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The Impact of Endothelial Dysfunction on the Course of Metabolically Associated Liver Disease in Combination with Subclinical Hypothyroidism

Olena Kolesnikova[1], Kira Vovk[2], Andriy Titarenko[1]

[1] L.T. Malaya Therapy National Institute of the National Academy of Medical Sciences of Ukraine, Kharkiv, Ukraine
[2] V.N. Karazin Kharkiv National University, Kharkiv, Ukraine

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ABSTRACT

Background. Intrahepatic endothelial dysfunction is involved in many diseases, including fatty liver disease, and in combination with other numerous mechanisms may play a role in atherosclerosis in hypothyroidism. It is possible that endothelial dysfunction markers may be important in assessing the risk of cardiovascular events in patients with metabolic-associated fatty liver disease (MAFLD) in combination with subclinical hypothyroidism.

Aims. The aim of this study was to evaluate changes in endothelial dysfunction markers in patients with MAFLD in combination with subclinical hypothyroidism.

Methods. We studied 298 patients aged 40-69 years with a history of MAFLD. Of these, 128 patients had a verified diagnosis of both MAFLD and subclinical hypothyroidism. In such cases, the morphological state of the vascular endothelium was assessed by counting circulating desquamated epithelial cells (CDEC) and vascular endothelial growth factor (VEGF-A) in the blood using phase-contrast microscopy and the value of these parameters was in comparison todepending on the level of thyroid stimulating hormone, insulin, age, and cardiovascular risk.

Results. It was found that the level of CDEC and VEGF-A was increased in patients with MAFLD and subclinical hypothyroidism. On the one hand, thyroid stimulating hormone levels were found to be associated with endothelial dysfunction and hyperinsulinemia. On the other hand, endothelial dysfunction indices also have links with the degree of cardiovascular risk. Patients with moderate and high cardiovascular risk have a relationship between CDEC and levels of triglycerides, C-reactive protein, and cardiovascular diseases. The levels of VEGF-A and CDEC were significantly higher in the adult age group. VEGF-A levels > 273 pg/mL were associated with abnormalities in the studied parameters, including those reflecting of the liver.

Conclusion. Patients with MAFLD in combination with hypertension have elevated levels of CDEC and VEGF-A, which affects the involvement of these factors in the mechanisms of endothelial dysfunction in this category. Markers of endothelial dysfunction can be used to assess the prediction of possible cardiometabolic risk.

Introduction

The endothelium plays an important role in vascular biology and the regulation of liver function. Healthy endothelial cells (EC) are involved in vasodilation by releasing nitric oxide (NO), which also inhibits platelet adhesion and aggregation, as well as leukocyte adhesion. Conversely, damaged EC can develop a vasoconstrictor, proinflammatory, and procoagulant phenotype[1]. In addition, EC transport glucose to the cells of the blood vessel wall and to the parenchymal tissue[2]. Vascular endothelial growth factor as a regulator of endothelial transit of free fatty acids (FFA), controls the expression of endothelial fatty acid transport proteins and thus lipid accumulation in tissues[3]. Hepatic sinusoidal EC demonstrate anti-inflammatory and antifibrogenic properties, preventing Kupffer and stellate cell activation and regulating intrahepatic vascular resistance and portal pressure, so in the early stages of MAFLD, hepatic sinusoidal EC dysfunction occurs, namely, loss of the ability to generate vasodilating agents[4]. Endothelial dysfunction (ED) in combination with other numerous mechanisms may explain atherosclerosis in patients with hypothyroidism, although the subclinical hypothyroidism (SCH) is associated with atherosclerosis is still controversial[5].

Endothelial dysfunction is associated with traditional cardiovascular risk factors, such as hypertension and diabetes, and predicts the progression of atherosclerosis and cardiovascular events (CVE) in the general population[1], so the assessment of ED, being an early biomarker, is useful for predicting cardiovascular risk (CVR) and evaluating treatment outcomes[6]. To prevent the development of CVE and its complications in patients with nonalcoholic liver steatosis in combination with SCH, it is essential to identify early predictors, which may include ED markers: VEGF-A, CDEC, the effect of which is still a subject of debate, despite the fact that over the past few years intrahepatic ED has been demonstrated in several models of liver disease, including fatty liver[7].

