Evaluation of Serum Thyroid Hormones and Thyroid Stimulating Hormone Levels among Non Alcoholic Fatty Liver Disease Patients in Khartoum State

A dissertation submitted in partial fulfillment for the requirements of M.Sc Degree in Medical Laboratory Science- Clinical Chemistry

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قال تعالى:

( قالوا سبحانك لا علم لنا إلا ما علمتنا إنك أنت العليم الحكيم )

صدق الله العظيم

سورة البقرة اية 32
DEDICATION

The reason of what I become today

To the soul of my great father

Whose affection, love, encouragement and prays of day and night to me

My beloved mother

To those who habitually support me and touched my life

My lovely brothers, sister and friends
Acknowledgment

Thank you my God, for giving me the ability and courage to bring research to light.

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<td>TM6SF2</td>
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<td>Ultrasoundgraphy</td>
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<td>VLDL</td>
<td>Very low density lipoproteins</td>
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<td>WBC</td>
<td>White blood cell</td>
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Abstract

Background: Non-alcoholic fatty liver disease (NAFLD) is one of the most common problems of liver worldwide. Recently, relationship between thyroid dysfunction and NAFLD has been discussed. The current study aims to evaluate serum levels thyroid hormones and thyroid stimulating hormone and its correlation with study variables among NAFLD patients.

Materials and methods: In a cross-sectional study (n 80) subjects were enrolled, they classified as (n 40) clinically diagnosed NAFLD and (n 40) apparently health as control group, age between 30 to 80 years old, during January to March 2017 in Khartoum State. Data was collected using standardized questionnaire. Serum TSH, free T₃ and free T₄ were measured using Automated Immune assay Analyzer 360 (TOSOH).

Results: Independent t-test analyses showed significant increase in TSH level in NAFLD p-value 0.002, while significant decreases were observed in mean concentration of free T₃ and free T₄ in comparison with healthy group p-value 0.000 and 0.000 respectively. In addition there was significant increase in TSH level in males with NAFLD p-value 0.010 while significant decrease were observed in mean concentration of free T₃ and free T₄ in comparison with female with NAFLD p-value 0.006 and 0.009 respectively. Also the study revealed significant positive correlation between TSH level and BMI p-value 0.000 and R-value 0.596 and significant negative correlation between free T₃ and free T₄ with BMI p-value 0.000 and 0.000 respectively, and R-value -0.620 and -0.835 respectively. And have no correlation between serum level of TSH, free T₃ and free T₄ with age group.

Conclusion: The data of present study concluded that, serum free T₃ and free T₄ level are lower in NAFLD, while TSH is higher. Females have higher free T₃, free T₄ and lower TSH than males. BMI correlate positively with TSH and negatively with free T₃ and free T₄. Thus monitoring of thyroid hormones is recommended for NAFLD patients.
مستخلص الدراسة

الخلفية: مرض الكبد الدهني غير الكحولي هو واحد من المشاكل الأكثر شيوعاً من امراض الكبد في جميع أنحاء العالم، وقد نوقشت مؤخرا العلاقة بين خلل الغدة الدرقية وهذا المرض. تهدف الدراسة الحالية إلى تقييم مستويات هرمونات الغدة الدرقية والهرمون المحفز للغدة الدرقية في الدم وارتباطها بمتغيرات الدراسة بين مرضى الكبد الدهني غير كحولي.

المؤول والطريق: في دراسة مستعرضة شارك فيها 80 شخص، وتم تصنيفهم إلى مجموعتين: الأولى 40 شخص مصاب بمرض الكبد الدهني غير كحولي و 40 شخص غير مصاب بالمرض، وتجاوز أعمارهم بين 30 إلى 80 سنة وقد أجريت هذه الدراسة خلال يناير إلى مارس 2017 في ولاية الخرطوم. تم جمع البيانات باستخدام استبيان موحد. وتم قياس TSH, free T3, free T4 في مصل الدم باستخدام محصل المناعة (TOSOH).

النتائج: أظهرت نتائج التحليل الإحصائي أن هناك زيادة معنوية في مستوى TSH (p-value 0.002) وانخفاض معنوي في متوسط تركيز free T3 و free T4 (p-value 0.000 و 0.000) على التوالي وتذكر تركيزات هرمونات الغدة الدرقية بين المصابين بالمرض بالمقارنة مع الأصحاء. بالإضافة إلى ذلك كان هناك زيادة كبيرة في مستوى TSH في الذكور المصابين بالمرض (p-value 0.010) بينما لوحظ انخفاض كبير في متوسط تركيز TSH في الإناث المصابات بالمرض (0.006 و 0.009) على التوالي. كما أظهرت الدراسة وجود علاقة ارتباط إيجابي بين مستوى TSH بالمقارنة مع الإناث المصابات بالمرض (0.006 و 0.009) ورتباط سلبي كبير بين الجسم (R-value 0.596 و 0.000) مع قيمة مؤشر كتلة الجسم و p-value 0.062 و 0.835 على التوالي.

وليس هناك أي علاقة بين مستوى مصل TSH و الفئة العمرية. 

الاستنتاج: من النتائج تم التوصل إلى أن هناك زيادة في مستوى TSH وانخفاض في مستويات TSH و انخفاض في مستوى TSH لدى المصابين بمرض الكبد الدهني غير كحولي بالمقارنة مع الأصحاء. الإناث لديها free T3, free T4 وتركيز أعلى من T3 و انخفاض T3, free T4 بالمقارنة مع الذكور. ومؤشر كتلة الجسم يرتبط TSH, free T3, free T4 وإيجابيا مع TSH وسلبية مع free T3, free T4 وبالتالي تقويم هرمونات الغدة الدرقية مهم لمرضى الكبد الدهني غير كحولي.
Chapter one

