diagnostics (electrocardiography, echocardiography, ultrasonography, ^{13}C-metacetin breath test, ELISA (adiponectin, leptin, IL-6). Patients' height and weight were measured, the body mass index was calculated according to Quetelet formula.

The level of office blood pressure was measured on the same arm in a sitting position at intervals of 2 min thrice in the morning; an average of 3 measurements was calculated. The heart rate was evaluated after the second measurement. AH was established according to the standards of its diagnostics and treatment and in accordance with the recommendations of the Ukrainian Association of Cardiology, the European Society of Hypertension and the European Society of Cardiology (ESH/ESC). While choosing the treatment strategy and follow-up plan, we also referred to the current orders and protocols of MHC of Ukraine, ESH/ESC recommendations, guidelines of American Gastroenterology Association (AGA) and American Association for the Study of Liver Diseases (AASLD) [11].

Informed consent to participate in the study was obtained from all patients (protocol of the Commission on Bioethics No. 1/18 of 15.01.2018).

To identify the ultrasonographic signs of non-alcoholic steatohepatitis, all individuals underwent ultrasonography (USG) of internal organs with particular attention paid to an increase in the liver size, density of its parenchyma, diffuse homogenous increase in echogenicity, distal shadowing and increase in the diameter of the portal vein. Fatty liver was determined in case of an increased liver size, diffuse homogeneous echogenicity and appearance of distal shadowing. To determine the functional state of microsomal enzyme systems of hepatocytes, we performed ^{13}C-metacetin breath test. This test provides an opportunity to non-invasively evaluate the percentage of functioning hepatocytes, classify liver failure into cirrhotic and non-cirrhotic type and establish its degree according to the Child-Pugh criteria. The sensitivity and specificity of ^{13}C-metacetin breath test is >90 % [12, 13]. The infrared spectroscope IRIS (Wagner, Germany) analyzed the air samples.

The results of ^{13}C-metacetin breath test evaluated the antitoxic function of the liver, determining its metabolic capac-

ity and metabolic rate of hepatocytes at 40 and 120 minutes. This method makes it possible to determine the proportion of functioning hepatocytes, as well as differentiate steatosis from steatohepatitis and fibrosis.

Echocardiography was performed in the M- and B-modes using the standard Siemens Acuson S3000 (Germany) ultrasonographic apparatus. According to the results of the echocardiogram, the mass of the myocardium (MM) (according to the Penn-Convention formula), the index of the myocardial mass (IMM) and the relative thickness of the myocardium (RTM) were calculated.

The level of adiponectin, level and IL-6 was evaluated using ELISA. All immuno-enzymatic methods were performed using the "Stat Fax 303 plus" analyzer (Awareness Technology, USA). The results were processed statistically using Student's test, Pearson correlation analysis with "Microsoft Excel" software.

3. Results

After the clinical examination and USG, 50 patients were selected to confirm the diagnosis of non-alcoholic steatohepatitis using ^{13}C-metacetin breath test. The control group was made up of 10 presumably healthy persons. According to the results of the ^{13}C-metacetin breath test, the metabolic rate was found in the range from 4.3 (%^{13}C/h) to 18.5 (%^{13}C/h), which averaged (13.66±0.39) (%^{13}C/h). This was significantly lower by 46.7 % compared with the control group ($p<0.01$). The cumulative dose for 40 minutes (CUM40) was (7.28±0.26) (%^{13}C) which decreased by 40 % ($p<0.01$), the cumulative dose for 120 minutes (CUM120) – (12.69±0.57) (%^{13}C) decreased by 46.8 % ($p<0.01$) compared to the control group (**Table 1**).

Among the patients who underwent the respiratory test, the expressed significant signs of steatohepatitis were detected in 21 (42.0 %) patients, while in the remaining 29 (58.0 %) the subjects, signs of steatosis transition into steatohepatitis were observed. Criteria for reliable signs of steatohepatitis included simultaneous changes of all 3 indicators, any 2 detected changes (metabolic rate and/or CUM40 and/or CUM120) were considered to be equivocal signs of steatohepatitis.

