EVALUATION OF HORMONAL AND METABOLIC PARAMETERS, ALONG WITH CARDIOVASCULAR RISK FACTORS IN WOMEN WITH NON-ALCOHOLIC FATTY LIVER DISEASE COMBINED WITH SUBCLINICAL HYPOTHYROIDISM DEPENDING ON AGE

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Abstract

Patients with NAFLD *(non-alcoholic fatty liver disease)* and subclinical hypothyroidism are at risk of cardiovascular complications that cause cardiometabolic changes, thus enabling to broaden our understanding of the cardiovascular events risk in a comorbid patient.

The aim: The study of hormonal and metabolic indicators and cardiovascular risk factors in women from NAFLD combined with SH *(subclinical hypothyroidism)* depending on the age.

Materials and methods: 128 patients with NAFLD were studied, which were divided into 2 groups: I group – patients with NAFLD and level of thyroid-stimulating hormone (TSH) – 4 to 10 mIU/mL ($n = 45$), II group – patients with NAFLD and level of TSH > 10 mIU/mL ($n = 49$). The control group consisted of 34 NAFLD patients without SH. Depending on the level of TSH and age, degree of cardiovascular risk, indicators of carbohydrate and lipid metabolism, as well as the indicators that reflect ED were evaluated.

Results: Comparison of metabolic parameters in two groups showed a significant difference ($p < 0.01$ between indicators depending on the TSH level, where patients were below 50 years of age: HbA1c, LDL cholesterol, HDL cholesterol, gamma-glutamyltranspeptidase (GGTP). The levels of CDEC (*circulating desquamated endothelial cells*), VEGF (*vascular endothelial growth factor*), CRP (*C-reactive protein*) and TNF-α (*tumor necrosis factor*-α) were dependent not only on TSH, but also on age. Significant differences ($p = 0.001$) were obtained in patients aged ≤ 50 years: CDEC; VEGF, CRP; TNF-α.

Conclusions: Patients from NAFLD combined with SH have hormonal-metabolic disorders, and their degree depends on the TSH level. Early cardiometabolic changes in women are formed already at the age under 50 years, which indicates the formation of early atherosclerotic vascular changes.

Keywords: non-alcoholic fatty liver disease, subclinical hypothyroidism, age, hormonal indicator, metabolic indicator, cardiovascular risk.

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1. Introduction

Non-alcoholic fatty liver disease (NAFLD) plays an important part in common non-communicable diseases combining clinicopathologic changes of various degrees: from hepatic steatosis to hepatocellular carcinoma and caused by lifestyle changes [1]. The current prevalence of NAFLD

in industrialized countries is estimated at 20 % to 35 % among general population. According to data from 2019, the prevalence of NAFLD in industrialized countries was estimated at 20 % to 35 % among general population. At the same time, in women aged about 40 years, non-alcoholic steatosis of the liver was observed in 75 % of cases [2, 3].

One of the main mechanisms for the implementation of metabolic disorders in the liver is the action of reproductive hormones that determines the gender specificities of NAFLD [1, 4]. Estrogens have a positive impact on liver function by inhibiting lipogenesis and gluconeogenesis, as well as facilitating the process of lipolysis. Whereas, androgenic hormones reduce lipogenesis and contribute to an increase of cholesterol production in the liver that leads to progression of NAFLD progresses accompanied by the accumulation of triglycerides together with cholesterol. For that reason, NAFLD develops usually at a young age (30–50 years) among men, and the incidence of NAFLD among women increases after menopause or in accordance with the hyperandrogenism, as a consequence of polycystic ovary syndrome and Cushing's syndrome. It should be noted that the alanine aminotransferase (ALT) level usually remains within the normal range in women suffer from NAFLD in contrast to men. At the same time, NAFLD in men is associated with a massive amount of visceral fat, larger increase in ALT level and a low level of adiponectin and leptin [4, 5].

According to research, low serum level of sex hormone binding globulin (SHBG) is also associated with NAFLD. This protein plays a suppressive role in hepatic lipogenesis. There is a feedback between SHBG and NAFLD: the accumulation of fat in the liver suppresses the synthesis of SHBG, and a decline in SHBG level results in increase of fatty infiltration of the liver [2]. Re-establishment of the optimal body weight in obese patients is an important component of therapy for NAFLD, because the level of SHBG increases in proportion to the degree of weight loss [1, 4].

In accordance to the Rotterdam study that assessed a random sample of women (mean age 69.0 ± 7.5 years), it was shown that 28 % of the patients suffered from NAFLD had signs of subclinical hypothyroidism (SH) together with the highest level of total cholesterol (TC) and the lowest level of HDL cholesterol [6, 7]. Changes in the lipid spectrum of blood serum in patients with NAFLD in combination with SH is stated as being due to the fact that in hypothyroidism the hepatic lipase activity is reduced, the transport and excretion of atherogenic lipids from bile is deteriorated. At the same time, there is a violation of the structure of HDL cholesterol, a decrease in the number and sensitivity of low-density lipoprotein cholesterol (LDL cholesterol) receptors in hepatocytes that reduces hepatic excretion of cholesterol and a further increase in LDL cholesterol and very-low-density lipoprotein cholesterol (VLDL cholesterol) [8, 9].