Introduction and literature review
1.1 Introduction:
Non alcoholic fatty liver disease (NAFLD) is characterized by excessive fat accumulation in the liver (hepatic steatosis). Non alcoholic steatohepatitis (NASH) is characterized by steatosis, liver cell injury, and inflammation. The mechanism of NAFLD is unknown but involves the development of insulin resistance, steatosis, inflammatory cytokines and oxidative stress. NAFLD is associated with physical inactivity, obesity and metabolic syndrome. Screening is not recommended in the general population. The diagnosis is usually made after an incidental discovery of unexplained elevation of liver enzyme levels or when steatosis is noted on imaging (Wilkins et al., 2013). NAFLD represents a broad clinical spectrum ranging from simple fatty liver to NASH, which may progress to liver fibrosis, cirrhosis and hepatocellular carcinoma. NAFLD is a rapidly growing diagnosis, and it is the most common cause of abnormal liver function tests worldwide. The growing pattern of NAFLD prevalence is generally attributed to a global increase in the prevalence of obesity and other metabolic risk factors. Advanced age and metabolic disorders, such as diabetes type 2, impaired glucose tolerance and central obesity, are among the risk factors for NAFLD. Cryptogenic cirrhosis is a term used for those patients with liver cirrhosis who lack any identifiable viral, alcoholic, autoimmune or drug-related cause of the condition. Many clinicians now believe that a considerable number of these patients have cirrhosis due to NASH. Considering the increasing incidence of NAFLD/NASH, especially in developed and developing countries, it is anticipated that cirrhosis due to these conditions may surpass other causes of cirrhosis in a near future. Therefore, understanding the pathophysiology, risk factors and new treatment options of NAFLD/NASH should be among the priorities in the field of hepatology (Eshraghian and Hamidian, 2014).

The thyroid gland is thoroughly involved in the cell metabolism, energy homeostasis, regulation of body weight, thermogenesis, lipid and carbohydrate metabolism and adipogenesis (Michalaki et al., 2006). Previous studies regarding
the association between thyroid dysfunction and NAFLD have yielded controversial results, varying from strong (Chung et al., 2012; Pagadala et al., 2012), to no association (Eshraghian et al., 2013; Mazo et al., 2011).

1.2 Liver:
The liver is the largest internal organ of the human body. It is a functionally complex organ that plays a critical biochemical role in the metabolism, digestion, detoxification and elimination of substances from the body (Bishop et al., 2010).

1.2.1 Anatomy of liver:
The liver is the largest organ, accounting for approximately 2% to 3% of average body weight. The liver has 2 lobes typically described in two ways, by morphologic anatomy and by functional anatomy. Located in the right upper quadrant of the abdominal cavity beneath the right hemidiaphragm, it is protected by the rib cage and maintains its position through peritoneal reflections, referred to as ligament as attachments. Although not true ligaments, these attachments are a vascular and are in the continuity with the Gilson capsule or the equivalent of the visceral peritoneum of the liver (Abdel-Misih and Bloomston, 2014).

1.2.2 Biochemical functions:
The liver performs four major functions: excretion/secretion, synthesis, detoxification and storage. The liver is so important that if the liver becomes nonfunctional, death will occur with 24 hours due to hypoglycemia (Bishop et al., 2010).

An essential function of the liver is protein synthesis and metabolism, including the metabolism of amino acids, carbohydrates, lipids and vitamins. However, the liver is also responsible for the removal of pathogens and exogenous antigens from the systemic circulation. The key position of the liver and its unique vasculature allow it to carry out the degradation of toxins and waste products. The role of the liver as the main metabolic organ increases the rate of exposure to newly produced neo-antigens and enhances the inherited risk of over activation of components of the immune system with potentially harmful consequences for
cell homeostasis. Thus, the immune system developed dedicated mechanisms to be able to “switch” from a tolerant to a responsive state at any given time. Early in the history of experimental transplantation, transplant surgeons were intrigued to note that while kidney, skin, pancreas and other allograft were rapidly rejected and allogeneic liver grafts were more tolerant. This prompted investigators to consider that the liver is predominantly an organ biased towards tolerance rather than a reactive state which would otherwise lead to rejections. The scientific basis for this tolerant state remained elusive for many years (Bogdanos et al., 2013).

1.2.3 Pathology of Liver:
Liver has a limited numbers of ways of responding to injury. Acute injury to the liver may be a symptomatic include acute hepatitis and cholestasis and chronic liver injury is long term complication include cirrhosis and hepatocellular carcinoma (HCC) (Buritis et al., 2008).

1.2.3.1 Liver Cirrhosis:
Cirrhosis is defined as the histological development of regenerative nodules surrounded by fibrous tissues in response to chronic liver injury that leads to portal hypertension and end stage liver disease. Cirrhosis is an advanced stage of liver fibrosis that is accompanied by distortion of the hepatic vasculature. It leads to shunting of the portal and arterial blood supply direct into the hepatic outflow (central veins), compromising exchange between hepatic sinusoid (Schuppan and Afdhal, 2009).

1.2.3.2 Hepatocellular Carcinoma (HCC):
HCC is the fifth most common cancer in men and seventh in women worldwide. Most of the burden of disease 85% is borne in developing countries with highest incidence rates reported in regions with hepatitis B virus is endemic, and HCC related to infection with hepatitis C virus has become the fastest rising cause of cancer related death in United State (El-serag, 2011). HCC has recently been linked to NAFLD, the hepatic manifestation of obesity and related metabolic
disorders such as diabetes. This association is alarming due to the globally high prevalence of these conditions and may contribute to the rising incidence of HCC witnessed in many industrialized countries (Buffy et al., 2012).

1.2.3.3 Hepatitis:
Means inflammation of liver, have different types include:
Hepatitis C virus (HCV) infection affects approximately 3% of the global population, or about 170 million people. Approximately 75-85% of infected persons will progress to chronic HCV infection and are at risk for development of extra hepatic manifestations, compensated and decompensate cirrhosis and HCC (Chen and Morgan, 2006).
Hepatitis B virus (HBV) a DNA virus transmitted percutaneously, sexually and prenatally. This affects 350 to 400 million peoples worldwide and HBV infection accounts annually for 1 million deaths worldwide from cirrhosis, liver failure and HCC. Progression from acute to chronic infection is influenced by patient's age at acquisition of the virus (Jules and Dienstag, 2008).
Hepatitis A is first introduced in 1967, is known to be a liver infection caused by hepatitis A virus (HAV) whose primary replication site is in hepatocyte, which is a positive sense, single stranded RNA virus that belong to family of picornaviridae. More than 70% of cases of HAV infection occur in children less than 6 years old are asymptomatic. However in older children and adult HAV infection causes more sever clinical illness including jaundice, malaise, fever and dark urine. Most cases of hepatitis A can explained by fecal-oral transmission of the virus(Yong and Son, 2009).

