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Relationship of climate components and hair cortisol level with thyroid functions

A Thesis Submitted to the Council of College of Medicine - University of Sulaimani in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in Medical Physiology

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This Thesis is Dedicated

to

- ***My Family …***
- ***My Colleagues …***
- ***All those who care for the development of my scientific and educational status…***

With love…

Darya

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Abstract

Background: Subclinical hypothyroidism (SCH) might reverse to euthyroidism without treatment. They might have many symptoms of hypothyroidism. Various changes in thyroxine (T4), tri-iodothyronine (T3) and thyroid stimulating hormone (TSH) levels were observed in different seasons. Hypothyroidism is associated with an increase in serum cortisol level while, long-term activity of hypothalamic-pituitary-adrenal (HPA) axis in hypothyroid and SCH subjects has not been studied.

Objectives: This study aimed to assess SCH subjects both clinically and biochemically and to compare them with Euthyroid subject with various TSH level. And to find the effect of temperate climate of the city of Sulaymaniyah on the seasonal variations of thyroid functions in euthyroid and SCH subjects. On the other hand, we aimed to assess the hair cortisol levels in euthyroid, SCH and hypothyroid groups and to examine the relation of different components of climate and hair cortisol levels on thyroid functions.

Methods: This prospective cross-sectional study included 233 subjects, 67 SCH and 166 euthyroid (48 with high-normal TSH vs. 118 with low-normal TSH). All subjects were examined clinically and biochemically, serum TSH, Free T3 (FT3), Free T4 (FT4), anti-thyroid peroxidase antibody (anti-TPO) and prolactin were measured. In 152 of healthy (euthyroid) volunteers and 25 SCH subjects the clinical and biochemical parameters were compared in both summer and winter season and correlated with the climate components. Hair sample were collected from 118 participants (including 27 hypothyroid), which prepared and measured in in pg/mg of hair. Hair cortisol levels were compared between groups and correlated with thyroid functions. The comparisons between groups were assessed using Independent Sample T-test and paired T-test, and correlations between most variables were evaluated using Pearson correlation ($P<0.05$).

Results: Euthyroid subjects with high-normal TSH were found to have higher anti-TPO than those with low-normal TSH (17.4% vs 7.6%). The Zulewski's clinical score and TSH were positively correlated with anti-TPO, $P < 0.05$. Small but statistically significant increased FT3 level and decreased FT4 level was observed during winter season in euthyroid and SCH subjects respectively. There was a significant negative correlation between FT3 and FT3/FT4 ratio with temperature and sunshine duration and a positive correlation with humidity and atmospheric pressure. Compared to Euthyroid subjects a significant higher hair cortisol was recorded in hypothyroid subjects (17.22 in euthyroid vs 24.17 in hypothyroid). Significant positive association was found between hair cortisol and serum TSH.

Conclusions: The SCH subject has higher Zulewski's clinical score than euthyroid subject and euthyroid with higher TSH levels had higher levels of anti-TPO.

The climate components contributed to the slight variance in hormone levels in different seasons and the effect was mostly on peripheral conversion of FT4 to FT3 rather than central effect, leading to slightly higher FT3 in winter.

Overt hypothyroidism but not SCH is significantly associated with higher hair cortisol levels compared to normal subjects, and a significant positive correlation between hair cortisol and TSH was found.

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List of Abbreviations and Acronyms

ACTH	**adrenocorticotrophic hormone**
ANCOVA	**analysis of covariate**
ANOVA	**analysis of variance**
BAT	**Brown adipose tissue**
BMI	**Body mass index**
BMR	**Basal metabolic rate**
CI	**Confidence interval**
CLIA	**Chemiluminescence immunoassay**
Cortisol log	**Cortisol logarithm**
CRH	**Corticotrophic releasing hormone**
CS	**Cushing's syndrome**
CVD	**Cardiovascular disease**
D1	**Diodothronine deiodinase 1**
D2	**Diodothronine deiodinase 2**
D3	**Diodothronine deiodinase 3**
ECLIA/ECLA	**Electrochemiluminescence immunoassay**
ELISA	**Enzyme linked immunosorbant assay**
EU	**Euthyroid**
FT3	**Free Tri-iodothryonine**
FT4	**Free Thyroxin**
HC	**Hair cortisol**
HNT	**High normal TSH**
HPA	**Hypothalamic- pituitary-adrenal axis**
HPT	**Hypothalamic-pituitary-thyroid axis**

LC/MS	**Liquid chromatography/mass spectrometry**
LNT	**Low normal TSH**
L-T4	**Levo-thyroxine**
mRNA	**Messenger Ribonucleic acid**
PBS	**Phosphate buffered saline**
Pg/mg	**Pictogram/milligram**
PTA	**Pituitary thyroid axis**
RIA	**Radio-immuno assay**
s.TSH	**Serum thyrotropin stimulating hormone**
SCH	**Subclinical hypothyroidism**
T3	**Tri-iodothyronine**
T4	**Thyroxine**
TFT	**Thyroid function test**
TH	**Thyroid hormone**
TPO	**Thyroid peroxidase**
Anti-TPO	**Thyroid peroxidase- antibody**
TRH	**Thyrotropine releasing hormone**
TSH	**Thyroid stimulating hormone**
UCP	**Uncoupling protein**

Introduction

The hypothalamic-pituitary-thyroid (HPT) axis is responsible for the biosynthesis and secretion of thyroid hormones (TH) thyroxine (T4) and triiodothyronine (T3) (1). This is regulated by the release of thyroid stimulating hormone (TSH) from the anterior pituitary, which in turn is stimulated by the release of thyrotropin releasing hormone (TRH) from the hypothalamus (1). Most of the serum T3 originates from the conversion of T4 by extra-thyroidal type 2 iodothyronine deiodinase (D2) (2). The TH are responsible for the regulation of the metabolic rate and thermogenesis (3,4).

Subclinical hypothyroidism (SCH) is a mild form of thyroid dysfunction (5). The presence of raised serum TSH in combination with a serum free T4 level that is within the reference range is sufficient to diagnose this condition (5). The SCH is more widespread than overt hypothyroidism, thus screening and early diagnosis are important (6). There are risks of development of some diseases in SCH and few studies revealed improvement after treatment (7–10).

Although studies have revealed that SCH does not create a specific symptom, a substantial number of these patients complain of some hypothyroid feature (11,12). Apart from biochemical results, the decision for treating such patients is determined by the physician's assessment of the clinical symptoms and risk factors (13). Therefore, the existence of a symptom-rating scale is important to assess the clinical feature and the potential effect of treatment (13). For this purpose, a clinical score was set by Zulewski et al. (12), who reevaluated the classically used hypothyroid clinical features in regards to more current laboratory investigations (12).

The most common cause of SCH is autoimmune thyroiditis with its association of raised anti-thyroid peroxidase antibodies (anti-TPO) (14). This antibody can also be found in a number of euthyroid subjects; these subjects might be at higher risk for developing SCH (13). As subjects with SCH develop frank hypothyroidism at a rate of 5% every year and euthyroid subjects have a risk of developing SCH, it is valuable to recognize the subjects at risk (15,16).

Some studies relate the conversion of SCH to euthyroid and vice versa to seasonal variation (17). Seasonal changes in TSH and/or TH levels have been observed among healthy individuals upon prolonged stay in arctic and Antarctic region (18–23). Most of the studies showed an increase in TSH in winter (19,22) or with prolonged exposure to cold temperatures (20); however, a few studies exhibited changes in T3 and T4 (24-26, 28). The cause of these seasonal changes in TH and TSH level varies; it could be due to a centrally mediated (21,24), change in thyroidal secretion (25), change in protein binding (26), or peripheral metabolism of TH during different seasons (27).

Circannual variations in the TH levels have also been studied in regions having temperate climate (28–30). Most of the studies were conducted before the development of new and efficient assays (24,30,31) or it was a retrospective study (17) or the sample size was small resulting in erroneous data analysis (28,30). The effect of higher temperatures on the hormones is still not known as earlier studies were focused in areas where the maximum temperature rarely exceeded 35^{o}C (17,29).

Studies in temperate climates (28,29,32) have reported an increase in TSH and decline of T4 level during the winter month (29). One study

demonstrated significant seasonal differences in serum TSH levels which led to a temporary transition between the euthyroid status and SCH (17).

Some variation in TSH secretion might be due to central cause. The HPA-axis functionally related to HPT- axis in animals and humans (33–40), especially in cases with hypothyroidism (39) or hyper-cortisolism (37,38). Cortisol is the product of HPA axis activation and it is elevated in stress and some disease condition (41–45). Studies reported that the HPT axis was inhibited at the level of hypothalamus and pituitary (34) and decreased peripheral conversion of T4 to T3 in subjects with increased cortisol level due to stress induction (35). Hypothyroidism have been found to be associated with increased cortisol levels (33,36,39). The positive TSH-cortisol association found in hypothyroidism (39) was detected in SCH subjects or even in euthyroid with high normal TSH range (40).

Previous reports measured the systemic cortisol levels to study the relationship of cortisol with TH (39,40,46), which does not represent long-term cortisol exposure. The systemic cortisol (the blood, urine, or saliva) is also affected by diurnal variations, pulsatile rhythm (40,47) and other shortcomings (36,48,49).

More recently measuring hair cortisol is regarded as a representative of long-term activity of the HPA axis (48,50,51). Moreover, this test has been supported by several animal and human researches (36,48,50–59). Free cortisol can be incorporated in hair matrix as it is a lipophilic substance (60) and growth of hair at a rate of about 10 mm/month can give a retrospective measure of cortisol levels over several months by one-time sample collection (61).

Collection of the scalp hair is an easy procedure (62). The hair sample can easily be stored and remains stable for several months (48,63–65). Several studies measured the hair cortisol in normal subjects and mean hair cortisol ranging from 7.7 to 46 pg/mg in different studies was recorded (45,57,58,65–67); however, in most studies of healthy adult subjects, the mean was within 20 to 30 pg/mg of hair (58,68).

In clinical studies of subjects with abnormal activity of HPA axis, hair cortisol demonstrated a change in response to a change in the activity of the HPA axis. In Cushing's syndrome, hair cortisol level followed the episode of the disease and correlated with early biochemical data (55,56,59). Hair cortisol is a marker of chronic stress (69) and risk of a cardiovascular event (70,71); it might be abnormal in psychiatric abnormalities such as depression and other (72); it is related to obesity (47) and metabolic syndrome (73)

Until now, no study exists on the clinical and biochemical evaluation of SCH and euthyroid subjects in Sulaymaniyah city; and to the best of our knowledge, prospective studies comparing SCH to euthyroid subjects or comparing euthyroid subjects with various TSH status for clinical and biochemical basis are scant. Few studies exist worldwide to assess SCH subjects for Zulewski's clinical score (12).

The absence of a thorough investigation on the relation of thyroid functions with atmospheric climate and weather parameters (climate components), and the variability in the results for the relation between TH and temperature and luminosity is a major drawback.

Moreover, hair cortisol has not been studied in SCH and hypothyroid subjects. Also, the association of hair cortisol as a long-term activation of

HPA axis with HPT axis was not assessed previously. To the best of our knowledge, no study measured hair cortisol level in Iraq.

The aims of this study are the following:

1. To assess the SCH and euthyroid subject clinically and biochemically. And to find the relationship between the Zulewski's clinical score and serum FT4, TSH, and anti-TPO levels and to know whether Zulewski's clinical score is helpful in the diagnosis of SCH subjects?

2. To evaluate whether euthyroid subjects with high-normal TSH have a higher frequency of clinical symptoms, anti-TPO positivity, than euthyroid subjects with low-normal TSH.

3. To assess the seasonal variations in thyroid functions in euthyroid and SCH subjects from the City of Sulaymaniyah, Iraq; a latitude still unexplored, with a wide seasonal difference where the maximum temperature in summer exceeds 45^{o}C and in winter it goes down to 0^{o}C, and sometimes as low as -5.6^{o}C (74). And to evaluate the effect of weather and climate parameters (temperature, humidity, sunshine duration, cloud cover, atmospheric pressure and the duration of outdoor exposure) on the hormone levels in these subjects.

4. To determine the average hair cortisol levels among a sample of healthy adult, SCH and hypothyroid subjects and to find the relation between hair cortisol as a long-term activity of HPA axis with thyroid functions.

Literature Review

1.1 Hypothalamic- pituitary- thyroid axis and thyroid hormone as an end product of this axis.

Hypothalamus release TRH which control anterior pituitary gland release of TSH, and TSH has receptors on thyroid gland and cause increase in the secretion of thyroid hormones, in turn free thyroid hormones (TH) negatively feeds back on the anterior pituitary to decrease TSH production, Figure 1-1. This negative feed-back is important in preventing over- or under production of TH to maintain proper metabolic activity (3).

Thyroid hormones include thyroxine (T4) and triiodothyronine (T3). Thyroid gland mainly secretes T4 (93%) (3), while most of the T4 (87%) is converted to T3 by peripheral de-iodination of T4 to T3 (75). Most of the TH binds to plasma protein in the blood especially to thyroxine binding globulin (TBG), and only about 0.02- 0.2 % of TH present freely inside blood, and only the free fraction of TH is active physiologically and has effect on anterior pituitary(3,75).

Thyroxine binding globulin elevated during pregnancy and estrogen treatment. Various drugs also have role in the elevation of this protein. A rapid and continuous increase in thyroid binding globulin level leads to a decrease in free TH, temporarily. This feeds back on the anterior pituitary gland to increase TSH release and returning free TH to normal level, and thus result in a net increase in the total quantity of the TH (75). The free hormone is not affected by pregnancy, other hormonal disturbances, drug or change in protein concentration, unlike the total TH. Thus assessing free TH (FT4 and FT3) is more accurate (3,75).

Thyroid hormones increase the rate of metabolism and activity throughout the body because it increases transcription of large number of genes and thus increase cell's protein production, which include enzymes, transporters, structural and other proteins (75).

