International Journal of Advanced Research in Biological Sciences
ISSN: 2348-8069
www.ijarbs.com
DOI: 10.22192/ijarbs Coden: IJARQG(USA) Volume 5, Issue 2 - 2018

Research Article

DOI: http://dx.doi.org/10.22192/ijarbs.2018.05.02.008

Study of Thyroid Functions in Egyptian Patients with Non-Alcoholic Fatty Liver Disease

Arafat Kassem[1], Farag Khalil[1], Mohamed Rashed[2]
[1]Department of Internal Medicine, Faculty of Medicine, Al-Azhar University, Cairo, Egypt
[2]Department of Clinical Pathology, Faculty of Medicine, Al-Azhar University, Cairo, Egypt

Abstract

Background: Thyroid dysfunction has been associated frequently with Non-alcoholic fatty liver disease (NAFLD) and the accumulation of fat mainly triglyceride exceeding 5 % of liver weight affecting approximately 20% of population in developed countries is called non-alcoholic fatty liver disease. The early subclinical hypothyroidism was detected more commonly in patients with (NAFLD). **Objective:** To evaluate the impact of Non-alcoholic fatty liver disease on thyroid function Testsand show the relation between non-alcoholic fatty liver disease and thyroid dysfunction. **Subjects and methods:** This study was done on sixty patients with non-alcoholic fatty liver disease and sixty controls without the disease. All subjects undergo a full medical history, physical examination, abdominal ultrasonography and routine laboratory tests. including liver function, GGT and thyroid function testing including FT3, FT4, TSH, and thyroid antibodies(anti-TG& anti-TPO), HbA1c,insulin, Leptin, Trigyceride and cholesterol**. Results:** Thirteen patients with NAFLD (21.7%) were found to have thyroid abnormalities in the form of subclinical hypothyroidism which is the predominant one in our study found in 10 (16.7%) NAFLD Patients followed by overt hypothyroidism in 3 patients (5%). There was statistically significant difference between patients and controls as regard to Leptin (p=0.002), insulin (p=0.002), IR (p=0.024), TSH (p=0.001), fT3 (P=0.043), FT4 (p=0.005), anti-TG (p=0.001) and anti-TPO (p=0.047). TSH concentration was significantly correlated with ALT (r= 0.359), GGT (r=0.354), FT4 (r=0.414), FT3(r=0.137), anti-TPO(r=0.298), serum insulin (r= 0.139), insulin resistance (IR) (r= 0.453), serum Leptin (r=0.360), , HbA1C (r=0.288), and triglyceride (r=0.368) levels. In our patients, hepatic steatosis in the form of bright hepatic echoes was present in almost all patients (100%), and enlarged liver in twenty-four patients (40%). Thus hepatic steatosis was correlated significantly with TSH, FT4, FT3, anti-TPO, anti-TG, serum insulin, insulin resistance (IR), serum Leptin, ALT, GGT, HbA1C, triglyceride, and HDL-C. **Conclusion:** Thyroid dysfunctions are associated with NAFLD with subclinical hypothyroidism being the most prevalent one with an evident correlation between hepatic steatosis with elevated TSH as well as thyroid autoantibodies.

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

Introduction

A pathological spectrum of chronic liver diseases ranging from simple steatosis to non-alcoholic steatohepatitis (NASH) with inflammation was called Non-alcoholic fatty liver disease (NAFLD), has a high risk for progression to cirrhosis (1-3). NAFLD is an early diagnosis and the most commonly encountered liver pathology in clinical practice (4,5). NAFLD is commonly asymptomatic and discovered incidentally. The most common cause of abnormal liver function results worldwide is NAFLD also it is incidence is increasing rapidly. (6). Only the exclusion criteria, such as alcohol consumption (more than 20 g/day), autoimmune liver disease, viral hepatitis infection, hemochromatosis, Wilson's disease, and drug

consumption is considered in the diagnosis of NAFLD. All of these must be excluded before considering NAFLD (7).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 (8). Liver cirrhosis who lack any identifiable viral, alcoholic, autoimmune or drug-related causes for the condition is defined as cryptogenic cirrhosis. Many clinicians now believe that a considerable number of these patients have cirrhosis due to nonalcoholic steato-hepatitis (NASH) (9). NAFLD has broad pathological spectrum, ranging from simple steatosis to NASH, and potentially progressing to fibrosis and cirrhosis (10). After fat infiltrates the liver, progression to hepatocellular inflammation and fibrosis may occur. Because of the hyperinsulinism, pro-thrombotic potential, and subclinical inflammation associated with NAFLD, patients with this condition are at increased risk for cardiovascular mortality (11). In addition, the correction of insulin resistance may not be sufficient to successfully treat NASH in the majority of patients, conflicting with previous studies on NAFLD pathogenesis (12).