In the general population, depending on the age and gender of the surveyed people, the incidence of SH ranges from 1.2 to 15 %. According to the American Association of Clinical Endocrinology, SH is observed in 3 % of male and 10 % of female (in the age group over 60 years it reaches 20 %) [1, 6]. In accordance to the Colorado study with 25.862 participants, an abnormally high thyroid-stimulating hormone (TSH) level was observed from 4 % to 21 % in women and from 3 to 16 % in men, depending on age. The significance of this disease is due to the fact that within one year SH reaches manifest form in 5–15 % of patients, and 70 % of the cases will be accompanied by the onset of cardiovascular system damage symptoms [10, 11].

A number of studies were devoted to the effect of hypofunctioning of the thyroid gland (TG) on lipid metabolism [10, 12]. Some research investigated the relationship between SH and dyslipidemia based on gender differences. For instance, the prospective EPIC-Norfolk study revealed significantly increased concentrations of serum TC, LDL cholesterol and triglycerides (TG) only in women having SH [8, 13]. According to the results of the population-based HUNT study, Norway, an association between thyroid stimulating hormone (TSH) level and blood lipids, even within the normal TSH range was detected in women with metabolic syndrome (MS) without thyroid disfunction, cardiovascular diseases or diabetes mellitus (DM). Moreover, the higher the TSH level, the higher the blood cholesterol level [14]. The research findings indicate that the lipid profile in women suffering from MS with SH is characterized by significant atherogenic disorders.

An increase of total cholesterol (TC) level and LDL cholesterol (in 98 % and 97 %, accordingly) was identified in female. At that time, the classic manifestations of dyslipidemia with MS without SH characterizing by hypertriglyceridemia and a decrease in HDL cholesterol, were

diagnosed in less than half of the women (47 % and 46 %, accordingly). It is important that women with TSH level above 10 mIU/mL have a significant increase in the level of LDL particles, which are associated with a higher atherogenic index [15]. This study also revealed a positive relationship between TSH levels within the reference values and the risk of death from coronary heart disease (CHD) in women without thyroid disorder [15].

The Norwegian Trøndelag Health Study conducted for 11 years with the number of participants $n = 15\ 020$ (euthyroid patients) has demonstrated a link between thyroid hormones and body mass index (BMI) [16, 17]. In women, an increase in TSH by 1 mIU/mL was accompanied by an increase in body weight by 0.9 kg and increase of BMI by 0.3 kg/m^2, while in men by 0.8 kg and 0.2 kg/m^2, accordingly, though, insulin resistance (IR) is a proven risk factor for NAFLD [14, 15].

Recently, scientists focus on the study of endothelial dysfunction (ED), as one of the basic mechanisms for the implementation of all factors of cardiovascular risk (CVR), and also as an early marker of the development and progression of atherosclerosis. One of the factors of endothelial dysfunction is the vascular endothelial growth factor (VEGF). VEGF is involved in the formation of MS at the nosological level that includes the development of atherosclerosis, hypertension, type 2 diabetes and other diseases. Release of VEGF facilitates the process of monocytes migration with subsequent transformation into macrophages.

The consequence of this process is the formation of foam cells, and then an atherosclerotic plaque (ASP). VEGF stimulates the expression of matrix metalloproteinase leading to dissolution of the extracellular matrix and migration of the endothelium into the collagen gel with the formation of endothelial tubes. Newly formed vessels contribute to the nutrition and growth of the plaque with its subsequent rupture and the development of vascular complications [18].

In one study, it was shown that the -634G/C (rs 2010963) polymorphism of the VEGF gene is more common in women having hypertension and obesity in premenopausal women than in the group of women after menopause. It was found that the level of VEGF was significantly higher in premenopausal and menopausal women with polymorphism of the GG-634G/C genotype (rs 2010963) of the VEGF gene compared with patients with the GC and CC genotypes ($p < 0.05$) [15]. The presence of polymorphism of the genotype GG-634G/C (rs 2010963) of the VEGF gene can be considered as an early marker of the development of cardiovascular risk of hypertension in combination with obesity in perimenopausal women [19, 20].

Thus, the study of hormonal-metabolic predictors and vascular markers in female suffering from NAFLD in combination with SH will expand the understanding of CVR formation mechanisms, as well as individualize the strategy for the prevention of cardiovascular events in a comorbid patients on this basis.

The aim of the research: The study of hormonal and metabolic indicators and cardiovascular risk factors in women suffering from NAFLD combined with SH depending on the age.

2. Materials and methods

The study was conducted at the SI «L. T. Malaya Therapy National Institute NAMS of Ukraine» during 2018–2019. The study included patients who were on inpatient and outpatient treatment.

A total 128 female patients were enrolled in the study diagnosed with NAFLD. The average age of the studied patients was 52.1 ± 5.8 years. Patients were divided into 2 groups: I group – patients with NAFLD and level of thyroid-stimulating hormone (TSH) – 4–10 mIU/mL ($n = 45$), II group – patients with NAFLD and level of TSH > 10 mIU/mL ($n = 49$). As a comparison group patients with isolated NAFLD ($n = 34$), identical in age and sex for the main group were studied. Under the study protocol, the patients were divided into three groups according to the level of total cardiovascular risk in accordance with SCORE: $n = 22$ women had a low risk; $n = 49$ – moderate risk; $n = 23$ – high risk.