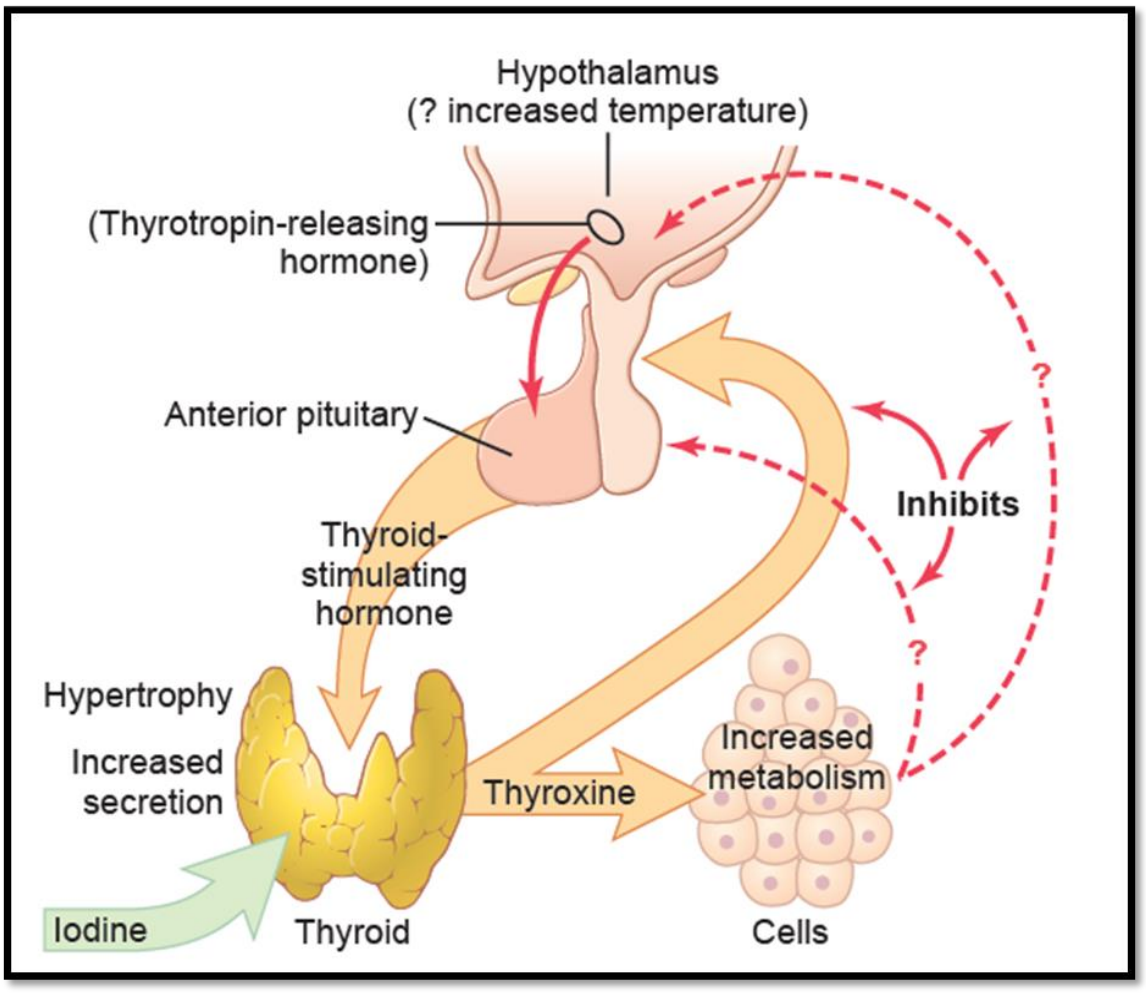

Figure 1-1. Hypothalamic Pituitary Thyroid-axis (HPT-axis).

"Regulation of thyroid secretion, Guyton and Hall text book of Medical Physiology, 13th Edition, 2015, Chapter 77 (3)".

1.2 Hypothyroidism, subclinical hypothyroidism and euthyroidism

1.2.1 Hypothyroidism

Hypothyroidism is mostly caused by primary hypothyroidism, and thus it is a decrease in TH secretion by thyroid gland with compensatory increase in serum TSH; the combination of these two biochemical finding is diagnostic of primary hypothyroidism (76).

The decreased TH in hypothyroidism associated with several clinical sign and symptoms but the extent of these clinical feature and complaint of the patient vary according to the degree and of decrease in TH and on the cause and rapidity of decrease in TH (slow or abrupt decrease). The clinical features are mostly related to decreased activity and metabolism in the whole body, which reveals as fatigue, sluggish movement, slow mentation, slow speech, cold intolerance, constipation and weight gain (77).

None of these manifestation are specific to hypothyroidism thus assessing the subjects with clinical scoring scale is important to combine symptoms and signs on this scale and accordingly with biochemical investigations diagnose and manage the subjects with hypothyroidism. While part of the clinical manifestations in hypothyroidism are due to accumulation of glycosaminoglycans in the interstitial space and appearance of dry, scaly thick skin and coarse hair, puffy faces, enlarge tongue and hoarseness of voice. These signs more visible in young individual and in older person might be obscured by aging process (77).

1.2.2 Subclinical hypothyroidism (SCH)

When the increase in TSH is accompanied by the normal TH, this condition defined as SCH. Its prevalence is about 4-15 % worldwide (78). The prevalence rises with age and it is higher in females than males (79,80).

Subclinical hypothyroidism is due to the same causes of overt hypothyroidism, and in both conditions most subjects might suffer from autoimmune thyroiditis (Hashimotos thyroiditis) and presence of thyroid peroxidase antibody (Anti-TPO). With destruction of the gland by the autoimmune disease (78) and gradual decrease in thyroid function and progressive changes in the thyroid function tests (TFT). However, SCH is more reversible, as it is sometime only a transient elevation of TSH (81).

At the beginning of mild thyroid abnormality (compensated hypothyroidism), the disturbance is first appear in serum TSH rather than TH, because TSH increase or decrease to compensate the decrease or increase in TH respectively, thus biochemically there will be change in TSH without change in TH, and thyroid function in these subjects were normal (81). This is all because of feedback effect that aim to maintain stable level of plasma free TH. However, when the elevation of the TSH continue the abnormality in the thyroid function will produce (81). In SCH the TSH levels is mostly <10 mIU/L. Thus the most common initial investigation is serum TSH, then if TSH is higher than normal (> 4.5 mIU/L according to the laboratories) then the investigation should be repeated few weeks later with the serum T4 in case of SCH (78).

Subclinical hypothyroidism might be associated with vague, non-specific symptoms of hypothyroidism, and some of these symptoms are

present in normal elderly persons, while in some individual more clinical symptoms of hypothyroidism appear (78). Thus, because SCH either has no or mild symptoms of hypothyroidism, previous studies fail to diagnose SCH clinically (82), and the diagnosis of this form of mild thyroid failure is mainly depend on biochemical tests (78,82).

There are controversies on whether to treat SCH subjects or not. Because SCH were associated with risk of development of some diseases it is important to monitor the SCH subjects (6) and some studies believe that it is important to treat these subjects to prevent progression to overt hypothyroidism and its clinical consequences (14,81).

Some cross sectional studies reported increased frequency of neurobehavioral abnormalities including depression, memory loss, ….etc in these subjects (81); SCH also associated with metabolic syndrome (TSH was significantly associated with triglyceride and fasting blood sugar level in SCH subjects) (83), impaired cardiovascular function and cardiovascular risk factors like increase serum cholesterol and low density lipoprotein in other studies (7,8).

It is important to measure thyroid function and exclude SCH in subjects who desire to conceive or in females who are pregnant, to prevent risk of abortion and adverse pregnancy outcomes, in comparison to euthyroid pregnant women, SCH pregnant women were three folds more susceptible for placental abruption complication, and premature delivery was about two times higher in SCH pregnant women (9), treatment in these subjects suggestive to correct and adjust TSH levels (9).

Levothyroxine (L-T4) therapy has been recommended for patients with TSH persistently >10 mIU/L, but controversy exists in cases of patients

with concentrations ≤10 mIU/L and progression to overt hypothyroidism is one of the factors considered in the decision to treat (84).

1.2.3 Euthyroidism

Euthyroidism is defined biochemically as normal serum TSH in combination with normal FT4 and FT3. Free TH is important to suggest if the subjects are euthyroid or has subclinical hyper or hypothyroid, as in abnormal plasma protein level, the total plasma T4 and T3 might be abnormal, although the subject is euthyroid (75).

1.3 Controversy on reference range of TSH

Recently, a controversy developed about the TSH level in SCH subjects (14,85). Euthyroid subjects with a serum TSH level of more than 3 mIU/L have higher anti-TPO titers and higher rates of progression to overt hypothyroidism, although their TSH is within the reference limit (86). Thus, reducing the upper limit of the normal TSH reference range from 5 to 3 or even 2.5 mIU/L has been suggested, and this affects the number of cases defined as SCH (85–89).

The reference ranges for TFT mostly come from the immunoassay manufacturer of the laboratory measuring these parameters. While studies assessing the oxygen consumption and clinical examination of euthyroid individual with the usual reference range found that the number of euthyroid subjects decreased, thus resulted in a narrower reference interval than that suggested by the manufacturer's range (TSH=0.76–3.90 mU/l) versus (TSH: 0.35–5.50 mU/l), respectively (89). Moreover, there were evidence of metabolic abnormality, and increased body fat in euthyroid subject and the presence of higher TSH and lower FT4 associated with higher risk of

cardiovascular disease and increased body fat composition. Higher TSH were associated with higher cholesterol, Triglyceride, and BMI (85). In another study with 6 years follow up of euthyroid subjects, they found that the risk factors for converting individual from euthyroid to SCH are glucose intolerance, elevated cholesterol, hypertension and obesity (90).

1.4 Clinical scoring system in hypothyroidism: Zulewski's clinical score

Studies who claimed that the clinical feature does not play role in diagnosis of SCH, they mostly evaluated elderly population, in whom most of the clinical feature of hypothyroidism is obscured by normal aging processes and elderly subjects has several complaints that mimic hypothyroid symptoms (78,82).

Although studies have revealed that SCH does not create a specific symptom, a substantial number of those patients complain of some hypothyroid feature such as fatigue, muscle cramps, cold intolerance, depressive symptoms, reduced quality of life, cognitive function, and memory loss (11,12). Apart from biochemical results, the decision for treating such patients is determined by the physician's assessment of the clinical symptoms and risk factors. Therefore, the existence of a symptom-rating scale is important to assess the clinical feature and the potential effect of treatment. The clinical score can judge the individual severity of metabolic hypothyroidism (13).

Decades ago, in 1969; a diagnostic clinical index was set by Billewicz et al. (91), by evaluating the existence or absence of several clinical feature components of hypothyroidism and scoring them in these individuals. But

the investigation that used to measure thyroid function was old at that time. Latter after development of newer laboratory technique and immunoassays, a newer clinical score was set by Zulewski et al. (1997), who reevaluated the classically used hypothyroid clinical features in regards to current laboratory investigations (12).

They selected 332 subjects from a cohort of hypothyroid and normal subjects. They correlated the clinical score with TSH, TH (FT4 and T3) and some other tests consistent with TH action on target tissues, like total cholesterol and clinical examination like relaxation time of ankle reflex. They assess the validity of this clinical score in reflecting tissue hypothyroidism. The best and significant correlation that was detected in SCH individual was between this new score and FT4 and TSH. The correlations of the new score with metabolic parameters of hypothyroidism were preserved in SCH: for total cholesterol and ankle reflex time (12). The clinical score set by Zulewski is shown in Table 1-1.

Table 1-1. Zulewski's clinical score for hypothyroidism (Scoring of symptoms and signs of hypothyroidism) (12)

On the basis of		New score	
		Present	**Absent**
Symptoms			
Diminished sweating	Sweating in the warm room or a hot summer day	1	0
Hoarseness	Speaking voice, singing voice	1	0
Paraesthesia	Subjective sensation	1	0
Dry skin	Dryness of skin, noticed spontaneously, requiring treatment	1	0
Constipation	Bowel habit, use of laxative	1	0
Impairment of hearing	Progressive impairment of hearing	1	0
Weight increase	Recorded weight increase, tightness of clothes	1	0
Physical signs			
Slow movements	Observe patient removing his clothes	1	0
Delayed ankle reflex	Observe the relaxation of the reflex	1	0
Coarse Skin	Examine hands, forearms, elbows for roughness and thickening of skin	1	0
Periorbital puffiness	This should obscure the curve of the malar bone	1	0
Cold skin	Compare temperature of hands with examiner's	1	0
Sum of all symptoms and signs present		12	0

For clinical judgment, add 1 point to the sum of symptoms and signs present in women younger than 55 yr.

Hypothyroid, more than 5 points; euthyroid, less than 3 points; intermediate, 3–5 points.

1.5 Reverting cases from SCH to euthyroidism or hypothyroidism

There is lack of a uniform guideline on screening for thyroid diseases through s. TSH level. While because of the high prevalence of SCH and its correlated risk factor for metabolic syndrome and other associated abnormalities, the recommendation were set by American thyroid Association to screen subjects for serum TSH from age 35 years for every 5 years (92). And suggestion of more frequent TSH assessment was made for subjects with clinical features of thyroid abnormalities, those who have risk factors (92) woman older than 50 years and women willing to conceive or pregnant women (87)

Subclinical hypothyroidism might remain as SCH throughout the years (16,93,94), while high rate of progression to overt hypothyroidism or reverting back to normal were recorded in previous studies (81,84,95).

Studies on normal population and those who assessed the natural history of SCH revealed various rate. The progression from SCH to overt hypothyroidism was in a rate of 4.3 % and 2.6 % per year in the presence or absence of anti-TPO, respectively (95) while higher rate of progression of 19% over 5 years and 35% over 10 years follow up were recorded in female patients (81,84).

In a study on natural history of SCH in women, the rate of normalization of 22.8% over 5 years and of 9% over 10 yr follow up were recorded (81). While higher rate of normalization were recorded, in a study on older population of age > 55 years, with about 3 years follow up, 52% of subjects experienced normalization of their TSH (93).

Although there is progression of a group of SCH to overt hypothyroidism and normalization of a group of them, a major group of SCH individual remain subclinical for several years (16,93,94).

The predictors of normalization of SCH or progression to overt hypothyroidism were detected to be baseline TSH level and presence or absence of anti-TPO, and history of radiation was also reported in some studies (81,84,87). This explains the difference in rate of normalization or progression of SCH throughout studies. Serum TSH more than 10 mIU/L and 20 mIU/L were regarded as a predictor of higher rate of progression (81,93), and TSH level of more than 8 mIU/L was regarded as predictor of the need of treatment (84).

When the controversy appears regarding the normal range of TSH level, and lowering the upper limit of normal from 5 to 3 mIU/L, subjects with the level of 3-5 were unlikely to complain of clear clinical symptoms or abnormality, although in comparison to SCH with TSH of less than 3 mIU/L, these subjects were at higher risk of progression to SCH (87).