The thyroid gland is significantly involved in lipid and carbohydrate metabolism, regulation of body weight andadipogenesis (13). Thyroid dysfunction in recent studies may play a role in NAFLD. Metabolic syndrome is a combination of cardiovascular mortality, and disturbance of lipid metabolism, insulin resistance plus subclinical hypothyroidism (14, 15). Thyroid dysfunctions in the form of overt or subclinical hypothyroidism are prevalent among patients with NAFLD/ NASH (16). Hypothyroidism is an endocrine as well as autoimmune disorder caused by a decrease in thyroid function. The patient of hypothyroidism is obese and 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 as well as delayed relaxation of ankle jerk and hypertension (17). As hypothyroidism is an autoimmune disease, it may be associated with other autoimmune disease like diabetes mellitus and pernicious anemia. In this study we try to find out any association between non-alcoholic fatty liver disease and thyroid dysfunctions and to assess the impact of non-alcoholic fatty liver disease on thyroid function tests in Egyptian patients.

Materials and Methods

This case–control prospective study was carried out on sixty adult men and women with non-alcoholic fatty liver disease in the period between August 2016 to July 2017 and sixty age- and sex-matched controls without the disease. The diagnosis of NAFLD was based on the results of abdominal ultrasonography, after excluding heavy alcohol consumption, viral, or other liver diseases. The subjects with NAFLD were collected from Outpatient Clinics 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 non-alcoholic fatty liver disease (NAFLD) of either sex age 18 years or more. However, the exclusion criteria include the following: (1) those with type 1 or 2 diabetes; glucocorticoid therapy, overt hypothyroidism, Cushing's disease, and those with renal disease; (2) those with known thyroid disease, current or past history of thyroid hormone or antithyroid drug intake, thyroid alterations in volume and morphology at ultrasound, (3) 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 (4) 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 alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), total bilirubin, and prothrombin time (PT). Total Cholesterol (TC) and high density lipoprotein (HDL cholesterol), serum triglycerides (TG), were measured photo-metrically using commercial kit provided from Spinreact, and LDL was calculated by the equation (LDL=total cholesterol - triglycerides/5 – HDL).Fasting blood glucose was measured according to glucose-oxidase method. Glycated haemoglobin 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%). **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. **Insulin level:** was estimated according to Angel (1988) (18) 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. **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), reference 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, Reference value was 2.57-4.43 pg/ml. Free serum thyroxin level (FT4) was measured using IMMULITE 2000 FT4, solid phase chemiluminescent competitive immunoassay method for the quantitative determination of FT4 in serum on an IMMULITE 2000 system, Reference value was 0.93-1.71 ng/ml. Anti-thyroid peroxidase (anti-TPO) and anti-thyreoglobulin (anti-TG) antibodies were assayed using an immunoradiometric Assay. The reference values were less than 20 IU/ml for anti-TG antibodies and less than 35 IU/ml for anti-TPO (17).

Statistical analysis

The data were analyzed using the statistical package for the Social Sciences Software (version 23.0; SPSS Inc., Chicago, IL) package. Independent Student's *t*-test was used to test the differences in the mean values for the continuous variables. Chi-square test was used to test the differences in the proportion of the categorical variables. The Pearson correlation coefficient (r) was used to determine the correlation between variables. Statistical significance was set at $P < 0.05$.

Results

The mean age of our patients was 35.3 ± 12.4 years. There were 40 females (66.7%) and 20 males (33.3%). No significant differences were found in gender and age as shown in table 1.

Table 1: Demographic data for patients and controls:

Demographic data	Patients	Controls	P value
Sex [No. (%)] Male Female	 20(33.3%) 40(66.7%)	 20(33.3%) 40(66.7%)	0.064
Age (years) Mean ± SD	35.3±12.4	37.1±13.2	0.426

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

Ultrasound of hepatobiliary system was done to assess the liver echotexture and hepatic steatosis and revealed that twenty-four patients (40%) had enlarged liver (hepatomegaly) and 100% of patients had both bright hepatic echoes (increased liver echogenicity) and hepatomegaly as shown in table 2.

