Non-Alcoholic Fatty Liver Disease and Hypothyroidism: Review of Clinical and Experimental Studies

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Abstract

Hypothyroidism is a widespread condition affecting people of different socio-economic background and geographical location. A lot of studies highlight the effect of hypothyroidism on the metabolic processes in various organs, including the liver. On the other hand, liver damage often results in the development of non-alcoholic fatty liver disease; however, the data on the impact of hypothyroidism on liver morphology, which can serve as a direct indicator and marker of liver condition and function, are limited and controversial. In this report, we reviewed the relationship between non-alcoholic fatty liver disease and hypothyroidism with an accent on morphological alteration of the liver discovered in clinical and experimental studies.

Keywords

Non-Alcoholic Fatty Liver Disease; Hypothyroidism; Ultrasonography; Histopathology

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Introduction

Thyroid gland diseases are common conditions characterized by a systemic effect on the human body [1]. The number of individuals with hypothyroidism significantly exceeds the prevalence rates of hypothyroidism, which is largely due to geographic dependency and residence in the iodine-deficient areas [2, 3]. Hypothyroidism is characterized by high serum levels of thyroid-stimulating hormone (TSH) and low serum levels of thyroid hormones and free thyroxine (fT4) [3]. On the other hand, increased TSH (> 4.0 to < 10 mU/l for hypothyroidism grade IA and ≥ 10 mU/l for hypothyroidism grade IB) can be accompanied by normal levels of free triiodothyronine (fT3) and fT4 with no clinical symptoms; (sub) hypothyroidism is defined biochemically [4]. At the same time, the upper normal limit of TSH, interpreted via laboratory studies, depends on many factors and conditions [5].

Reduced thyroid hormone levels are usually associated with hypometabolism. This condition is defined as weight gain, decline in resting energy expenditure, reduced gluconeogenesis, and lipolysis. Thyroid dysfunction can provoke obesity, lipid metabolism disorders, which are the components of metabolic syndrome [6, 7]. Hypothyroidism reduces lipolysis and gluconeogenesis, thereby impairing triglyceride clearance and fatty acid β -oxidation, as well as increasing hepatic triglyceride accumulation and low-density lipoprotein reuptake [8].

Non-alcoholic fatty liver disease (NAFLD) is a new global health challenge and its association with other metabolic pathologies has been one of the main research topics in the last decade [9]. The etiological spectrum of NAFLD development involves the presence of comorbidity, viral infections, sociodemographic factors [10, 11]. Furthermore, most patients with NAFLD suffer from undiagnosed primary hypothyroidism [6], which can serve as an inducing factor. Therefore, hypothyroidism-induced NAFLD is regarded as a separate clinical entity [12]. At the same time, the mechanisms of liver dysfunction and morphological changes observed in its damage are not fully understood [7, 8]. This review is aimed to summarize the data regarding the relationship between hypothyroidism and NAFLD, with an accent on morphological alteration discovered in clinical and experimental studies.

Materials and Methods

The search was carried out in Web of Science Core Collection database in March 2021 and updated in October 2021. English-language papers published from 2016 to 2021 were considered. Clinical studies were considered for analysis if they involved patients with hypothyroidism (including sub-clinical hypothyroidism) and coexistent (ultrasound- and/or biopsy-proven) NAFLD. Experimental studies involving animals (rats) with experimentally induced hypothyroidism were included in the review if liver morphology was evalu-

ated by histological methods.

Clinical Studies

Clinical studies play a significant role in understanding NAFLD prevalence (Table 1), its relationship with thyroid pathology; they induce further studies on the pathogenetic mechanisms and the development of new diagnostic and therapeutic methods.