Thus studies evaluating natural history of SCH revealed progression, normalization or stable SCH and more rate of normalization were detected recently (16,81,84,93–95). And studies evaluating normal population reported intra-individual variation of thyroid function tests. They explained these variations to circadian rhythm, slight seasonal variation in TSH secretion (96), however much of this intra-individual variation in TSH secretion are unknown and couldn’t be explained especially in mild SCH (17).

The baseline TSH of less than 8 or 10 mIU/L and the absence of thyroiditis are among the predictor of spontaneous normalization of SCH

(84), and TSH level of < 6 mIU/L is a predictor of lower possibility of progression (93). Studies revealed that reverting of SCH to euthyroid is about 20-50% (81,84,93). While in subjects with TSH level of more than 8 mIU/L this level decreased to 5% of cases (84). Thus level of TSH at baseline is important predictor of progression or normalization of SCH subjects (84).

1.6 Hyperprolactinemia in hypothyroidism

Previous studies related the presence of anti-TPO antibody with hyperprolactinemia (97). Hyperprolactinemia is a usual finding in frank hypothyroidism, and mild prolactin elevation is found in SCH, particularly those with higher TSH values (13). A different mechanism is stated to be responsible for the development of hyperprolactinemia in hypothyroidism (97).

One of the proposed mechanism of hyperprolactinemia in hypothyroidism is as follow, decreased TH secretion in hypothyroidism negatively feeds back on the hypothalamus to increase TRH secretion, and TRH secretion stimulate release of prolactin in addition to TSH secretion (75). Decreased prolactin clearance and reduced sensitivity of the anterior pituitary cells to dopamine in hypothyroidism has also been proposed as mechanisms of elevated prolactin (97).

1.7 Season, climate and thyroid function

1.7.1 Effect of season on TSH, FT4 and FT3.

These hormones increase the basal metabolic rate and body heat production. Complete lack of these hormones, cause the basal metabolic rate (BMR) to fall 40-50% below normal (3).

Despite the effect of age and sex on thyroid hormone level (as the concentration of hormones decreases with age in both sexes but the drop is more in female than males), the effect of season on T3, T4 and TSH have also been noted. Higher levels of TSH are noted in winter than in summer (17,98).

One of the best-known stimuli for increasing the rate of TRH secretion by the hypothalamus, and therefore TSH secretion by the anterior pituitary gland, is exposure of an animal to cold. This effect almost certainly results from excitation of the hypothalamic centers for body temperature control. Exposure of rats for several weeks to severe cold increases the output of TH sometimes to >100% of normal and can increase the BMR as much as 50%. Interestingly, persons moving to arctic regions have been known to develop BMR 15-20% above normal (3). Moreover, prolonged Antarctic residence increases TSH by approximately 30%, which suggests that prolonged cold exposure could affect TSH levels in human adults (20). This modest effect on TSH could partly explain changes in thyroid function in patients whose TSH levels are near the upper limit of normal (20).

Although most of the results suggested that season has effect on the thyroid function tests even if small, different results were recorded though out the literature. Some studies revealed effect on T3 only, other suggests effect on TSH, or both (19,20,22,23). While there is study that revealed no significant annual difference in thyroid functions (29).

As these hormones facilitate adaptation to temperature variation, seasonal changes in TSH and/or TH levels have been observed among healthy (euthyroid) individuals exposed to extreme environmental conditions, as in the arctic and sub-arctic regions and those with prolonged

stay in Antarctica (18–23). Most of the studies showed an increase in TSH in winter (19,22) or with prolonged exposure to cold temperatures (20); however, a few studies exhibited an increase in T3 and/or T4 (20,23), while a decrease in the TH levels were also observed (19–21). Different factors were supposed to be the causes of these seasonal variation in thyroid functions; thus the etiology varies, it could be due to a centrally-mediated response of the HPT axis (21,24), change in thyroidal secretion (25), change in protein binding (26), ambient luminosity (19), or peripheral metabolism of thyroid hormone during different seasons (27).

In temperate climate; with greater difference in temperature between summer and winter compared to polar region, seasonal variations in TH levels have also been observed (28–30). However, most of these studies lacked reliability because they were conducted before the development of new and efficient assays (24,30,31) or the sample size was small resulting in erroneous data analysis (28,30) or it was a retrospective study (17). Moreover, the effect of higher temperatures on the hormones are still not known as earlier studies were focused in areas where the maximum temperature rarely exceeded 35^{o}C (17,29).

Studies on the seasonal changes in the TSH levels in euthyroid and hypothyroid residents of temperate climates (28,29,32) have reported an increase in serum TSH and decline of serum T4 during the winter month; in some cases, these changes may be clinically relevant (29).

1.7.2 Atmospheric climate (climate components): Its effect on TSH and thyroid hormones

One of the part of climate system is atmosphere which is the most dynamic and changeable, it is the air that envelops the earth's surface (99).

Most weather phenomena occur at the lowest level of atmosphere; the troposphere, which is closest to the earth's surfaces. Nearly all water vapor (humidity) and clouds are present in this layer. Earth has diverse regional climate, ranging from extreme cold at the polar region to tropical heat at the equator (100). Thus for assessing regional weather and climate, the following parameters were used: temperature, humidity (%), wind, , sunshine duration /luminosity (hours), cloud cover (okta), precipitation, dew point and atmospheric (ambient) pressure (mmHg) etc (101,102).

At winter there are differences in the temperature (both minimum and maximum temperature), sunshine duration, humidity and cloud cover in comparison to summer. In some region and latitude as tropical area and polar region, the difference in climatic parameters between winter and summer are not significant, while in temperate climate there is hot summer and cold winter although not reaching extreme like continental climate. In continental climate the difference between winter and summer temperature is even greater (103).

Antarctic studies reported increased TSH, T3 with decrease FT4 as a cold adaptation in these regions, when the individuals remain for more than five months (20) while only T3 increased in subarctic region (104). A part of the increase in T3 in these cold region were linked to increase in thyroid synthetic activity (20). The effect of photoperiod on TH level has also been suggested in Antarctica, where exposure to bright light cause greater reduction in TSH and greater increase in FT3 in winter than summer(105).

Few studies directly correlated the weather and climate variables with thyroid functions especially in area with temperate climate (28,29,106). Inverse relationship between temperature and T3 were found in some studies

(28,106), while no correlation was found between climatic component and TSH in other study (29). In a recent study by Yoshihara et al, who retrospectively studied the patient data of 6 years in a hospital of Japan regardless of thyroid disease and treatment, they found negative correlation of temperature with TSH and FT3 levels (106).

1.7.3 Effect of season on reverting of SCH cases

As previously discussed SCH is mild thyroid failure and management of this condition is complicated because subclinical hypothyroidism is reversible in more cases than previously thought; one study of more than 400 000 individuals showed that in 60% of patients with mild TSH elevation (5.5 to -10 mIU/L), TSH levels normalized without any intervention (96).

Although some predictors of persistent TSH elevation have been suggested, including a higher TSH level with the presence of anti-thyroid peroxidase (TPO) antibodies (93), much of the variability in TSH remains unexplained, especially in cases with mild TSH elevation (17).

In a retrospective longitudinal study in Korea evaluating effect of season on transition from SCH to euthyroid and vice versa, the researchers conclude that the season in which thyroid investigations were assessed was independently related to the transition between SCH and euthyroid status. And they suggested that seasonal variations in TSH level should be considered before deciding on treatment of SCH, especially in the regions with a wide range of temperature differences between the seasons (17).

Overall, the TSH levels increased during winter-spring and decreased during the summer-fall season. The normalization of SCH increased 1.4-fold on follow-up tests performed during the summer-fall, whereas SCH

increased 1.4-fold in euthyroid subjects during the winter-spring follow-up (17).

1.8 Peripheral conversion of FT4 to FT3 and role of deiodinases

Conversion of T4 to T3 is account for about 80% of the plasma T3 and iodothyronine deiodinase is responsible for this conversion. There are three types of iodothyronine deiodinase, D1, D2 and D3. These deiodinases remove a specific iodine from its precursor molecule of T4 to regulate TH activity (107,108). They either activate or inactivate the TH through working on the type of rings of tetra-iodothyronine (108).

Decades earlier, D1 assumed to be responsible for the large fraction of circulating T3, and they identified D1 in the liver and kidney of rat and human. While recently D2 mRNA discovered in human skeletal muscle and its activity was quantified (107).

The D2 generates the active form T3 via deiodination of T4. The D3 has opposite effect by inactivating T3 and to the lesser extent T4, while the D1 is an inefficient enzyme kinetically and it activates or inactivates T4. The role of D1 in health is not clarified yet (107).

Researchers conclude that D2 is a major contributor of serum T3 in euthyroid and hypothyroid human, while D1 is the major source when FT4 is high (at thyrotoxic level). And they suggested that the T3 produced from conversion by D2 is 2-3 times more powerful in gene transcription than T3 produced by D1 (2,107). The deiodinase in extra-thyroidal tissues contribute a major determinant of circulating T3, because all of the T3 produced in the

cytoplasm will eventually exit the cell, unless if it is metabolized in its course (109).

Studies on euthyroid subjects in temperate climate showed no change in TSH and TH level apart from small increase in FT3 in winter. But they couldn't identify if the increase in FT3 is due to thyroidal secretion or increased extrathyroidal T4 to T3 conversion (29).

1.9 Skeletal muscle, brown adipose tissue and thyroid: Their relation to thermogenesis

In a study on human skeletal muscle culture, they revealed that the D2 in myoblasts of different skeletal muscle types has the same activities on conversion of T4 to T3 (2). Studies shown that Beta adrenergic responsive D2 activity is detectable in skeletal muscle of human (110).

Thyroid hormone plays a major role in adaptive thermogenesis; an important target for T3 (111). Mitochondrial uncoupling protein (UCP3) in skeletal muscle and the brown adipose tissue (BAT) is significantly related to cold-induced adaptive thermogenesis by the dissipation of heat due to uncoupling of the respiratory chain from oxidative phosphorylation (112). The UCP3 protein is induced by T3 in addition to fatty acid (113). The contribution of muscle is responsible, on an average for 40% of thermogenesis in humans (111).

Cold exposure increases the sympathetic nervous system activity and increases the potential for cold tolerance by nonshivering thermogenesis in humans (114,115). It was previously thought that BAT are functional only in rodents and newborns (116), but recently functional BAT was discovered in

adult human, the primary location being the supraclavicular area of the body and the uncoupling protein present is UCP1 (117).

Thus, studies on the BAT, skeletal muscle and thermogenesis suggestive of the increase in D2 activity by cold exposure (111,112,114–117).

1.10 Hypothalamic-Pituitary-Adrenal axis and cortisol as a major glucocorticoid

Production and secretion of glucocorticoid is regulated well by the anterior pituitary and hypothalamus under negative feedback (118). Cortisol bind to the glucocorticoid receptor (118) and regarded as a most common glucocorticoid in human (48).

Anterior pituitary gland synthesizes ACTH, a major regulator of cortisol production, and hypothalamus release CRH which is a stimulator of ACTH. In a negative feedback, cortisol has effect on both hypothalamus and pituitary to inhibit CRH and ACTH production (119). This is called hypothalamic pituitary adrenal axis. This axis is the strict connection between the hypothalamus (CRH), pituitary (ACTH) and adrenal gland (cortisol) (50,119) as demonstrated in Figure 2. This axis works to prevent great fluctuation in cortisol level and control the appropriate level of cortisol production (119).

The hormone cortisol which is regulated by this axis is regarded as a biomarker and one of the indicators of stress (50). The time of the day the cortisol is measured should take into consideration (119) because cortisol level is variable throughout the day. In healthy subjects, it peak in the early morning, and gradually decrease thereafter (48). Cortisol level increase with

physical and psychological stress and an increase of up to 10 times have been recorded after acute severe stress (47,118). Exogenous glucocorticoid is another factor that has role in changing cortisol level (119).

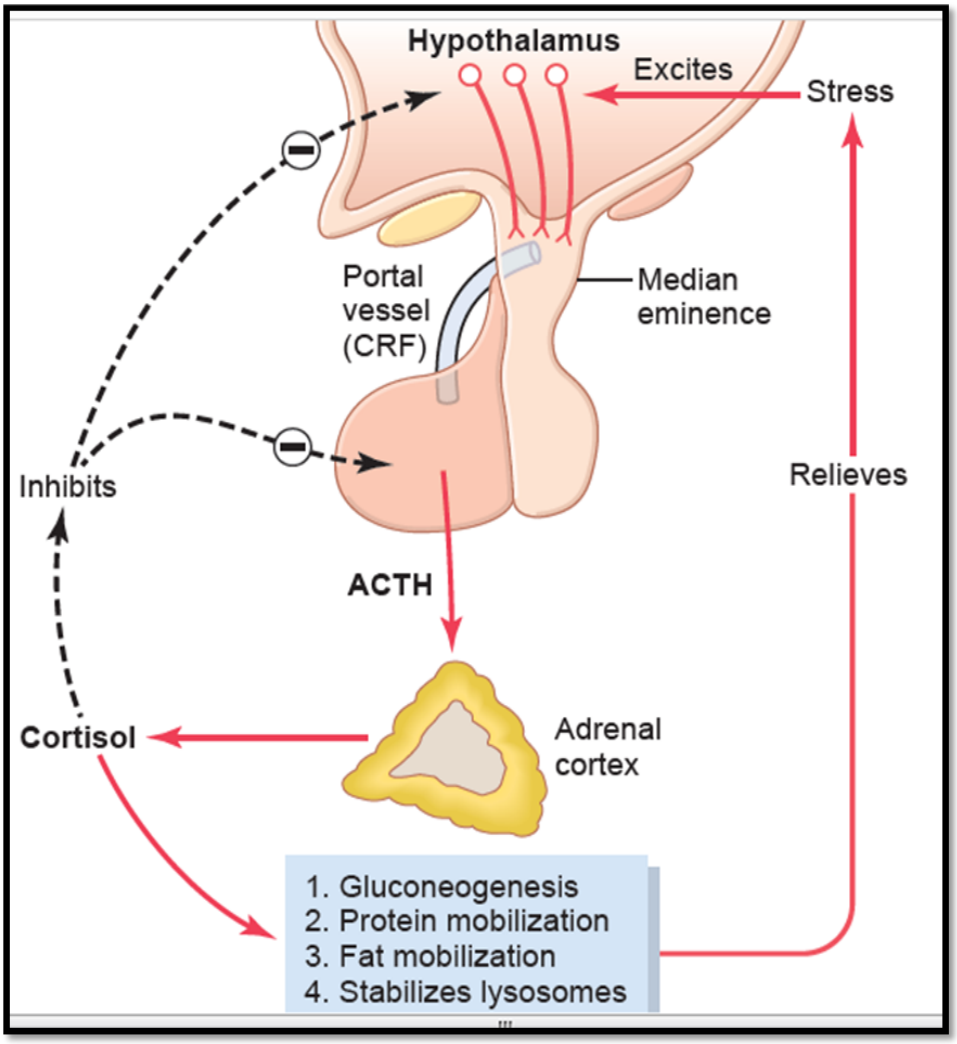

Figure 1-2. Hypothalamic Pituitary Adrenal-axis.