Thus, the study of markers of ED in patients with MAFLD in combination with SCH will provide an opportunity to expand our understanding of the mechanisms of CVR formation, to individualize on this basis the strategy for the prevention of CVE in a comorbid patient. At the same time, understanding the mechanisms of ED formation in patients with MAFLD in combination with SCH expands our understanding of early CVE in the combined course of diseases.

Materials and methods

On the basis of the State Institution «L.T. Malaya National Therapy Institute of the National Academy of Medical Sciences of Ukraine», 298 people aged 40-69 years with a history of MAFLD were studied. Of these, 128 people had a verified diagnosis of MAFLD and SCH. The diagnosis of MAFLD was established according to the recommendations of the European Association for the Study of the Liver (EASL, 2021)[8]. Subclinical hypothyroidism was diagnosed according to the guidelines of the European Thyroid Association (ETA, 2015)[9]. Waist circumference (WC) was measured with a flexible tape at the level of the navel. Cardiovascular risk was calculated using the SCORE2 scale. In all patients, the level of total cholesterol (TC), high-density lipoprotein (HDL), and triglycerides (TG) was determined by the enzymatic method on a «Humalayzer» biochemical analyzer (№ 2106-1709) using a set of reagents from «Human» (Germany). The cholesterol content of LDL cholesterol was calculated by the formula of Friedewald W.T. Glycated hemoglobin (HbA1c%) was determined from venous blood by ion-exchange chromatography using a biochemical semi-automatic analyzer «ERBA CHEM-7-RU». The concentration of C-reactive protein (CRP) and fasting insulin in the blood serum was studied by enzyme-linked immunosorbent assay on a semi-automatic enzyme-linked immunosorbent microplate analyzer «ImmunoChem - 2100» (HighTechnology, Inc., USA). The HOMA-IR index was used to quantify the severity of insulin resistance. The morphological state of the vascular endothelium was assessed by counting CDEC in the blood using phase-contrast microscopy. To diagnose the presence of thyroid pathology, ultrasound was performed using the ultrasound diagnostic system «LOGIQ-5». To assess TCIM in duplex scanning, the ultrasound diagnostic system «Philips IU» was used. Statistical analysis of the results was performed using a package of application programs for Windows. To determine differences, Student's t-test was used for dependent and independent samples. The frequency of signs in the groups was compared using the χ^2 test. To determine the presence and nature of the relationship between various manifestations and pathogenetic factors of various processes, correlation analysis was performed using Pearson's test. The study was approved by the Bioethics Committee of the State Institution «L.T. Malaya National Therapy Institute» (№2 19 March 2024).

Results

According to the observation data in patients with combined course of MAFLD and SCH, significant changes in the vascular endothelium at the cellular level were observed, which was expressed in a significant increase in the index of CDEC in patients with MAFLD compared with the control group (10.8±1.6 cells/100 μL vs 7.5±1.2 cells/100 μL, $p<0.01$) and a significant increase in the desquamated fraction in patients with MAFLD in combination with SCH vs the MAFLD group - 15.4±2.2 cells/100 μL vs 10.8±1.6 cells/100 μL ($p<0.01$), respectively. Also, in patients with a combined course of MAFLD and SCH, there were significant changes in another marker that reflects vascular endothelial dysfunction - vascular endothelial growth factor VEGF-A: 610±112.27 pg/ml vs 428.24±74.28 pg/ml ($p<0.01$).

The ED indices were significantly higher in the group of patients with MAFLD in combination with SCH with thyroid stimulating hormone (TSH) levels >10 mU/L compared with patients with TSH levels in the range of 4-10 mU/L: CDEC - 13.11±0.50 cells/100μL vs 8.83±1.08 cells/100μL, respectively ($p=0.012$); VEGF-A - 486.99±22.17 pg/mL vs 319.94±66.48 pg/mL, respectively ($p=0.029$) (Table 1).