Table 2: Ultrasonography of hepatobiliary system

	No. of patients	Percent
Hepatomegaly	24	40%
Bright hepatic echoes	60	100%

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), serum insulin, insulin resistance (IR), and serum leptin, thyroid stimulating hormone (TSH), free thyroxine (FT4), free tri-idothyronin (FT3), anti-thyroid peroxidase (anti-TPO), anti-thyroglobulin (anti-TG), fasting blood sugar (FBS), glycated hemoglobin (HbA1C), total cholesterol, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C) and triglycerides, 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 ± SD		P value
	Patients	Controls	
Total bilirubin	0.94±0.18	0.69±0.13	1.381
AST(IU/L)	49.73 ± 7.57	30.17 ± 2.07	1.186
ALT (IU/L)	61.68±7.38	25.7± 5.46	0.004*
GGT (IU/L)	94.53±19.97	64.1±9.9	0.001*
Prothrombin time (seconds)	13.67±1.41	11.57±0.5	0.542
Leptin (ng/ml)	21.8 ±3.69	10.4 ± 1.8	0.002*
Insulin(mIU/ml)	13.4 ± 2.1	7.1 ± 2.08	0.002*
HOMA-IR	5.59 ± 1.16	3.25± 1.21	0.024*
FT4 (ng/ml)	0.93±0.18	1.48±0.2	0.005*
FT3 (pg/ml)	3.12±0.89	3.34±0.32	0.043*
TSH (IU/ml)	4.45±1.39	2.31±0.81	0.001*
Anti-TG (IU/ml)	26.37±7.8	17.6±1.3	0.001*
Anti-TPO (IU/ml)	33.43±7.1	21.3±2.35	0.047*
FBS (mg/dl)	109.57±7.52	94.47±5.63	0.037*
HbA1C (%)	6.16±0.24	4.48±0.46	0.034*
Total cholesterol (mg/dl)	221.17±15.7	168.07±7.6	0.009*
LDL-C (mg/dl)	150.17±15.4	73.47±2.74	0.003*
HDL-C (mg/dl)	31.47±3.6	62.3±7.47	0.006*
Triglycerides(mg/dl)	196.19±40.43	139.43±5.94	0.001*

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

Laboratory parameters of thyroid dysfunction were present in 13 patients (21.7%):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
Euthyroid state	47		78.3
Subclinical hypothyroidism	10		16.7
Hypothyroidism	3		5

There were statistically significant correlation between abdominal ultrasonography results (hepatic steatosis) in the form of bright hepatic echoes 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 5.

Table 5: Correlation between the results of abdominal ultrasound (in the form of bright hepatic echoes alone, or both bright hepatic echoes and hepatomegaly) with laboratory parameters of our patients

Variables	Hepatic steatosis		
	r	P value	
ALT	0.201	0.002	S
AST	0.039	0.557	NS
GGT	0.206	0.002	S
Total bilirubin	0.098	0.138	NS
Prothrombin time	0.039	0.557	NS
FBS	0.022	0.738	NS
HbA1C	0.272	0.001	HS
Triglyceride	0.444	0.001	HS
Total cholesterol	0.097	0.142	NS
LDL-C	0.005	0.937	NS
HDL-C	-0.334	0.001	HS
TSH	0.519	0.001	HS
FT4	-0.401	0.001	HS
FT3	-0.237	0.001	HS
Anti-TPO	0.205	0.002	S
Anti-TG	0.345	0.001	HS
IR	0.201	0.002	HS
Insulin	0.202	0.002	S
Leptin	0.436	0.001	HS

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 6.

Table 6: Correlation between serum TSH With laboratory parameters of our patients:

Variables	TSH		
	r	P value	
ALT	0.359	0.001	HS
AST	0.039	0.557	NS
GGT	0.354	0.001	HS
Total bilirubin	0.097	0.142	NS
Prothrombin time	0.022	0.738	NS
FBS	0.098	0.138	NS
HbA1C	0.288	0.001	HS
Triglyceride	0.368	0.001	HS
Total cholesterol	0.120	0.070	NS
LDL-C	0.039	0.557	NS
HDL-C	0.099	0.134	NS
FT4	0.414	0.001	HS
FT3	0.137	0.039	S
Anti-TPO	0.298	0.001	HS
Anti-TG	0.089	0.138	NS
Leptin	0.360	0.001	HS
Insulin	0.139	0.023	S
IR	0.453	0.001	HS

Discussion

NAFLD is associated with a wide range of metabolic abnormalities, including glucose intolerance, dyslipidemia, and insulin resistance, and also with atherosclerosis in coronary arteries (19). 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 (20). 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 (21). 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 (22). The prevalence of NASH related cirrhosis and hepatocellular carcinoma (HCC) is also high among patients with diabetes, as in obesity (23). As a risk factor, hypertriglyceridemia is also associated with insulin resistance and NAFLD, even in patients without obesity (24, 25).

Hypothyroidism is autoimmune thyroid disease with antithyroid peroxidase antibodies positive (Hashimoto's thyroiditis) and is the most common cause in the United States. 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 as manifested by 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, bradycardia, delayed relaxation of deep tendon reflexes, and hypertension (27, 28).

The present study show a statistically significant increased levels of thyroid stimulating hormone (TSH), anti-thyroid peroxidase (anti-TPO), and anti-thyroglobulin (anti-TG), with low levels of both free thyroxin (FT4) and free tri-idothyronin (FT3) in our patients as compared with controls with a high 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 with a significant higher prevalence of hypothyroidism in patients with NAFLD when compared to controls. Our study was in agreement with a study done by (29) in which the prevalence of hypothyroidism in patients with non-alcoholic steatohepatitis was 27.4% ($n = 349$). The serum thyroxin (TT4) concentration in subjects with hepatic steatosis was reduced ($p = 0.0004$). Adjusting for age, or BMI, there was an increased prevalence of hepatic steatosis in subjects with reduced TT4 concentrations ($p = 0.0143$; $p = <.0001$).