Table 1
Results of metacetin ^{13}C-breath test

Indexes	Control group (n=10)	NAFLD (n=50)
Metabolic rate (Dose/h %)	25.63±1.36	13.66±0.39*
CUM40 (%)	12.10±0.50	7.28±0.26*
CUM120 (%)	23.88±0.73	12.69±0.57*

*Note: *– p<0.01 compared with the control group*

Results of metacetin ^{13}C-breath test indicated the presence of fatty liver infiltration in the patients as provoked by obesity, which, in turn, leads to changes in the indicators of functional state of the liver. Thus, ^{13}C-metacetin breath test was able to reliably establish a reduced function of hepatocytes and confirm the diagnosis of non-alcoholic steatohepatitis. On the basis of these data, patients were divided into groups with a pronounced decrease in the activity of hepatocytes and with a moderate decrease in the activity of hepatocytes.

The results of the study showed that leptin levels in patients with hypertension in combination with obesity and non-alcoholic fatty liver disease (NAFLD) ranged from 9.7 to 90.8 ng/ml and averaged 41.85±3.34 ng/ml. An increase in the level of leptin above the reference values was found in 80 % of the examined patients, indicating a metabolic process disorder with combined pathology.

The level of IL-6 in patients with hypertension in combination with obesity and NAFLD ranged from 1.8 to 25 pg/ml and averaged (9.12±0.73) pg/ml. An increase in the level of IL-6 above the reference values was found in 84.0 % of the examined patients, indicating the development of subclinical inflammation with comorbidity.

The adiponectin level ranged from 6.20 to 24.68 μg/ml (averaged 16.02±0.57 μg/ml). The decrease in adiponectin levels below the reference values was found in 88.0 % of the examined patients, indicating an inadequate protective activity of the body in hypertension combined with obesity and NAFLD.

As seen in **Table 2**, the level of leptin in patients with non-alcoholic steatohepatitis (NASH) with pronounced decrease in hepatocyte activity in the ^{13}C-metacetin breath test index was 48.86±4.51 ng/ml and significantly exceeded the similar figure twice as compared to those with non-alcoholic steatohepatitis with moderate decrease in hepatocyte activity ((24.15±2.36) ng/ml) ($p<0.05$).

The level of adiponectin in patients with a combined pathology associated with a moderate hepatocyte activity decreased reaching (19.14±0.36) μg/ml on average and was significantly higher than that of patients with non-alcoholic steatohepatitis with a pronounced decrease in hepatocyte activity by 48.4 %; the median values was (12.90±0.56) μg/ml ($p<0.01$), (**Table 2**). Indicators of IL-6 in patients with combined a pathology and a pronounced decrease in hepatocyte activity were 1.7 times higher compared to the respective indices in patients with non-alcoholic steatohepatitis and a moderate decrease of hepatocyte activity ($p<0.01$) (**Table 2**).

Table 2
Levels of leptin, adiponectin and interleukin-6 depending on the activity of the inflammatory process

Indexes	NASH with a moderate decrease in the activity of hepatocytes (n=27)	NASH with pronounced decrease in the activity of hepatocytes (n=23)
Leptin, ng/ml	24.15±2.36	48.86±4.51*
Adiponectin, μg/ml	19.14±0.36	12.90±0.56**
Interleukin-6, pg/ml	7.31±0.97	12.38±1.07**

*Note: * – p<0.05 among a group of patients with non-alcoholic steatohepatitis with a moderate decrease in hepatocyte activity and a pronounced decrease in the activity of hepatocytes; ** – p<0.01 among a group of patients with non-alcoholic steatohepatitis with a moderate and severe decrease in the activity of hepatocytes*

In our opinion, an elevated level of leptin and IL-6 and lowered adiponectin levels can be used to determine the degree of activity of non-alcoholic steatohepatitis and predict the course of NAFLD in combination with hypertension and obesity.

The level of leptin in patients with hypertension in combination with obesity and NAFLD with an index of myocardial mass of less than 100 g/m^2 was (34.04±3.22) ng/ml on average and exceeded a similar figure in case of an index of myocardial mass greater than 100 g/m^2 by 23.8 % (**Table 3**).