Table 1: Comparison of ED indices depending on TSH levels in patients with MAFLD in combination with SCH

ED rates in subgroups with TSH up to 4 mU/L and TSH in the range of 4-10 mU/L			
Indicators	TSH subgroup <4 mU/L	TSH subgroup 4-10 mU/L	Significance, P
CDEC, cells/100μL	8,83±1,08	13,11±0,50	p=0,012
VEGF-A, pg/mL	319,94±66,48	486,99±22,17	p=0,029
ED rates in subgroups with TSH 4-10 mU/L and TSH > 10 mU/L			
Indicators	TSH subgroup 4-10 mU/L	TSH subgroup>10 mU/L	Significance, P
CDEC, cells/100μL	13,11±0,50	16,50±7,50	p>0,05
VEGF-A, pg/mL	486,99±22,17	861,49±372,49	p>0,05

At the same time, the levels of CDEC and VEGF-A depended not only on TSH but also on age. Significant differences were obtained in patients aged >50 years and <50 years: CDEC - 9.88±0.52 cells/100μL vs 6.67±0.33 cells/100μL (p=0.006); VEGF-A - 398.94±25.74 pg/ml vs 97.08±19.39 pg/ml (p=0.001), i.e. there was a significant prevalence of CDEC and VEGF-A in the older age group (p<0.01), which may indicate the role of age in the development of vascular events in patients with MAFLD in combination with SCH (Table 2).

Table 2: Comparison of ED rates depending on TSH of different ages in patients with MAFLD in combination with SCH

Indicators	TSH subgroup <4 mU/L	TSH subgroup 4-10 mU/L	Significance, P
ED rates in subgroups aged <50 years			
CDEC, cells/100μL	6,67±0,33	9,88±0,52	p=0,006
VEGF-A, pg/mL	197,08±19,39	398,94±25,74	p=0,001
ED rates in subgroups aged >50 years			
CDEC, cells/100μL	11,00±1,00	13,58±0,54	p>0,05
VEGF-A, pg/mL	442,79±81,36	499,79±24,69	p>0,05

Significant changes in the level of CDEC were obtained depending on the TSH values in patients with MAFLD in combination with SCH, which indicates the influence of TSH levels on endothelial function in patients with MAFLD in combination with SH (Table 3).

Table 3: Influence of TSH level on CDEC in patients with MAFLD in combination with SCH

Indicators		Sum of squares	degree of freedom	root mean square	F-test	P
CDEC	between groups	217,791	2	108,895	4,463	0,013
	within groups	3050,139	125	24,401		
	together	3267,930	127			

Significant differences were found depending on hyperinsulinemia in the indicators reflecting ED. There was a 1.5-fold increase in the indexes of CDEC and VEGF-A in the group of patients with MAFLD in combination with SCH with an insulin level of >30 mU/L, which amounted to 17.67±2.13 cells/100μL vs 12.15±0.42 cells/100μL (p=0.003), respectively, and 718.33±106.63 pg/mL vs 449.32±19.16 pg/mL (p=0.036) (Table 4).

Table 4: ED indicators depending on the level of hyperinsulinemia in patients with MAFLD in combination with SCH

Indicators	Insulin <30 mIU/L	Insulin >30 mIU/L	Significance, P
CDEC, cells/100μL	12,15±0,42	17,67±2,13	P=0,003
VEGF-A, pg/mL	449,32±19,16	718,33±106,63	P=0,036

We analyzed the content of ED markers depending on the CVR in patients with MAFLD in combination with SCH. Comparison of moderate and low-risk groups showed significant differences in the indexes of CDEC, which amounted to 11.93±.541 cells/100μL vs 8.83±1.10 cells/100μL (p=0.060), as well as probable differences in VEGF-A - 422.82±10.01 pg/ml vs 319.94±66.47 (p=0.461). At the same time, a comparison of ED indices in the groups of high and moderate CVR demonstrated the presence of significant differences in both indicators: CDEC was 15.68±1.08 cells/100μl vs 11.93±.541 cells/100μl (p=0.004); VEGF-A - 646.44±58.11 pg/ml vs 422.82±10.01 pg/ml (p=0.001).