1.2.3.4 Fatty liver:
Fatty liver disease is potentially a reversible condition where in large vacuoles of triglyceride fat accumulate in liver cells via the process of steatosis. Despite having multiple causes fatty liver can be considered a single disease that occur worldwide in those with excessive alcohol intake and the obese with or without
effect of insulin resistance. The condition is also associated with other disease that influence fat metabolism (Reddy and Rao, 2006).

1.2.3.5 Nonalcoholic fatty liver disease:
NAFLD is defined as the presence of hepatic steatosis with no evidence of hepatocellular injury in the form of ballooning of the hepatocytes. NASH is defined as the presence of hepatic steatosis and inflammation with hepatocyte injury with or without fibrosis (Chalasani et al., 2012).
And also characterized by excessive hepatic fat accumulation, associated with insulin resistance, and defined by the presence of steatosis in >5% of hepatocytes according to histological analysis or by a proton density fat fraction (providing a rough estimation of the volume fraction of fatty material in the liver) (Byrne and Tragher, 2016).

**Epidemiology:** The prevalence of NAFLD has increased as more patients develop a sedentary lifestyle, metabolic syndrome, and obesity. It is correlated with many factors, including body mass index (BMI), fat distribution, race, ethnicity and sex. It is the most common liver disease in Western countries, with a prevalence of 27% to 38%. In the United States, the prevalence in the community-based Framingham heart study population was 17% (19% in men and 15% in women), but the prevalence approaches 90% in patients considering bariatric surgery. Patients with non alcoholic fatty liver disease have 26% higher health care costs at five-year follow-up (Wilkins et al., 2013).

**Etiology:** The etiology of NAFLD and its progression is complex and remains incompletely understood. It is clearly multifactorial. Many cases are related to a “Western lifestyle,” i.e., nutrient abundance coupled with a sedentary lifestyle however, it is likely that genetic predisposition plays an important, if not decisive, role in determining which individuals are at increased risk for development of NAFLD and for its progression. The first recognized stage of NAFLD, “simple” benign steatosis, can be viewed as indicative that adipose tissue fat storage capacity has been exceeded, particularly in the case of visceral
Adiposity, a major risk factor for NAFLD and its progression. Adipose tissue, particularly visceral adipose tissue, has been recognized as an endocrine organ and it secretes a variety of hormones, cytokines and chemokines, both pro- and anti-inflammatory, some of which have been suggested to play a role in progression of NAFLD to its less benign stages. Many of the molecular pathways implicated in NAFLD and its progression appear similar to those found in other “injured” organs and tissues, regardless of original insult. In addition to dysregulation in lipid metabolism, the innate immune system is likely to play an important role in the initial response of the liver to insult/injury. A fibrotic response also is likely to have similar molecular mechanisms. It is likely that liver-specific modes of regulation are involved in the development of NAFLD and its progression as well as host factors (Erickson, 2008).

**Pathophysiology:** NAFLD is characterized by the accumulation of triglycerides, which are formed from the esterification of free fatty acid (FFA) and glycerol within the hepatocyte. FFAs arise in the liver from three distinct sources as follow, lipolysis (the hydrolysis of FFA and glycerol from triglyceride) within adipose tissue, dietary sources and de novo lipogenesis. In contrast, FFA may be utilized either through b-oxidation, re esterification to triglycerides and storage as lipid droplets, or packaged and exported as very low density lipoprotein (VLDL). Hence hepatic fat accumulation can occurs as a result of increased fat synthesis, increased fat delivery, decreased fat export, and/or decreased fat oxidation. This establishes the relative contribution of lipid accumulation in patients with NAFLD (Dowman et al., 2010).

**Clinical Feature:** Most patients with NAFLD have no symptoms or signs of liver disease and the diagnosis are usually made after the finding of abnormal liver blood tests performed during routine investigations. Around 50% of patients will have fatigue and some right upper abdominal pain. The only abnormality on examination prior to the development of cirrhosis will be enlarged liver (Beckett et al., 2005).
Extra Hepatic Complications of NAFLD: There are many complications extended from liver to different organs include:

Cardiovascular disease (CVD): CVD is the commonest cause of death worldwide, with greater than 75% of events being attributed to coronary heart disease (CHD) and stroke. The risks of CVD can be mitigated by addressing recognized risk factors, such as smoking cessation, having a healthy diet, increasing physical activity, and aggressive management of comorbidities including Type 2 diabetes mellitus, dyslipidemia and hypertension. CVD and NAFLD share common risk factors, including insulin resistance (peripheral and hepatic) and central adiposity (Chacko and Reinus, 2016). The relationship between NAFLD and CVD seem to be due to an atherosclerotic effect of hepatic steatosis and steatohepatitis. Putative mechanisms for accelerated atherosclerotic disease in patients with NAFLD include a systemic proinflammatory state and disordered lipid metabolism (Chacko and Reinus, 2016). Impaired endothelial function is an early step in the process of atherosclerosis, a head of development of fatty streaks or plaque inflammation and hence crucial in CVD development (Francque et al., 2016).

Diabetes Mellitus (DM): 65 to 87% of patients with type 2 diabetes have NAFLD. NAFLD is the second most common cause of being on a waiting list for a liver transplant and the most common cause of HCC. The metabolic/insulin resistance syndrome is a well-established predictor of type 2 DM, although overt hyperglycemia only develops in those whose beta-cells fail to sustain hyperinsulinemia in the face of insulin resistance. The liver is the site of production of glucose and very low-density lipoprotein (VLDL) - triglycerides. In subjects with ‘metabolic/obese NAFLD, the liver is insulin resistant leading to over production of both glucose and VLDL. Glucose in turn stimulates insulin secretion there by inducing hyperinsulinemia. The increase in VLDL leads to lowering of the concentration of high-densitylipoprotein (HDL) cholesterol. These changes are often observed in obese subjects, but are also observed
independently of obesity. NAFLD is thus closely linked to the pathogenesis of the metabolic syndrome raising the possibility that NAFLD predicts type 2 DM, even independently of obesity. In addition to the association of NAFLD with the metabolic/insulin resistance syndrome, two common genetic variants increase the risk of NAFLD. A variant in the patatin-like phospholipase domain-containing 3 (PNPLA3) confers to NAFLD susceptibility by increasing liver fat content, risk of inflammation, and fibrosis (‘PNPLA3 NAFLD’). Genetic variation in the transmembrane 6 super family member 2 (TM6SF2) is also associated with liver fat accumulation and increased risk of NASH (‘TM6SF2 NAFLD’). Insulin resistance is not a characteristic of these two conditions, although genetic and metabolic causes of NAFLD may both exist in the same person (Lakhan et al., 2011).

