



Association and impact of non-alcoholic fatty liver disease on thyroid function

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Abstract

Nonalcoholic fatty liver disease (NAFLD) means accumulation of fat mainly triglyceride exceeding 5 % of liver weight, affecting approximately 20% of population in developed countries. Thyroid dysfunction have been frequently associated with non-alcoholic fatty liver disease. The aim of this study is to determine any association between non-alcoholic fatty liver disease and thyroid dysfunction and to evaluate the impact of non-alcoholic fatty liver disease on thyroid function tests. Subjects and methods: This study was carried out on sixty adult men and women with non-alcoholic fatty liver disease and sixty age- and sex-matched males and females without the disease. NAFLD patients were subjected to a full assessment of medical history, physical examination, abdominal ultrasonography as well as routine laboratory tests. Patients and controls underwent liver function and thyroid function testing including thyroid antibodies. Results: Thirteen patients with NAFLD (21.7%) were found to have thyroid dysfunction. The most common thyroid dysfunction was subclinical hypothyroidism which was found in 10 (16.7%) NAFLD patients followed by overt hypothyroidism in 3 patients (5%). There were statistically significant difference between patients and controls as regard to TSH ($p=0.0001$), FT4 ($p=0.005$), FT3 ($P=0.043$), anti-TPO ($p=0.047$), anti-TG ($p=0.001$), IR ($p=0.024$), insulin ($p=0.002$), and Leptin ($p=0.002$). TSH concentration was significantly correlated with ALT ($r=0.381$), GGT ($r=0.354$), HbA1C ($r=0.288$), triglyceride ($r=0.368$), FT4 ($r=0.414$), FT3($r=0.231$), anti-TPO($r=0.298$), serum insulin ($r=0.287$), insulin resistance (IR) ($r=0.453$), and serum Leptin ($r=0.360$) levels. In our patients, hepatic steatosis in the form of increased liver echogenicity was present in almost all patients (100%), and hepatomegaly in twenty-four patients (40%). Thus. Hepatic steatosis were correlated significantly with ALT, GGT, HbA1C, triglyceride, HDL-C, TSH, FT4, FT3, anti-TPO, anti-TG, serum insulin, insulin resistance (IR), and serum Leptin. Conclusion: Thyroid dysfunctions are common in NAFLD patients, with subclinical hypothyroidism hypothyroidism being the most prevalent one. Hepatic steatosis has shown an evident positive correlation with elevated TSH as well as thyroid autoantibodies.

Keywords: Non-alcoholic fatty liver disease, Hypothyroidism, Insulin resistance, Hyperlipidemia, Leptin.

Introduction

Nonalcoholic fatty liver disease (NAFLD) is characterized by excessive hepatic accumulation of triglycerides and free fatty acids in the liver (1). The incidence of NAFLD is increasing rapidly, and it is the most common cause of abnormal liver function results worldwide (2). The increase in the prevalence of NAFLD has been attributed to the global increase in the prevalence of obesity and other metabolic risk factors. Advanced age and metabolic disorders such as type 2 diabetes mellitus, impaired glucose tolerance, and central obesity, are among the risk factors for NAFLD (3). Cryptogenic cirrhosis is a term used for patients with liver cirrhosis who lack any identifiable viral, alcoholic, autoimmune or drug-related causes for the condition. Many clinicians now believe that a considerable number of these patients have cirrhosis due to nonalcoholic steatosis hepatitis (NASH) (4). NAFLD has broad pathological spectrum, ranging from simple steatosis to NASH, and potentially progressing to fibrosis and cirrhosis (5). After fat infiltrates the liver, progression to hepatocellular inflammation and fibrosis may occur.