The study was performed in compliance with the basic provisions of the Helsinki Declaration of the World Medical Association on ethical principles of scientific and medical research involving human (1964–2000) and the order of the Ministry of health of Ukraine dated 23.09.2009 No. 690. The study was approved by the commission on bioethics at the SI «L. T. Malaya Therapy National Institute NAMS of Ukraine», *(protocol No. 12, 19/Nov/2020)* consistent with the principles outlined in the Helsinki Declaration.

The diagnosis of NAFLD was grounded consisted with the current unified clinical protocol. To verify the diagnosis of SH on the background of Hashimoto's disease, the concentration of thyroid-stimulating hormone (TSH), free thyroxine (T4 free) and thyroid peroxidase antibodies (TPO) in blood serum were studied using enzyme immunoassay. The state of carbohydrate and lipid metabolism as well as indicators of the functional state of the liver was assessed using a generally accepted technique *(Fasting glucose levels were examined to assess the control of carbohydrate metabolism. Blood glucose was determined by the glucose oxidase method. Determination of glucosylated hemoglobin (HbA1c, %) (Human, Germany). From venous blood by ion exchange chromatography was used as an informative method for characterizing long-term glycemic control. In all patients, the level of total cholesterol, high density lipoprotein cholesterol and triglycerides was determined by the enzymatic method on a biochemical analyzer «Humanayzer» using a set of reagents from «Human» (Germany). Very low density lipoprotein cholesterol content was calculated using the Friedewald W. T. taking into account the measurement of the indicator in mmol/l. The indicators of the functional state of the liver were determined according to standard generally accepted methods: the activity of aminotransferase (AST, ALT), gamma-glutamine transpeptidase, alkaline phosphatase (AF), the content of bilirubin in the blood using the enzyme assay method on the biochemical).*

The condition and degree of damage to the vascular endothelium were assessed by counting the circulating desquamated endothelial cells (CDECs) using phase contrast microscopy and VEGF using. The concentration of C-reactive protein (CRP) and tumor necrosis factor-α (TNF-α) in the serum were used as markers of inflammation by enzyme immunoassay.

The statistical processing of the data was carried out using the «Statistica software package, version 8.0». The data resulting from the study are presented as the mean ± standard deviation from the mean (M ± SD). To assess the differences between groups in a distribution close to normal, the «Student´s test» was used. Differences were considered statistically significant at $p < 0.05$.

3. Results

The metabolic changes in the two groups depending on the TSH level were associated with the age of the women.

In particular, the results of the comparison showed that in 2 groups patients under the age of 50, there were significant differences depending on the level of TSH on such indicators as: fasting glucose level – 5.67 ± 0.27 mmol/l against 4.14 ± 0.23 mmol/l, $p = 0.010$; level of HbA1c – 6.54 ± 0.25 % against 5.02 ± 0.10 %, $p < 0.001$; LDL cholesterol – 4.32 ± 0.24 mmol/L versus 3.25 ± 0.19 mmol/L ($p = 0.030$), HDL cholesterol – 1.00 ± 0.02 mmol/L versus 1.09 ± 0.04 mmol/L ($p = 0.038$). Detection of statistical differences in the indicator of gamma-glutamyltransferase (GGT) – 78.0 ± 3.85 U/l against 53.33 ± 9.35 U/l, $p = 0.016$ (**Table 1**) constitutes an important finding.

Table 1

The metabolic values depending on the level of TSH (from 4 to 10 mIU/mL and more than 10 mIU/mL) in women having NAFLD combined with SH aged ≤ 50 years, M ± m

Indicator	TSH from 4 to 10 mIU/mL) *n* = 23	TSH> 10 mIU/mL) *n* = 24	*P*-value
Fasting glucose, mmol/L	4.14 ± 0.23	5.67 ± 0.27	*P* = 0.010
Insulin (mIU/mL)	16.42 ± 0.76	16.42 ± 0.76	*P* = 0.02
HbA_{1C}, %	5.02 ± 0.10	6.54 ± 0.25	*P* < 0.001
VLDL-CH, mmol/L	0.76 ± 0.03	0.85 ± 0.02	*P* = 0.029
HDL-CH, mmol/L	1.09 ± 0.04	1.00 ± 0.02	*P* = 0.038
LDL-CH, mmol/L	3.25 ± 0.19	4.32 ± 0.24	*P* = 0.030
GGTP, IU/L	53.33 ± 9.35	78.0 ± 3.85	*P* = 0.016

The results achieved in patients suffering from NAFLD in combination with SH indicate that metabolic changes are formed under the age of 50 and they are significantly influenced by TSH level. The study revealed a correlation between TSH and CRP – $r = 0.88$, $p = 0.02$, insulin – $r = 0.82$, $p = 0.046$ and GGTP $r = 0.87$, $p = 0.025$.

We believe that it is important that changes in the level of GGTP depending on the level of TSH in the presence of NAFLD in combination with SH not only characterizes the liver enzyme GGTP as a membrane-specific enzyme, but also confirms its indirect participation in the formation of atherosclerotic plaque [10, 11].