A significant direct correlation was found in patients with low CVR between CDEC and gamma-glutamyl transpeptidase (GGT) levels - r=0.73 (p=0.099).

In patients with moderate CVR, there were significant correlations of CDEC with the indicators of WC - r=0.40 (p=0.088); TG - r=0.43, (p=0.004) and CRP - r=0.30 (p=0.052). The data obtained are probably due to the fact that in the transition from the category of low to moderate CVR, its formation in patients with MAFLD in combination with SCH is influenced by a large number of factors.

The analysis of correlations between patients with MAFLD in combination with SCH with high CVR revealed the presence of relationships not only with CDEC, but also with other metabolic parameters. A direct relationship between CDEC and VEGF-A was demonstrated - r=0.53 (p=0.011) and total cholesterol - r=0.48 (p=0.025).

By comparing the groups of patients with MAFLD in combination with SCH and isolated MAFLD with VEGF-A

< 273 pg/ml did not reveal statistically significant differences in lipid and carbohydrate metabolism, liver function, TSH, CRP and TNF-α levels (p>0.01). According to the analysis of significant differences (p=0.001) obtained by comparing the groups of patients with MAFLD in combination with SCH and isolated MAFLD who had VEGF-A > 273 pg/ml, significant abnormalities in the studied parameters, including those reflecting the state of the liver, were obtained (Table 5).

Table 5: Comparison of parameters in patients with MAFLD in combination with SCH with VEGF-A > 273 pg/ml and the group of isolated MAFLD

Indicators	MAFLD ± SCH, n=68			MAFLD, n=24			Mann–Whitney U-test	P
	Median	Percentiles		Median	Percentiles			
		25	75		25	75		
Age	60,00	52,00	67,00	54,00	47,00	58,50	33,50	0,001
TC	6,72	4,97	7,48	5,64	5,51	5,80	199,00	0,001
TG	1,73	1,18	2,30	1,09	0,88	1,99	139,00	0,001
VLDL	0,74	0,527	1,02	0,42	0,34	0,50	164,50	0,001
HDL	1,07	0,87	1,37	1,54	1,43	1,60	185,00	0,001
LDL	3,73	3,18	4,33	2,72	2,53	2,85	195,00	0,001
AC	4,19	3,37	5,42	2,14	1,89	2,27	92,00	0,001
HbA1c	6,89	6,12	7,58	5,240	3,85	5,56	0,00	0,001
Glucose	6,50	5,31	8,11	5,30	5,09	5,54	20,00	0,001
Insulin	15,92	10,20	25,31	8,23	6,90	10,17	156,00	0,001
CDEC	11,00	8,00	14,00	8,50	6,50	9,00	204,50	0,001
VEGF-A	488,22	227,7	530,3	298,20	199,1	335,2	253,00	0,001
CRP	9,70	6,92	13,07	6,88	6,50	7,25	167,00	0,001
IMT	0,96	0,90	1,12	0,78	0,74	0,81	200,00	0,001

Discussion

The results of our study indicate that patients with MAFLD in combination with SCH showed signs of ED. This is in line with the results of the study by Lekakis J. et al. in which it was found that ED is detected even within normal TSH values and worsens with increasing TSH levels, although, unlike our study, asymmetric dimethylarginine (ADMA) was used as a marker of ED and oxidized low-density lipoprotein (oxLDL)[10]. These data are consistent with the study by Ciccone M. et al. according to which autoimmune thyroiditis, which occurs more often in the setting of SCH, may be associated with arterial stiffness[11]. Moreover, Cho K. and Lee J. proved that the presence of antibodies to thyroid tissue correlated with arterial stiffness[12].

We have found an increase in VEGF-A in patients with MAFLD and SCH. This can be explained by the fact that VEGF-A is a profibrogenic factor[13]. This is consistent with the findings of Yang L. et al. according to which in a mouse model VEGF-A promoted liver fibrogenesis, as well as Shen H. et al. according to which the production of VEGF-A by hepatocytes promoted fibrosis[14]. At the same time, the role of VEGF-A in liver tissue repair and fibrosis disappearance remains unclear. The results of the study by Musso G. et al. indicate that in patients with MAFLD, along with a higher prevalence of traditional risk factors for CVE (obesity, DM, MS, etc.), new risk factors such as ED and thickening of the intima-media complex are found[15].