**Chronic Kidney Disease (CKD):** The evidence regarding the pathophysiology of NAFLD, CKD is an inflammatory disorder. These mechanisms include the role of obesity, the renin-angiotensin system, and dysregulation of fructose metabolism and lipogenesis in the development of both disorders (Marcuccilli and Chonchol, 2016).

**1.2.3.6 Nonalcoholic Steatohepatitis (NASH):**
NASH represents the most severe histologic form of nonalcoholic fatty liver disease (NAFLD), which is defined by fat accumulation in the liver exceeding 5% of its weight. Uniform criteria for diagnosing and staging NASH are still debated. Insulin resistance is related to obesity and is central to the pathogenesis of NAFLD. In addition, oxidative stress and cytokines are important contributing factors, together resulting in steatosis and progressive liver damage in genetically susceptible individuals. Key histologic components of NASH are steatosis, hepatocellular ballooning, and lobular inflammation; fibrosis is not part of the histologic definition of NASH. However, the degree of fibrosis on liver biopsy (stage) is predictive of the prognosis, whereas the degree of inflammation and necrosis on liver biopsy (grade) are not. The disease can remain asymptomatic for
years, or can progress to cirrhosis and hepatocellular carcinoma. One global hypothesis for the pathogenesis of NASH is the “multi-hit hypothesis with metabolic syndrome playing a major role, due to insulin resistance and the proinflammatory process mediated by different proteins and immune components. The identities of the multiple “hits” are different in each patient and largely undefined at present (Takahashi et al., 2012).

1.2.3.7 Diagnosis of NAFLD and NASH:

The diagnosis of disease depends on:

Serum Biomarker: Large adult series have suggested scoring systems using age, BMI, insulin resistance, aspartate aminotransferase/alanine aminotransferase, platelet count and albumin to differentiate mild from severe disease. These simple markers are neither sensitive nor specific enough in. A growing understanding of the pathophysiology of the disease has allowed the investigation of more specific, mechanism-based biomarkers. These biomarkers focus on the specific pathways involved in the progression of the disease process: hepatocyte apoptosis, oxidative stress, inflammation and fibrosis (Fitzpatrick and Dhawan, 2014). And other techniques which include: Ultrasound (US), Magnetic resonance (MRI), Computed tomography (CT) and Liver biopsy (Tovo et al., 2015; Koplay et al., 2015; AlShaalan et al., 2005; Nalbantoglu and Brunt, 2014; Noureddin and Loomba, 2012).

1.3 Thyroid:

The thyroid gland is responsible for the production of two hormones: thyroid hormone and calcitonin. Calcitonin is secreted by parafollicular C cells and is involved in calcium homeostasis. Thyroid hormone is critical in regulating body metabolism, neurologic development and numerous other body functions (Bishop et al., 2010).

1.3.1 Anatomy of thyroid gland:

The thyroid is butterfly shaped gland located in the front of the neck just above the trachea in the adult human. The fully developed thyroid gland in the human
weight approximately about 15-20 grams and composed of two lobes and connected by isthmus. The thyroid follicle is secretory unit of thyroid gland. Each unit of follicle has an outer layer of epithelial cells that enclose an amorphous material called colloid. Colloid is mainly composed of thyroglobulin (Tg) and small quantities of iodinated thyroalbumin. The important reactions of thyroid hormone synthesis, such as iodination and initial phase of hormone secretion are believed to take place at or near the surface of epithelial cells. The thyroid gland also contains other type of cells known as parafollicular cells or C cells. These cells have been shown to produce poly peptide hormone known as calcitonin. These cells are confined within basement lamina or exist in cluster in the interfollicular space (Burits et al., 2008).

1.3.2 Thyroid hormones:
The thyroid gland secretes two hormones, thyroxin(3,5,3,5-L-tetraiodothyronine) and triiodothyronine (3,5,3-L-triiodothyronine ) and which are commonly known as T₄ and T₃ respectively. In addition the thyroid gland secretes small amounts of biologically inactive (3, 3, 5-L-triiodothyronine) reverse T₃ and minute quantities of monoiodotyrosine(MIT) and diiodotyrosine(DIT), which are precursors of T₃ and T₄ (Burits et al., 2008).

1.3.3 Biological function:
The thyroid hormones have many important biological effects. A major function is their control of the basal metabolic rate and calorigenesis through increased oxygen consumption in tissue via the effect of thyroid hormones on membrane transport (cycling of Na⁺\K⁺ATPase with increased synthesis and consumption of adenosine triphosphate ) and enhanced mitochondrial metabolism (stimulation of mitochondrial respiration and oxidative phosphorylation ). Thyroid hormones are known to: stimulate neural development and normal growth, promote sexual maturation, stimulate adrenergic activity with increased heart rate and myocardial contractility, stimulate protein synthesis and carbohydrate metabolism, increase synthesis and degradation of cholesterol and triglycerides, increase the
requirement for vitamins, increase the calcium and phosphorus metabolism and enhance sensitivity of adrenergic receptors to catecholamine's. These effects are typically magnified in patients with either an overactive thyroid gland, such as in hyperthyroidism or reduced in patients with sluggish thyroid such as hypothyroidism (Burits et al., 2008).