"Mechanism for regulation of glucocorticoid secretion. ACTH, adrenocorticotropic hormone; CRF, corticotropin-releasing factor .Guyton and Hall. Textbook of Medical Physiology, 13th Edition, Chapter 78 (120)."

1.11 Relationship of hypothalamic-pituitary-thyroid (HPT) axis with hypothalamic pituitary adrenal (HPA) axis

Relation between HPA-axis and hypothalamic-pituitary-thyroid (HPT) axis have been recorded in several human and animals studies (33–40), most of these studies find relation in hypothyroid cases (39) or hypercortisolism (37,38). In a study of stress induction, the increased cortisol level cause inhibition of HPT axis at both hypothalamus and pituitary level (34), and in another study the increased cortisol level associated with the decrease in the peripheral conversion of T4 to T3 (35).

Studies found that cortisol levels increased in both hyperthyroid and hypothyroid subjects (33,36,39). In hyperthyroidism, the increase in cortisol levels was related to increased metabolism and thus increased activity of HPA axis (33), while decreased clearance, decreased effectiveness of the negative feedback of cortisol on the HPA axis and metabolic stress are all reported to be responsible for the increased cortisol level in hypothyroid subjects (39,40). Thus positive relation between TSH and cortisol found when the primary disease is in HPT or when thyroid hormone level affected (39,40).

In contrast when the primary disease affecting HPA axis and cortisol level is abnormal, negative association between TSH and cortisol were recorded. Both endogenous (stress and Cushing's syndrome) and exogenous corticosteroids suppress TSH in these studies (37,38). Thus a physiological feedback loop has been suggested when these studies taken into

consideration, which means that low thyroid hormone increases cortisol level while high cortisol feeds back to reduce serum TSH (40).

1.12 Systemic cortisol measurement:

Cortisol measurement in serum, saliva and urine:

Previous reports measured the systemic cortisol levels to study the relationship of cortisol with TH (39,40,46), which does not represent long-term cortisol exposure (48).

Systemic cortisol measurements include cortisol measured in any of the saliva, serum or urine sample. In healthy individual, cortisol is associated with pulsatile release and diurnal variation under effect of ACTH and CRH, thus systemic cortisol do not represent chronic cortisol level, they may represent cortisol level in a single time point or affected by acute cortisol secretion. Moreover some of these samples that used for systemic cortisol assessment are associated with other shortcomings (48,53). The shortcomings associated with each sample type are summarized below.

1.12.1 Serum cortisol:

1. Serum cortisol might be associated with stress during venipuncture and high cortisol level during sample taking (53,121).
2. Serum cortisol measure total cortisol, rather than its free fraction in plasma. Total cortisol level affected by cortisol binding protein (48).
3. Because cortisol level highest in the morning and lowest in the night (120), multiple sampling is required to get cortisol level throughout a day which is an invasive procedure (122).

1.12.2 Salivary cortisol:

The collection of saliva for cortisol measurement is less invasive procedure and evaluates free cortisol, significant association between serum and salivary cortisol was found previously (48,121). However multiple sampling throughout the day is needed to overcome fluctuation which makes the procedure difficult and uncomfortable by the patient, might be affected by patient's compliance, especially in a study with large sample size (48).

1.12.3 Urine cortisol (24 hour urinary cortisol):

The 24-hr urine cortisol has been emerged to overcome the daily fluctuation and frequent sample taking. This test will measure the free cortisol; however it might be associated with difficulty in collecting the sample by the patients and couldn't be done in patients with renal failure and patients on hemodialysis (48). Although 24-hr urine cortisol represents more prolonged cortisol secretion in comparison to serum and saliva sample, it still assesses the cortisol level in one day (48), and couldn't be used for measure of long term or chronic cortisol release (57).

1.13 Hair cortisol detection and mechanism of incorporation of cortisol into hair:

Decades ago, hair analysis was used to detect exposure to environmental toxins (heavy metals, organic and pesticides), drug abuse, and dosage of prescribing drug (123–125). Recently, several studies measured cortisol and other steroids in hair, and hair cortisol used as a biomarker of stress and a long term activity of HPA axis (64,122,126). Hair cortisol is also related to some psychological problems (68). More recently hair cortisol

assessed in obese individual (47), and related with BMI (47,67), metabolic syndrome (127) and high hair cortisol in Cushing's syndrome (59,128) were reported. The data on some of these studies is presented in Table 1-2.

The mechanism by which compounds incorporated into hair is simple passive transfusion as was discussed by the Boumba first time, who proposed a multi-compartment model (125) as shown in the Figure 1-3 (48).

Blood from capillaries that present in contact with follicular bulb; the part that hair cells developed from, supply the growing hair cells and the compound in the blood incorporated into these cells. These cells have the ability to divide every 24-72 hours. As the cells division continue, the cells leave follicle and go upward to the keratogenous zone Here the hair cells will die, thus the cells up not perfused with blood and the exposure to the blood compounds will end and when they move up bring the already containing incorporated compound (that keratinized and bind to them tightly) up with them (125).

Hair grow at an average of 0.44mm/day and 1 cm/month in vertex of scalp (125,129). This constant growth rate make the segmental analysis of hair to be a useful procedure for assessing the time-course of drug used and compound incorporating into hair (125).

Human hair growth cycle consists of three phases; anagen, catagen and telogen. In angenic phase the cell in bulb divided rapidly, during this phase capillary blood supplying follicle in addition to nutrient provide any compound present inside blood. Several mechanisms have been proposed for cortisol incorporation into hair, but the primary mechanism is by passive diffusion to medulla from blood capillaries (125).

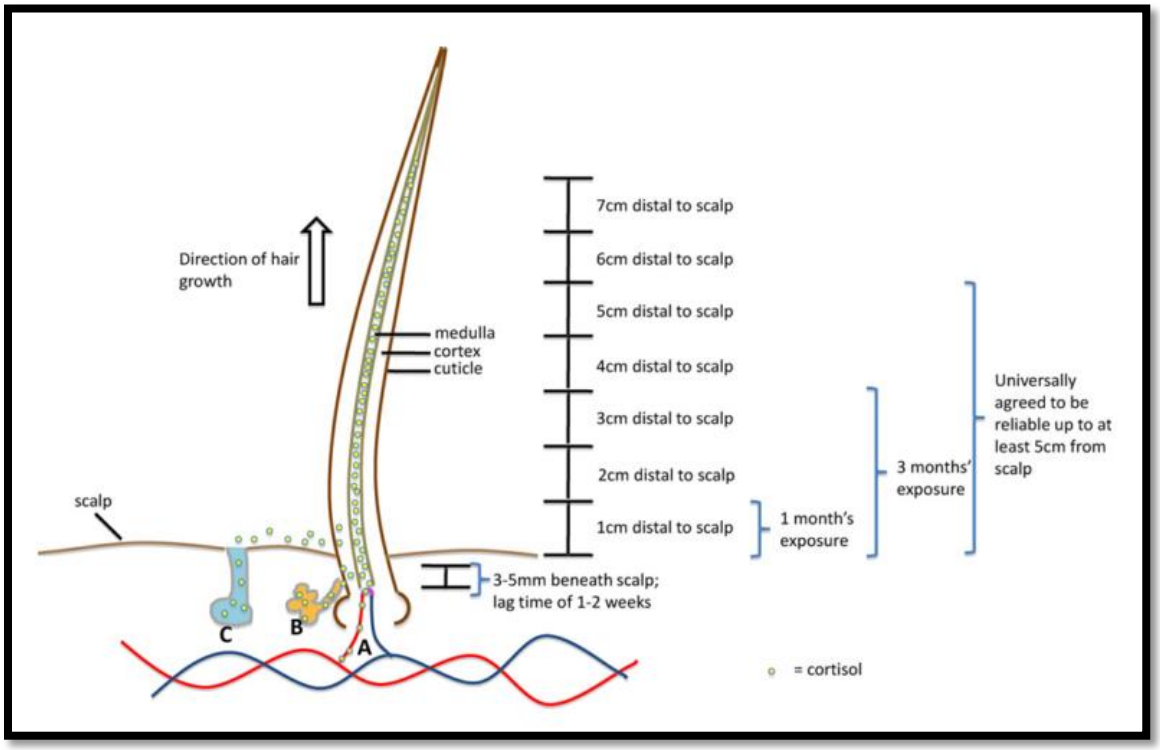

Fig.1-3: Proposed mechanisms for incorporation of cortisol into hair via blood (A), and sebum (B), and sweat (C). Russell et al, 2012 (48)

Some studies suggested that cortisol in the hair is regulated partly by the local (peripheral) HPA axis under the effect of CRH (130,131). Although these studies associated with shortcoming of small sample size, and in some of them hair cortisol of periphery (Arm and legs) were used, the hair in these area may be different in growth and regulation (131).

Moreover the presence of high hair cortisol in Cushing's syndrome and those treated with high dose of hydrocortisone (128), and low hair cortisol in primary adrenal insufficiency all inconsistent to the significant local cortisol production, and all support that most of the hair cortisol is primarily from central HPA axis activity (55) and systemic exposure

(diffusion from blood), rather than from local production, sweat and sebum (48).

Table 1-2. Studies measured Hair cortisol in healthy and diseased subjects.

Year	Author	Condition or Disease	Hair cortisol: (pg/mg hair) Range Mean or Median	Hair cortisol association
2004	Raul et al. (132)	Healthy adult human	5-91 Mean: 18	n.s
2006	Davenport et al.(133)	rhesus macaques Before and after stress (relocation)	Mean 81.1 vs. 129.6	Hair cortisol level significantly increased after prolonged and significant stress
2007	Sauve et al.(57)	Adult non-obese	1.7—153.2 Median: 46.1	Positive with 24 hrs urine cortisol
2014	Veldhorst et al.(47)	Obese children *vs.* Control	Median: 25 *vs.* 17	Positive with BMI and waist circumference
2010	Stalder et al.(134)	1. Alcoholics in acute withdrawal, 2. abstinent alcoholics and 3.controls	51.99 13.98 12.59	n.s
2011	Manenschijn et al.(67)	Shift work *vs.* Daily work	47.32 *vs.* 29.72	Positive with BMI
2012	Manenschijn et al. (56)	CS *vs.* Control	399.7 *vs.* 27.3	Early recognition of cyclic Cushing

Year	Author	Condition or Disease	Hair cortisol: (pg/mg hair) Range Mean or Median	Hair cortisol association
2012	Manenschijn et al.(68)	Psychiatric co-morbidity *vs.* control	44.87 *vs.* 31.41	Positive with mean cortisol level in saliva
2017	Hodes et al. (59)	Cushing's Syndrome Control	266.6 *vs.* 38.9	Proximal hair cortisol ass. With early biochemical test
2018	Penz et al.(135)	Burnout syndrome	n.s	Hyper-cortisolism found in subject with burnout

n.s: the association is not significant, or the data were unknown

1.14 The Advantage of hair cortisol measurement over other methods

1. One of the benefits of hair cortisol measurement is the ability to overcome the pulsatile release, diurnal variation and short term cortisol release thus it omit intra- and inter-day variation in cortisol secretion. By taking the most proximal 6 cm sample from scalp, we can measure cortisol secretion of up to 6 months period, instead of one time point measure or measurement in 24 hour, only (63).

2. It can assess cortisol level in subjects and patients retrospectively, for example before and after stress, before or after particular disease or particular management. This is because of predicted average

growth rate of hair as described by Wennig et al (129), as hair grow at an average rate of 1cm/month, thus segmental analysis of hair can be done.

3. The procedure of sample taking is simple and non-invasive, thus doesn't affect the test result like acute cortisol elevation associated with venipuncture (63).

4. Cortisol in hair is stable and does not need special storage and easily transfer, the sample can be stored at room temperature in a dark dry place for one year (63).

Significant positive correlation was found between hair cortisol and 24 hour urine cortisol, while comparing hair cortisol with salivary and serum cortisol fail to show significant association (57).

1.15 The technique of hair cortisol analysis

Generally the same technique were used for measurement and extraction of hair cortisol similar to the method used by Sauvé et al (57), with slight difference in some steps between Laboratories in the type of cutting and drying and immunoassay that used (58).

1.16 Effect of hair dying, hair washing, environmental damage, gender and ethnicity on hair cortisol level

Hair cortisol level is affected by hair treatment products (57) and it might be affected by sweating, natural hair colour and type (57), frequency of hair washing (127), and some environmental factors, thus considering these factors during hair cortisol assessment is important to prevent falsely low or high cortisol level due to hair dying or excessive sweating in some subjects, respectively (125).

Studies reported that cosmetic treatment such as hair dying affects the hair cortisol level (55,57) and significantly lower hair cortisol level were found in subject with dyed hair than subjects with natural hair colour (57). The proposed mechanism is increased porosity of hair and leach out of cortisol due to effect of bleaching used during hair dying (125) or due to increased hair weight and thus dilution like effect (48).

Excessive sweating is another causative factor that affect hair level, higher hair cortisol level were found in athletes and in other subjects with excessive sweating. This was explained by presence of cortisol in the sweat that may add to the cortisol level in hair and affecting the results (66).

Researchers segmented the long hair sample to compare the hair cortisol level between distal and proximal hair segments and they revealed lower hair cortisol in distal segment in comparison to proximal segment (136). The significantly lower hair cortisol difference were found after the 6 cm length of hair, thus the first 5 (137) or 6 cm (136) is reliable for hair cortisol assessment in individual while after the 6 cm length of hair the hair cortisol gives falsely lower level. The effect of washing out of cortisol from hair due to environmental damage was proposed as the causative factor for lower hair cortisol level in the most distal hair segments (136). While no significant decrease in hair cortisol were observed along the 10-14 cm hair shaft in some studies (55,128).