Also hypothyroidism is an independent risk factor for NAFLD. This indicates that hypothyroidism may directly result in NAFLD irrespective of other metabolic risk factors (30). The findings of our study were correlated with a study done by (31) 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.

Previous studies have investigated the association between NAFLD and thyroid function in adult or elderly patients (16, 17, 29, 30, 31). The study by(17) showed a strong association between thyroid function tests and serum liver enzyme activity concentrations. In particular 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 (32).

In our study, there was a significant positive association between hepatic steatosis (defined by the presence of bright hepatic echoes and increased ALT concentrations) with TSH concentration, but inverse associations with FT4 and FT3 concentrations, thus both overt (5%) and subclinical hypothyroidism (16.7%) respectively was associated with hepatic steatosis. In contrast to (30, 35) show a significant inverse association between the FT4 concentration of NAFLD could be demonstrated, while no significant association could be identified for TT3 or TSH. This underscores the importance of the TT4 or FT4 concentration as a marker for hepatic steatosis in the

general population. In contrast, (33, 34) the TT3 concentration had no identified value as a marker. The explanation of these studies could be related to an inhibition of the conversion of TT4 to TT3, possibly explaining the subordinate diagnostic role of the TT3 or FT3. In a study (35) they founded that there is a clear evidence of the association between hypothyroidism and NAFLD and did not ascribe any diagnostic value to the TT3 concentration. Beside the negative association with FT4 there is a positive correlation between NAFLD and TSH. This correlation was observed in other studies (29, 34, 36). Multiple studies founded that elevated TSH level with normal FT3 and FT4 concentrations in obese subjects with NAFLD may occur as a result of peripheral hormonal resistance and a decreased relationship between TSH and peripheral thyroid hormones (29, 37, 38, 39). Subclinical hypothyroidism is also reported to be related to the relative low level of T4 binding protein in obese children (40). Subclinical hypothyroidism has been found in NAFLD, and it has been reported that hypothyroidism is closely associated with NAFLD (35).

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. Subclinical hypothyroidism is the most widely spread thyroid dysfunction with normal serum levels of free thyroxine (FT4) and elevated serum thyroid stimulating hormone (TSH) levels (41). The most common cause of subclinical hypothyroidism is autoimmune thyroiditis mainly Hashimoto's thyroiditis. Antibodies against thyroglobulin and thyroid peroxidase are present in almost all patients with Hashimoto's thyroiditis (HT) (41). TPO antibodies can be used to help predict development of hypothyroidism, particularly when combined with measurement of TSH levels (35).

The results of this study (31) 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 subjects with subclinical hypothyroidism, the increased liver fat content, and not low thyroid function, is more predictive of metabolic abnormalities. In another study by (42) they concluded thatin non-diabetic patients, insulin resistance (IR) and thyroid dysfunction have strong correlations with NAFLD and the role of thyroid autoimmunity in this relationship needs further assessment (42). 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 despite of elevated or low thyroid hormones. The observed changes in TSH and free T3 levels may attribute to alterations in thyroid hormones due to sick euthyroid syndrome in NAFLD (38, 40).

In our study, there was an elevated levels of total cholesterol, LDL-C, triglycerides with significant low levels of HDL-C in patients as compared with controls. As regard thyroid stimulating hormone (TSH), its concentration was significantly correlated with triglyceride level but not correlated with total cholesterol, LDL-C, and HDL-C. Also hepatic steatosis in patients with NAFLD was significantly correlated with both triglyceride and HDL-C levels. Which was agree with (43) as they revealed a significant positive correlation of the presence of NAFLD with triglyceride and significantly decreasing HDL-C. This is explained by thyroid hormones induce their effects on lipid metabolism via thyroid hormone receptor β, which is expressed in liver (42). 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 (42, 43). Our study would confirm the correlation between NAFLD and thyroid dysfunction and a correlation between the TSH level and triglycerides (P =0.001). These observations agree with the findings of other studies that suggest a correlation between hypothyroidism and hyperlipidemia (43, 44).The increase in triglycerides in patients with hypothyroidism is explained by the reduced hepatic activity of triglyceride lipase and increased fatty acid oxidation (45, 46). Liver steatosis will reduce after treatment of animal models with liver-targeted thyroid hormone receptor agonist (47). Furthermore, hypothyroidism and elevated TSH result in diminished hepatic lipoprotein lipase activity and cause elevated serum triglyceride levels (48). Hepatic steatosis may develop from hypothyroidism induced hyperlipidemia and overweight (49). Thus for hepatic steatosis to occur it may be related to an increase influx of triglycerides and an imbalance between the in-and outflow of other lipids in the liver.