Ultrasound imaging is widely used in clinical diagnosis, being a rather specific and sensitive method for detecting

hepatic steatosis; its non-invasiveness allows for obtaining real-time results [13, 14]. In fatty infiltration in less than 30% of hepatocytes, however, the accuracy of ultrasound imaging has been proven to decrease significantly that justifies the advisability of using the pathohistological method (biopsy) [15, 16]. At the same time, development of fibrosis in NAFLD is a common pathohistological finding as well [17]. This highlights the importance of applying elastography to assess significant and advanced fibrosis [18, 19].

Table 1. NAFLD and hypothyroidism: clinical studies.

#	Authors (year)	Study	Liver Morphology Assessment	Thyroid Function Assessment	Key Findings	Ref
1	D'Ambrosio R et al. (2021)	Retrospective single center study, 52 subjects (ET (38) – 55 (28–72) years, H (14) – 50 (30–73) years)	- Liver biopsy: NAFLD activity score, fibrosis assessment (Kleiner classification) - Stiffness measurement (transient elastography)	TSH, fT3, fT4, rT3, Tg-Ab, TPO-Ab	- Prevalence of NAFLD is higher among patients with hypothyroidism. - No association between hypothyroidism and severity of steatosis and fibrosis was found. - Liver stiffness does not significantly differ in hypo- and euthyroid patients.	[6]
2	Choi SY et al. (2021)	Retrospective multi-center study, 428 subjects (ET (370) – 12.16 ± 2.97 years, SH (58) – 12.19 ± 3.31 years)	- Ultrasonography - APRI score	TSH, fT4	- Subclinical hypothyroidism was found in 13.6% patients with NAFLD. - Subclinical hypothyroidism is significantly associated with steatosis grade (assessed by liver ultrasonography).	[20]
3	Kim HJ. (2020)	Retrospective study, 77 subjects (ET (60) – 9 (1–17) years, SH (17) – 5 (1–17) years)	- Ultrasonography	TSH, fT4.	- The incidence of NAFLD was similar among euthyroid patients and patients with subclinical hypothyroidism. - The presence and severity of NAFLD are not significant risk factors for subclinical hypothyroidism.	[25]
4	Grewal H et al. (2020)	Prospective cross-sectional study, 200 subjects (ET (100) – 46.18 ± 13.90 years, H (100) – 43.79 ± 13.41 years)	- Ultrasonography (evidence and grade of hepatic steatosis)	TSH, T3, T4	- Ultrasonography-proven NAFLD is more prevalent in hypothyroid patients. - The severity of fatty liver disease is higher in hypothyroid patients with higher values of body mass index. - Hypothyroidism is significant risk factor for NAFLD development.	[21]
5	Tahara K et al. (2019)	Cross-sectional study, 140 subjects (ET (70) – 68.3 ± 7.3 years, SH (70) – 69.1 ± 8.1 years)	- Ultrasonography - FIB-4 index	TSH, fT4	- The prevalence of NAFLD and FIB-4 index are significantly higher among patients with subclinical hypothyroidism. - TSH levels are significantly associated with NAFLD.	[22]

#	Authors (year)	Study	Liver Morphology Assessment	Thyroid Function Assessment	Key Findings	Ref
6	Kim D et al. (2018)	Cross-sectional study, 425 subjects (strict-normal TSH level (282) – 52.4±14.9 years, low-normal TSH level (84) – 51.6±16.8 years, SH (59) – 55.4±15.4 years)	- Liver biopsy (hematoxylin and eosin and Masson's trichrome): NAFLD (presence of 5% macrovesicular steatosis) and fibrosis assessment (Kleiner classification) - Ultrasonography	TSH, T4	- Higher grades of hepatic steatosis and more severe degrees of hepatocyte balloon degeneration and fibrosis stage are associated with higher levels of TSH. - Risks of NASH and advanced fibrosis are significantly associated with higher TSH levels.	[23]
7	Bano A et al. (2016)	Prospective cohort study, 9,419 subjects (64.7±9.7 years; H 536)	- Ultrasonography - Stiffness measurement (transient elastography)	TSH, fT4, TPO-Ab	- Increasing NAFLD and risk of clinically relevant fibrosis is associated with increasing TSH levels. - Higher levels of fT4 decrease the risk of NAFLD.	[24]
8	Gökmen F et al. (2016)	Cohort study, 115 subjects (ET (61) – 48.44 ±13.19 years, H (54) – 47.98 ±11.87 years)	- Ultrasonography	TSH, fT3, fT4	- The prevalence of NAFLD did not significantly differ between euthyroid and hypothyroid patients. - The fT3/fT4 ratio significantly differs in patients with and without NAFLD and may serve as NAFLD predictor.	[26]