1.3.4 Biochemistry:
Approximately 40% of secreted $T_4$ is deiodinated in peripheral tissues by enzyme deiodinases to yield $T_3$, and about 45% is deiodinated to yield $rT_3$, a biologically inactive metabolite. The biosynthesis of thyroid hormones involves the (1) trapping of circulating iodide by thyroid gland, (2) incorporation of iodide into tyrosine and (3) coupling of iodinated tyrosyl residues to form thyronines within the protein backbone of Tg in the follicular lumen. Endocytosis followed by proteolytic cleavage of Tg releases the iodothyronine into circulation. Dietary iodine is basic element involved in synthesis of thyroid hormones. It is normally ingested in form of iodide. Iodide transport to the follicles is the first and rate limiting step in synthetic process. The follicular cells of thyroid concentrate iodide to some 30 to 40 times the normal plasma concentration by means of energy dependent pump mechanism. The synthesis of $T_3$, $T_4$, DIT and MIT in Tg molecules occurs mainly at the follicular cell-colloid interface but also within colloid. Tg is present in highest concentrations within the colloid where it is stored. The follicular cells engulf colloid globules by endocytosis. These globules then merge with lysosomes in the follicular cells. Lysosomal proteases break the peptide bonds between iodinated residues and Tg and $T_4$, $T_3$, DIT and MIT are released into cytoplasm of the follicular cell. $T_4$ and $T_3$ diffuse into the systemic circulation after their liberation from Tg. DIT and MIT are deiodinated by an intracellular microsomal iodothyrosine dehydrogenase. The feed iodide is then reused for thyroid hormone synthesis. Each step in the synthesis of thyroid hormones is regulated by pituitary thyroid stimulating hormone (TSH). TSH stimulate (1) the iodide pump (2) Tg synthesis and (3) colloidal uptake by
follicular cells. TSH also regulates the rate of proteolysis of Tg for the liberation of T₄ and T₃. In addition TSH induces an increase in the size and number of thyroid follicular cells. Prolonged TSH stimulation leads to increased vascularity and eventual hypertrophic enlargement of the thyroid gland (goiter) (Bruits et al., 2008).

1.3.5 Regulation of thyroid hormones:

The most important regulator of thyroid homeostasis is TSH or thyrotrophin. This peptide hormone comprises a specific B- subunit, which is required for binding to the TSH receptor, and α- subunit which is common for gonadotrophins both subunits are required for bioactivity. The subunits contain carbohydrate chains that are essential for biological activity of the molecule. Modifications to these carbohydrate residues occur in different thyroid states, which alter both the bioactivity of the molecule and its plasma half life. The production of TSH is controlled by stimulatory effect of hypothalamic tripeptide thyrotropin releasing hormone (TRH) mediated by a negative feedback from circulating free T₃ and free T₄. It is thought that the hypothalamus via TRH sets the level of thyroid hormone production required physiologically and that the pituitary acts as a thyroid state to maintain the level of thyroid hormone production that has been determined by hypothalamus. Dopamine, somatostatin and glucocorticoids also appear to be in inhibiting the release of TSH, and these agents together with interleukins may be important modifiers of TSH release in non thyroidal illness (NTI) (Beckett et al., 2005).

1.3.6 Pathology of thyroid gland:

Thyroid disorder is a general term representing several different diseases involving thyroid hormones and the thyroid gland. Thyroid disorders are commonly separated into two major categories, hyperthyroidism and hypothyroidism, depending on whether serum thyroid hormone. Levels (T₄ and T₃) are increased or decreased, respectively. Thyroid disease generally may be sub-classified based on etiologic factors, physiologic abnormalities. The
prevalence and incidence of thyroid disorders is influenced primarily by sex and age. Thyroid disorders are more common in women than men, and in older adults compared with younger age groups. The prevalence of unsuspected overt hyperthyroidism and hypothyroidism are both estimated to be 0.6% or less in women, based on several epidemiologic studies (Smith and Bennett, 1992).

1.3.6.1 Hypothyroidism:

Hypothyroidism is a condition in which the thyroid gland is underactive and produces too little thyroid hormone. When the condition is very severe it is sometimes called myxedema because a substance collects in subcutaneous tissues (under the skin) that cause nonpitting edema. About 95% of the time hypothyroidism is the result of malfunction of the thyroid gland itself (primary hypothyroidism). Causes of primary hypothyroidism can be either, congenital (something an individual is born with) or acquired (something an individual gets). The most common causes of primary hypothyroidism are acquired and are the result of destruction of the gland by an autoimmune disease (such as Hashimoto’s thyroiditis) or by radioactive iodine therapy or surgery for hyperthyroidism. Other causes of primary hypothyroidism include goiter due to iodine deficiency and too much iodine in individuals with thyroid disease. Some drugs can also cause hypothyroidism including lithium carbonate, para-aminosalicylic acid, thiourea drugs, sulfonamides, phenylbutazone and others. Decades ago congenital hypothyroidism was a common cause of mental retardation and severe disability in affected children. About 5% of the time hypothyroidism is the result of a problem outside of the thyroid gland itself (secondary hypothyroidism). Tumors or other abnormalities of the hypothalamus or pituitary gland are examples of conditions that can cause secondary hypothyroidism. Rarely an individual’s tissues are resistant to thyroid hormone (Smith and Bennett, 1992).

Symptoms: Too little thyroid hormone causes the body’s metabolism to slow down. Symptoms of hypothyroidism usually develop slowly and can be fairly
subtle at first. Often the individual seems depressed. The facial expression seems
dull and their face looks puffy or swollen. There is often weight gain. The skin
becomes coarse, dry and scaly while the hair becomes dry and sparse. The voice
sounds hoarse, and hearing may be impaired. There is constipation, cold
intolerance, generalized aches and pains, weakness, tiredness and sleepiness.
Some people appear confused, forgetful or even demented. In case of very severe
hypothyroidism can cause anemia, low body temperature, heart failure, and life
threatening myxedema coma (Smith and Bennett, 1992).

**Treatment:** Hypothyroidism is an easily treated disease. The most frequently
prescribed is Synthroid (levothyroxine). Other preparations include Liotrix,
Desiccated Thyroid, and Levotriiodothyronine. Regulating the dose of thyroid
replacement hormone may take a few weeks and several blood tests to determine
if the correct amount of medication is being given. After thyroid function is back
to normal the doctor will want to monitor therapy by checking serum free T₄,
TSH and free T₃ levels annually (Smith and Bennett, 1992).