It seems interesting that in patients over 50 years old significant differences in groups depending on TSH level were different with the prevalence of changes in the carbohydrate profile: HbA_{1C} – 7.07±0.14 % versus 4.74±0.24 %, $p < 0.001$; insulin – 18.70±1.28 mIU/mL versus 15.10±0.49 mIU/mL, $p < 0.001$), along with changes in GGTP (79.44±1.89 IU/L versus 62.67±5.23 IU/L) and alkaline phosphatase (AP) levels, $p = 0.025$ (**Table 2**).

Table 2

The metabolic values depending on the level of TSH (from 4 to 10 mIU/mL and more than 10 mIU/mL) in women having NAFLD in combination with SH over 50 years, M±m

Indicator	TSH from 4 to 10 mIU/mL *n* = 22	TSH > 10 mIU/mL *n* = 25	*P*-value
Fasting glucose, mmol/L	4.26±0.17	6.66±0.17	$p < 0.001$
Insulin mIU/mL	15.10±0.49	18.70±1.28	$p < 0.001$
HbA_{1C}, %	4.74±0.24	7.07±0.14	$p < 0.001$
GGTP, IU/L	62.67±5.23	79.44±1.89	$p = 0.025$
AP, IU/L	1540.33±130.95	1857.11±31.31	$p = 0.025$

The data obtained indicate about the impact of TSH level in women over 50 years having NAFLD combined with SH on the formation of changes in carbohydrate metabolism, as well as the progression of changes in the liver tissue.

The presence of a correlation between AST and HbA1c ($r = 0.39$, $p = 0.006$); GGTP and fasting glucose ($r = 0.59$, $p = 0.001$); total, direct bilirubin and antibody levels to thyroperoxidase, which was ($r = 0.89$, $p = 0.04$ and $r = 0.99$, $p = 0.001$), respectively, has been proof of the influence of liver functional status on the metabolic parameters of patients with NAFLD in combination with SH.

The dependence of ED and inflammation was analyzed in women with NAFLD in combination with SH, taking into account the level of TSH and age. Significant differences were obtained for all indicators in women aged ≤ 50 years: circulating desquamated endothelial cells (CEC) – 9.88±0.52 cells/100 μl versus 6.67±0.33 cells/100 μl, $p = 0.006$; VEGF – 398.94±25.74 pg/ml versus 97.08±19.39 pg/ml, $p = 0.001$; CRP – 10.64±1.02 mg/L versus 7.58±1.14 mg/L, $p = 0.001$; TNF-α – 11.93±0.92 versus 8.6±0.54, $p = 0.001$ (**Table 3**) that may indicate the role of age in the development of vascular events in patients with NAFLD in combination with SH.

Table 3

Comparison of ED indicators and inflammatory markers depending on TSH in women of different ages suffering from NAFLD in combination with SH, M±m

Indicators	Endothelial dysfunction indicators and inflammatory markers in subgroups ≤ 50 years		
	TSH from 4 to 10 mU/L *n* = 23	TSH > 10 mU/L *n* = 24	*P*-value
CECs, cells/100 μl	6.67±0.33	9.88±0.52	0.006
VEGF, pg/ml	197.08±19.39	398.94±25.74	0.001
CRP, mg/L	7.58±1.14	10.64±1.02	0.001
TNF-α	8.60±0.54	11.93±0.92	0.002
Indicators	**Endothelial dysfunction indicators and inflammatory markers in subgroups > 50 years**		
	TSH from 4 to 10 mU/L *n* = 22	**TSH > 10 mU/L *n* = 25**	***P*-value**
CECs, cells/100 μl	11.00±1.00	13.58±1.54	>0.05
VEGF, pg/ml	442.79±81.36	499.79±24.69	>0.05
CRP, mg/L	7.68±1.25	8.70±0.66	>0.05
TNF-α, pg/ml	6.90±0.86	8.90±0.44	0.02

Thus, in the age group of 50 years and above, only TNF-α was statistically higher in female group with TSH level from 4 to 10 mIU/mL – 8.90 ± 2.14 pg/ml versus 6.90 ± 1.06 pg/ml, $p = 0.02$.

Comparison of indicators of vascular endothelium in the group of women with NAFLD in combination with SH significantly differed depending on age (>50 years and ≤50 years), with a predominance of CECs and VEGF indicators in the older age group, $p < 0.01$.

The relationship between the lipid profile and metabolic parameters demonstrates a moderate inverse correlation between HDL–CH and the level of CRP – $r = –0.33$, $p = 0.029$; hip – $r = –0.39$, $p = 0.050$; intima–media complex thickness – $r = –0.34$, $p = 0.026$; straight line – between TSH and CRP – $r = 0.34$, $p = 0.025$. Among the indicators of the lipid profile there was a direct correlation: between the indicator of total cholesterol (TC) and CRP – $r = 0.49$, $p = 0.022$; CDECs – $r = 0.49$, $p = 0.025$; VLDL-CH – $r = 0.47$, $p = 0.028$; TSH – $r = 0.74$, $p = 0.001$, between the index VLDL-CH and CECs – $r = 0.69$, $p = 0.001$; VEGF – $r = 0.59$, $p = 0.004$; CRP – $r = 0.43$, $p = 0.048$; TSH – $r = 0.54$, $p = 0.038$.