Notes: ET - euthyroid patients; H - hypothyroid patients; SH - patients with subclinical hypothyroidism.

Regarding liver damage in hypothyroidism, ultrasound and/or biopsy-proven NAFLD is more commonly observed in patients with (subclinical) hypothyroidism [6, 20–22]. Several authors have found that increased TSH concentrations are associated with the severity of pathological changes in the liver such as steatosis, balloon degeneration and fibrosis [20, 21, 23]. In addition, thyroid hypofunction has been noted as a statistically significant risk factor for NAFLD development [21–24]. At the same time, other studies have demonstrated no relationship between the prevalence of NAFLD among euthyroid individuals or patients with (subclinical) hypothyroidism [25, 26], as well as between the severity of steatosis and fibrosis and hypothyroidism [6].

Experimental Studies

Experimental studies play an important role in the determination of morphological alterations or investigation of induced pathological conditions [27]. Thus, Chalouati H *et al.* have found irreversible thyroid changes and hypothyroid state caused by subchronic hexachlorobenzene exposure [28]. According to Jiang LQ *et al.*, amiodarone may affect lipid profile and cause hypothyroidism [29]. Experimental research conducted by Sarkar D *et al.* has shown that biochemically proven hypothyroidism may result from a long-term exposure to excessive iodine [30]. On the other hand,

according to Duan J *et al.*, high iodine and di-n-butyl phthalate exposure exacerbates autoimmune thyroid disease [31].

At the same time, there have been only a few experimental studies on morphologic alterations of the liver in hypothyroidism (Table 2). Such studies differed significantly in their design. Thus, the Wistar and Sprague-Dawley rats were mainly used in the experiment; however, they differed in body weight and age. Hypothyroidism was induced by subcutaneous [32, 33] or intraperitoneal administration [34] of propylthiouracil (PTU), or by adding the reagent to water [35–38], or using intragastric intubation [39]. The duration of administration was quite variable, from 15–21 days [34, 38] to 12 weeks [40].

The assessment of thyroid function is based on the determination of fT3, fT4 and TSH levels. At the same time, the spectrum of biochemical assays of the liver is quite wide, from the determination of basic biochemical markers (AST, ALT) [34] to the assessment of the lipid profile and the state of the prooxidant and antioxidant systems [32, 33, 35, 37, 40]. In most studies, the parameters were determined in both serum and liver homogenates. At the same time, the results were quite different. Several studies indicated no changes in ALT level [32, 33, 37] or its increase [35, 40]. AST level mostly increased [32, 33, 40], underwent no changes [37], or decreased, according to Panda S *et al.* [35]. Regarding the prooxidant and antioxidant systems, in experimental hypothyroidism, the increase

Table 2. Experimental studies on morphological changes in the liver in hypothyroidism.