**1.3.6.2 Hyperthyroidism:**

In hyperthyroidism the thyroid gland is overactive and produces too much
thyroid hormone. Over 2 ½ million Americans have hyperthyroidism. It is much
more common in women than in men. There are several causes of
hyperthyroidism. The most common include immunologic conditions (like
Graves’ disease and thyroiditis) toxic thyroid nodules (adenomas) and toxic
multinodular (many nodules or adenomas) goiter (enlargement of the thyroid
gland). Graves's disease is a syndrome of hypermetabolism, enlarged thyroid
gland and exophthalmous (bulging of the eyeballs due to the collection of
abnormal substances in the tissues of the orbit). Autoimmune thyroiditis is an
inflammation of the thyroid gland that may cause damage to the gland and
eventually result in hypothyroidism. Thyroid nodules (one or many) are areas of
abnormal thyroid tissue within the thyroid gland. They can be benign or malignant (cancer) but most are benign (not cancer) (Smith and Bennett, 1992).

Symptoms: Too much thyroid hormone speeds up metabolism and the symptoms can be dramatic, the heart pounds rapidly and may develop an irregular beat (arrhythmia), blood pressure goes up, the individual loses weight despite an increased appetite, there is usually heat intolerance and excess sweating, diarrhea, sleep disturbance, and muscle weakness. Many individuals feel nervous, tremulous (shaky) and emotionally labile. The skin feels smooth, warm and moist. With Graves’ disease there may be mild or marked bulging of the eyes that leads to irritation, drying, inflammation, and increased sensitivity to light. Some individuals with hyperthyroidism develop a very severe form of this condition that is called thyroid storm. Thyroid storm may be brought on by stress or infection. All the symptoms of hyperthyroidism seem to be exaggerated. The individual may also have a fever and abdominal (stomach) pain. He/she may be delirious, obtunded ("out of it") or psychotic. Twenty to 40% of people with thyroid storm die. This is a true medical emergency that must be treated intensively in a hospital setting (Smith and Bennett, 1992).

Treatment: Treatment of hyperthyroidism depends upon the cause of the condition. Several options are available including medications, radioactive iodine therapy and surgery. Antithyroid medications used to treat hyperthyroidism include propylthiouracil (PTU) and methimazole (Tapazole). They have several unpleasant side effects including rash, damage to white blood cell production (agranulocytosis) liver damage and inflammation of blood vessels (vasculitis). The doctor will monitor the white blood cell count (WBC) and liver function tests (LFTs) on a regular basis as long as the patient is taking one of these drugs. Very often treatment will be done in two stages. In the short-term, antithyroid drugs will be prescribed to decrease the production and release of thyroid hormone. Plenty of rest, good nutrition and stress management are also important pieces of the initial management of hyperthyroidism. Sometimes B-adrenergic
blocking agents (like Propanolol) are prescribed to reduce the symptoms of hyperthyroidism during this phase of treatment. These medications slow down the heart rate, reduce the shakiness and control anxiety. Some patients (10-20%) may go into remission with this therapy. Most will need to be reassessed between 12-18 months and a decision made about long-term management Options for long-term management include continuation of antithyroid medication, surgery or radioactive iodine therapy. It is up to the individual and the physician to decide the best method of treatment in each case. Individuals with eye involvement will need to be followed by an ophthalmologist (Smith and Bennett, 1992).

1.3.7 Diagnosis of thyroid disorders:
When screening for abnormalities of thyroid gland function most doctors will order blood tests that measure the level of TSH (thyroid stimulating hormone) and freeT₄. Depending upon the results of these tests and upon the specific thyroid disorder, a number of other tests may be ordered. Some other tests include levels of T₃, thyroxin-binding protein, antithyroid antibodies and serum thyroglobulin. Occasionally tests that evaluate functional responses to stimulation of the thyroid or pituitary glands may be performed. The anatomy of the thyroid gland can be evaluated by a thyroid ultrasound or by a thyroid scan. The scan is done by injecting a minute amount of radioactive iodine into the individual, waiting 30 minutes and then measuring the radioactivity over the thyroid gland (which has the unique capacity of trapping iodine). The scan produces a picture of the active part of the thyroid gland. A needle biopsy (taking a sample of tissue) or needle aspiration (taking a sample of fluid) from nodules in the thyroid gland can also be done by a physician (Smith and Bennett, 1992).

The previous studies showed that hypothyroidism is more prevalent in patients with NAFLD when compared with healthy controls. They also demonstrated that hypothyroidism is more likely to happen in NASH patients in comparison to patients without NASH. Therefore, hypothyroidism may not only predicts the presence of NAFLD but also may occur in more severe pathologic form of
NAFLD (NASH) (Pagadala et al., 2012). Also hypothyroidism was closely associated with NAFLD independently of known metabolic risk factors, confirming a significant clinical relationship between these two diseases (Parikh et al., 2015).

Another studies showed the thyroid hormones abnormality in patients with NAFLD may simply due to alterations in thyroid hormones occurring in non thyroidal illnesses (Eshraghian et al., 2013).

NAFLD/NASH is supposed to be a hepatic feature of metabolic syndrome and insulin resistance (Collantes et al., 2006). Hypothyroidism has been reported to be associated with obesity and metabolic syndrome (Waring et al., 2012) so the association between hypothyroidism and metabolic syndrome which accompanied by NAFLD which strengthen between the hypothyroidism and NAFLD.
1.4 Rationale:
NAFLD is one of the most common problems worldwide and prevalence of it in Sudan is about 15%. Few studies were done in that group of people to determine prevalence of NAFLD in Sudan. In fact that, an association between the levels of TSH, free T₃ and free T₄ with NAFLD which previously reported, gives us an interest to conduct this research, moreover, extend our objective to assess thyroid hormones level among NAFLD and its association with study variables. Concurrent with previous study done in the Sudan which designed as epidemiological researches, the current study hypothesized that there is an association between thyroid hormones levels, NAFLD and its related complications.

1.5 Objectives:
1.5.1 General Objective:
To evaluate serum thyroid hormones level among non alcoholic fatty liver disease patients.