We have individually analyzed the features of hormonal and metabolic changes depending on the degree of CVR. Depending on the level of CVR in patients having NAFLD with SH, significant differences were found between the group of moderate and low CVR that was characterized by a significant increase in body weight – 79.50 ± 0.50 kg versus 75.57 ± 1.42 kg, $p = 0.010$; indicators of the carbohydrate profile – glucose – 6.23 ± 0.17 mmol/l versus 4.20 ± 0.13 mmol/l, $p = 0.048$; HbA_{1C} – 6.86 ± 0.67 % versus 4.88 ± 0.73 %, $p = 0.089$; fasting insulin 16.42 ± 0.76 uIU/mL versus 14.23 ± 0.65 uIU/mL, $p = 0.018$; GGTP activity – 73.47 ± 5.67 U/L versus 58.00 ± 5.23 U/L, $p = 0.048$ with the increase of TSH – 6.50 ± 0.17 mIU/mL versus 3.06 ± 0, 15 mIU/mL, $p = 0.045$ (**Table 4**).

Table 4

Comparison of hormonal and metabolic parameters in women with NAFLD combined with SH depending on the degree of CVR in the groups of low and moderate CVR, M ± m

Indicators	Low CVR *n* = 22	Moderate CVR *n* = 49	*P*-Value
Fasting glucose, mmol/L	4.20 ± 0.13	6.23 ± 0.17	0.048
Insulin, uIU/mL	14.23 ± 0.65	16.42 ± 0.76	0.018
HbA_{1C}, %	4.88 ± 0.73	6.86 ± 0.67	0.089
Total cholesterol (TC), mmol/L	5.68 ± 0.10	5.86 ± 0.13	0.93
TG, mmol/L	2.10 ± 0.04	2.25 ± 0.08	0.834
VLDL-CH, mmol/L	0.76 ± 0.15	0.87 ± 0.01	0.784
HDL-CH, mmol/L	1.03 ± 0.095	0.92 ± 0.04	0.831
LDL-CH, mmol/L	3.19 ± 0.73	3.62 ± 0.17	0.854
AC	3.96 ± 0.96	4.59 ± 0.31	0.737
AST, IU/L	31.83 ± 4.65	35.19 ± 1.64	0.322
ALT, IU/L	42.00 ± 4.92	48.02 ± 2.11	0.483
GGTP, IU/L	58.00 ± 5.23	73.47 ± 5.67	0.048
AP, nmol/h·L	1583.50 ± 66.85	1751.19 ± 62.64	0.405
TSH, mIU/mL	3.06 ± 0.15	6.50 ± 0.17	0.045
Free T4, mU/L	11.54 ± 0.15	13.03 ± 0.71	0.288

When comparing hormonal and metabolic parameters in the groups of moderate and high CVR, significant differences in the lipid profile were revealed: TC – 7.24 ± 0.22 mmol/L versus 5.86 ± 0.13 mmol/L, $p = 0.001$; TG – 2.75 ± 0.11 mmol/L versus 2.25 ± 0.08 mmol/L, $p = 0.001$; VLDL-CH – 1.03 ± 0.05 mmol/L versus 0.87 ± 0.01 mmol/L, $p = 0.008$. More significant changes were observed in indicators of the functional state of the liver (ALT – 64.05 ± 2.74 IU/L versus 48.02 ± 2.11 IU/L, $p = 0.001$; GGTP – 90.95 ± 2.31 IU/L versus 73.47 ± 5.67 IU/L, $p = 0.001$; AP – 2026.68 ± 33.38 nmol/h·L versus 1751.19 ± 62.34 nmol/h·L, $p = 0.001$). The increase in the degree of CVR occurred against the background of the deepening of the degree of SH (8.26 ± 0.30 mU/L versus 6.50 ± 0.17 mU/L, $p = 0.001$) (**Table 5**).

Table 5

Comparison of hormonal and metabolic parameters in women having NAFLD in combination with SH, depending on the degree of CVR, in the groups of moderate and high CVR, M ± m

Indicators	Moderate CVR *n* = 49	High CVR *n* = 23	*P*-Criterion
Fasting glucose, mmol/L	6.23 ± 0.17	7.09 ± 0.27	0.01
Insulin, uIU/mL	16.42 ± 0.76	23.59 ± 2.62	0.02
HbA_{1C}, %	6.86 ± 0.67	7.23 ± 0.18	0.13
Total cholesterol (TC), mmol/L	5.86 ± 0.13	7.24 ± 0.22	0.001
TG, mmol/L	2.25 ± 0.08	2.75 ± 0.11	0.001
VLDL-CH, mmol/L	0.87 ± 0.01	1.03 ± 0.05	0.008
HDL-CH, mmol/L	0.92 ± 0.04	0.90 ± 0.05	0.76
LDL-CH, mmol/L	3.62 ± 0.17	4.31 ± 0.27	0.04
AC	4.59 ± 0.31	5.59 ± 1.99	0.43
AST, IU/L	35.19 ± 1.64	38.73 ± 2.67	0.27
ALT, IU/L	48.02 ± 2.11	64.05 ± 2.74	0.001
GGTP, IU/L	73.47 ± 5.67	90.95 ± 2.31	0.001
AP, nmol/h·L	1751.19 ± 62.64	2026.68 ± 33.38	0.001
TSH, mIU/mL	6.50 ± 0.17	8.26 ± 0.30	0.001
Free T4, pmol/L	13.03 ± 0.71	16.63 ± 0.37	0.001

In the group of NAFLD patients with SH with low CVR, there was a correlation between lipid metabolism indicators (TG indicator correlated with insulin indicator $r = 0.84$, $p = 0.035$) and an inverse correlation (HDL-CH with CRP indicator – $r = –0.97$, $p = 0.002$; TG – $r = –0.95$, $p = 0.004$; insulin – $r = –0.87$, $p = 0.024$; TSH – $r = –0.88$, $p = 0.022$).