Table 3
Levels of leptin, adiponectin and IL-6 depending on the mass index of myocardium

Indexes	The myocardial mass index is less than 100 g/m^2 (n=37)	The myocardial mass index is more 100 g/m^2 (n=13)
Leptin, ng/ml	34.04±3.22	42.15±4.21
Adiponectin, μg/ml	16.42±0.72	15.20±0.94
Interleukin-6, pg/ml	8.81±0.70	9.93±0.98

*Note: *– p<0.05 – probability between groups*

The adiponectin level at an IMM less than 100 g/m^2 reached 16.42±0.72 μg/ml on average and was 1.08 times higher than with an IMM greater than 100 g/m^2 (p>0,05). The level of IL-6 was 12.7 % higher with an IMM greater than 100 g/m^2 compared with a similar index in the IMM of less than 100 g/m^2 (p>0.05), (**Table 3**). In women, the average level of leptin was (45.63±4.06) ng/ml and was 1.3 times higher than that of men (p<0.05) (**Table 4**). In women, the average level of leptin was (45.63±4.06) ng/ml and was 1.3 times higher than that of men (p<0.05) (**Table 4**).

Table 4
Levels of leptin, adiponectin and IL-6 according to gender characteristics

Indexes	Norm	Female (n=31)	Male (n=19)
Leptin, ng/ml	For female – 0.5–13.8 For male – 1.1–27.6	45.63±4.06	35.94±3.12*
Adiponectin, μg/ml	For female – 11.7 For male – 7.9	16.60±1.26	15.40±0.76
Interleukin-6, pg/ml	1.5–7	8.46±0.82	7.98±0.86

*Note: * – p<0.05 – probability between groups*

In women, the average adiponectin level was (16.60± ±1.26) μg/ml and was 1.1 times higher than that of men (15.40±0.76) μg/ml, but it was not significantly different from that of men (p>0.05) (**Table 4**).

4. Discussion

Thus, according to the results of ^{13}C-metacetin breath test, a moderate decrease in the detoxifying function of the liver was observed, indicating a decelerated metabolic rate by 46.7 %, in particular, CUM40 decreased by 40 % and CUM120 decreased by 46.8 % in hypertensive patients with obesity and NAFLD at the stage of steatohepatitis, in comparison with the control group.

According to the results of ELISA in patients with hypertension associated with obesity and NAFLD at the stage of steatohepatitis, levels of leptin and IL-6 increased while levels of adiponectin decreased. The elevated levels of leptin and IL-6 and lowered adiponectin levels can be used to determine the degree of activity of non-alcoholic steatohepatitis and predict the course of NAFLD in combination with hypertension and obesity. It was more pronounced in case of a significant reduction in the activity of hepatocytes compared with a moderate decrease in their activity; this indicates the effect of these biologically active compounds on the functional state of the liver.

We established an adverse effect of an increased level of leptin and IL-6 and a reduced level of adiponectin upon the course of hypertension, which adversely affects the increase of IMM in patients with comorbid pathology and aggravates the condition and course of this pathology [10, 14].

Limitations of the study. In accordance with the limitations of this study, the results can be determined as preliminary. For further detailed analysis, we plan to continue this study with a large number of patients.

5. Conclusions

The results of the study involving patients with hypertension and obesity combined with non-alcoholic fatty liver disease showed a moderate decrease in the detoxifying function of the liver, increased levels of leptin, IL-6 and decreased levels of adiponectin.

Conflict of interests

The authors declare that they have no conflicts of in-terest.