We observed an increase in the level of ED indicators in patients with MAFLD in combination with SCH, depending on the level of hyperinsulinemia. This is consistent with the publicly available evidence that MAFLD is closely associated with features of the metabolic syndrome, especially insulin resistance. According to Nasiri-Ansari N. et al., hyperinsulinemia, hyperglycemia and altered adipocytokine secretion can activate such harmful processes as inflammation, oxidative stress, endoplasmic reticulum stress and apoptosis, which leads to the development of MAFLD, demonstrating its multifactorial etiology[16]. Currently, there are two points of view regarding the relationship between ED and insulin resistance: some believe that ED is secondary to insulin resistance, i.e. it is a consequence of the factors that characterize insulin resistance, carbohydrate and lipid metabolism disorders; others believe that ED is not a consequence but a cause of insulin resistance[17]. According to Nasiri-Ansari N. et al., insulin resistance observed in MAFLD can lead to vascular endothelial dysfunction through various mechanisms, including an imbalance in NO production, which can lead to decreased blood flow. This in turn worsens insulin resistance, creating a vicious circle[16].

Significant changes in ED markers in patients with MAFLD in combination with SCH can be considered as one of the risk factors for the development of atherosclerosis and its complications, which form the background for the development of cardiovascular complications. This is confirmed by the presence of correlations between the ED markers - CDEC, VEGF-A, inflammatory markers - CRP, TNF-α and proatherogenic lipids, which indicates their involvement in the development and progression of early atherosclerotic vascular changes. This is consistent with the results of several studies that have shown that subclinical hypothyroidism is associated with atherosclerotic changes and, therefore, may increase the risk of cardiovascular disease[5]. Thus, the results of the study by Niknam N. et al. showed a link between SCH and ED in terms of impaired endothelium-dependent vasodilation[18], and Shavdatuashvili T. reported that higher levels of serum TSH, cholesterol, and LDL are associated with greater ED. Similar findings were observed in a 10-year study in Taiwan, which included 115746 participants in the absence of thyroid disease,

in which SCH was defined as a serum TSH levels of 5.0-19.96 μIU/mL with normal T4 levels, and which demonstrated that individuals with SCH have an increased risk of CVE mortality[19]. However, there are studies that have not found a clear association between hypothyroidism, increased risk of cardiovascular disease, and ED[20].

According to Lopez-Yus M. et al., pathogenetically, the above processes are associated with the activation of systemic inflammation[21]. Proinflammatory cytokines mediate intercellular interactions and maintain local inflammation in atherosclerotic plaque, activate endothelial cells and induce the expression of adhesion molecules, endothelial prothrombotic activity, have cardioprotective effects, increase myocardial ischemia and thus significantly change the clinical course of the disease, and are markers of poor prognosis and high CVR. However, the study by Raposo L. et al. showed that ED in patients with SCH causes a decrease in the availability of nitric oxide, which can be eliminated by levothyroxine therapy[22], and according to Falkevall A. et al. the basis of ED in SCH is lipid infiltration[3].

Conclusion

Patients with MAFLD in combination with SCH have elevated levels of CDEC and VEGF-A, which indicates the involvement of these factors in the mechanisms of ED in this category of patients. On the one hand, significant changes in the level of CDEC and VEGF-A depending on TSH and hyperinsulinemia in patients with MAFLD in combination with SCH were demonstrated, which indicates a link between TSH levels and indicators of ED and hyperinsulinemia. On the other hand, ED indices also have links with the degree of CVR. Patients with moderate and high CVR had an link between CDEC and metabolic parameters (TG, CRP, and TC). The association of CDEC and VEGF-A levels with age may indicate the influence of age in the development of CVE in patients with MAFLD in combination with SCH. The value of VEGF-A > 273 pg/mL as a marker of ED may predict the likelihood of developing CVE and can be used to assess the prediction of possible cardiometabolic risk.

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