Effect of gender and ethnicity on hair cortisol level were also reported (138). Women has lower hair cortisol level than men while black population has higher cortisol level than white (138). Difference in hair growth rate were found among populations with different ethnic group (African, Asian and Caucasian) (139), thus hair cortisol level might also

affected due to difference in duration of cortisol exposure from blood to the hair, in subjects with difference in hair growth rate, thus African has higher hair cortisol level due to the slower hair growth rate than Caucasian (139).

Subjects and methods

2.1 Study design and ethical consideration

This prospective cross-sectional study conducted at the Department of Physiology, College of Medicine, University of Sulaimani and Shar hospital, Sulaymaniyah city- Iraq, during the period of two years, from July 2016 till July 2018.

The study obtained approval from the ethical committee of the College of Medicine/University of Sulaimani.

2.2 Inclusion and Exclusion criteria

Inclusion criteria includes: any adult healthy subjects (apart from hypothyroidism for hypothyroid group), with the age range of 17-70 years old.

Exclusion criteria includes: (i) abnormal serum TSH levels (<0.5 mIU/L or >10 mIU/L); (ii) abnormal free thyroid hormones T4 (FT4) or T3 (FT3); (iii) those with HPA axis abnormality (Cushing Syndrome as an example) (140) or previously diagnosed thyroid problem other than hypothyroidism for hypothyroid subjects; (iv) presence of thyroid nodule; (v) Those already on any sort of treatment for thyroid disease or treatment affecting HPA axis; (vi) chronic diseases such as diabetes; (vii) acute illness; (viii) hyperprolactinemia (ix) inpatients and (x) those who had aparent psychological problem (141).

Exclusion criteria for hair sample taking and measurement apart from HPA axis abnormality, incude hair dying (142), insufficient hair in the

vertex region of scalp (140), skin disease and subjects who received steroid treatment few months before the sample taking (143).

2.3 Study participants: Enrolment and informed consent

The flow diagram of the study participants throughout the study demonstrated in Figure 2-1

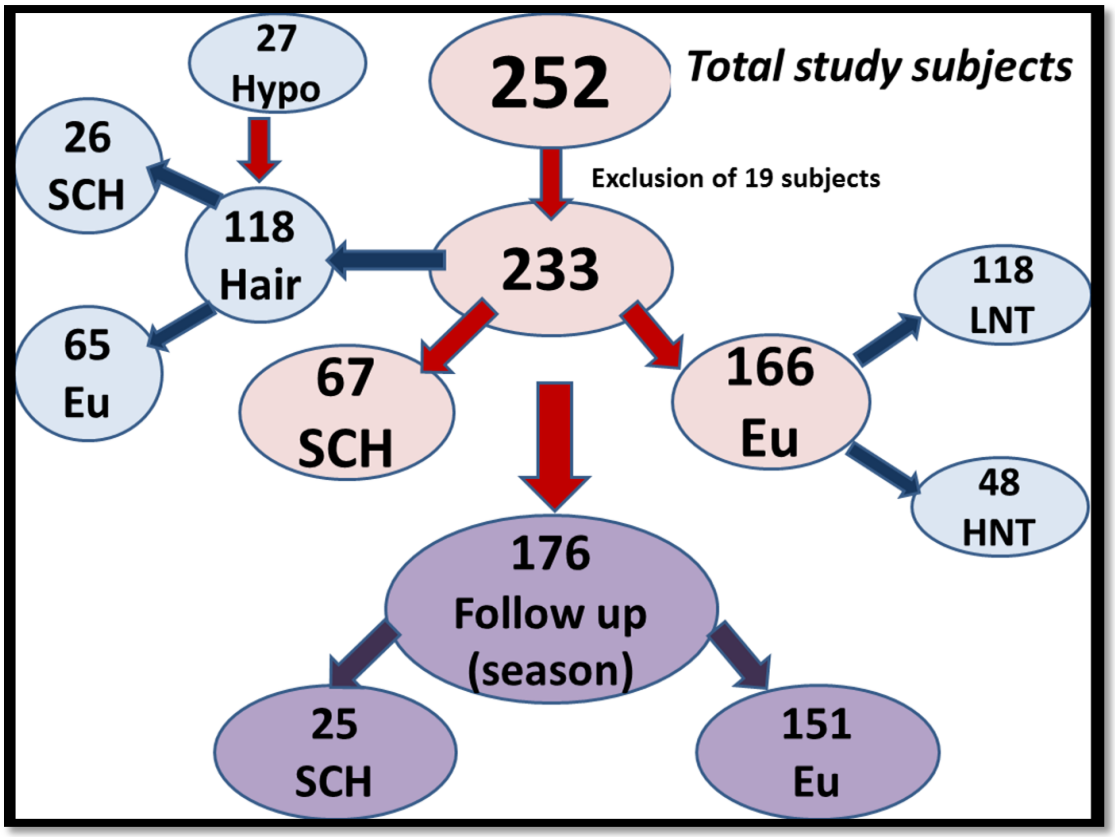

Figure 2-1. The flow diagram of the study participants

2.3.1 Informed consent

Written informed consent was taken from all study participants.

2.3.2 Subject enrolment at baseline

A total of 252 (45 male *vs*. 188 female) study subjects were participated in the study with the mean age of 34.09 years, they were evaluated prospectively for biochemical and clinical parameters. They included those who visited Shar Hospital/Sulaimaniyah city for their health check-up. Normal healthy hospital employees, friends, and relatives were also included. These subjects were sent for thyroid function tests and other required investigations and asked about hypothyroid symptoms, and examined by the authors for signs of hypothyroidism.

After exclusion of 19 subjects, who had at least one of the exclusion criteria, the 233 subjects were separated into two groups according to their serum TSH level. The groups included 67 SCH (SCH defined as having two laboratory readings of elevated serum TSH and normal serum FT4 level with 2 weeks apart) and 166 euthyroid subjects (normal TSH and FT4 levels); the euthyroid subjects were further divided into euthyroid subjects with high-normal TSH (TSH 2.5-4.2 mIU/L) and euthyroid subjects with low-normal TSH (TSH value of 0.5-2.49 mIU/L), for comparison of these groups clinically and biochemically.

During summer season, underwent thyroid function tests and separated according to the TSH level, and investigated for the proportion of anti-TPO in the serum.

2.3.3 Follow up for seasonal assessment

In the follow-up study in winter season, total 53 subjects were excluded either due to the development of overt hypothyroidism, thyrotoxicosis, due to pregnancy, or as they did not turn up for the follow up test. Among the remained 176 subjects, 25 subjects with subclinical hypothyroidism and 151 subjects with euthyroidism were included in the final analysis of seasonal variation. All biochemical investigation of thyroid function test (TSH, FT4, and FT3) was repeated for these subjects.

2.3.4 Enrolment for hair sample collection

Hair sample were taken from 118 study participants (those who agreed to give hair sample). After exclusion of those with exclusion criteria for hair sample measurement 91 subjects remain, with 65 euthyroid and 26 SCH subjects. Moreover, a group of overt hypothyroid which include 27 subjects were included for hair analysis to compare with the euthyroid and SCH groups for hair cortisol level.

The hypothyroid group include any subject with newly diagnosed hypothyroidism who didn't receive treatment yet, who didn't take their treatment for at least 3 months or any subjects with uncontrolled hypothyroidism. Any subjects with systemic disease other than hypothyroidism or subjects on any sort of treatment were excluded from the study.

Witten informed consent for hair sample collection was taken separately after detailed explanation of the sample collection procedure with illustration by image.

2.4 Questionnaire: Socio-demographic and anthropometric measures

A detailed questionnaire was filled out for each participant. The questionnaire contained questions about socio-demographic status; the subjects were asked about age, smoking history, education, detail of work and lifestyle including sleep duration per 24 hour. It also included the degree of natural exposure to the outdoor atmosphere, based on which the subjects were divided into two groups; good exposure group and slight exposure group:

Good exposure: being outside building for more than 3 hours per 24 hours

Slight exposure: being outside building for less than or equal to 3 hours per 24 hours

The questionnaire also includes questions about history of systemic diseases, thyroid disease, adrenal insufficiency or hyper-function, drug and surgical history, and history of stress or psychological abnormality, the questionnaire was demonstrated in Appendices section, Appendix A.

Anthropometric measures: The subjects assessed for anthropometric measures. Height, weight were recorded, BMI were calculated by dividing weight in kg by height in square meter (144).

Waist circumference for each subject was evaluated by a tape measure around the waist at the midpoint between the last palpable rib and the top of the iliac crest (145).

2.5 Clinical assessment:

2.5.1 Assessment of participants for the Zulewski's clinical score

The subjects were assessed for Zulewski's clinical score (12) which includes 7 symptoms and 6 signs of hypothyroidism (Table 1-1). The Zulewski's score form was filled out by the author. The subjects were asked for the symptoms and examined for the physical signs included within the score. The sums of the score were calculated later for each participant.

2.5.2 Assessment of participant for other clinical sign and symptoms

Apart from Zulewski signs and symptoms, the patient was asked for other common hypothyroid symptoms that include fatigue, muscle cramp, menstrual disturbance, cold intolerance, memory loss, alopecia, hirsutism, and galactorrhea and they were examined for goiter.

2.6 Biochemical assessment

2.6.1 Laboratory measurement of blood samples

The participants were asked to fast on the day of investigations and to come in the morning at 9:00 - 11:00 am for blood drawing. Blood were drawn intravenously from the forearm, and inserted into the gel tube, and kept in a cool place for 15 minutes. The blood samples were centrifuged in a speed of 5300 rpm for 10 minutes, and the serum was separated; the sera were analyzed for FT3, FT4, and TSH. Part of the serum was separated and put in to eppendorf tubes and stored in deep freeze at -70˚C.

Anti-TPO antibody and serum prolactin were determined on the stored serum sample. All biochemical tests were analyzed by the recent immunoassay method, which is electrochemiluminescence immunoassay (ECLIA), with the use of the same type of kits from Roche Diagnostics GmbH, Germany and using the same device, the Cobas e 411 analyzer GmbH, Germany (Hitachi High-Technologies Corporation). The data were analyzed with running of quality control.

2.6.2 Reference ranges (146)

FT3: 2.0-4.4 pg/ml

FT4: 0.93-1.70 ng/dl

TSH: 0.27-4.2 mIU/L

Prolactin:

In males, up to15 ng/ml was regarded as normal

In females, up to 23 ng/ml, was regarded as normal

Thyroid peroxidase antibody (anti-TPO): up to 34 IU/ml were regarded as normal.

2.7 Term Definition

Euthyroid: was defined as a subject with TSH and FT4 within the reference range(17).

Euthyroid with low-normal TSH: was defined as euthyroid subjects with TSH values of 0.5-2.49 mIU/L.

Euthyroid with high-normal TSH: were euthyroid subjects with TSH of 2.5-4.2 mIU/L.

SCH: was defined as subjects who had elevated TSH (> 4.2 and up to 10 mIU/L) in the presence of FT4 within the reference range (with having two laboratory readings of elevated serum TSH and normal serum FT4 level with 2 weeks apart) (76).

Overt hypothyroidism: was defined as subjects who had a combination of elevated TSH and low FT4 (TSH > 4.2 and FT4 < 0.93ng/ml) (76).

Thyroid peroxidase positivity (TPO-positive subjects): was defined as having anti-TPO above 34 IU/mL (76).

Hyperprolactinemia: was defined as having prolactin level of more than the upper limit for males and females (147).

Normal weight: any subject with BMI between 18.5 and 24.9 Kg/m^2.

Overweight: any subject with BMI between 25 and 29.9 kg/m^2.

Obese: any subject with BMI 30 kg/m^2 and above (147).

2.8 Hair Sample collection, Preparation, Extraction, and Analysis

Scalp hair was taken from a posterior vertex in all participants who accepted to give the hair sample. About a half centimeter (cm) thickness of hair was strapped and cut as proximal to the scalp above the strapped area with clean scissors. Then, the proximal 3 cm of the cut hair with marked proximal and distal end was placed on to an aluminum foil, and then in a coded envelope paper. It was then stored at room temperature in a dark and dry place until the analysis. Exclusion criteria for hair sample collection included dying hair or insufficient hair.

A laboratory protocol follow the one described by Sauvé et al. (57) with few modifications. Before analysis, the most proximal segment of hair samples were cut with surgical scissors into 2 to 3 mm length and weighed with an electronic analytical balance.

About 20 to 50 mg of each hair sample was put into a clean glass test tube, coded, and 1.5 mL of methanol was added to it. The test tubes were then incubated with shaking incubator at 52-degree centigrade for 16 hours.

After incubation, the test tubes with incubated methanol were centrifuged at 5000 rpm for 10 minutes. The supernatant was then separated and pipetted using a digital micropipette and transferred into another clean glass test tube; methanol was evaporated at 60 ^{o} C till complete dry.

After the process of drying, 0.2 to 0.25 mL of phosphate buffered saline (PBS) was added to the precipitate and vortexed with a vortex mixer for 30 seconds. The vortexing was repeated twice, and then hair cortisol was analyzed by electrochemiluminescence assay (ECLA) Roche Diagnostic Cobas e-411, with Roche diagnostic cortisol kit, as described by Iglesias et al. (142).

The measured hair cortisol was recorded and the amount of cortisol within each mg of hair was calculated in pg/mg. Final results of cortisol in pg/mg of hair sample were obtained by using this calculation:

Cortisol result of immunoassay (in microgram)/ Weight of the hair (in mg)] X [methanol added (ml)/methanol extracted (ml)] X Buffer added (ml) X 10,000 (54).

In 24 hair samples from 12 hypothyroid and 12 SCH subjects, both proximal and distal hair segments were prepared using the same procedure.

The hair divided into the proximal (first cm) segment, and distal (third cm) segment in these subjects and the hair cortisol in each segment were measured and compared.

2.9 Weather and climatic data

The detailed climatic data for each day during the study period was obtained from the Metrological Station of Sulaimani, which includes temperature, humidity, cloud cover, sunshine duration and atmospheric pressure & etc. Seasons were defined by their respective solstices and equinoxes, i.e. winter: 21 December–20 March and summer: 21 June–20 September (28). For both summer and winter seasons, the mean of each climatic parameter (component) assessed in this study and are demonstrated in Chapter 3, Results.