#	Authors (year)	Experimental Design	Biochemical Assay	Morphological Assay	Key Biochemical and Morphological Findings	Ref
1	Ayuob NN et al. (2019)	Male Wistar rats (180–200 g) Hypothyroidism (PTU 6 mg/kg/BW by intragastric intubation for 6 weeks)	- T3, T4, TSH - GSH, NO, MDA, SOD, CAT, GPX - Gene expression of CAT in supernatant	- Hematoxylin and eosin - Immunohistochemical (streptavidin–biotin–peroxidase technique) - Semi-quantitative analysis (NAFLD activity score)	- Significant increase in MDA and NO. - Steatosis (fatty degeneration), diffusely located micro- and macrovesicles in the hepatocytes. - Steatotic lesion accompanied by intralobular inflammatory reaction (NASH). - CD68+ cell infiltration in the portal and lobular regions.	[39]
2	Tasci HI et al. (2017)	Female Wistar rats (260–320 g) Hypothyroidism + sepsis (10 mg/kg PTU, intraperitoneally for 15 days + sepsis modeling (cecal ligation and perforation))	- fT3, fT4, TSH - AST, ALT	- Hematoxylin and eosin - Semi-quantitative evaluation of inflammation, congestion, Kupffer cell hyperplasia, and hydropic degeneration.	- Increasing AST and ALT levels in both groups. - Negative correlation between fT3 and AST, ALT levels. - Inflammatory cell infiltration and congestion were more severe in sepsis + hypothyroidism rather in sepsis + hyperthyroidism.	[3]
3	Panda S et al. (2021)	Female Wistar rats (165±10 g, 7 weeks old) Hypothyroidism (0.05% PTU in drinking water for 6 weeks)	- T3, T4, and TSH - ALT, AST, TC and TG - Liver tissue: MDA, SOD, CAT, GPX, GSH, protein content	- Hematoxylin and eosin	- Decreasing antioxidants followed by increasing lipid peroxidation (MDA). - Increasing ALT levels accompanied by decreasing AST levels. - Increasing TC and TG levels. - Liver histopathology: irregular orientation of hepatic cords, necrosis in the centrilobular area, and inflammatory cell infiltration.	[4]
4	Bunker SK et al. (2018)	Male Wistar rats (328±19.23 g, age: 330±10 days) Hypothyroidism (0.05% PTU in drinking water for 30 days)	- T3, T4, TSH - Liver tissue: thiobarbituric acid reactive substance (lipid peroxidation)	- Hematoxylin and eosin - Morphometry: counting hepatocytes	- The level of thiobarbituric acid reactive substance significantly increased. - The number of hepatocyte nuclei decreased (17%). - Congestion of hepatocytes and reduction in the sinusoidal space were observed.	[5]
5	Mohibbullah M et al. (2019)	Male SPF/VAF outbred and Sprague-Dawley rats (264–318 g) Hypothyroidism (PTU 10 mg/kg subcutaneously for 28 days)	- T3, T4, TSH - AST, ALT, TC, LDL, HDL, TG - Liver tissue: MDA, H ₂ O ₂ , CAT, SOD	- Hematoxylin and eosin - Morphometry: counting hepatocytes	- Significantly increasing HDL levels and decreasing TG levels. - Significantly increasing H ₂ O ₂ and SOD levels, followed by decreasing CAT levels. - Increasing AST levels, without affecting ALT levels. - Swelling and reduced number of hepatocytes, lipid droplet accumulation.	[1]