1.5.2 Specific Objectives:
1. To estimate the serum levels of TSH, free T₃ and free T₄ among study group (Non alcoholic fatty liver disease patients) and control group.
2. To compare mean concentrations of TSH, free T₃ and free T₄ among study and control group.
3. To compare mean concentrations of TSH, free T₃ and free T₄ among male and female of case group.
4. To correlate between serum levels of TSH, free T₃ and free T₄ and study variables (age, gender and BMI).
Chapter two
Materials and methods
2.1 Materials:

2.1.1 Study Design:
This is cross sectional, hospital based study.

2.1.2 Study Area:
The study was conducted in Khartoum State, the patients enrolled in this study were from Advanced Diagnostic Center, during January 2017 to March 2017.

2.1.3 Study Population:
The study was conducted on the Sudanese patients with Non Alcoholic Fatty Liver Disease (males and females) as test group, and apparently healthy individual (males and females) as control group. Males and females were matched for age (30-80 years).

2.1.4 Inclusion Criteria:
Test group are Sudanese patients with non alcoholic fatty liver disease (males and females).

2.1.5 Exclusion Criteria:
Patients with any liver diseases, and patients that have been taken alcohol or certain medications use to treat hypertensions had been excluded.

2.1.6 Sample Size:
Forty Sudanese patients with non alcoholic fatty liver disease as a test group and forty apparently healthy Sudanese individuals were enrolled in this study.

2.1.7 Ethical consideration:
All participants were told about the research importance and all of them were accept to participate, and an informed consent had been taken.

2.1.8 Sampling Technique:
After an informed consent had been taken a local antiseptic was used to clean the skin. Venous blood were taken from each participant by standard procedures, and placed in plan container after clotting centrifuged at 3000 rpm for 3 minutes, and then obtained serum for thyroid stimulating hormone and thyroid hormones (free
T3 and free T4) were separated in other plain container and kept at -20 C until use.

2.2 Methodology:

2.2.1 Estimation of Thyroid hormones and Thyroid Stimulating Hormone:

TSH, free T₃ and free T₄ were estimated using Automated Immune assay Analyzer 360 (TOSOH).

**Principle:** competitive immunoassay for free T₃ and free T₄ which the radio-labeled antigen or tracer (Ag*) competed with unlabeled antigen (Ag) for a limited number of binding sites of antibodies (Ab). The proportion of Ag and Ag* binding with the Ab is related to the Ag and Ag* concentration and requires limited Ab in the reaction. In the competitive assay, the Ag* concentration is constant and limited, as the concentration of Ag increases, more binds to the Ab, resulting in less binding of Ag*.

The sandwich assay for TSH: is another noncompetitive assay used to detect Ab, in which the immobilized Ag captures specific Ab. After washing, the labeled detector Ab is added and binds to the captured Ab. The amount of bound-labeled Ab is directly proportional to the amount of specific Ab present.

2.2.2 Quality Control:

The precision and accuracy of method used in this study were checked and analyzed by commercially prepared control sera.

2.2.3 Statistical Analysis:

Data was analyzed by using the SPSS computer program. The means and standard deviations of serum levels of TSH, free T₄ and free T₃ were detected and t-test was used for comparison (p-value of < 0.05 is considered to be significant). And correlation test was used to assess correlation between the levels of TSH, free T₃ and free T₄ and study variables (BMI, age and gender).
Chapter three

Results
3. Results

A eighty randomly samples were collected to evaluate the levels of TSH, free T_3, free T_4 and BMI among study group, then classified as 40 healthy apparently as control group and 40 with NAFLD as cases, males account 18 and female 22 and participants age between (30-80).

Figure (3.1) Shows frequencies of gender among NAFLD, results expressed as percentage (%).

Table (3.1): Shows mean concentration of TSH, free T_3 and free T_4 in case (2.99±3.00),(1.09±0.709) and (0.622±0.171) versus control group (1.23±0.906),(2.66±0.333) and (1.25±0.369), with p-value=0.002,0.000 and 0.000 respectively.

Table (3.2): Shows mean concentration of TSH, free T_3 and free T_4 in males (4.44±3.64), (0.761±0.553) and (0.546±0.175) versus females group (1.81±1.65), (1.36±0.717) and (0.684±0.144), with p-value=0.010, 0.006 and 0.009 respectively.

Figure (3.2): Shows no person correlation results between TSH and age with R-value 0.209 and p-value 0.196.

Figure (3.3): Shows no person correlation results between free T_3 and age with R-value -0.165 and p-value 0.310.

Figure (3.4): Shows no person correlation results between free T_4 and age with R-value-0.052 and p-value 0.750.

Figure (3.5): Shows significant positive person correlation between TSH and BMI with R-value 0.596 and p-value 0.000.

Figure (3.6): Shows significant negative person correlation between freeT_3 and BMI with R-value - 0.620 and p-value 0.000.

Figure (3.7): Shows significant negative person correlation between free T_4 and BMI with R-value - 0.835 and p-value 0.000.
Fig. 3.1 Shows frequencies of gender among NAFLD patients, results expressed as percentage (%).
Table 3.1 Shows mean concentration of TSH, free T₃ and free T₄ in case versus control groups, the result show as (Mean±SD) and significant differences consider, p-value <0.05.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Cases Mean±SD</th>
<th>Controls Mean±SD</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSH µIU/ml</td>
<td>2.99±3.00</td>
<td>1.32±0.906</td>
<td>0.002</td>
</tr>
<tr>
<td>free T₃ pg/ml</td>
<td>1.09±0.709</td>
<td>2.66±0.333</td>
<td>0.000</td>
</tr>
<tr>
<td>free T₄ µg/dl</td>
<td>0.622±0.171</td>
<td>1.25±0.369</td>
<td>0.000</td>
</tr>
</tbody>
</table>
Table 3.2 shows mean concentration of TSH, free T₃ and free T₄ in males versus females, the result show as (Mean±SD) and significant differences consider, $p$-value <0.05

<table>
<thead>
<tr>
<th>Variables</th>
<th>Males Mean±SD</th>
<th>Females Mean±SD</th>
<th>$p$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSH µIU/ml</td>
<td>4.44±3.64</td>
<td>1.81±1.65</td>
<td>0.010</td>
</tr>
<tr>
<td>free T₃ pg/ml</td>
<td>0.761±0.553</td>
<td>1.36±0.717</td>
<td>0.006</td>
</tr>
<tr>
<td>free T₄ µg/dl</td>
<td>0.546±0.175</td>
<td>0.684±0.144</td>
<td>0.009</td>
</tr>
</tbody>
</table>
Fig (3.2) shows person correlation results no correlation between TSH and age with R-value 0.209 and p-value 0.196.