In the course of the study the content of endothelial dysfunction indicators and inflammatory markers were analyzed depending on CVR in women suffering from NAFLD in combination with SH of moderate and low risk, significant differences in CECs and TNF-α indicators were revealed (11.93 ± 0.54 cells/100 μl versus 8.83 ± 1.10 cells/100 μl, p = 0.010 and 8.9 ± 0.37 pg/ml versus 6.9 ± 0.66 pg/ml, $p = 0.010$, respectively). At the same time, there were significant differences in CECs and TNF-α (15.68 ± 1.08 cells/100 μl versus 11.93 ± 0.54 cells/100 μl, $p = 0.001$; VEGF – 646.44 ± 58.11 pg/ml versus 422.82 ± 10.01 pg/ml, $p = 0.001$)in women having NAFLD combined with SH of high and moderate CVR.

An inverse correlation in patients with low CVR was observed between CECs and GGTP level – $r = 0.73$, $p = 0.099$. Connections of CECs were not so expressed in patients with moderate CVR and were distributed as follow: with TG index – $r = 0.43$, $p = 0.004$ and CRP – $r = 0.30$, $p = 0.050$. It is likely that the obtained data caused by the fact that during the transition from the category of low to moderate CVR, a large number of factors influence its formation in patients with NAFLD combined with SH.

Analysis of correlations in patients suffering from NAFLD combined with SH having a high CVR revealed the presence of a direct relationship between CECs and VEGF – $r = 0.53$, $p = 0.011$ and total cholesterol – $r = 0.48$, $p = 0.025$.

Thus, the formation of CVR in women having NAFLD combined with SH occurs under the conditions of complex interactions between hormonal and metabolic parameters and indicators, each can be important and, even, essential for the further course of the disease.

4. Discussion

Our study demonstrated that in females under 50 years old who have NAFLD in combination with SH, prevailed changes in the lipid spectrum of blood serum with a significant increase in GGTP. Furthermore, in females over 50 years old dominated changes in carbohydrate metabolism with changes in liver parameters, which indicates the progression of the course of NAFLD.

Probably, these changes are influenced not only by age, but also by the aggravation of hypothyroidism degree. Thyroid hormones are well known to regulate a wide range of metabolic parameters, including lipoprotein metabolism and CVD risk factors.

It was found that, depending on the level of TSH, age of patients, the degree of CVR in patients with NAFLD in combination with SH, changes were distinguished in metabolic parameters in the form of a significant increase in TG ($p < 0.001$), HDL cholesterol ($p = 0.023$), GGTP ($p < 0.001$), insulin ($p = 0{,}003$), CRP ($p < 0.05$), which indicates the contribution of thyroid dysfunction in the formation of cardiometabolic risk. During our study, it was found that women under 50 years old with NAFLD in combination with SH had metabolic changes, the indicators of which are significantly affected by TSH levels > 10 mIU/mL. Increasing the level of GGTP depending on the level of TSH in women with NAFLD in combination with SH, in our opinion, confirms the indirect relation of the membrane-specific enzyme GGTP in the formation of atherosclerotic plaque. At the same time in women older than 50 years, there were significant differences in groups depending on the level of TSH > 10 mIU/mL and were dominated by changes in the carbohydrate profile, vascular endothelium and inflammatory markers showed a significant prevalence of CDEC and VEGF, $p < 0.01$. Our data coincide with a study showing that cases of SH are risk factors for dyslipidemia compared with the control group [21]. In another study by Sharma R. et al., it was shown that patients with SH had significantly increased levels of highly sensitive CRP, lipoprotein A, CHD and LDL compared with these parameters of the control group [21]. Teran V. S. and Calle M. A. showed that TSH levels were statistically related to CHD and LDL levels [22]. Some reviews have examined the relationship between SH and dyslipidemia with varying results. A prospective EPIC-Norfolk study found significantly elevated serum levels of cholesterol, LDL cholesterol, and TG in SH only in female [23].

Thus, the combination of NAFLD with SH forms an abnormal metabolic phenotype, which is characterized by the presence of dyslipidemia, hyperinsulinemia on the background of oxidative stress and ED. From the perspective of endothelial dysfunction and vascular aging, we proved the influence of the thyroid gland condition and age on the vascular endothelium state in patients with NAFLD. Similar changes have been found by some researchers. In a study by Erman H. et al. it was found that ED is detected even within normal TSH values and worsens with increasing TSH levels [24]. There are suggestions about the influence of a woman's postmenopausal status on the indicators of vascular stiffness of the arterial wall, which depend on the condition of the thyroid gland [25].

There is no doubt that the mechanisms of ED development in comorbid pathology, namely NAFLD in combination with SH, are complex and insufficiently studied. Determination of circulating endothelial cells in the blood as a marker of vascular endothelial damage is currently one of the simplest, most informative and accessible methods. Some authors have proved the increase in these cells in various pathological conditions, including CVD [26].