2.10 Statistical method:

The data were entered and analyzed statistically using SPSS 22 (IBM Corporation, New York, USA). All demographic, biochemical and climatic data were represented as mean ± SD[a] or median (range)[b] for non-parametric data and frequency (%) for categorical data[c]. All continuous data are parametric except hair cortisol and TSH value of study participants for hair cortisol analysis. The hair cortisol log transformed to normalize the distribution. After log transformation, the skewness was corrected, mean of hair cortisol log transformed (hair cortisol log) was used for the comparison and correlations.

The frequency of clinical symptoms, anti-TPO positivity and hyperprolactinemia were assessed between groups using chi-square test.

The mean of the parametric variables (anti-TPO level, TSH, FT4, prolactin and clinical score) were analyzed for each different group, and the means were compared between the groups by Independent Sample T-test and ANCOVA, with the exception of using the Kruskal-Wallis H test[d] and Mann Whitney U test for comparison of median among non-parametric variables. $P \leq 0.05$ was regarded as statistical significance.

The mean of each climatic component and the hormone levels between summer and winter seasons and mean of hair cortisol level between proximal and distal segment were compared using Paired samples T test. Correlation between variables was evaluated using Pearson's correlation coefficient for parametric variables and Spearman's correlation[e] between non-parametric variables. P value of ≤ 0.05 regarded as significant.

Results

Part 1

Clinical score and anti-TPO assessment in SCH compared with euthyroid with different TSH levels.

The results of this part were based on the analysis of 233 participants, 67 were defined as SCH and 166 were defined as euthyroid subjects. The euthyroid subjects were further subdivided into euthyroid with low-normal TSH (LNT) n=118 and euthyroid with high-normal TSH (HNT) (n = 48). The frequency of SCH among normal subjects who volunteered for the study was 14.79% (29 out of 196).

3.1 Socio-demographic characteristics of the study participants

Socio-demographic status for the study participants is shown in Table 3-1. The mean age was 34.09 years with an age range of 17-70 years; 80.69% of the participants were female.

Table 3-1. Socio-demographic state of the study participants.

Parameter	Frequencies N (%) Mean (± SD)
Age in year [a] **Age range (y)**	34.09 (±12.34) 17-70
Gender • Female • Male	 188 (80.69%) 45 (19.31%)
Body mass index (BMI) [a]	27.28 (±4.84)
Waist circumference (in cm) [a]	84.88 (±12.14)
Smoker (yes %)	20 (8.6%)
Work • Student • No work • Employee • Free work	 35 (14.8%) 68 (29.1%) 86 (37%) 44 (19.2%)
Marital status • Married • Single • Divorced	 140 (60.1%) 89 (38.2%) 4 (1.7%)

3.2 Comparison of socio-demographic, biochemical and clinical parameters between SCH and euthyroid subjects.

The comparison of characteristics, biochemical and clinical data between SCH and euthyroid subjects is shown in Table 3-2.

3.2.1 The socio-demographic data

There were no statistically significant differences in sex, age, BMI, and waist circumference between SCH and euthyroid groups, $P > 0.05$.

3.2.2 The biochemical data

The serum TSH and anti-TPO titer were significantly higher in the SCH compared to the euthyroid group (6.23 vs 2.03 mIU/L, $P < 0.001$ and 95.7 vs 23.32 IU/ml, respectively. A significantly higher rate of TPO positivity among SCH subjects were found compared to euthyroid (43.8% vs 10.2%, $P < 0.001$). However, the frequency of hyperprolactinemia is similar between the two groups, and there is no significant difference in prolactin levels.

3.2.3 The clinical data

Regarding the clinical data, the SCH group has a significantly higher frequency of hypothyroid sign and symptoms (Goiter, fatigue, muscle pain and cold intolerance), $P < 0.05$; and higher mean Zulewski's score than euthyroid subjects, $P < 0.001$.

Table 3-2.A Comparison of socio-demographic and biochemical parameters between SCH and euthyroid subjects.

Parameters	SCH (n = 67) Mean (±SD) or %	Euthyroid (n = 166) Mean (±SD) or %	P-value
Age (years)	35.13 (±13.69)	33.67 (±11.77)	0.415
Gender (female) [c]	85.1%	78.9%	0.281
BMI (Kg/m^2)	27.95 (±4.35)	27.00 (±5.01)	0.183
Waist circumference (cm)	86.77 (±13.19)	84.12 (±11.66)	0.202
Biochemical Data			
TSH (mIU/L)	6.23 (±1.69)	2.03 (±0.89)	**0.001**
FT4 (ng/dl)	1.33 (±1.36)	1.26 (±0.18)	0.512
FT3 (pg/ml)	3.39 (±0.46)	3.37 (±0.60)	0.864
Anti-TPO titer(IU/ml)	95.70 (±158.11)	23.32 (±61.49)	**0.001**
Prolactin (ng/ml)	19.74 (±11.43)	17.75 (±9.48)	0.183
male	19.59 (±14.6)	16.53 (±7.94)	0.384
female	19.77 (±10.9)	18.09 (±9.88)	0.315
TPO-positivity [c]	43.8%	10.2%	**0.001**
hyperprolactinemia [c]	27%	28%	0.873

Table 3-2.B Comparison of clinical parameters and Zulewski's score between SCH and euthyroid subjects.

Parameters	SCH (n = 67) N (%) Mean (±SD)	Euthyroid (n = 166) N (%) Mean (±SD)	P-value
Zulewski's score[a]	3 (6)	2 (7)	**0.001**
Clinical data[c]			
Goiter	15.1%	4.9%	**0.017**
Menstrual disturbance	51.2%	39.7%	0.193
Decreased appetite	29.8%	19.9%	0.126
Fatigue	80.3%	58.2%	**0.002**
Muscle pain	62.3%	38.9%	**0.002**
Memory loss	50.8%	43%	0.304
Alopecia	46.6%	40.4%	0.42
Hirsutism	24.5%	22.1%	0.723
Cold intolerance	37.3%	23.8%	**0.05**
Galactorrhea	3.6%	1.9%	0.473

3.2.3.1 Assessment of types of menstrual disturbances

When the types of menstrual disturbances (slight, heavy, irregular, short interval and long interval) were compared between SCH and euthyroid groups (Figure 3-1), the frequency of heavy menstrual period among SCH group was found to be highest when compared to euthyroid group, P < 0.009.

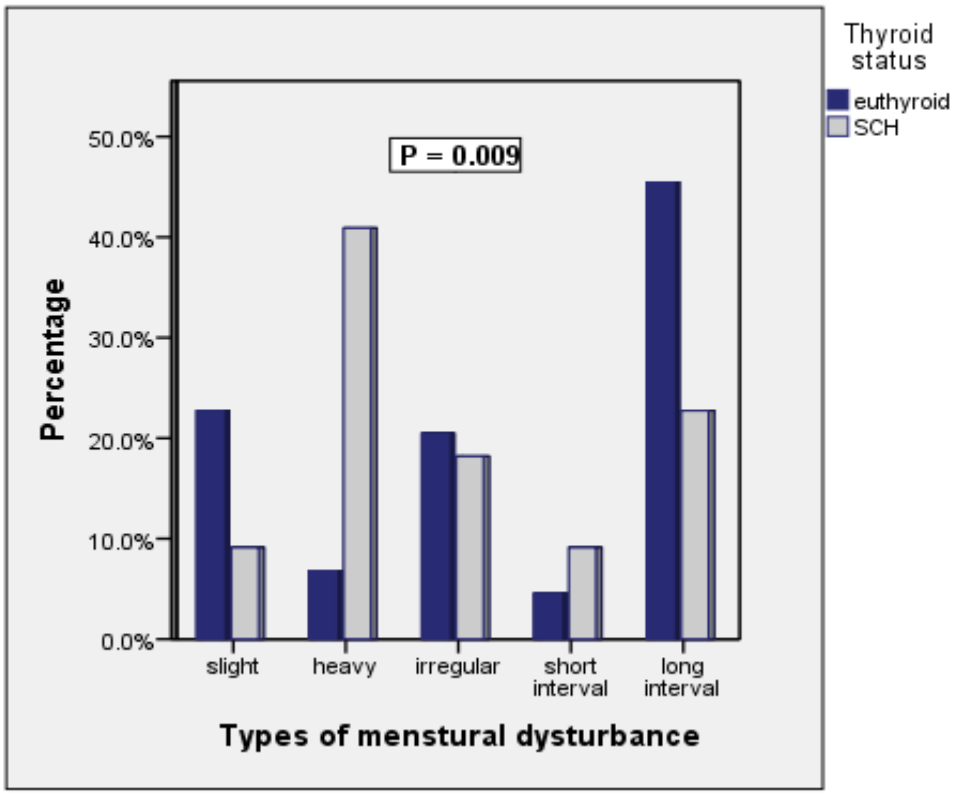

Figure 3-1. Frequency of menstrual disturbance types in SCH and euthyroid subjects.

3.2.3.2 Assessment of Zulewski's clinical score

When each component of the Zulewski's clinical score analyzed individually, Paraesthesia was the most frequent symptom (50.7 %) followed by Dry skin (28.4%), increased weight (26.9%) and constipation (25.3). Among the physicals signs of Zulewski, slow movement is the most frequent signs (20.9%).

For assessing validity of Zulewski's clinical score, the frequency of subjects who has intermediate score (3-5) that is suggestive of mild hypothyroidism (SCH) were examined among biochemically euthyroid and SCH subjects. It was found that the frequency of SCH subjects with the intermediate score was 42%, while 80% of Euthyroid subjects have a normal score (of less than 3). This will give the 2.9 times (OR= 2.89) higher risk of abnormal Zulewski's score, intermediate score (3-5) among subject with high TSH (SCH).

3.3 Comparison of socio-demographic, biochemical and Zulewski's score between euthyroid subjects with LNT and HNT levels.

In Table 3-3, a comparison between euthyroid with LNT and HNT is demonstrated. There was higher anti-TPO positivity in HNT in comparison to LNT, but the difference is not statistically significant. The anti-TPO titer and TSH were significantly higher (40.76 vs 16.59 IU/ml, $P = 0.024$ and 3.18 vs 1.57 mIU/L, $P < 0.001$) respectively. FT3 and FT4 are significantly lower in the HNT euthyroid group compared to LNT euthyroid group. The differences in clinical data between the two groups were statistically not significant, and Zulewski's score was same in both groups.

Table 3-3. Comparison of socio-demographic, biochemical parameters and Zulewski's score between Euthyroid subjects with LNT and HNT levels.

Parameter	Euthyroid with LNT (TSH 0.5-2.49) (n = 118)	Euthyroid with HNT (TSH 2.5-4.2) (n = 48)	P-value
Socio-demographic data			
Age	33.53 (±12.09)	34.33 (±10.98)	0.697
BMI	26.99 (±4.96)	27.23 (±5.12)	0.782
Waist circumference	84.13 (±11.92)	84.69 (±10.76)	0.812
Biochemical data			
TSH (mIU/L)	1.57 (±0.51)	3.18 (±0.51)	**0.001**
FT4 (ng/dl)	1.28 (±0.19)	1.21 (±0.14)	**0.023**
FT3 (pg/ml)	3.48 (± 0.39)	3.02 (±0.95)	**0.014**
Anti-TPO titer (IU/ml)	16.59 (±37.44)	40.76 (±98.51)	**0.024**
Prolactin (ng/ml)	17.63 (±9.23)	17.35 (±9.12)	0.864
TPO-positivity [c]	9 (7.6%)	8 (17.4%)	0.065
Hyperprolactinemia [c]	37 (31.4%)	9 (20.5%)	0.365
Zulewski's clinical score	2 (±7)	2 (±7)	0.149

3.4 Comparison between TPO-positive and TPO-negative subjects.

When TPO-positive and negative subjects were compared for socio-demographic parameters, no significant difference was observed between them in age, sex and waist circumference, while BMI was higher in TPO-positive group compared to TPO-negative group (28.4 kg/m^2 vs 26.9 kg/m^2, P =0.052).

Comparison between the two groups in biochemical data (Table 3-4) showed no significant difference in prolactin level, and hyperprolactinemia, however, TSH was higher and FT4 was lower in TPO-Positive groups, $P < 0.05$.

When clinical parameters were evaluated between the two groups (Table 3-5), the occurrences of goiter, menstrual disturbance, and cold intolerance were higher in the TPO-positive group than in the TPO-negative groups, $P < 0.05$. Zulewski's score was higher among TPO positive groups (1.89 vs 1.56) while the value not reaching statistically significant level, P = 0.178.

Table 3-4. Comparison of biochemical parameters between TPO-negative and TPO-positive subjects.

Variables	TPO-negative N= 186 Mean ±SD	TPO-positive N= 45 Mean ±SD	P-value
TSH (mIU/L)	2.81 ± 1.92	4.96 ± 2.64	**0.001**
FT4 (ng/dl)	1.31 ± 0.83	1.17 ± 0.16	**0.043**
FT3 (pg/ml)	3.64 ± 0.57	3.54 ± 0.56	0.546
Anti-TPO titer (IU/ml)	8.47 ± 5.7	190.88 ± 170.6	**0.001**
Prolactin (ng/ml)	18.32 ± 10.3	18.42 ± 9.1	0.951

Table 3-5. Comparison of clinical parameters between TPO-negative and TPO-positive subjects.

Clinical data	TPO-negative N= 186 Frequency %	TPO-positive N= 45 Frequency %	P-value
Goiter	5.6%	17.1%	**0.02**
Menstrual disturbance	37.7%	60%	**0.019**
Decreased appetite	23.2%	20%	0.663
Fatigue	61.1%	76.7%	0.056
Muscle pain	42.9%	54.8%	0.165
Memory loss	45.7%	41.9%	0.654
Alopecia	41.3%	43.9%	0.764
Hirsutism	22.9%	22.2%	0.931
Cold intolerance	22.8%	48.8%	**0.001**
Galactorrhea	2.3%	2.7%	0.88

3.5 Correlations between some socio-demographic, biochemical and clinical parameters in all participants:

Correlations between variables were assessed; a significant positive correlation between serum TSH level and median Anti-TPO titer was found ($r = 0.321$, $P < 0.0001$). A significantly positive correlation was observed between the Zulewski's score and TSH ($r = 0.272$; $P < 0.001$) and significant positive correlation were observed between BMI and TPO ($r = 0.155$; $P = 0.020$). There was a significantly negative correlation between age and each of the FT3 and prolactin hormone levels (age and FT3: $r = - 0.247$; $P = 0.03$, age and prolactin: $r = -0.274$; $P < 0.001$). However, significant correlations were not observed between prolactin and TSH or with TPO.