The thyroid gland is significantly involved in energy homeostasis, lipid and carbohydrate metabolism, regulation of body weight and adipogenesis. Subclinical and overt hypothyroidism has been associated with metabolic syndrome, cardiovascular mortality and disturbance in lipid metabolism (6). Hypothyroidism is an endocrine as well as autoimmune disorder. It is caused by decrease in thyroid function. The patient of hypothyroidism is obese. There may have diabetes, hypercholesterolemia, and may present with weight gain, puffy face, hoarse and croaky voice, non-pitting edema in legs, loss of outer third of eyebrow, dry skin and bradycardia (7). There is also delayed relaxation of ankle jerk and hypertension. Laboratory finding of hypothyroidism include low FT3 and FT4 but raised serum level of TSH. Since hypothyroidism is an autoimmune disease, it may be associated with other autoimmune disease like pernicious anemia and diabetes. In this study we try to find out any association between non-alcoholic fatty

liver disease and thyroid abnormalities and to evaluate the impact of non-alcoholic fatty liver disease on thyroid function tests in Egyptian patients.

Materials and Methods

This case-control study consisted of sixty adult men and women with non-alcoholic fatty liver disease in the period between May 2015 to April 2016 and sixty age- and sex-matched males and females without the disease. The diagnosis of NAFLD was based on the results of abdominal ultrasonography, after excluding heavy alcohol consumption, and viral, or other liver diseases. The subjects with NAFLD were drawn from Outpatients Clinic of the Internal Medicine Department of El-Husein University Hospital and the controls were members of staff of the hospital. The Ethical Research and Review Committee of the Hospital approved the study protocol, and informed consent was obtained from the participants.

The inclusion criteria included NAFLD of either sex age 18 years or more. However, the following subjects were excluded: (a) those with renal disease; type 1 or 2 diabetes; glucocorticoid therapy, overt hypothyroidism, and Cushing's disease); (b) those with known thyroid disease, current or past history of thyroid hormone or antithyroid drug intake, thyroid alterations in volume and morphology at ultrasound, (c) those with any laboratory or clinical evidence suggesting an alternate or coexistent underlying chronic liver disease including hepatic virus infections (Hepatitis A-E), autoimmune hepatitis, metabolic hepatic disease, and (d) those with history of alcohol consumption).

All patients were subjected to full medical history and were clinically examined for establishment of fatty liver. Ultrasonography of hepatobiliary system of all patients was done. Blood was examined for total bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), and prothrombin time (PT). Serum triglycerides (TG), Total Cholesterol (TC) and high density lipoprotein (HDL cholesterol)

were measured photo metrically using commercial kit provided from Spinreact, and LDL was calculated by the equation (LDL=cholesterol - triglycerides/5 –HDL). Fasting blood glucose was measured according to glucose-oxidase method. Glycated hamoglobin A1C was measured using a column chromatography method by commercial kit provided from Biosystem Company. After final elution the result was determined photometrically using Biosystem photometer (Reference range 5.1-6.4%). %).

Insulin level: was estimated according to Angel (1988) (8) using a commercially available ELISA kit which was modified for use in microtiter plates. The adapted assay, is based on the binding of porcine anti-guinea pig insulin antibodies to microtiter plates and uses insulinperoxidase conjugate as displacer. IR was calculated using the homeostasis model assessment for IR formula: fasting glucose (mg/dL) X fasting insulin (µU/mL) / 405. **Serum leptin level:** was measured by a commercially available ELISA kit (The DSL- 10-23100 Human Leptin ELISA Kit; Diagnostic System laboratories, Webster, Texas). This assay is a direct Sandwich ELISA based, sequentially, on capture of human leptin molecules from samples to the wells of a microtiter plate coated by pre-titered amount of polyclonal rabbit anti-human leptin antibodies, and wash away of unbound materials from samples, then, binding of a biotinylated monoclonal antibody to the captured human leptin, and conjugation of alkaline phosphatase to