In this study, there were significant increased levels of fasting plasma glucose, glycated A1c, serum insulin, insulin resistance (IR), and serum leptin in NAFLD 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 (19) that identified a significant association between thyroid dysfunction, a hypothyroid metabolic state and the metabolic syndrome. Metabolic syndrome is associated with NAFLD and would point indirectly to a possible correlation between thyroid dysfunction and NAFLD (19). Non-alcoholic fatty liver disease (NAFLD) is supposed to be a hepatic feature of metabolic syndrome and insulin resistance (7, 12). Hypothyroidism has been reported to be associated with obesity and metabolic syndrome (55, 56). Insulin resistance in the context of hypothyroidism and its relief with treatment of hypothyroidism has been reported (7, 12).

Hypothyroidism was more common in patients with type 2 diabetes and in some studies was associated with diabetic microangiopathy (8, 38, 39, 42). Associations of hypothyroidism with these metabolic abnormalities in NAFLD, intensify the idea of association between hypothyroidism and NAFLD.

The role of adipocytokines in NAFLD 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. In our study, both hepatic steatosis and thyroid stimulating hormone were correlated significantly with serum Leptin level ($r < 0.001$). Our results were in agreement with (50) who stated that 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. Others failed to find an association between serum levels of adiponectin and hypothyroidism (51). Leptin is an adipocytokines 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 (52, 53, 54). Our study was correlated with (55) who concluded that TSH plays a relevant role in regulation of leptin metabolism independent of thyroid hormones and the pituitary thyroid axis might be linked to the Leptin system in both thyroid diseases.

In our study, hepatic steatosis and TSH levels were correlated significantly with insulin resistance (HOMA-IR) ($R<0.001$). The results of our study were correlated with the results of (56) they stated that insulin resistance (IR), leading to impaired hepatic glucose production and glucose uptake in muscle is a component of the MS. Recent studies have revealed that subclinical hypothyroidism worsens insulin resistance (IR). It has been reported that increasing levels of TSH and decreasing levels of FT4 are associated with increased IR (57). In a study by Singh et al, they demonstrated that hypothyroidism leads to a state of insulin resistance (58). However in our study patients with hypothyroidism either overt or SCH had significantly higher HOMA- IR values with a significant correlation of TSH with HOMA-IR. (59) was demonstrate a significant interactions between insulin resistance and thyroid function in euthyroid nondiabetic adults. The findings of our study are in accordance with the above mentioned investigations. Obese hypothyroid females that has high TSH levels are associated with the dyslipidemia observed in metabolic syndrome (60). (66) show a significant correlation of triglyceride and HDL levels with HOMA-IR values in human subjects with insulin resistance syndrome (61).

The present study has some limitations, first it is a prospective study with a relatively small number of subjects, which permitted an examination of association, so its ability to infer causality is limited. The diagnosis of NAFLD in our study was based on ultrasound data and the severity of liver disease was not confirmed by liver biopsy for histopathological evaluation. Despite these limitations, the associations we found were highly significant and consistent with other studies addressing the above mentioned association.

Conclusion

The results of the present study confirm an association between increased TSH concentrations and 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 metabolic dysfunction, hepatic insulin resistance as well as of hepatic steatosis and may be related to increased 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 investigated as a part of initial clinical assessment in patients with NAFLD and to screen females with hypothyroidism for evidence of metabolic syndrome. Future studies should further clarify the impact of non-alcoholic fatty liver disease on the causation of thyroid hormone dysfunction and to explain the underlying pathophysiologic mechanisms and to provide an important diagnostic data for both therapeutic and preventive measures.