These correlations were demonstrated in the Table 3-6 and Figures 3-2, 3-3, 3-4 and 3-5 in pages 59-61.

Table 3-6. Pearson's correlations between socio-demographic, biochemical and clinical parameters in all participants

Correlations	Age	BMI	TSH	FT3	FT4	Anti-TPO	Zulewski's score
Age		.433**	.027	-.400**	-.240**	.126	.158*
BMI	.433**		.047	-.146	-.074	.155*	.226**
TSH	.027	.047		-.075	-.275**	.321**	.287**
FT3	-.400**	-.146	-.075		-.30	-.006	-.128
FT4	-.240**	-.074	-.275**	-.30		-.203**	-.149*
Anti-TPO	.126	.155*	.321**	-.006	-.203**		.200**
Prolactin	-.274**	-.209**	.124	-.069	-.10	-.012	.004

*P value <0.05, ** P value < 0.01.

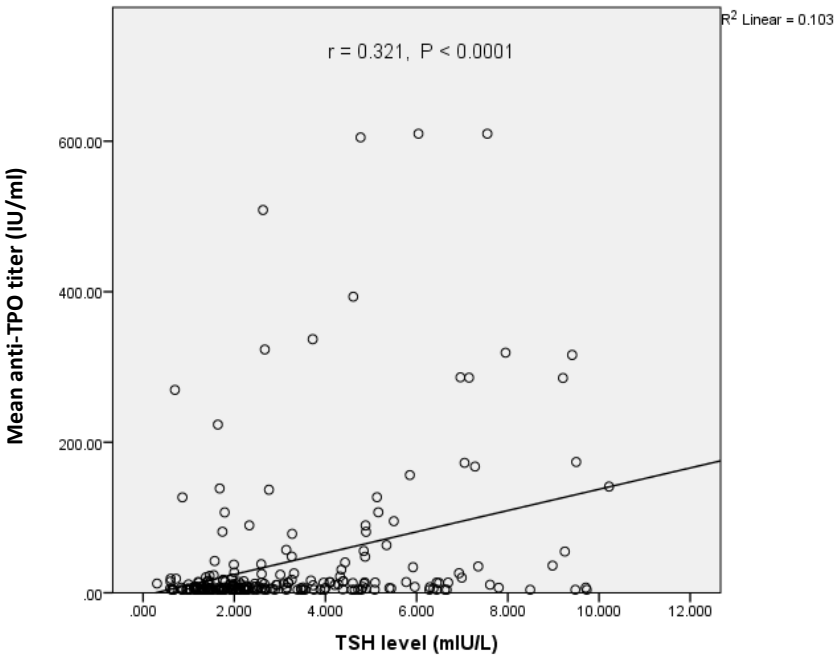

Figure 3-2. Correlation between mean TSH level (mIU/L) and mean anti-TPO titer (IU/ml).

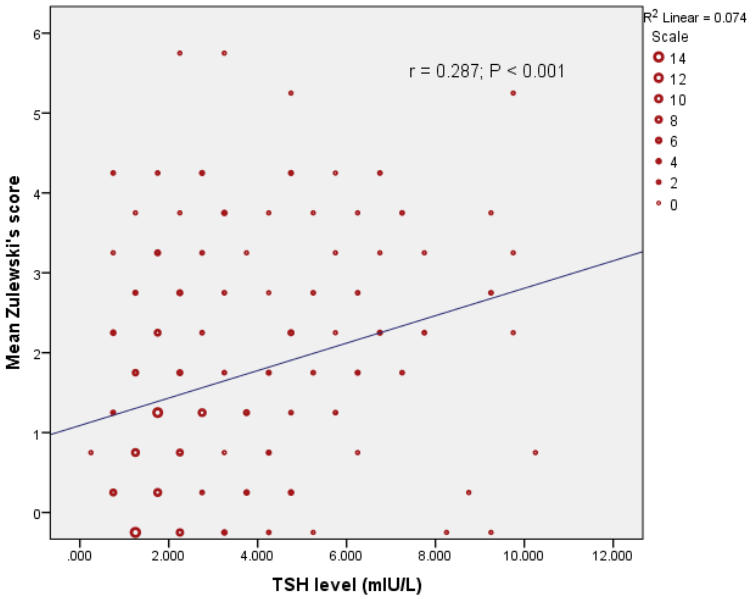

Figure 3-3. Significant correlations between mean Zulewski's score and mean TSH levels (mIU/L).

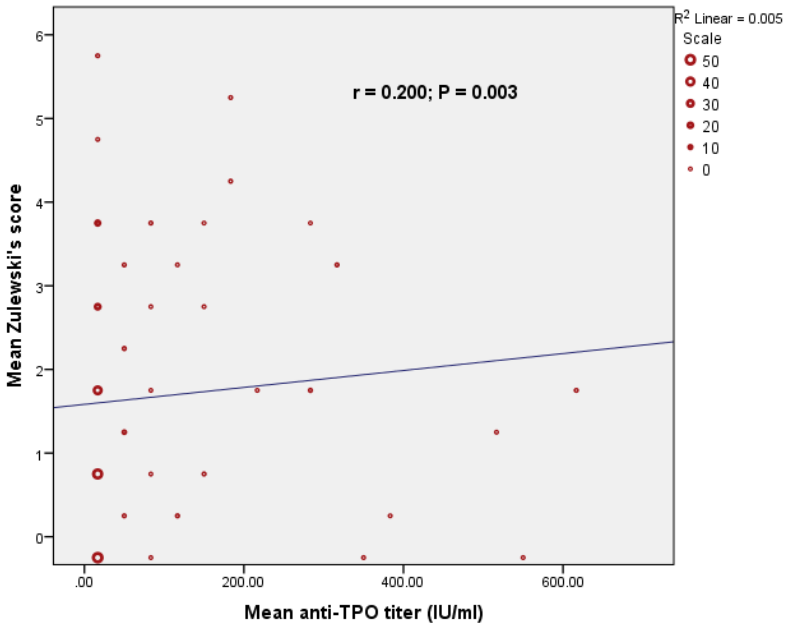

Figure 3-4. Significant correlations between the Zulewski's score and anti-TPO level (IU/ml).

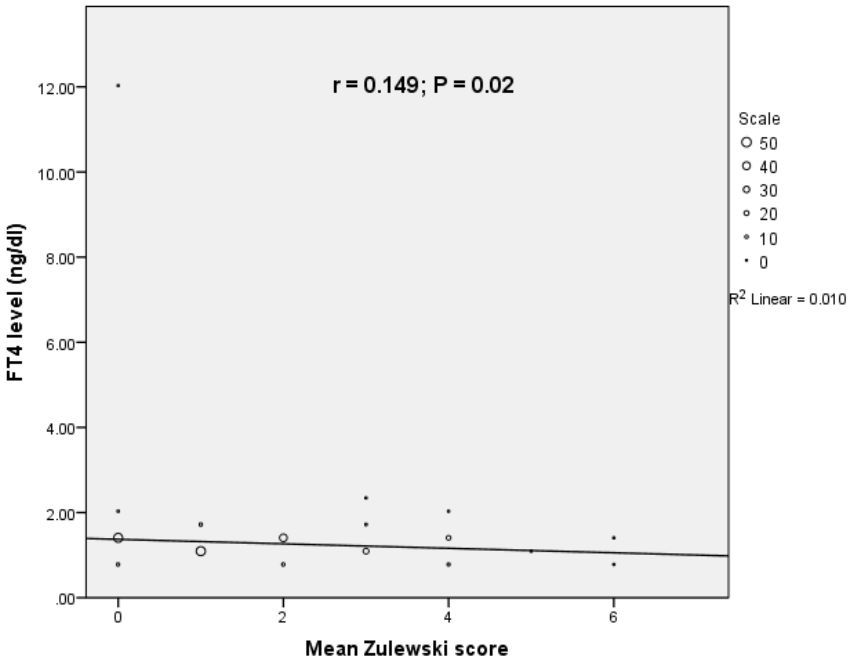

Figure 3-5. Significant correlations between the Zulewski's score and FT4 level (ng/dl).

Part 2

Effect of summer and winter seasonal climate on thyroid functions:

3.6 Study subjects entering for analysis of seasonal variation:

Result of this part derived from analysis of 176 subjects who enter the follow up study of season from the total of 252 participants. From the 252 subjects after exclusion of the subjects either due to their failure in returning for the follow-up or due to the development of thyroid diseases or pregnancy, the number of study subjects entering for the analysis was 176.

Table 3-7 illustrates the demographic characteristics of SCH and euthyroid subjects who participated in the seasonal follow up of thyroid functions assessment. Waist circumference and BMI were higher among SCH compared to euthyroid group, $P < 0.05$. While there were no statistically significant differences in the age and gender of the studied participants.

Table 3-7. Demographic characteristics of SCH and euthyroid subjects.

Characteristics		Euthyroid n=151 mean±SD n (%)	SCH n=25* mean±SD n (%)	P value
Age		34.24±12.05	36.44±14.82	0.635
Gender	Male	37 (24.3%)	2 (8%)	0.336
	Female	114 (75.7%)	23 (92%)	
BMI		26.78 ± 4.57	27.69 ± 4.63	**0.026**
Waist circumference (cm)		84.64 ± 11.27	89.48 ± 15.40	**0.038**

*This number is based on those who were subclinical hypothyroid at summer and winter seasons, the subjects who are Euthyroid at either season were excluded.

Table 3-8. Number of SCH and euthyroid studied subjects in summer and winter season.

Characteristics		Total; n	Euthyroid n (%)	SCH n (%)
Season	Summer	176	136 (77.3)	40 (22.7)
	Winter	176	140 (79.5)	36 (20.5)

3.7 Climatic components of seasonal variations in Sulaymaniyah city during period of study.

The mean of each climatic component has been demonstrated in Table 3-9. Statistically significant differences were observed (P <0.001) between the measured components during summer and winter season.

Table 3-9. Mean climate characteristics in summer and winter

Mean climate parameters	Summer (mean±SD)	Winter (mean±SD)	P value
Maximum temperature (°C)	41.67±2.61	12.09±4.05	0.001
Minimum temperature (°C)	26.5±2.63	2.5±2.56	0.001
Average temperature (°C)	34.11±2.32	7.28±2.58	0.001
Average humidity (%)	25.53±3.57	66.85±11.63	0.001
Cloud cover (okta)	0.083±0.129	0.408±0.311	0.001
Sunshine duration (Hours)	10.76±1.34	4.87±3.14	0.001
Atmospheric pressure (mmHg)	1005.73±12.56	1024±3.65	0.001

In Figure 3-6, the average temperature means of different months were demonstrated throughout summer and winter seasons. There were no significant differences in average temperature during different months of the same season, $P < 0.05$.

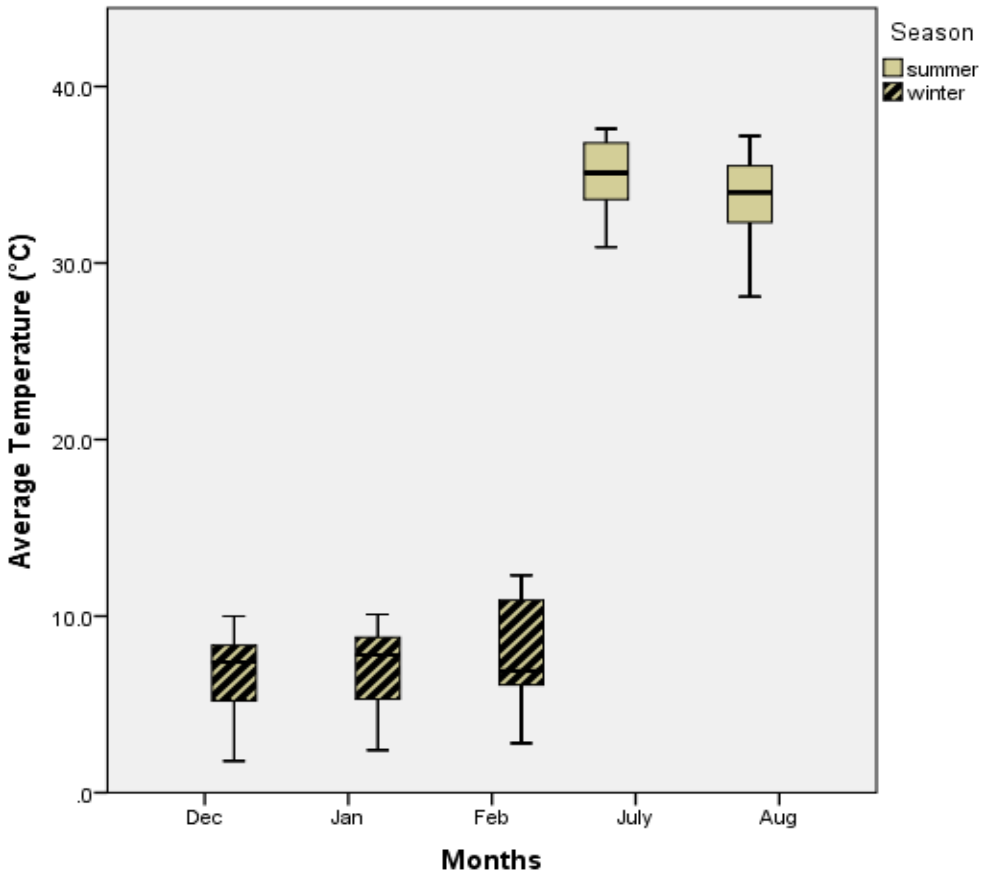

Figure 3-6. The boxplots of average temperature, during each month of sample taking in summer and winter seasons.

3.8 Comparison between thyroid functions during summer and winter season in euthyroid and SCH subjects.

These results are shown in Table 3-10. When all participants are taken together (n=176), serum FT4 was statistically significantly higher during summer (1.23±0.57) compared with winter (1.2±0.16), P = 0.007. However both FT3 and FT3/FT4 ratio were significantly lower during summer compared with winter (FT3 3.23 vs 3.37, P < 0.001) and (FT3/FT4 ratio 2.68 vs 2.87, P < 0.001) respectively. Meanwhile, TSH level was not significantly different during summer and winter.

In euthyroid subjects (n =151), both FT3 and FT3/FT4 ratio were significantly lower in summer compared with winter (P <0.001), however comparison between TSH and FT4 in euthyroid subjects during summer and winter turned out to be statistically not significant.