biotinyiated antibodies, after that, wash away of free antibody-enzyme conjugates. Assessment of thyroid function was performed using the following: Thyroid stimulating hormone (TSH): was measured using immunometric assays (IMMULITE 2000 Third Generation; Diagnostic Products Corporation, Los Angles, California, USA), Refrence value was 0.27-4.2 IU/ml. Free serum tri-iodothyronin (FT3) level was determined using IMMULITE 2000FT3, competitive, analog-based immunoassay for quantitative estimation of FT3 in serum on an IMMULITE 2000 system, Refrence value was 2.57-4.43 pg/ml. Free serum thyroxin level (FT4) was measures IMMULITE 2000 FT4, solid phase chemiluminescent competitive immunoassay method for the quantitative determination of FT4 in serum on an IMMULITE 2000 system, Refrence value was 0.93-1.71 ng/ml. Anti-thyreoglobulin (anti-TG) antibodies and anti-thyroid peroxidase (anti-TPO) were assayes using an Immunoradiometric Assay. The refrence values were less than 20 IU/ml for anti-TG antibodies and less than 35 IU/ml for anti-TPO (7).

Results

The mean age of patients was 35.3 ± 12.4 years. Baseline clinical data of patients are shown in table 1.

Table 1: Demographic data for patients and controls:

Demographic data	Patients	Controls	P value
Age (years)	35.3± 12.41	37.0 ± 13.14	0.426
Mean ± SD			
Sex [No. (%)]			
Male	20(33.3%)	20(33.3%)	0.064
Female	40(66.7%)	40(66.7%)	

This table shows no statistically significant difference between patients and controls as regard age and sex.

There were 40 females (66.7%) and 20 males (33.3%).

Ultrasonography of hepatobiliary system to assess the liver echotexture and hepatic steatosis was

done and revealed that twenty-four patients (40%) had hepatomegaly and 100% of patients had both increased liver echogenecity and hepatomegaly as shown in table 2.

Table 2: Ultrasonography of hepatobiliary system

	No. of patients	Percent
Increased liver echogenicity	60	100%
Hepatomegally	24	40%

Baseline laboratory parameters of our patients and controls are shown in table 3. There were statistically significant difference between patients and controls as regard to alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), fasting blood sugar (FBS), glycated hemoglobin (HbA1C), total cholesterol, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C),

triglycerides, thyroid stimulating hormone (TSH), free thyroxine (FT4), free tri-iodothyronine (FT3), anti-thyroid peroxidase (anti-TPO), anti-thyroglobulin (anti-TG), serum insulin, insulin resistance (IR), and serum leptin, but there were no statistically significant difference between our patients and controls as regard to aspartate aminotransferase (AST), total bilirubin, and prothrombin time as shown in table 3.

Table 3: Laboratory parameters of patients and controls:

Parameter	Range \pm SD		P value
	Patients	Controls	
ALT (IU/L)	61.68 \pm 7.38	25.7 \pm 5.46	0.004*
AST(IU/L)	49.73 \pm 7.57	30.17 \pm 2.07	1.186
GGT (IU/L)	94.53 \pm 19.97	64.1 \pm 9.9	0.001*
Total bilirubin (mg/dl)	0.94 \pm 0.18	0.69 \pm 0.13	1.381
Prothrombin time (seconds)	13.67 \pm 1.41	11.57 \pm 0.5	0.542
FBS (mg/dl)	109.57 \pm 7.52	94.47 \pm 5.63	0.037*
HbA1C (%)	6.16 \pm 0.24	4.48 \pm 0.46	0.034*
Total cholesterol (mg/dl)	221.17 \pm 15.7	168.07 \pm 7.6	0.009*
LDL-C (mg/dl)	150.17 \pm 15.4	73.47 \pm 2.74	0.003*
HDL-C (mg/dl)	31.47 \pm 3.6	62.3 \pm 7.47	0.006*
Triglycerides(mg/dl)	196.19 \pm 40.43	139.43 \pm 5.94	0.0001*
TSH (IU/ml)	4.45 \pm 1.39	2.31 \pm 0.81	0.0001*
FT4 (ng/ml)	0.93 \pm 0.18	1.48 \pm 0.2	0.005*
FT3 (pg/ml)	3.12 \pm 0.89	3.34 \pm 0.32	0.043*
Anti-TPO (IU/ml)	33.43 \pm 7.1	21.3 \pm 2.35	0.047*
Anti-TG (IU/ml)	26.37 \pm 7.8	17.6 \pm 1.3	0.001*
Leptin (ng/ml)	21.8 \pm 3.69	10.4 \pm 1.8	0.002*
Insulin(mIU/ml)	13.4 \pm 2.1	7.1 \pm 2.08	0.002*
IR	5.59 \pm 1.16	3.25 \pm 1.21	0.024*