References

1. Adolph, T. E., Grander, C., Grabherr, F., Tilg, H. (2017). Adipokines and non-alcoholic fatty liver disease: multiple interactions. International Journal of Molecular Sciences, 18 (8), 1649. doi: http://doi.org/10.3390/ijms18081649
2. Kumar, R., Prakash, S., Chhabra, S., Singla, V., Madan, K., Datta Gupta, S. et. al. (2012). Association of pro-inflammatory cytokines, adipokines & oxidative stress with insulin resistance & non-alcoholic fatty liver disease. Indian Journal of Medical Research, 136 (2), 229–236.
3. Polyzos, S. A., Kountouras, J., Zavos, C., Tsiaousi, E. (2010). The role of adiponectin in the pathogenesis and treatment of non-alcoholic fatty liver disease. Diabetes, Obesity and Metabolism, 12 (5), 365–383. doi: http://doi.org/10.1111/j.1463-1326.2009.01176.x
4. Fang, H., Judd, R. L. (2018). Adiponectin Regulation and Function. Comprehensive Physiology, 8 (3), 1031–1063. doi: http://doi.org/10.1002/cphy.c170046
5. Andrabi, K., Dar, R., Rasool, S., Waza, A., Ayoub, G., Qureshi, M. et. al. (2019). Polymorphic analysis of leptin promoter in obese/diabetic subjects in Kashmiri population. Indian Journal of Endocrinology and Metabolism, 23 (1), 111. doi: http://doi.org/10.4103/ijem.ijem_164_18
6. Lemoine, M., Ratziu, V., Kim, M., Maachi, M., Wendum, D., Paye, F. et. al. (2009). Serum adipokine levels predictive of liver injury in non-alcoholic fatty liver disease. Liver International, 29 (9), 1431–1438. doi: http://doi.org/10.1111/j.1478-3231.2009.02022.x

7. Procaccini, C., Galgani, M., De Rosa, V., Carbone, F., La Rocca, C., Ranucci, G. et. al. (2010). Leptin: The Prototypic Adipocytokine and its Role in NAFLD. Current Pharmaceutical Design, 16 (17), 1902–1912. doi: http://doi.org/10.2174/138161210791208884
8. Ghantous, C. M., Azrak, Z., Hanache, S., Abou-Kheir, W., Zeidan, A. (2015). Differential Role of Leptin and Adiponectin in Cardiovascular System. International Journal of Endocrinology, 2015, 1–13. doi: http://doi.org/10.1155/2015/534320
9. Barrios, V., Escobar, C., Calderon, A., Böhm, M. (2009). Blood pressure goal achievement with olmesartan medoxomil-based treatment: additional analysis of the OLMEBEST study. Vascular Health and Risk Management, 5, 723. doi: http://doi.org/10.2147/vhrm.s7003
10. Hashizume, M., Mihara, M. (2011). IL-6 and lipid metabolism. Inflammation and Regeneration, 31 (3), 325–333. doi: http://doi.org/10.2492/inflammregen.31.325
11. Chalasani, N., Younossi, Z., Lavine, J. E., Diehl, A. M., Brunt, E. M., Cusi, K. et. al. (2012). The diagnosis and management of non-alcoholic fatty liver disease: Practice Guideline by the American Association for the Study of Liver Diseases, American College of Gastroenterology, and the American Gastroenterological Association. Hepatology, 55 (6), 2005–2023. doi: http://doi.org/10.1002/hep.25762
12. Gorowska-Kowolik, K., Chobot, A., Kwiecien, J. (2017). 13C Methacetin Breath Test for Assessment of Microsomal Liver Function: Methodology and Clinical Application. Gastroenterology Research and Practice, 2017, 1–5. doi: http://doi.org/10.1155/2017/7397840
13. Park, G. J.-H., Wiseman, E., George, J., Katelaris, P. H., Seow, F., Fung, C., Ngu, M. C. (2011). Non-invasive Estimation of Liver Fibrosis in Non-alcoholic Fatty Liver Disease Using the 13C-Caffeine Breath Test. Journal of Gastroenterology and Hepatology, 26 (9), 1411. doi: http://doi.org/10.1111/j.1440-1746.2011.06760.x
14. Bochar, O., Sklyarov, E., Bochar, V. (2017). Leptin and interleukin-6 level in patients with hypertension and obesity combined with non-alcoholic steatohepatitis during treatment with sartans and statins. Current Issues in Pharmacy and Medical Sciences, 30 (2), 57–60. doi: http://doi.org/10.1515/cipms-2017-0011

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