Attention should be given not only to age depending factors, but also to the increase in inflammation markers. CRP is an integral indicator that reflects the inflammation of low degrees of gradation, as it is an indirect marker of ED. Significant increase in the group of women under 50, in our opinion, can be regarded as a factor that initiates «vascular» aging, along with the expression of ED indicators – VEGF and CECs, cells.

Study limitations. Our study included patients of only certain age groups (40–49 and 50–59 years old). Perhaps in other age groups (wider age range) metabolic and hormonal parameters will have different characteristics. The limitation of our work was also the fact that the patients' intake of hormone replacement therapy was not taken into account.

Prospects for further research in the development of methods for early drug correction of hormonal and metabolic changes in women with NAFLD in combination with SH of different age groups in order to prevent «vascular» aging.

5. Conclusion

In women suffering from NAFLD combined with SH, metabolic changes develop already at a young age (less than 50 years) and their values are reliably influenced by TSH level < 10 mIU/mL.

Increasing the level of GGTP depending on the level of TSH in women with NAFLD in combination with SH, in our opinion, confirms the indirect participation of the membrane-specific enzyme GGTP in the processes of atherosclerotic plaque formation.

At the same time, in women over 50 years old, extensive differences in the groups depending on the TSH level > 10 mIU/mL were found with their distinquishing features including predominant carbohydrate profile changes, a significant predominance of CEC and VEGF, $p < 0.01$ on indicators for endothelial dysfunction and inflammatory markers.

The results of the study have demonstrated that in women, younger than 50 years old, with NAFLD in combination with SH, changes that are associated with endothelial dysfunction, immune inflammation are formed, the severity of which depends on the level of TSH.

Conflict of interest

The authors declare that they have no conflicts of interest.

References

[1] Anokhina, H. A., Kharchenko, V. V., Dynnyk, O. B. (2018). Rol zapalennia ta metabolichnykh porushen u prohresuvanni khronichnykh zakhvoriuvan pechinky: profilaktyka ta likuvannia. Zdorovia Ukrainy, 15-16, 60–62.

[2] Athyros, V. G., Alexandrides, T. K., Bilianou, H., Cholongitas, E., Doumas, M., Ganotakis, E. S. et. al. (2017). The use of statins alone, or in combination with pioglitazone and other drugs, for the treatment of non-alcoholic fatty liver disease/non-alcoholic steatohepatitis and related cardiovascular risk. An Expert Panel Statement. Metabolism: clinical and experimental, 71, 17–32. doi: http://doi.org/10.1016/j.metabol.2017.02.014

[3] Mehran, L., Amouzegar, A., Azizi, F. (2019). Thyroid disease and the metabolic syndrome. Current Opinion in Endocrinology & Diabetes and Obesity, 26 (5), 256–265. doi: http://doi.org/10.1097/med.0000000000000500

[4] Anokhina, H. A. (2019). Henderni osoblyvosti perebihu y terapii nealkoholnoi zhyrovoi khvoroby pechinky. Zdorovia Ukrainy. Tematychnyi nomer «Hastroenterolohiia. Hepatolohiia. Koloproktolohiia», 2, 21.

[5] Danese, M. D., Gleeson, M., Kutikova, L., Griffiths, R. I., Khunti, K., Seshasai, S. R. K., Ray, K. K. (2017). Management of lipid-lowering therapy in patients with cardiovascular events in the UK: a retrospective cohort study. BMJ Open, 7 (5), e013851. doi: http://doi.org/10.1136/bmjopen-2016-013851

[6] Liu, L., Yu, Y., Zhao, M., Zheng, D., Zhang, X., Guan, Q. et. al. (2017). Benefits of Levothyroxine Replacement Therapy on Nonalcoholic Fatty Liver Disease in Subclinical Hypothyroidism Patients. International Journal of Endocrinology, 2017, 1–10. doi: http://doi.org/10.1155/2017/5753039

[7] Selmer, C., Olesen, J. B., Hansen, M. L., von Kappelgaard, L. M., Madsen, J. C., Hansen, P. R. et. al. (2014). Subclinical and Overt Thyroid Dysfunction and Risk of All-Cause Mortality and Cardiovascular Events: A Large Population Study. The Journal of Clinical Endocrinology & Metabolism, 99 (7), 2372–2382. doi: http://doi.org/10.1210/jc.2013-4184

[8] Ludwig, U., Holzner, D., Denzer, C., Greinert, A., Haenle, M. M. et. al. (2015). Subclinical and clinical hypothyroidism and non-alcoholic fatty liver disease: a cross-sectional study of a random population sample aged 18 to 65 years. BMC Endocrine Disorders, 15 (1). doi: http://doi.org/10.1186/s12902-015-0030-5

[9] Aksoy, N., Yeler, M. T., Ayan, N. N., Ozkeskin, A., Ozkan, Z., Serin, N. O. (2015). Association between thyroid hormone levels and insulin resistance and body mass index. Pakistan journal of medical sciences, 31 (6), 1417–1420. doi: http://doi.org/10.12669/pjms.316.7560

[10] Chatrath, H., Vuppalanchi, R., Chalasani, N. (2012). Dyslipidemia in Patients with Nonalcoholic Fatty Liver Disease. Seminars in Liver Disease, 32 (1), 22–29. doi: http://doi.org/10.1055/s-0032-1306423