References

1. Pagadala MR, Zein CO, Dasarathy S, Yerian LM, Lopez R, McCullough AJ. Prevalence of hypothyroidism in nonalcoholic fatty liver disease. Dig Dis Sci. 2012;57(2):528-34, http://dx.doi.org/10.1007/s10620-011-2006-2.
2. Contos MJ, Choudhury J, Mills AS, Sanyal AJ. The histologic spectrum of nonalcoholic fatty liver disease. Clin Liver Dis. 2004;8(3):481-500, vii. http://dx.doi.org/10.1016/j.cld.2004.04.013.
3. Tarantino G, Finelli C, Colao A, Capone D, Tarantino M, Grimaldi E, et al. Are hepatic steatosis and carotid intima media thickness associated in obese patients with normal or slightly elevated gamma-glutamyl-transferase? J Transl Med. 2012;10:50, http://dx.doi.org/10.1186/1479-5876-10-50.
4. Angulo P. GI epidemiology: nonalcoholic fatty liver disease. Aliment Pharmacol Ther. 2007;25(8):883-9, http://dx.doi.org/10.1111/j.1365-2036. 2007.03246.x.
5. Postic C, Girard J. The role of the lipogenic pathway in the development of hepatic steatosis.Diabetes & Metabolism. 2008;34(6, Part 2):643-8, http://dx.doi.org/10.1016/S1262-3636(08)74599-3.
6. Corte CD, Ferrari F, Villani A, Nobili V. EPIDEMIOLOGY AND NATURAL HISTORY OF NAFLD. J Med Biochem 2015; 34 (1): 13 –17.
7. Asrih M, Jornayvaz FR. Metabolic syndrome and nonalcoholic fatty liver disease: Is insulin resistance the link? Mol Cell Endocrinol. 2015.
8. Yamada T, Fukatsu M, Suzuki S, Wada T, Yoshida T, Joh T. Fatty liver predicts impaired fasting glucose and type 2 diabetes mellitus in Japanese undergoing a health checkup. J Gastroenterol Hepatol 2010;25:352-356.
9. Caldwell SH, Lee VD, Kleiner DE, Al-Osaimi AM, Argo CK, Northup PG, et al. NASH and cryptogenic cirrhosis: a histological analysis. Ann Hepatol 2009;8:346-352.
10. Matteoni CA, Younossi ZM, Gramlich T, Boparai N, Liu YC, McCullough AJ. Nonalcoholic fatty liver disease: a spectrum of clinical and pathological severity. Gastroenterology 1999;116:1413-1419.
11. Maurantonio M, Ballestri S, Odoardi MR, Lonardo A, Loria P. Treatment of atherogenic liver based on the pathogenesis of nonalcoholic fatty liver disease: a novel approach to reduce cardiovascular risk? Arch Med Res. 2011;42(5):337-53, http://dx.doi.org/10.1016/j.arcmed.2011.08.004.
12. Lonardo A, Bellentani S, Ratziu V, Loria P. Insulin resistance in nonalcoholic steatohepatitis: necessary but not sufficient - death of a dogma from analysis of therapeutic studies? Expert Rev Gastroenterol Hepatol. 2011; 5(2):279-89, http://dx.doi.org/10.1586/egh.11.19.
13. Michalaki MA, Vagenakis AG, Leonardou AS, Argentou MN, Habeos IG, Makri MG, et al. Thyroid function in humans with morbid obesity. Thyroid. 2006;16(1):73-8, http://dx.doi.org/10.1089/thy.2006.16.73.
14. Rodondi N, den Elzen WP, Bauer DC, Cappola AR, Razvi S, Walsh JP, et al. Subclinical hypothyroidism and the risk of coronary heart disease and mortality. JAMA. 2010;304(12):1365-74, http://dx.doi.org/10.1001/ jama.2010.1361.
15. Amarapurkar D, Kamani P, Patel N, Gupte P, Kumar P, Agal S, et al. Prevalence of non-alcoholic fatty liver disease: population based study. Ann Hepatol. 2007;6(3):161-3.
16. HASAN B F , IBRAHIM N A, DUAA A A. ESTIMATION OF THYROID HORMONES AND LIVER ENZYMES LEVELS IN HYPO AND HYPERTHYROIDISM IN IRAQI WOMEN. Int J Pharm Bio Sci 2016; 7(4): (B) 707 – 713.
17. Alam MJ, Rahman S, Al-Matab M. Association of fatty liver and hypothyroidism.Euroasian J Hepato-gastroenterol 2013, 3(1): 8-9.
18. Angel I (1988) The use of microtiter plates for the simple and sensitive determination of insulin by an ELISA method. Experientia 44: 877-879.