#	Authors (year)	Experimental Design	Biochemical Assay	Morphological Assay	Key Biochemical and Morphological Findings	Ref
6	Baki AM et al. (2020)	Male Sprague-Dawley rats (250-350 g) Hypothyroidism (PTU 500 mg/L in drinking water for 10 weeks)	- fT3, fT4 - Glucose, TC, TG, albumin, ALT, AST - Liver tissue: ROS, MDA, SOD, GPX, ferric reducing antioxidant power, oxidative protein damage	- Hematoxylin and eosin	- Decreasing glucose and TG levels. - Increasing TC levels. - No changes in ALT, AST, and albumin levels. - No changes in the levels of MDA and ROS and oxidative protein damage. - No changes in ferric reducing antioxidant power, SOD, GPX levels. - Hydropic degeneration of hepatocytes around central veins followed by sinusoidal congestion.	[37]
7	Lee W-Y et al. (2017)	Male Sprague-Dawley rats (6 weeks old) Hypothyroidism (PTU 10 mg/kg subcutaneously for 28 days)	- ALT, AST, TC, TG, HDL, LDL - Liver tissue: MDA, CAT, SOD, H ₂ O ₂	- Hematoxylin and eosin - Morphometry: counting hepatocytes	- Increasing H ₂ O ₂ , SOD levels and decreasing CAT levels; no changes in MDA levels. - No changes in ALT levels followed by increasing AST levels. - Increasing HDL levels, without changing LDL levels; decreasing TG levels, with no changes in TC levels. - Swelling of hepatocytes with lipid droplet deposition. - Decrease in hepatocyte number/mm ² .	[2]
8	Ustun YB et al. (2018)	Male Wistar rats (200–250 g) Hypothyroidism (PTU in drinking water for 21 days)	- Liver tissue: CAT, GPX, SOD, and MDA	- Hematoxylin and eosin - Immunohistochemical analysis (Ki67, insulin-like growth factor-I) - Semiquantitative analysis + cells counting	- No changes in MDA, CAT, SOD and GPX levels. - The presence of cytoplasmic vacuolization, hypereosinophilia, nuclear pyknosis, necrosis with impairment of hepatocyte cords, bleeding, and neutrophilic infiltrations have been evaluated; however, semiquantitative analysis did not show significant changes.	[7]
9	Demir S et al. (2016)	Male Wistar rats (8-12 weeks, 250-330g) Hypothyroidism (methimazole 0.025% in drinking water for 12 weeks)	- fT3, fT4, TSH - Serum glucose, insulin, visfatin, ALT, AST, TG, HDL, LDL	- Hematoxylin and eosin - Oil Red-O staining	- Increasing AST and ALT, LDL, fasting glucose levels. - Mild steatosis, microscopic hepatosteatosis, microvesicular lipid vacuoles with sharp edges in hepatocytes. Mild parenchymal degeneration. Mild mononuclear cell infiltrations in the portal regions.	[40]

Notes: CAT – catalase; GSH – reduced glutathione; GPX – glutathione peroxidase; HDL, LDL – high- and low-density lipoprotein cholesterol; MDA – malondialdehyde; NO – nitric oxide; PTU – propylthiouracil; SOD – superoxide dismutase; TG – triglycerides; TC – total cholesterol; ROS – reactive oxygen species.

in lipid peroxidation [35, 36, 39] and deterioration in the antioxidant state were observed [32, 33, 35]. At the same time, several studies have found no significant changes in these systems [37, 38].

On the other hand, morphological studies are typical

and are limited to using hematoxylin-eosin. Only several studies used the other histological [40] and immunohistochemical methods [38, 39]. Morphological confirmation is descriptive; the semi-quantitative methods or cell counting are sometimes used [32, 33, 37, 38]. Most studies indicate

hepatocyte swelling, cytoplasmic vacuolization, accumulation of lipid inclusions [32, 33, 37, 38, 40] followed by a decrease in the number of hepatocytes [32, 33, 36] and inflammatory cell infiltration [34, 35, 38–40].

Limitations

This review has several limitations according to its objective. First, the search was carried out in Web of Science Core Collection database only and was limited to the articles published from 2016 to 2021. Second, studies reported liver damage confirmed by biochemical assays and/or surrogate markers only were not considered. In addition, this review did not include the results of studies involving euthyroid patients or subjects with elevated thyroid hormone levels (without hypothyroidism).

Conclusions

According to the results of clinical studies, hypothyroidism is one of the risk factors for NAFLD development. However, it is difficult to make comparison between these studies as they involve groups of subjects who differ in age, gender, geographical location. Experimental morphological studies are mainly focused on descriptive or semi-quantitative histopathological analysis. Therefore, time-dependency between hypothyroidism, severity of NAFLD and progression of morphological alteration is still unclear and requires future studies.

Ethical Statement

This report does not include any human subjects and animals.

Conflict of Interest

The author declares that no conflicts exist.

Financial Disclosure

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