[11] Ross, D. S., Burch, H. B., Cooper, D. S., Greenlee, M. C., Laurberg, P., Maia, A. L. et. al. (2016). 2016 American Thyroid Association Guidelines for Diagnosis and Management of Hyperthyroidism and Other Causes of Thyrotoxicosis. Thyroid, 26 (10), 1343–1421. doi: http://doi.org/10.1089/thy.2016.0229

[12] Cahill, P. A., Redmond, E. M. (2016). Vascular endothelium – Gatekeeper of vessel health. Atherosclerosis, 248, 97–109. doi: http://doi.org/10.1016/j.atherosclerosis.2016.03.007

[13] Newsome, J. S. (2016). Evaluation of Statin Treatment for Nonalcoholic Fatty Liver Disease. Journal of Pharmacy Technology, 32 (4), 169–173. doi: http://doi.org/10.1177/8755122516640800

[14] Yahmur, V. B. (2013). Nealkoholna zhyrova khvoroba pechinky: suchasnyi pohliad na patohenez, diahnostyku ta likuvannia. Hastroenterolohiia, 3 (49), 138–147.

[15] Iaresko, M., Kolesnikova, E. (2016). The role of polymorphism-634 G/C (rs 2010963) of VEGF gene in the development of hypertension and obesity in premenopausal women. Georg. Med News, 256-257, 33–37.

[16] Zhao, T., Chen, B., Zhou, Y., Wang, X., Zhang, Y., Wang, H., Shan, Z. (2017). Effect of levothyroxine on the progression of carotid intima-media thickness in subclinical hypothyroidism patients: a meta-analysis. BMJ Open, 7 (10), e016053. doi: http://doi.org/10.1136/bmjopen-2017-016053

[17] Svare, A., Nilsen, T. I. L., Bjøro, T., Åsvold, B. O., Langhammer, A. (2011). Serum TSH related to measures of body mass: longitudinal data from the HUNT Study, Norway. Clinical Endocrinology, 74 (6), 769–775. doi: http://doi.org/10.1111/j.1365-2265.2011.04009.x

[18] Bonci, E., Chiesa, C., Versacci, P., Anania, C., Silvestri, L., Pacifico, L. (2015). Association of Nonalcoholic Fatty Liver Disease with Subclinical Cardiovascular Changes: A Systematic Review and Meta-Analysis. BioMed Research International, 2015, 1–11. doi: http://doi.org/10.1155/2015/213737

[19] Sinn, D. H., Cho, S. J., Gu, S., Seong, D., Kang, D., Kim, H. et. al. (2016). Persistent Nonalcoholic Fatty Liver Disease Increases Risk for Carotid Atherosclerosis. Gastroenterology, 151 (3), 481–488.e1. doi: http://doi.org/10.1053/j.gastro.2016.06.001

[20] Bano, A., Chaker, L., Plompen, E. P. C., Hofman, A., Dehghan, A., Franco, O. H. et. al. (2016). Thyroid Function and the Risk of Nonalcoholic Fatty Liver Disease: The Rotterdam Study. The Journal of Clinical Endocrinology & Metabolism, 101 (8), 3204–3211. doi: http://doi.org/10.1210/jc.2016-1300

[21] Sharma, R., Sharma, T. K., Kaushik, G. G., Sharma, S., Vardey, S. K., Sinha, M. (2011). Subclinical hypothyroidism and its association with cardiovascular risk factors. Clinical laboratory, 57 (9-10), 719–724.

[22] Sarzosa Terán, V., Astudillo Calle, M. A. (2012). Relationship of thyroid-stimulating hormone levels to development of dyslipidemia and determination of an ideal cut-off point to start replacement therapy. Endocrinologia y nutricion, 59 (10), 575–582. doi: http://doi.org/10.1016/j.endonu.2012.07.006

[23] Boekholdt, S. M., Titan, S. M., Wiersinga, W. M., Chatterjee, K., Basart, D. C. G., Luben, R. et. al. (2010). Initial thyroid status and cardiovascular risk factors: The EPIC-Norfolk prospective population study. Clinical Endocrinology, 72 (3), 404–410. doi: http://doi.org/10.1111/j.1365-2265.2009.03640.x

[24] Erman, H., Gelisgen, R., Cengiz, M., Tabak, O., Erdenen, F., Uzun, H. (2016). The association of vascular endothelial growth factor, metalloproteinases and their tissue inhibitors with cardiovascular risk factors in the metabolic syndrome. European review for medical and pharmacological sciences, 20 (6), 1015–1022.

[25] Cho, K. I., Lee, J. H. (2014). The impact of thyroid autoimmunity on arterial stiffness in postmenopausal patients with fibromyalgia. International Journal of Rheumatic Diseases, 20 (12), 1978–1986. doi: http://doi.org/10.1111/1756-185x.12257

[26] Sun, J., Yao, L., Fang, Y., Yang, R., Chen, Y., Yang, K., Tian, L. (2017). Relationship between Subclinical Thyroid Dysfunction and the Risk of Cardiovascular Outcomes: A Systematic Review and Meta-Analysis of Prospective Cohort Studies. International Journal of Endocrinology, 2017, 1–15. doi: http://doi.org/10.1155/2017/8130796

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