19. Ginsberg HN, Zhang YL & Hernandez-Ono A. Metabolic syndrome: focus on dyslipidemia. Obesity 2006 14 (Suppl 1) 41S–49S. (doi:10.1038/oby.2006.281)
20. Ahmed A, Wong RJ, and Harrison SA. Nonalcoholic Fatty Liver Disease Review: Diagnosis, Treatment, and Outcomes. Clinical Gastroenterology and Hepatology 2015;13:2062–2070.
21. Rentapalli BR, Siraj AK, Dawood S, Iqbal A , Jaya LG, Laxminarayana V. Study of Lipid Profile in Non-Alcoholic Fatty Liver Disease. IOSR Journal of Dental and Medical Sciences (IOSR-JDMS). 2017:16, 11: 46-49.
22. Bacon BR, Faravash MJ, Janny CG, et al. Non-alcoholic steatohepatitis: An expanded clinical entity. Gastroenterology 1994; 107:1103.
23. Yang SQ, Lin HZ, Lane MD, Clemens M, Diehl AM. Obesity increases sensitivity to endotoxin liver injury: implications for the pathogenesis of steatohepatitis. Proc Natl Acad Sci USA 1997; 94: 2557-62.
24. Miyake T, Kumagi T, Hirooka M, et al. Significance of exercise in nonalcoholic fatty liver disease in men: a community-based large cross-sectional study. J Gastroenterol 2014;25: 127-32.
25. Tilg H, Moschen AR. Evolving therapies for non-alcoholic steatohepatitis. Expert Opin Drug Discov 2014; 9: 687-96.
26. Bello F and Bakari A. G. Hypothyroidism in adults: A review and recent advances in management. Journal of Diabetes and Endocrinology 2012;3(5):57-69.
27. Nicoloff JT, LoPresti JS (2007). Hypothyroidism. In: Rakel RE, Bope ET, (eds), Conn's Current Therapy. Saunders Elsevier, Philadelphia. pp. 766-771.
28. Biondi B, Cooper DS. The clinical significance of subclinical thyroid dysfunction. Endocr Rev. 2008;29(1):76–131.
29. Ludwig U, Holzner D, Denzer C, Greinert A, Haenle MM, Oeztuerk S, Koenig W, Boehm OB, Mason RA, Kratzer W, Graeter T, and the EMIL-Study. 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 Endocr Disordv.15; 2015. PMC4536732
30. Eshraghian A, Hamidian Jahromi A. Non-alcoholic fatty liver disease and thyroid dysfunction: A systematic review**.** *World J Gastroenterol* 2014; 20(25): 8102-8109.
31. Carlos PR, Esteban JG, Rosalinda PS, Jorge AV, Juan GRojas, Eric KH, Aida MU and Guillermo CS. Fatty liver largely explains associations of subclinical hypothyroidism with insulin resistance, metabolic syndrome, and subclinical coronary atherosclerosis. European Journal of Endocrinology (2014) 171, 319–325.
32. Targher G, Montagnana M, Salvagno G et al., "Association between serum TSH, free T4 and serum liver enzyme activities in a large cohort of unselected outpatients," *Clinical Endocrinology*, vol. 68, no. 3, pp. 481–484, 2008.
33. Prabhakar A, Ambili N. R., Kartha U.T. D., Renymol B. Prevalence of non-alcoholic fatty liver disease (NAFLD) in patients with type 2 diabetes mellitus and its correlation with coronary artery disease (CAD). Int J Res Med Sci. 2017 Dec;5(12):5175-5181
34. Moustafa AH, Ali EM, Mohamed TM, Abdou HI. Oxidative stress and thyroid hormones in patients with liver diseases. Eur J Intern Med. 2009;20:703–8.
35. G. E. Chung, D. Kim, W. Kim et al., "Non-alcoholic fatty liver disease across the spectrum of hypothyroidism," *Journal ofHepatology*, vol. 57, no. 1, pp. 150–156, 2012.
36. L. Carulli, S. Ballestri, A. Lonardo et al., "Is nonalcoholic steatohepatitis associated with a high-though-normal thyroid stimulating hormone level and lower cholesterol levels?" *Internal and Emergency Medicine*.*Intern Emerg Med* 2013; **8**: 297-305.
37. Torun E, Ozgen IT, Gokce S, Aydın S, Cesur Y. *Thyroid Hormone Levels in Obese Children and Adolescents with Non-Alcoholic Fatty Liver Disease*.J Clin Res Pediatr Endocrinol 2014;6(1):34-39
38. Park HT, Cho GJ, Ahn KH, Shin JH, Hong SC, Kim T, et al. Thyroid stimulating hormone is associated with metabolic syndrome in euthyroid postmenopausal women. Maturitas. 2009;62:301–305. doi: 10.1016/j.maturitas.2009.01.007. [PubMed] [Cross Ref]
39. Roos A, Bakker SJ, Links TP, Gans RO, Wolffenbuttel BH. Thyroid function is associated with components of the metabolic syndrome in euthyroid subjects. J Clin Endocrinol Metab. 2007;92:491–496. doi: 10.1210/jc.2006-1718. [PubMed] [Cross Ref]
40. Bilgin H, Pirgon O. *Thyroid Function in Obese Children with Non-Alcoholic Fatty Liver Disease.* J Clin Res Pediatr Endocrinol 2014;6(3):152-157.

41. Cooper, D., 2001. "Subclinical hypothyroidism", N Engl J Med., Vol. 345(4), pp.260-265, http://dx.doi.org/10.1056/NEJM200107263450406
42. AssemM, FawziM, IbrahimA & SaifA. Assessment of thyroid functions, thyroid autoimmunity and insulin resistance in non-diabetic patients with non-alcoholic fatty liver disease**.** Endocrine Abstracts (2017) 49 EP1240.
43. Mahmoud H'S, Helal MM, Hassan MH, Sherif MF. Correlation between Anthropometric Measures, Lipid Profile and Serum Adiponectin and Steatosis in Nondiabetic Nonalcoholic Fatty Liver Disease. British Journal of Medicine and Medical Research, 2015; 7(9):771- 8. https://doi.org/10.9734/BJMMR/2015/1659448.
44. Hulbert AJ. Thyroid hormones and their effects: a new perspective. Biol Rev Camb Philos Soc. 2000;75:519–631. [PubMed]
45. Grover GJ, Mellström K, Ye L, Malm J, Li YL, Bladh LG, Sleph PG, Smith MA, George R, Vennström B, et al. Selective thyroid hormone receptor-beta activation: a strategy for reduction of weight, cholesterol, and lipoprotein (a) with reduced cardiovascular liability. Proc Natl Acad Sci USA. 2003;100:10067–10072.[PMC free article] [PubMed]
46. Erion MD, Cable EE, Ito BR, Jiang H, Fujitaki JM, Finn PD, Zhang BH, Hou J, Boyer SH, van Poelje PD, et al. Targeting thyroid hormone receptor-beta agonists to the liver reduces cholesterol and triglycerides and improves the therapeutic index. Proc Natl Acad Sci USA. 2007;104:15490–15495.[PMC free article] [PubMed]
47. Cable EE, Finn PD, Stebbins JW, Hou J, Ito BR, van Poelje PD, Linemeyer DL, Erion MD. Reduction of hepatic steatosis in rats and mice after treatment with a liver-targeted thyroid hormone receptor agonist. Hepatology. 2009;49:407–417. [PubMed]
48. Brenta G, Berg G, Arias P, Zago V, Schnitman M, Muzzio ML, Sinay I, Schreier L. Lipoprotein alterations, hepatic lipase activity, and insulin sensitivity in subclinical hypothyroidism: response to L-T(4) treatment. Thyroid. 2007;17:453–460. [PubMed]
49. Loria P, Carulli L, Bertolotti M, Lonardo A. Endocrine and liver interaction: the role of endocrine pathways in NASH. Nat Rev Gastroenterol Hepatol. 2009;6:236–47.
50. Kautzky W A, Ludwig C, Nowotny P, Roden A, Huemer C, Widhalm K, Vierhapper H, Waldhäusl W, Roden M. Elevation of plasma leptin concentrations in obese hyperinsulinaemic hypothyroidism before and after treatment. Eur J Clin Invest. 1999;29:395–403. [PubMed]
51. Leonhardt U, Ritzel U, Schäfer G, Becker W, Ramadori G. Serum leptin levels in hypo- and hyperthyroidism. J Endocrinol. 1998;157:75–79. [PubMed]
52. Oswal A, Yeo G. Leptin and the control of body weight: a review of its diverse central targets, signaling mechanisms, and role in the pathogenesis of obesity. Obesity (Silver Spring) 2010;18:221–229.[PubMed]
53. Saxena NK, Ikeda K, Rockey DC, Friedman SL, Anania FA. Leptin in hepatic fibrosis: evidence for increased collagen production in stellate cells and lean littermates of ob/ob mice. Hepatology. 2002;35:762–771. [PMC free article] [PubMed]
54. Benomar Y, Wetzler S, Larue-Achagiotis C, Djiane J, Tomé D, Taouis M. In vivo leptin infusion impairs insulin and leptin signalling in liver and hypothalamus. Mol Cell Endocrinol. 2005;242:59–66.[PubMed]
55. OGE A, BAYRAKTAR F, SAYGILI F, GUNEY E, and DEMIRS. TSH influences serum Leptin levels independent of thyroid hormones in hypothyroid and hyperthyroid patients. Endocrine journal 2005, 52 (2): 213-217.
56. Pacifico L, Anania C, Ferraro F andreoli GM, Chiesa C. Thyroid function in childhood obesity and metabolic comorbidity. Clin Chim Acta 2012;413:396-405. Epub 2011 Nov 27
57. Maratou E, Hadjidakis DJ, Kollias A, Tsegka K, Peppa M, Alevizaki M, Mitrou P, Lambadiari V, Boutati E, Nikzas D, Tountas N,Economopoulos T, Raptis SA, Dimitriadis G. Studies of insulin resistance in patients with clinical and subclinical hypothyroidism. Eur J Endocrinol 2009;160:785-790. Epub 2009 Jan 13.
58. Singh BM. GoswamiB, and Mallika V ASSOCIATION BETWEEN INSULIN RESISTANCE AND HYPOTHYROIDISM IN FEMALES ATTENDING A TERTIARY CARE HOSPITAL. Indian Journal of Clinical Biochemistry, 2010 / 25 (2) 141-145

59. Bakker SJL, Ter Maaten JC, Popp-Snijders C, Slaets JPJ, Heine RJ, Gans ROB. The relationship between thyrotropin and low density lipoprotein cholesterol is modified by insulin sensitivity in healthy euthyroid subjects. J Clin Endocrinol Metab 2001; 86: 1206-11.
60. Demidova T, Galieva OR. The role of thyroid hypofunction in development of metabolic syndrome. Ter Arkh 2009; 81(4): 69-73. 24.
61. Khan SH, Khan FA, Ijaz A, Sattar A, Dilawar M, Hashim R. Spectrum of lipid and lipoprotein indices in human subjects with insulin resistance syndrome. J Ayub Med Coll Abbottabad 2008; 20(3): 17-22.

Access this Article in Online	
	Website: www.ijarbs.com
Quick Response Code	Subject: Medical Sciences
DOI:10.22192/ijarbs.2018.05.02.008	

How to cite this article:
Arafat Kassem, Farag Khalil, Mohamed Rashed. (2018). Study of thyroid functions in Egyptian patients with non-alcoholic fatty liver disease. Int. J. Adv. Res. Biol. Sci. 5(2): 70-81.
DOI: http://dx.doi.org/10.22192/ijarbs.2018.05.02.008