*Statistically significant, ALT= Alanine aminotransferase, AST= Aspartate aminotransferase, GGT= Gamma glutamyl transferase, FBS= Fasting blood sugar, HbA1c=Haemoglobin A1c, LDL-C= Low density lipoprotein, HDL-C= High density lipoprotein, TSH= Thyroid stimulating hormone, FT4= Free thyroxine, FT3= Free tri-iodothyronine, Anti-TPO= Anti-thyroid peroxidase, Anti-TG= Anti-thyroglobulin.

Laboratory thyroid abnormalities were present in 13 patients (21.7%), from whom 10 patients (16.7%) had subclinical hypothyroidism and the

other three patients (5%) had overt hypothyroidism as shown in table 4.

Table 4: Pattern of thyroid profile in fatty liver patients

TSH	Frequency	Percent
Subclinical hypothyroidism	10	16.7
Euthyroid state	47	78.3
Hypothyroidism	3	5

There were statistically significant correlation between serum thyroid stimulating hormone (TSH) in our patients with ALT, GGT, HbA1C, triglyceride, FT4, FT3, anti-TPO, serum insulin, insulin resistance (IR), and serum Leptin

levels but there were no statistically significant correlation between serum TSH with AST, total bilirubin, prothrombin time, FBS, total cholesterol, LDL-C, HDL-C, and anti-TG as shown in table 5.

Table 5: Correlation between serum TSH With laboratory parameters of our patients:

Variables	TSH	P value	
	r		
ALT	0.381	0.002	HS
AST	0.599	1.297	NS
GGT	0.354	0.005	HS
Total bilirubin	0.577	1.390	NS
Prothrombin time	0.770	2.531	NS
FBS	0.637	2.215	NS
HbA1C	0.288	0.04	S
Triglyceride	0.368	0.003	HS
Total cholesterol	0.747	1.661	NS
LDL-C	0.712	1.734	NS
HDL-C	0.590	1.193	NS
FT4	0.414	0.001	HS
FT3	0.231	0.05	S
Anti-TPO	0.298	0.01	S
Anti-TG	0.935	0.782	NS
Leptin	0.360	0.003	HS
Insulin	0.287	0.038	S
IR	0.453	0.002	HS

There were statistically significant correlation between abdominal ultrasonography results (hepatic steatosis) in the form of increased liver echogenicity with or without hepatomegaly with ALT, GGT, HbA1C, triglyceride, HDL-C, TSH, FT4, FT3, anti-TPO, anti-TG, serum insulin,

insulin resistance (IR), and serum leptin but there were no statistically significant correlation between hepatic steatosis with AST, total bilirubin, prothrombin time, FBS, total cholesterol, and LDL-C as shown in table 6.