(R= Regression correlation, P= Strength of correlation)
Fig (3.3) shows person correlation results no correlation between free $T_3$ and age with R-value - 0.165 and $p$-value 0.310.

(R= Regression correlation, P= Strength of correlation)
Fig (3.4) shows person correlation results no correlation between free T₄ and age with R-value -0.052 and p-value 0.750. 
(R= Regression correlation, P= Strength of correlation)
Fig (3.5): shows person correlation results significant positive correlation between TSH and BMI with $R$-value 0.596 and $p$-value 0.000.

($R$=Regression correlation, $P$=Strength of correlation)
Fig (3.6): shows person correlation results significant negative correlation between free T₃ and BMI with R-value -0.620 and p-value 0.000.

(R= Regression correlation, P= Strength of correlation)
Fig (3.7): shows person correlation results significant negative correlation between free T₄ and BMI with R-value -0.835 and p-value 0.000.

(R= Regression correlation, P= Strength of correlation)
Chapter four

Discussion, conclusion and recommendation
4.1 Discussion

NAFLD have high prevalence in Sudan which account of 15% with high rates of morbidity and mortality, researchers' link between thyroid hormones levels and complications of NAFLD. Accordingly, the present study aims to evaluate thyroid hormones levels and thyroid stimulating hormone among NAFLD patients and its correlation with study variables.

The results of frequencies revealed that, NAFLD is common in females 55% than males which account of 45% with ratio of 1.2:1 the findings were similar to pervious reported in India with ratio of females to males 1.8:1 (Parikh et al., 2015). This finding attributed to hormonal and genetic variations that related to cholesterol and estrogen hormones increasing risk of gallstones formation in females, moreover, alter lipid metabolism, resulting in fatty liver.

In the current study there was significant increase in mean TSH level of NAFLD when compared with healthy control group p-value 0.002. This finding in agreement with pervious study which reported that serum TSH level in patients with NAFLD is significantly higher than controls (Zhang et al., 2012). In contrast, Eshraghian et al contradict our finding, which report, serum TSH level was not significantly different in participants with NAFLD and those without NAFLD (Eshraghian et al., 2013). Therefore, contradiction justified by that patients included in our study have variation regarding duration of disease form acute to chronic status which impact differently on TSH level. Eshraghian included alcoholic cirrhosis in his study a group which excluded from current study.

The present study provides evidence that, mean concentration of free T_3 and free T_4 were significantly decreased in NAFLD when compared with healthy control group (p-value 0.000 and 0.000) respectively, this finding in agreement with previous report by Ittermann et al(2012). In contrast, contradict with other study which showed that, serum free T_3 and free T_4 levels were not significantly different in participants with NAFLD and those without NAFLD (Eshraghian et al., 2013). This finding justified as liver dysfunction altered thyroid hormones level.
In fact, serum TSH level was significantly higher in females than in males with NAFLD (Zhang et al., 2012), which contradicted the current study finding that, there was significant increase in mean TSH level of NAFLD males in comparison with NAFLD females (p-value 0.010). This finding justified as estrogen hormone in females increase level of thyroxin binding globulin which act as reservoir of thyroid hormones level that are responsible for feedback inhibition of TSH.

In the present study, there was significantly decrease in mean free T₃ and free T₄ of males with NAFLD when compared with females (p-value 0.006 and 0.009) respectively. The finding contradicted with previous study which showed that serum free T₃ and free T₄ levels were significantly lower in females than in males (Rubina et al., 2011). Therefore, contradiction justified by females have estrogen hormone which increase level of thyroxin binding globulin that prevent loss of free T₃ and free T₄.

The results of correlation revealed that, there was no correlations between serum TSH, free T₃ and free T₄ levels and age of patients with NAFLD (p-value 0.196, 0.310 and 0.750) respectively and (R-value 0.209, -0.195 and -0.052) respectively. The findings that contradicted with previous study showed that serum TSH level was significantly positive correlation with age (Carulli et al., 2013) and serum free T₃ and free T₄ level were significantly negative correlation with age (Xu et al., 2011). The result attributed to decrease functions of thyroid gland in geriatric population.

In addition, the results of study give evidence that, there was significant positive correlation between serum TSH level and BMI p-value 0.000 and R-value 0.596 and significant negative correlations between free T₃, free T₄ and BMI (p-value 0.000 and 0.000) respectively and (R-value -0.620 and -0.835) respectively. The finding that are in agreement with previous article showed that serum TSH values were positively correlated to BMI in addition lower free T₄ values were accompanied by
higher BMI values and the findings contradicted to previous result in free T₃ which have no relationship with BMI(Iacobellis et al., 2005). The result attributed to higher BMI due to obesity was associated with increased risk of hypothyroidism in NAFLD.
4.2 Conclusion

The study concludes that:

TSH is higher, free T$_3$ and free T$_4$ are lower in patients with NAFLD compared to healthy individuals. In addition TSH is higher, free T$_3$ and free T$_4$ are lower in males than in females with NAFLD. Also there is no correlation between serum levels of TSH, free T$_3$ and free T$_4$ with age of patients, but there is significant positive correlation of serum level of TSH and significant negative correlation of serum levels of free T$_3$ and free T$_4$ with BMI of patients with NAFLD.
4.3 Recommendations

From the result of this study it is recommended that:

- Health education programs to improve people awareness about serious health effects of complications of NAFLD.
- Inclusion of other parameters to find out mechanism of alteration.
- Further studies should be done to measure the serum levels of TSH, free T<sub>3</sub> and free T<sub>4</sub> with inclusion of other parameters like thyroxin binding protein and thyroglobulin.
References
References:


Chen SL, Morgan TR. (2006). The national history of hepatitis C virus (HCV)


Appendix
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Questionnaire

Evaluation of thyroid hormones and thyroid stimulating hormone among Non Alcoholic Fatty Liver disease patient In Khartoum State

**Personal Information:**

Serial No: ............

Gender: ..................

Age: ..................

Hight: ...........

Weight...

BMI: ...............  

Residence: ............

Other disease: .................

**Lab Information:**

Serum TSH:

Serum FT4:

Serum FT3: