Current Research in Microbiology and Biotechnology
Vol. 6, No. 1 (2018): 1411-1419
Research Article
Open Access

ISSN: 2320-2246

Detection of some Physiological and Immunological parameters in Iraqi clinical and subclinical hypothyroid patients: Relationship study

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Received: 25 November 2017 Accepted: 23 December 2017 Online: 01 January 2018

ABSTRACT

The present study was performed to evaluate the level of some risk factors (biochemical and immunological) in hypothyroid Iraqi patients considering the different thyroid functional states (hypothyroidism and subclinical hypothyroidism).The study includes 82 patients clinically diagnosed with hypothyroidism**.** Three study groups have been investigated: (47 clinical hypothyroid patients, 12 subclinical hypothyroid patients 23 healthy individuals) of different ages. This study, show that the proportion of females (83.3 %), (87.2%) in subclinical and clinical hypothyroidisim respectively higher than the proportion of males (16.7%),(12.8%) in subclinical and clinical hypothyrodism respectively of the total patients.The majority of subclinical hypothyroidism patients were found to be obese (61.7%) followed by normal weight class (21.3%).While in the case of clinical hypothyroidism the majority of patients are from the class of normal weight (66.7%) followed by over weight class (33.3%). The statistical study showed no significant differences in the TSH level between the weight categories among each of the studied groups, but the weight between 25-29.9 kg/m^2 showed a highly significant increase and a significant increase in the level of TSH in the clinical and subclinical cases in comparison to its level in the healthy group. No significant differences were found in the level of HDL cholesterol (Good cholesterol) and triglyceride among the patient،s groups in comparison with its level of the control LDL cholesterol (Bad cholesterol) level increased in the subclinical and the clinical hypothyroid patients in comparison with its level in the healthy group, statistical analysis showed no significant differences in LDL–C. level between the subclinical and healthy groups. In contrast, there is a significant difference in LDL–C. level between clinical and healthy groups.The same relationships were noticed in the total chol. level and atherogenic agent.(nonsignificant increase in the subclinical hypothyroid patients and a significant increase in the clinical cases in comparison with its level in the healthy persons). No significant differences were found in the level of IL-12 and IL-33 between the studied groups. Serum IL-33 level was lower in subclinical hypothyroid patients in comparison to its level in the other groups. In conclusion, as long as cholesterol and TSH continue high, the patients become more susceptible to coronary heart disease as proved through calculating atherogenic agent in each group. According to the positive relationship between thyroid hormones and IL-12, the latter may act as immunomodulating agents. The decrease of IL-33 in subclinical hypothyroid group suggests humeral immunological failure which may need more studies and evaluating for the other related cytokines.

Keywords: Hypothyroidism, TSH, IL-12, IL-33, Lipid profile.

1. INTRODUCTION

Hypothyroidism is the decrease in Triioidothyronine (T3) and Thyroxine (T4) secretion and reduced thyroid gland function [1]. It affects hundreds of millions around the world [2]. Biochemically, reducing the secretion of T3 and T4 may be correlated with the amplified secretion of Pituitary TSH, this is a key laboratory finding, particularly in the early detection of thyroid failure [3].

Clinical hypothyroidism (HO) is an insulin-resistant state, this is due to defects in the ability of insulin to increase glucose utilization in peripheral tissues, mainly muscle.Patients in this group have distinct signs and symptoms with high TSH but low T3 and low T4 levels in serum, while Subclinical hypothyroidism (SHO) has high TSH values but normal T3 and T4 levels in serum as compared to normal reference levels. The patients in the latter group have very few or no clinical signs and symptoms [4,5].

It is well-known and according to many studies that overt thyroid disorder associate with a cardiovascular risk factor, dyslipidemia, insulin resistance, hypertension, inflammation, oxidative stress, endothelial dysfunction, coagulation disorders and, athero-sclerosis [6]. A number of recent studies have to associate subclinical hypo-thyroidism with an increased number of cardiovascular risk factors, including hypertension [7], weight gain [8], insulin resistance [4], hypercholesterolaemia, dyslipidemia [9], and coronary and ischaemic heart diseases [10].

The present study was performed to evaluate the level of some risk factors (biochemical and immunological) in hypothyroid Iraqi patients considering the different thyroid functional states (hypothyroidism and subclinical hypothyroidism) and to find its possible role in disease pathogenesis through the study the correlation between these risk factors.

2. MATERIALS AND METHODS

A prospective study has been carried out in (Endocrine gland and diabetic center) in Baghdad during the period between March 2017 and September 2017, include: 82 patients clinically diagnosed as hypothyroid**.** Three study groups have been investigated: (47 clinical hypothyroid patients, 12 subclinical hypothyroid patients 23 healthy individuals) of different ages.

Venous blood samples (4 ml) were taken from all participants of both hypothyroid patients and healthy control groups after 12-14 hours fasting, centrifuged at 3000 rpm for 10 minutes. The collected serum used to determine the biochemical and immunological parameters according to the manufacturer's protocol. As indicators of thyroid function, T3, T4, TSH level were estimated using Biomerieux (France) kits. Lipid profile was estimated using Linear (Spain) kits and cytokines (IL-12 and IL-33) were estimated using.R&D (USA) ELISA kits. Body Mass Index (BMI) was calculated based on the following formula:

BMI = Weight (Kg) / Square of weight (m^2)

Body weight was measured using an analog scale and height was measured as into four groups according to their BMI: Underweight (BMI<18.5), Normal (18.5 ≤ BMI<25), Overweight (25 ≤ BMI < 30) and Obesity (BMI ≥ 30).

Atherogenic index (AI) was calculated by the following equation:

AI = Triglyceride / HDL cholesterol

All calculations were made using a standard statistical package (SPSS for Windows software- V.24), used to test the difference between three groups on the study parameters. Oneway analysis of variance was used, and Least Significant Difference –LSD was used to significantly compare among means in this study where data were expressed as (mean ± SE) with $P<0.05$ considered to be significant and $P<0.01$ considered to be highly significant.

3. RESULTS AND DISCUSSION

Clinical hypothyroidism is a common disease, and it has been found to be higher in adult women than in adult men. Sawin *et al*., 1985 [11] pointed out that the proportion of women with hypo-thyroidisim is about 2% of the general society compared to a lower proportion of men is about 0.2%, This was observed in our study, it was found that the proportion of females (83.3%), (87.2%) in subclinical and clinical hypothyroidisim respectively higher than the proportion of males (16.7 %),(12.8 %) in subclinical and clinical hypothyroidisim respectively of the total patients (Figure 1) Several statistical studies, witch explain this phenomenon in different ways. Yamada *et al*.,1984 [12] pointed to the high incidence of the disease in women who are over 40 years old and attributed that to the increase of Antithyroglobulin antibodies and Antithyroid peroxidase antibodies with a rise in concentration level of TSH, and the rate of TRH hormone increase with age, especially in women. Baha, 2001 [13] mentioned that hypothyroidisim is more common in women because of disorders in the hormones reproduction, especially Progesterone and Estrogen hormones, as a result of fatigue or stress or during pregnancy or menstrual cycle, it has been found that estrogen hormone found to increase concentration of the thyroxine-carrier proteins (TBG) and stimulates the immune system to increase the production of antithyroglobulin anti-bodies. High female predominance in thyroid autoimmunity might be associated with the X chromosome containing a number of sex and immune related genes which are an important key in the preservation of immune tolerance [14].

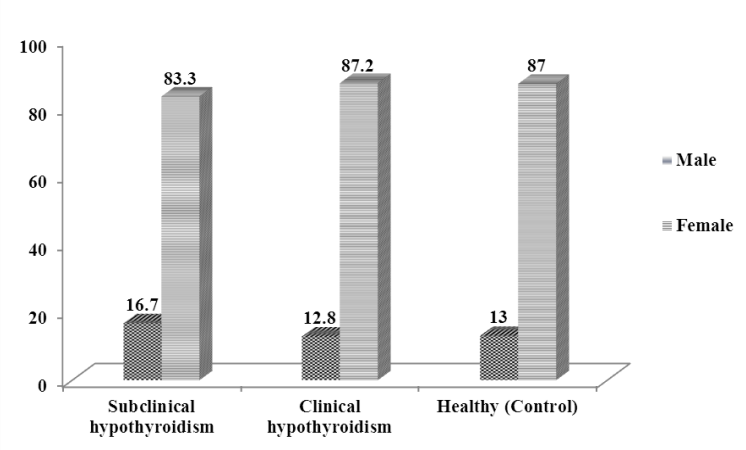

Figure 1: Gender percentage among study groups.

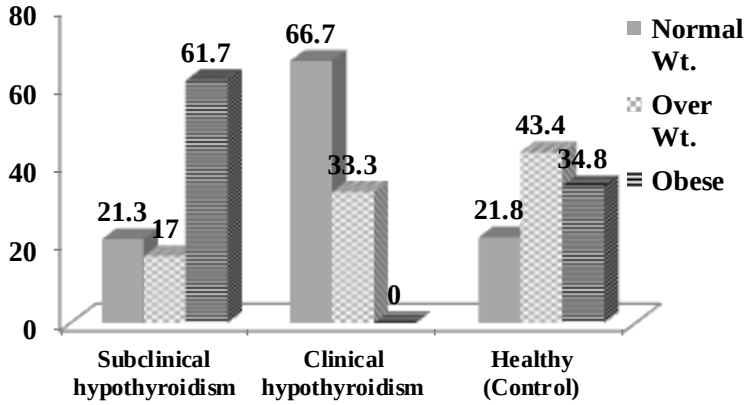

Figure 2: Weight categories percentage among study groups.

In our study, it has been found that the majority of subclinical hypo-thyroidism patients were found to be obese (61.7%) followed by normal weight class (21.3%). While in the case of clinical hypothyroidism the majority of patients are from the class of normal weight (66.7%) followed by overweight class (33.3%) (Figure 2). Most cases belong to subclinical hypothyroidism are recently discovered so they never had the chance to take a treatment and submit to its effect, that is why the majority of them are obese, unlike the old discovered cases which follow a restrict treatment for a long time.

Thyroid hormones (TH) play a key role in regulating metabolism through the modulation of thermogenesis and energy expenditure. The putative relation-ships between TH, body weight, and adipose tissue homeostasis have been the focus of several studies in recent years [15,16]. A cross-sectional study found a higher BMI among women with subclinical hypothyroidism Changes in thyroid function, are associated with changes in BMI, result from long-standing minor alterations in energy expenditure. This is more pronounced when mild hypo- or hyperthyroidism is present because thyroid function may importantly influence the prevalence of obesity in a population [17]. Besides, it was found that even a slightly elevated serum TSH levels .are associated with an increase in the occurrence of obesity [18].

Table 1: Serum concentration of thyroid function test in the study groups expressed as (mean± standard deviation)

Group	Thyroid function test		
	T3	T4	TSH
Healthy	1.35 ± 0.083	83.08 ± 1.82 b†	1.50 ± 1.15 b
Subclinical hypothyroid	1.53 ± 0.192	97.51 ± 6.72 ab	10.23 ± 3.76 ab
Clinical hypothyroid	1.32 ± 0.074	74.53 ± 3.53 c	18.24 ± 3.63 a
P-Value	0.422	0.004**	0.004**

Data presented as mean ± SE , (*) significant differences (p<0.05), (**)highly significant differences (p<0.01). †Means that do not share a letter (vertically) are significantly different.

Table 1 showed no significant differences in the T3 level between the three groups, no significant differences in T4 level in the subclinical group in comparison with its level in the healthy group.The clinical hypothyroid patients show the lowest level of T4 with a significant difference when compared with its level in the other two groups. The clinical hypothyroid patients also show the highest level of T4 with a significant difference when compared with its level in the other two groups.

Hypothyroidism is characterized by high serum level of TSH hormone than normal level and occurs according to the mechanism of negative feedback, due to the reduction of thyroid hormones (T3, T4) in the blood [19]. One of the most important reasons that may lead to hypothyroidism and low level of T3 and T4 hormones is the occurrence of immune reactions against the thyroid gland leading to the destruction of glandular cells. At first, the thyroiditis occurs and then damage the tissues usually ending up with the damage of the gland, as a result there is a deficiency in its ability to produce and release thyroid hormones [20]. The disease also occurs as a result of modified expression of the deiodinases which is responsible for converting T4 into T3 in peripheral tissues), modified entry of thyroid hormone into tissue (damage membrane) [21].

Table 2: Serum concentration of Thyroid stimulating hormones in the study groups expressed as (mean± standard deviation) according to body mass categories.

BMI (Kg/m²)	TSH			P-value
	Healthy	Subclinical	Clinical hypothyroid	
<25	1.26±0.39	10.3±1.16	17.75±5.94	0.104 N.S
25-29.9	1.37±0.41 c	9.3±2.16 b	33.93±13.46 a	0.009**
≥30	1.87±0.39	14.3±0.0	14.74±4.25	0.360 N.S
P-Value	0.603 N.S	0.528 N.S	0.188 N.S	

Data presented as mean ± SE , N.S not significant (*) significant differences (p<0.05), (**)highly significant differences (p<0.01). †Means that do not share a letter (Horizontally) are significantly different (according to Least Significant Test (LSD).

The statistical study showed no significant differences in the TSH level between the weight categories among each of the studied groups ,but the weight between 25-29.9 kg/m² show a highly significant increase in TSH level in the clinical and subclinical groups in comparison to its level in the healthy group (Table 2). It was found that there is a highly significant relationship (p<0.00) between TSH levels and BMI, when the BMI levels were 30 or more the TSH levels were significantly high on comparison with the TSH level in patients with BMI < 18.55 [22].

Bastemir *et al*.,2007 [23] suggested in his research a link between the level of TSH hormone and the degree of obesity that may result in some metabolic changes in the body as he observed a significant elevating in the level of TSH hormone in overweight people (25-29.9 BMI) more than in normal weight people (<25 BMI), and a positive correlation between TSH hormone level and body weight especially in overweight people.

Another hypothesis, the association between serum TSH and body weight may be due to leptin [24]. A recent study in treated thyroid cancer patient found an increase in serum leptin after acute recombinant human TSH administration, which was proportional to adipose mass [25]. Several studies have also suggested that adipocytes and pre-adipocytes possess special receptors for TSH hormone, and the association of TSH hormone with these receptors stimulates differentiation of pre-adipocyte cells to adipocyte cells and as a result, fatty tissue expands [26,27]. Also, the highest correlations in the association between BMI and serum TSH concentration was shown among morbidly obese participants [28], therefore, measuring the level of TSH hormone is considered a pressing medical necessity in many studies, especially the study of links between thyroid function and obesity or adiposity [23].

Table 3: Serum concentration of Lipid profiles in the study groups expressed as (mean± standard deviation).

Group	Lipid profiles				
	HDL-C	LDL-C	Trig.	VLDL-C	Total Chol.
Healthy	47.74 ± 2.96	67.2 ± 11.3 b	104.4 ± 14.08	20.9 ± 2.81	135.4 ± 12.0 b
Subclinical hypothyroid	44.00 ± 3.29	104.3 ± 14.6 b	93.0 ± 8.5	18.6 ± 1.70	165.5 ± 14.2 b
Clinical hypothyroid	45.85 ± 2.03	191.6 ± 15.3 a	127.5 ± 16.9	21.97 ± 2.32	266.0uu15.4 a
P-Value	0.732	<0.001**	0.432	0.761	<0.001**

Data presented as mean ± SE, (*) significant differences (p<0.05), (**) highly significant differences (p<0.01). †Means that do not share a letter (vertically) are significantly different.

No significant differences were found in the level of HDL cholesterol and triglyceride between the patients groups in comparison with its level of the control. LDL cholesterol level increased in the subclinical and the clinical hypothyroid patients in comparison with its level in the healthy group, statistical analysis showed no significant differences in LDL–C. level between the subclinical and healthy groups. In contrast, there is a significant difference in LDL–C. level between clinical and healthy groups. The same relationship was noticed in the total chol. level (nonsignificant increase in the subclinical hypothyroid patients and a significant increase in the clinical cases in comparison with its level in the healthy persons).

LDL-C levels in subjects with subclinical hypothyroidism were significantly increased while HDL-C levels were decreased when compared to euthyroid subjects, after adjustment for age, sex, and BMI [29]. Many studies, however, showed that HDL-C and TG levels were similar between subclinical hypothyroidism patients and control groups. Although TC and LDL-C levels were higher in patients with subclinical hypothyroidism than in controls, the difference was not statistically significant [30,31].

The results in this research showed higher levels of TC and LDL-C in hypothyroid patients, which is similar to the findings of previous studies ,which reported higher total cholesterol, LDL cholesterol and apolipoprotein B in overt hypothyroidism [32,33].The study of Chen et al.,2016 [34] showed a significant increase in serum levels of TG, TC, and LDL-C in patients with hypothyroidism, which is also compatible with our results. Dyslipidemia has been reported in patients with thyroid dysfunction [35,36]. Its found that the decrease in thyroid hormones level will attenuate the lipoprotein lipase (LPL) activity, this enzyme responsible for TG-rich lipoproteins clearance [37], as a result, serum level of TG will increase. In hypothyroidism, decreased thyroid hormones lead to reduced expression of LDL receptors, which may attenuate cellular uptake of LDL-C from circulation and catabolism of LDL-C and finally lead to increase in TC level [35,38].

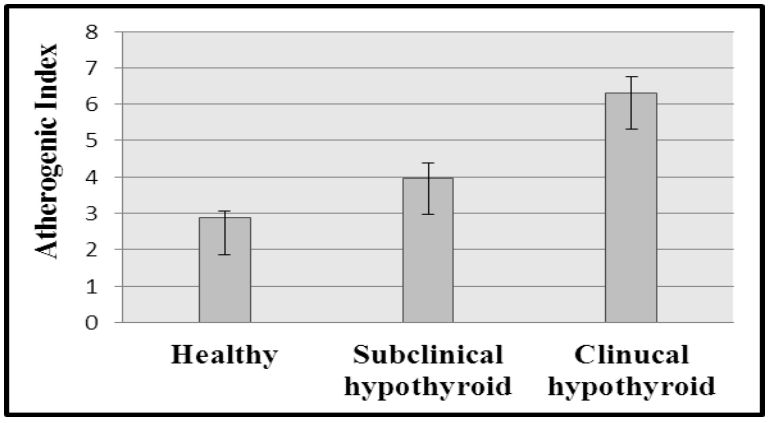

Figure 3: Show the atherogenic Index (AI) value in the patients groups in comparison with the control.

On the other hand, thyroid hormones change the lipid profiles by stimulating hydroxymethyl-glutaryl coenzyme A (HMG CoA), the key enzyme of cholesterol biosynthesis, and induce an increased synthesis of cholesterol. Besides, the LDL-C receptor gene has a thyroid hormone responsive element (TRE) that could allow T3 hormone to modulate the gene expression of the LDL-C receptor and cause an increase of LDL-C receptor synthesis [39].

The clinical hypothyroid group has the highest value of Atherogenic Index (AI) when compared with its values in the other groups, as it was obvious from the figure 3. AI value in the subclinical hypothyroid group was higher than its value in the healthy group at the same time less than its value in the clinical hypothyroid group. No significant differences were found between AI value in subclinical hypothyroid patients and healthy group, while its value is highly significant in the clinical hypothyroid group in comparison to the healthy group.

The atherogenic index is a predictive value for atherosclerosis related with the different disease in different degree of illness as cleared through many types of research.Our results show that as long as T3 continue in low, the illness becomes more stabilized, the patient becomes more susceptible to cardiac problems.

Lipid profile consists of a group of biochemical tests often used in predicting, diagnosing and treating lipid-related disorders including atherosclerosis [40]. Several studies show that overt hypothyroidism is associated with abnormalities of lipid metabolism such as elevated levels of total Chol., LDL-C, and TG, thereby predisposing to cardiovascular diseases [41,42,43]. Some other studies have been indicated that small changes in thyroid hormone levels within the reference range may influence the severity of atherosclerosis [44].

The strong association between the risk of coronary artery diseases (CAD), high levels of LDL-C and low levels of HDL-C has been well established [45,46]. The ratio of LDL-C to HDLC is considered as a prognostic marker for cardiovascular disease. The elevated ratio of LDL-C to HDL-C occurred in patients with hypothyroidism due to a significant increase in LDL-C levels and a slight decrease in HDL-C levels, indicating an increase in risk of cardiovascular disease [35,38].

The atherogenic index of plasma which is a mathematical relationship between TG and HDL-C has been successfully used as an additional index when assessing cardiovascular risk factors [47]. Additionally, the low-T3 syndrome is a strong predictor of death in cardiac patients and might be directly implicated in the poor prognosis of cardiac patients [22].

IL-12 is immunoregulatory cytokines with antagonistic effect on T-helper lymphocytes differentiation. IL-12 is a pro-inflammatory cytokine composed of two subunits, p40 and p35, encoded by the IL12B and IL12A genes, respectively. IL-12 induces the differentiation of CD4C T lymphocytes from a Th0 to a Th1 phenotype and promotes cell-mediated immunity [48,49].The role of IL-12 in the pathogenesis of autoimmune thyroid diseases has been widely investigated and it is well established that they have a pivotal role in the pathogenesis of the disease [50,51].

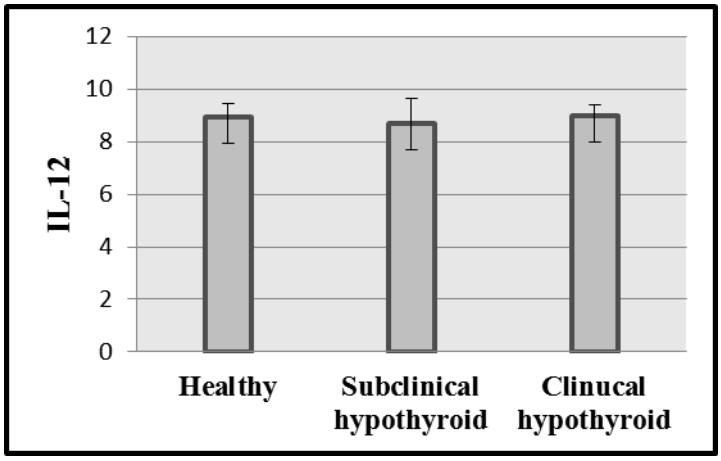

Figure 4: Serum concentration of IL-12 in the patients groups in comparison with the control.

Our results show no significant differences in the level of IL-12 between the study groups. The results of other investigators concerning the IL-12 serum levels in different stages of hypothyroidism are contradictory. The study of Phenekos *et al*.,2004 [51] have shown that IL-12 serum concentrations were significantly higher than those in normal controls in hypothyroid patients. Zhang *et al*.,2006 [52] found that the mean levels of IL-12 in euthyroid hypothyroid patients were significantly higher than those in normal controls; however, they observed no differences in IL-12 serum levels between hypothyroid patients and normal controls as compared to our results. The study of Guclu *et al*.,2009 [53] showed a statistically significant decrease in the IL-12 serum levels in hypothyroid patients after treatment with levothyroxine for about 12 weeks.

During iodine-induced autoimmune (lymphocytic) thyroiditis in non-obese diabetic mice, IL-12 is produced in the thyroid gland early and throughout the course of the disease and local production of IL-12 in the thyroid enhances the expression of sodium-iodide symporter and inhibits thyroid hormonogenesis, thus inducing primary hypothyroidism; also the disease-promoting effect of IL-12 was independent of interferon-g. On the basis of our results and data from experimental models and clinical studies, it may be suggested that the effect of IL-12 on the initiation and regulation of immune responses and also on thyroid function is crucial in hypothyroidism [54,55].

Tamaru *et al.,* 1999 [56] investigated serum total interleukin-12 (IL-12) levels in patients with Graves' disease and Hashimoto's thyroiditis. The serum IL-12 levels in Graves' disease were significantly increased in the hyperthyroid state and were decreased during treatment with antithyroid drugs (methimazole or propylthiouracil) in accordance with the decline of free tri-iodothyronine (T3) levels, free thyroxine levels and thyroid-binding inhibiting immunoglobulin (TBII) levels. When T3 was administered orally to normal subjects, serum IL-12 levels were slightly increased. These results suggest that IL-12 might be increased due to prolonged stimulation with thyroid hormone, and thyroid hormone by itself might be a self- perpetuating factor of Graves' disease via increased IL-12 production.

Kimura *et al*., 2006 [55] reported a novel role of IL-12 on thyroid hormonogenesis and implicated it in the pathogenesis of organ-specific autoimmune diseases, such as Hashimoto's thyroiditis. IL-12 transgenic mice after immunization with a dose of mouse thyroglobulin, developed a lymphocytic thyroiditis which is more frequent and severe than that occur in wild-type mice.

There was no statistically significant difference between groups for serum IL-33 levels. Serum IL-33 level was lower in subclinical hypothyroid patients in comparison to its level in the other groups as shown in (Figure 5).

It was found that serum IL-33 concentrations were significantly higher in Graves' disease group compared to the other groups and there was a positive correlation between serum IL-33 and free T3 and free T4. Also, negative correlation between serum IL-33 and TSH was statistically significant. These findings correlation of serum IL-33 with thyroid hormone levels may be useful as an indicator for Graves' disease and may help to make evident to the pathophysiologic processes of the autoimmune thyroid diseases [57].

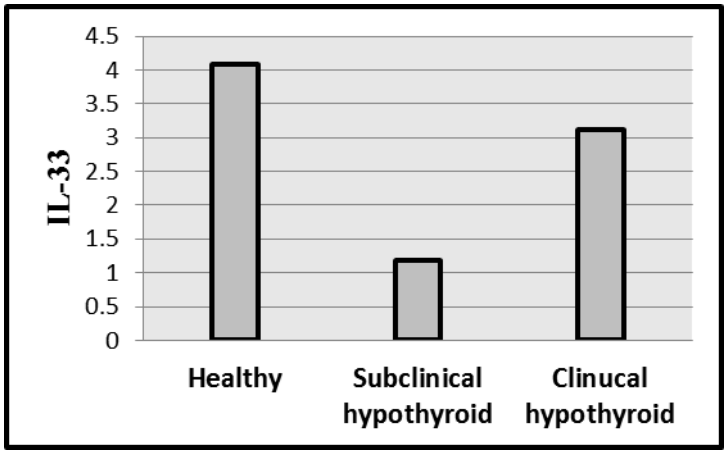

Figure 5: Serum concentration of IL-33 in the patients groups in comparison with the control.

These findings agree with our results because the positive relationship between T3 and T4 with IL-33 mean that the decrease in the thyroid hormone as in hypothyroidism accompanied by a decrease in the IL-33 level and vice versa.

Interleukin-33 (IL-33), a 30 kDa cytokine, is a member of the IL-1 family. It is considered to be an autoimmune biomarker associated with T helper 2 (Th 2) response [57]. There are studies investigating the levels of IL-33 and IL-1 receptor family members in several pathological conditions. IL-33 levels are increased in patients with disorders of inflammation such as ankylosing spondylitis, rheumatoid arthritis, systemic lupus erythematosus, and multiple sclerosis [58].

T helper 1 (Th1) cells produce INF-γ, IL-2, and TNF-β which activate macrophages and are responsible for cell-mediated immunity and phagocyte-dependent protective responses. By contrast, T helper (Th2) cells produce IL-4, IL-5, IL-10, and IL-13, which are responsible for strong antibody production, eosinophil activation, and inhibition of several macrophage functions, thus providing phagocyte-independent protective responses [59].

Cytokines play important roles in mediating autoimmune processes in thyroid disease. *In vivo*, IL-33 induces the expression of IL-4, IL-5, and IL-13 and leads to severe pathological changes in mucosal organs [60].

4. CONCLUSION

Dyslipidemia is more common in clinical hypothyroidism than other groups and more severe than subclinical hypothyroidism. As long as cholesterol and TSH continue high, the patients become more susceptible to coronary heart disease as proved through calculating atherogenic agent in each group.

The positive relationship between thyroid hormones and IL-12 may refer to the involvement of the latter in endocrine signaling as immunomodulation. The decrease of IL-33 in subclinical hypothyroid group suggests humeral immunological failure which may need more studies and evaluating for the other related cytokines.

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