Table 6: Correlation between the results of abdominal ultrasound (in the form of increased liver echogenicity alone, or both increased liver echogenicity and hepatomegaly) with laboratory parameters of our patients

Variables	Hepatic steatosis		
	r	P value	
ALT	0.267	0.039	S
AST	0.720	1.045	NS
GGT	0.206	0.024	S
Total bilirubin	0.718	1.029	NS
Prothrombin time	0.486	0.087	NS
FBS	0.814	2.592	NS
HbA1C	0.272	0.03	S
Triglyceride	0.444	0.0003	HS
Total cholesterol	0.830	2.371	NS
LDL-C	0.680	2.246	NS
HDL-C	0.415	0.001	HS
TSH	0.413	0.001	HS
FT4	0.382	0.002	HS
FT3	0.273	0.04	S
Anti-TPO	0.296	0.01	S
Anti-TG	0.345	0.002	HS
IR	0.380	0.002	HS
Insulin	0.270	0.02	S
Leptin	0.410	0.001	HS

Discussion

Nonalcoholic fatty liver disease (NAFLD) defines a spectrum of histological abnormalities, from simple fatty liver to nonalcoholic steatohepatitis (NASH), in a person consuming no alcohol (9, 10,11). The main element of NAFLD is the accumulation of triglycerides (TG) as fat droplets within the cytoplasm of hepatocytes, which is a prerequisite for subsequent events of NASH, as more than 5%-10% of hepatocytes have fat droplets, as evident on liver biopsy (10). Increased delivery of both free fatty acids (FFA) and TG to the liver, diminished hepatic utilization of FFA, diminished export of TG from the liver, and impaired beta-oxidation of FFA within hepatocytes cause TG accumulation within the cytoplasm of hepatocytes (12, 13, 14). Excess carbohydrate, either from dietary sources or de novo gluconeogenesis in the liver, is also a major stimulus for de novo fatty acid synthesis in the liver. Thus, fatty liver is caused by failure of normal hepatic fat metabolism either due to a defect within the hepatocyte or to delivery of excess fat, fatty acid and carbohydrate beyond the

secretory capacity for lipids by the liver cells (15). Nonalcoholic fatty liver disease occurs equally in both genders (12). It is generally found within the fourth or fifth decade of life. Patients with NAFLD may have hypertension, besides obesity, type 2 diabetes, and hypertriglyceridemia (14, 16, 20). Many patients have no symptoms. The most frequent symptoms are right upper quadrant pain and dullness in a small number. It may present as diffuse smooth hepatomegaly such as obesity and diabetes mellitus (16,17). The prevalence of NAFLD and NASH is 10% to 40% and about 2% to 5% in the American general adult population, respectively and 20% and 1.2%-4.8% in other developed countries. Hispanics may progress to cirrhosis more frequently than either blacks or whites (18, 19, 20). Historically, an autopsy study showed a prevalence of NASH of 18.5% in markedly obese and 2.7% in lean subjects (20). Studies performed in general populations also showed that the prevalence of NASH is 3% in nonobese and 20% in obese subjects (10,19, 22). The prevalence of NASH related cirrhosis and hepatocellular carcinoma

(HCC) is also high among patients with diabetes, as in obesity (13, 14, 16). As a risk factor, hypertriglyceridemia is also associated with insulin resistance and NAFLD, even in patients without obesity (11, 23).

Hypothyroidism is a common endocrine disorder resulting from deficiency of thyroid hormone or, more rarely, from their impaired activity at tissue level. Prevalence is 1.9% in women, and it increases with age. . It may be a primary process in which the thyroid gland produces insufficient amounts of thyroid hormone (e.g. autoimmune thyroiditis, previous radio-iodine or surgical treatment of hyperthyroidism), but can also be secondary, that is, lack of thyroid hormone secretion due to inadequate secretion of either thyrotropin (that is, thyroid stimulating hormone [TSH]) from the pituitary gland or thyrotropin-releasing hormone (TRH) from the hypothalamus (secondary or tertiary hypothyroidism). The patient's presentation may vary from asymptomatic to, rarely, coma with multisystem organ failure (myxedema coma). The most common cause in the United States is autoimmune thyroid disease with antithyroid peroxidase antibodies (Hashimoto's thyroiditis). Individuals with subclinical hypothyroidism are often asymptomatic, but clinical manifestations can include non-specific complaints or symptoms similar to those seen in overt hypothyroidism, such as fatigue, weakness, weight gain, cold intolerance, and constipation (26). Hypothyroidism is characterized by a generalized reduction in metabolic function that most often manifests as a slowing of physical and mental activity with obesity. They may have diabetes and hypercholesterolemia and may present with weight gain, puffy face, hoarse and croaky voice, nonpitting edema in lower limbs, loss of outer third of eyebrow, dry skin, and bradycardia. There is also delayed relaxation of deep tendon reflexes, and hypertension (24, 25).

The findings from the present study showed a significant increased levels of thyroid stimulating hormone (TSH), anti-thyroid peroxidase (anti-TPO), and anti-thyroglobulin (anti-TG), but a significant low levels of both free thyroxin (FT4) and free tri-iodothyronine (FT3) in our patients as

compared with controls. Also, there was a higher prevalence of thyroid dysfunction in the form of overt and subclinical hypothyroidism among patients with NAFLD. The prevalence of overt and subclinical hypothyroidism in this study was reported to be 5 % and 16.7 % respectively among patients with NAFLD. As compared to healthy controls there was a significantly higher prevalence of hypothyroidism in patients with NAFLD compared to the controls. Our study was in agreement with a study done by Liangpunsakul and Chalasani in which the prevalence of hypothyroidism in patients with non-alcoholic steatohepatitis was 15% was significantly higher than in the controls (7.2%, $P < 0.001$). By multivariate analysis, the prevalence of hypothyroidism in the non-alcoholic steatohepatitis group was significantly higher than in control group (27).

Several studies demonstrated that hypothyroidism is an independent risk factor for NAFLD. This indicates that hypothyroidism may directly result in NAFLD irrespective of other metabolic risk factors. Considering the results of these studies, hypothyroidism may be added to risk factors of NAFLD (28). The findings of our study were correlated with a study done by Chung et al who concluded that subclinical hypothyroidism, even in the range of upper normal TSH levels, was found to be related to NAFLD in a dose-dependent manner. Hypothyroidism is closely associated with NAFLD independently of known metabolic risk factors, confirming a relevant clinical relationship between these two diseases (29).

Previous studies have investigated the association between NAFLD and thyroid function in adult or elderly patients (29, 30, 32, 33). The study by Targher et al. involving a large cohort of unselected adult outpatients was the first to report a strong association between thyroid function tests and serum liver enzyme activity concentrations (33). In particular, Targher et al. found a significant positive relationship between serum TSH, ALT, and GGT activities throughout the normal and high TSH ranges, and a similar inverse relationship between FT4 and serum liver enzyme activity concentrations (33).

In our study, there was a significant positive association between hepatic steatosis (defined by the presence of a hyperechogenic ultrasound pattern of the liver and increased ALT concentrations) with TSH concentration, but inverse associations with FT4 and FT3 concentrations, thus both overt (5%) and subclinical hypothyroidism (SH) (16.7%) in our study was associated with hepatic steatosis. In another two studies, a significant inverse association between the FT4 concentration of NAFLD could be demonstrated, while no significant association could be identified for TT3 or TSH (30, 35). This underscores the importance of the TT4 or FT4 concentration as a marker for hepatic steatosis in the general population. By contrast, the TT3 concentration, in studies done by Ittermann et al. and Xu et al., had no identified value as a marker (31, 34). This finding could related to an inhibition of the conversion of TT4 to TT3, possibly explaining the subordinate diagnostic role of the TT3 or FT3. Even the study of Chung et al., which presented clear evidence of the association between hypothyroidism and NAFLD, did not ascribe any diagnostic value to the TT3 concentration (29). Beside the inverse association with FT4, Chung et al. and Xu et al. identified a positive association between NAFLD and TSH. This association was observed in other studies (32, 35).

In another study by Torun et al, he founded that elevated TSH level with normal FT3 and FT4 concentrations in obese subjects with NAFLD may occur as a result of resistance to thyroid hormones of peripheral tissues and a decreased negative feedback relationship between TSH and peripheral thyroid hormones (37). Subclinical hypothyroidism is also reported to be related to the relative low level of T4 binding protein in obese children (39). Chung et al stated that subclinical hypothyroidism has been found in NAFLD, and it has been reported that hypothyroidism is closely associated with NAFLD (29).

Subclinical hypothyroidism (SCH) is defined as serum TSH level over 4.2 mIU/L with normal FT4(0.93 – 1.71 ng/ml) or FT3(2.57-4.43 pg/ml) concentrations. The most common cause of

subclinical hypothyroidism is autoimmune thyroiditis especially Hashimoto's thyroiditis. Antibodies against TG and TPO are present in almost all patients with Hashimoto's thyroiditis (HT) (40). In addition to aiding the diagnosis, TPO antibodies can be used to help predict development of hypothyroidism, particularly when combined with measurement of TSH levels (41). In our study there was a positive correlation between TSH with anti-TPO antibodies but not correlated with anti-TG antibodies especially in patients with subclinical hypothyroidism, thus the most common cause of subclinical hypothyroidism in our patients may be related to Hashimoto's thyroiditis. Our study was in agreement with a study done by Assem et al. The results of this study revealed that a significant positive correlation between NAFLD and thyroid functions with higher prevalence of subclinical thyroid dysfunction in NAFLD patients. TPO-antibodies in subclinical hypothyroid patients showed significant positive correlation with IR within the total study population. This study concluded that, in non-diabetic patients, IR and thyroid dysfunction have strong correlations with NAFLD and the role of thyroid autoimmunity in this relationship needs further assessment (42). Other studies fail to demonstrate any association between markers of thyroid dysfunction and presence of NAFLD. Despite lower free T3 levels among the NAFLD group, serum TSH, free T4, free T3, and markers of thyroid autoimmunity were not different in the participants with NAFLD and those without NAFLD. However, NAFLD patients were more likely to have low TSH levels although hypothyroidism and hyperthyroidism were not associated with NAFLD. The observed changes in TSH and free T3 levels may attribute to alterations in thyroid hormones due to sick euthyroid syndrome in NAFLD(28, 43).

In our study, there was a significant increased levels of total cholesterol, LDL-C, triglycerides with significant low levels of HDL-C in our patients as compared with controls. Also, thyroid stimulating hormone (TSH) levels were significantly correlated with triglyceride levels but not correlated with total cholesterol, LDL-C, and HDL-C. Lastly, hepatic steatosis in our patients was significantly correlated with triglycerides and HDL-C levels.

Our results correlated with a study by Musso et al, they stated that patients with NAFLD/NASH have abnormal lipid profiles notable for elevated cholesterol, low density lipoprotein and triglyceride levels (44). Thyroid hormones induce their effects on lipid metabolism via thyroid hormone receptor , which is expressed in liver (45). Thyroid hormone receptor activation results in a reduction in body weight and fat as well as a decrease in cholesterol and triglyceride levels, which takes place only in hepatocytes (46, 47). Our study would confirm the correlation between NAFLD and thyroid dysfunction and a correlation between the TSH level and triglycerides (P =0.003). These observations agree with the findings of other studies that suggest a correlation between hypothyroidism and hyperlipidemia (48, 49). The increase in triglycerides in patients with hypothyroidism is explained by the reduced hepatic activity of triglyceride lipase (48, 50) and increased fatty acid oxidation. Loria et al. come to the conclusion that hepatic steatosis may develop from hypothyroidism induced hyperlipidemia and overweight (51). The development of hepatic steatosis may be explained by an increase influx of triglycerides and an imbalance between the in- and outflow of other lipids in the liver.

The findings from our study revealed that, there were significant increased levels of FBS, HbA1C, serum insulin, insulin resistance (IR), and serum leptin in our patients as compared with controls. Plasma levels of TSH were correlated significantly with levels of HbA1C, leptin, insulin and insulin resistance. Also, hepatic steatosis was significantly correlated with HbA1C, serum insulin, insulin resistance and serum leptin levels in our patients.

Our results were in agreement with a study done by Roos et al, and Park et al that identified a significant association between thyroid dysfunction, a hypothyroid metabolic state and the metabolic syndrome (48,50). Metabolic syndrome, in turn, is associated with NAFLD (44, 52, 53) and would point indirectly to a possible correlation between thyroid dysfunction and NAFLD. Non-alcoholic fatty liver disease (NAFLD) is supposed to be a hepatic feature of metabolic syndrome and insulin resistance (54,

55). Hypothyroidism has been reported to be associated with obesity and metabolic syndrome (55, 56, 57). Insulin resistance in the context of hypothyroidism and its alleviation with treatment of the hypothyroidism has been reported (58).

Hypothyroidism was more prevalent in patients with type 2 diabetes and in some reports was associated with diabetic microangiopathy (59). Associations of hypothyroidism with these metabolic abnormalities, which are frequently accompanied by NAFLD, strengthen the notion of association between hypothyroidism and NAFLD. While the underlying pathophysiology for this association is still not clear, several mechanisms have been proposed. The role of adipocytokines in NAFLD (60, 61) has been established previously, and some studies aimed to find a relationship between adipocytokines and hypothyroidism to clarify the mechanism of thyroid dysfunction and NAFLD. An increased level of leptin has been identified in patients with hypothyroidism, and it may be responsible for the development of NAFLD/NASH in this context (62, 63). Leptin is an adipocytokine involved in the regulation of appetite, with an increased level seen in cases of obesity, can induce collagen synthesis in the liver and promotes hepatic insulin resistance (64, 65, 66). Our study was correlated with the study by Aeberli et al. who prospectively examined the associations between changes in thyroid function, IR, and other metabolic risk factors in obese children undergoing weight and fat loss in a well-controlled 8-week inpatient program, changes in TSH did not correlate with losses of weight, fat, or lean tissue (as assessed by dual energy X-ray absorption), but they significantly correlated with fasting insulin and HOMA-IR, independently of body weight and body composition (67). In our study, IR increased as the degree of steatosis increased parallel to increased TSH levels and there was a relationship between TSH and IR in correlation analysis. The results of our study were correlated with the results of Pacifico et al, they stated that IR, leading to impaired hepatic glucose production and glucose uptake in muscle is a component of the MS (68). Recent studies have revealed that subclinical hypothyroidism worsens IR (69). It has been reported that increasing levels of TSH

and decreasing levels of FT4 are associated with increased IR (68).

The present study has some limitations, first it is a short prospective design which permitted an examination of association, not causation. The diagnosis of NAFLD in our study was based on ultrasound data and the severity of liver disease was not confirmed histologically.

Conclusion

The results of the present study confirm an association between increased TSH concentrations and hepatic steatosis. The prevalence of hepatic steatosis rises significantly with elevations in TSH concentrations. An increased TSH concentration appears to be an independent risk factor for hepatic steatosis. There were significant correlations between TSH with fasting insulin, HOMA-IR and Leptin. We found that elevated TSH is a significant predictor of lipid and glucose dysmetabolism, hepatic insulin resistance as well as of hepatic steatosis, independently of the degree of total and central obesity. Nonetheless, the possibility that NAFLD and thyroid hormones share common genetic, autoimmune or environmental influences accounting for the observed association cannot be discounted. However, from this study thyroid hormone profiles may be tested as a part of initial clinical assessment in patients with NAFLD and if treatment of hypothyroidism in patients with NAFLD with thyroid replacement therapy will improve disease progression and outcome or not. Future studies should further elucidate the impact of NAFLD on thyroid hormone parameters. This could serve to clarify the underlying pathogenetic mechanisms and provide potentially important data for diagnostic, therapeutic and preventive measures.

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