In SCH subjects (n = 25), serum FT4 was significantly higher in summer compared with winter (1.21 vs 1.14, P = 0.009). However, FT3/FT4 ratio was significantly lower in summer compared with winter (2.81 vs 2.99, P = 0.037), but TSH and FT3 level were not significantly different in SCH subjects during summer and winter.

Numbers of subjects who were SCH in summer and revert to euthyroidism during winter season were 11, and numbers of subjects who convert to overt hypothyroidism were 5, and 9 subjects were convert from euthyroidism to SCH, while 5 subjects developed thyrotoxicosis, after 6 months follow up.

Table 3-10. Comparison of the serum TSH, FT4, FT3 and FT3/FT4 ratio during summer and winter season in euthyroid and SCH subjects.

Parameters	Seasons	Total	P value	Euthyroid	P value	SCH	P value
TSH(mIU/L)	**Summer vs Winter**	2.93±1.96 vs 2.77±1.79	0.136	2.34 ±1.25 vs 2.25±1.25	0.382	6.64±1.60 vs 6.04±1.17	0.171
FT4 (ng/dl)	**Summer vs Winter**	1.23±0.57 vs 1.20±0.16	**0.007**	1.23±0.15 vs 1.21±0.16	0.063	1.21±0.15 vs 1.14±0.14	**0.009**
FT3 (pg/ml)	**Summer vs Winter**	3.23±0.44 vs 3.37±0.39	**0.001**	3.22±0.44 vs 3.39±0.40	**0.001**	3.27±0.45 vs 3.25±0.32	0.853
FT3/FT4 ratio	**Summer vs Winter**	2.68±0.45 vs 2.87±0.43	**0.001**	2.65±0.45 vs 2.84±0.43	**0.001**	2.81±0.44 vs 2.99±0.43	**0.037**

3.9 Comparison of thyroid functions according to outdoor stay hours during summer and winter.

Comparison of subjects on the basis of duration of outdoor exposure revealed an increase in FT3 during winter in subjects with good exposure (P = 0.023), with no significant differences between other variables as illustrated in Table 3-11.

Table 3-11. Comparison of the serum TSH, FT4, FT3 and FT3/FT4 ratio between slight and good exposure groups in two different seasons.

Parameters	Seasons	Outdoor stay hour (n = 170)		P value
		Slight (≤ 3 hours) n = 120	Good (> 3 hours) n = 50	
TSH (mIU/L)	Summer	3.02±2.01	2.72±1.81	0.385
	Winter	2.86±1.91	2.52±1.41	0.275
FT4 (ng/dl)	Summer	1.23±0.15	1.22±0.15	0.858
	Winter	1.19±0.16	1.21±0.15	0.491
FT3 (pg/ml)	Summer	3.18±0.42	3.30±0.46	0.191
	Winter	3.30±0.34	3.49±0.45	**0.023**
FT3/FT4 ratio	Summer	2.64±0.442	2.76±0.45	0.196
	Winter	2.84±0.44	2.94±0.35	0.275

3.10 Correlation between climate parameters and thyroid functions in euthyroid subjects.

Correlation analysis between the different climate components measured one day, one week and one month before blood sample collection and the hormone levels was carried out using the Pearson's correlation analysis as shown in Table 3-12.

3.10.3 One day before sampling

In correlation of climatic weather of one day before sampling with thyroid functions, FT3 showed negative correlation with temperature and sunshine duration while a positive correlation were observed with humidity and atmospheric pressure. Free T4 showed a negative correlation with the cloud cover ($r=-0.136$, $P < 0.05$). There were significant correlations between most climate components of the day before testing with the FT3/FT4 ratio.

3.10.4 One week before sampling

When climatic components of one week before sampling were correlated with thyroid functions, significant negative correlation was found between FT3 and temperature and significantly positive correlation were found between each of the FT3 and FT3/FT4 ratio and humidity.

3.10.5 One month before sampling

Significantly positive correlation was found between FT3 and atmospheric pressure of one month before samplings, and most climate component of one month before sampling has significant correlation with FT3/FT4 ratio.

Thus, in euthyroid subjects there were significant correlations between climate components of the day before blood testing and FT3 and climate components of the day and month before testing with the FT3/FT4 ratio. Temperature and sunshine duration were negatively correlated while humidity and atmospheric pressure were positively correlated to FT3. Positive correlation was observed between the cloud cover and FT3/FT4 ratio, though it was negatively correlated to FT4.

3.11 Correlation between climate parameters and thyroid functions in SCH subjects.

In the SCH group, no significant correlation was observed between climate components and FT3 or FT3/FT4 ratio level. The only significant correlations that found were the negative correlation between TSH and the cloud cover (r=-0.332, P=0.006) and FT4 and humidity (r=-0.247, P=0.040), a day and a week earlier respectively.

Table 3-12. Pearson's correlations of climate components of one day, one week and one month before blood sampling with the mean TSH, FT3, FT4, FT3/FT4 in euthyroid subjects throughout the study period.

Duration from sampling	Climate parameters (Summer and Winter)	Euthyroid			
		FT3 (pg/ml)	FT4 (ng/dl)	FT3/FT4 ratio	TSH (mIU/L)
One day	Temperature	**-0.195***	0.029	**-0.193***	0.027
	Humidity	**0.204***	-0.032	**0.222***	-0.046
	Sunshine duration (Hours)	**-0.203***	0.098	**-0.264****	0.077
	Cloud cover (Octa)	0.140	**-0.136***	**0.254****	-0.085
	Atmospheric pressure	**0.174***	-0.02	0.151	-0.001
One week	Temperature	**-0.220****	0.058	-0.239	0.007
	Humidity	**0.227****	-0.050	**0.212****	-0.033
	Sunshine duration (Hours)	-0.141	0.033	-0.142	0.07
	Cloud cover (Octa)	-0.041	0.001	-0.042	-0.021
	Atmospheric pr	0.067	-0.004	0.057	-0.014

Duration from sample taking	Climate parameters (Summer and Winter)	Euthyroid			
		FT3 (pg/ml)	FT4 (ng/dl)	FT3/FT4 ratio	TSH (mIU/L)
One Month	**Temperature**	-0.143	0.045	**-0.172***	0.003
	Humidity	0.124	-0.02	0.136	-0.037
	Sunshine duration (Hours)	-0.127	0.05	**-0.202***	0.023
	Cloud cover (Octa)	0.108	-0.021	**0.174***	-0.005
	Atmospheric pressure	**0.168***	-0.035	**0.186***	0.001

*P value <0.05, **P value <0.01

Part 3

Assessment of Hair cortisol in Euthyroid, hypothyroid and SCH subjects.

Among the 152 subjects who participated in the study, 124 subjects were left for hair sample collection and hair cortisol measurement, after applying the exclusion criteria. In six individuals, hair cortisol exceeded the 3 SD of the mean, further exclusion done. This resulted in a final sample of 118 subjects; 65 euthyroid, 26 SCH and 27 hypothyroid subjects.

Table 3-13 and 3-14 lists the demographic characteristics and biochemical parameters of the study participants.

Table 3-13. Demographic characteristics of all subjects participated in hair cortisol assessment.

Variables	Mean (SD) Number (%)
Total number	118
Age	34.24(±9.88)
Gender	
Male	11(9.3%)
Female	107(90.7%)
Weight	
Normal	45(38.1%)
Overweight	30(25.5%)
Obese	43(36.4%)
BMI (Kg/m^2)	29.52(±5.25)
Waist circumference (cm)	91.94(±16.12)
Mean sleep duration per 24 hour (hours)	6.74(±1.89)

Table 3-14. Biochemical parameters of all subjects participated in hair cortisol assessment.

Variables	Mean (SD) Median [range]
TSH (mIU/L) [b]	3.14 [99.36]
FT4 (ng/dl)	1.102(± 0.278)
FT3 (pg/ml)	3.187(± 0.526)
Anti-TPO titer (IU/ml)	91.82(± 161.05)
Cortisol (pg/mg hair)[b]	20.94 [63.18] (5.75-69.18)
Cortisol Log	1.279 (± 0.271)

3.12 Comparison of socio-demographic characteristics in euthyroid, SCH and hypothyroid subjects

The comparison between euthyroid, SCH and hypothyroid subjects in demographic characteristics are presented in Table 3-15.

Weight significantly higher among hypothyroid subjects compared to euthyroid subjects, and waist circumference were significantly higher in SCH and hypothyroid subjects compared to euthyroid subjects.

Table 3-15. Comparison of socio-demographic characteristics in euthyroid, SCH and hypothyroid subjects

Variables	**Euthyroid (N=65) Mean (**±SD)	***P*** [a]	**SCH (N=26) Mean (**±SD)	***P*** [b]	**Hypothyroid (N=27) Mean (**±SD)	***P*** [c]
Age (years)	33.12(10.38)	0.329	36.85(13.44)	0.948	37.97(10.91)	0.134
Weight (Kg)	64.51(13.94)	0.073	70.47(9.33)	0.928	72.49(11.71)	**0.027**
BMI (Kg/m^2)	26.36(5.74)	0.198	28.25(4.36)	0.888	29.32(4.53)	0.062
Waist circumf. (cm)	82.24(12.62)	**0.048**	89.9(15.24)	0.917	92.82(11.03)	**0.009**

P-value < 0.05 was regarded as significant (Bold)

[a] P value between euthyroid and SCH groups

[b] P value between SCH and hypothyroid groups

[c] P value between hypothyroid and euthyroid groups

3.13 Comparison of thyroid functions and hair cortisol in euthyroid, SCH and hypothyroid subjects

The comparison between euthyroid, SCH and hypothyroid subjects in their biochemical data are presented in Table 3-16.

TSH were significantly higher in hypothyroid compared to SCH and euthyroid subjects, and SCH compared to euthyroid subjects. While FT3 and FT4 were significantly lower in hypothyroid compared to both euthyroid and SCH. The anti-TPO demonstrates significantly higher value in hypothyroid subjects compared to SCH and euthyroid subjects.

Hair cortisol was slightly higher in the SCH group, but the difference was not statistically significant (P = 0.783), however it was significantly higher in hypothyroid subjects compared to euthyroid subjects, P = 0.047, Figure 3-7.

Table 3-16. Comparison of thyroid functions and hair cortisol in euthyroid, SCH and hypothyroid subjects

Variables	Euthyroid (N=65)	*P* [a]	SCH (N=26)	*P* [b]	Hypothyroid (N=27)	*P* [c]
TSH (uIUml) [d]	1.95 [0.82]	**<0.001**	6.41 [0.65]	**<0.001**	16.4 [98.64]	**<0.001**
FT4 (ng/dl)	1.25(±0.169)	0.219	1.17(±0.15)	**<0.001**	0.829(±0.34)	**<0.001**
FT3 pg/ml	3.29(±0.42)	0.210	3.49(±0.46)	**<0.001**	2.56(±0.73)	**<0.001**
TPO titer (IU/ml)	23.59(±73.86)	0.124	74.0(±137.22)	**<0.001**	228.27 (±192.26)	**<0.001**
Cortisol (pg/mg hair) Median[d]	16.74 [63.3]	0.417	19.86[42.84]	0.115	26.79 [57.65]	**0.010**
Cortisol Log Mean (anti-log) Range	1.236(±0.299) 17.22 5.75-69.18	0.783	1.277(±0.249) 18.92 7.41-50.12	0.322	1.383(±0.193) 24.17 10-67.61	**0.047**

[a] P value between euthyroid and SCH groups, [b] P value between SCH and hypothyroid groups, [c] P value between hypothyroid and euthyroid groups

[d] Median of non-parametric data between the groups were compared using Kruskal-Wallis H test and Mann-Whitney-U test.

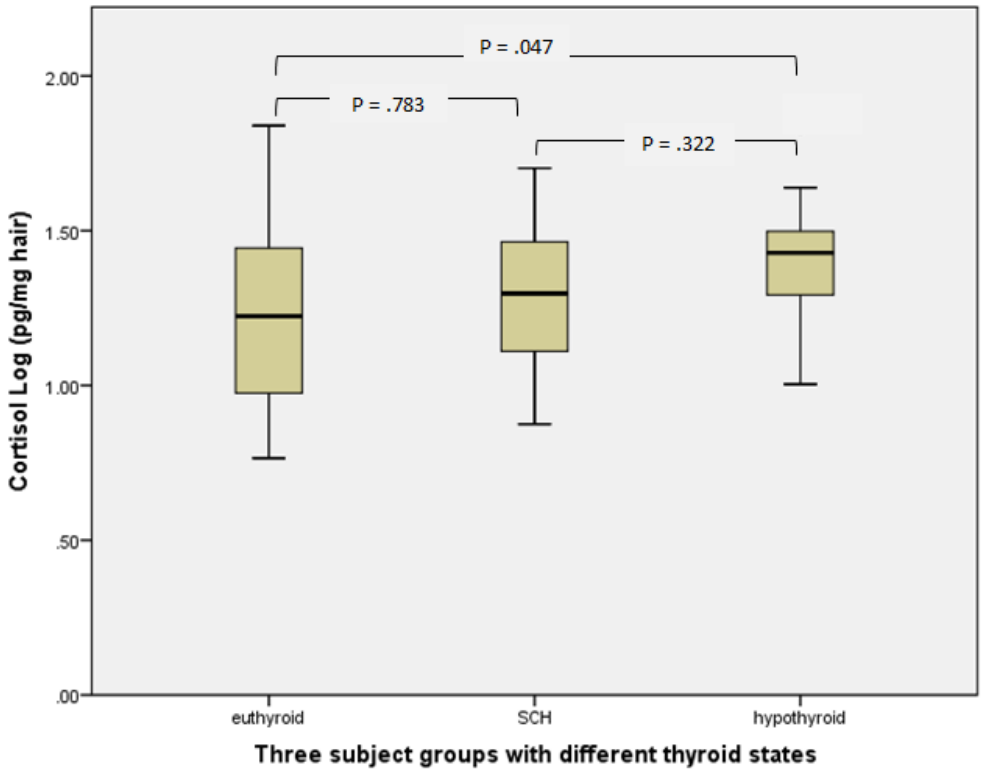

Fig 3-7. Mean hair cortisol level (Hair cortisol log) in the euthyroid, SCH and hypothyroid subjects.

3.14 Comparison of hair cortisol levels between normal weight subject and overweight/obese subjects.

Comparison of subjects on the basis of BMI demonstrated a significantly higher hair cortisol level in overweight/obese group than in the normal weighted person (P = 0.009), as illustrated in Figure 3-8.

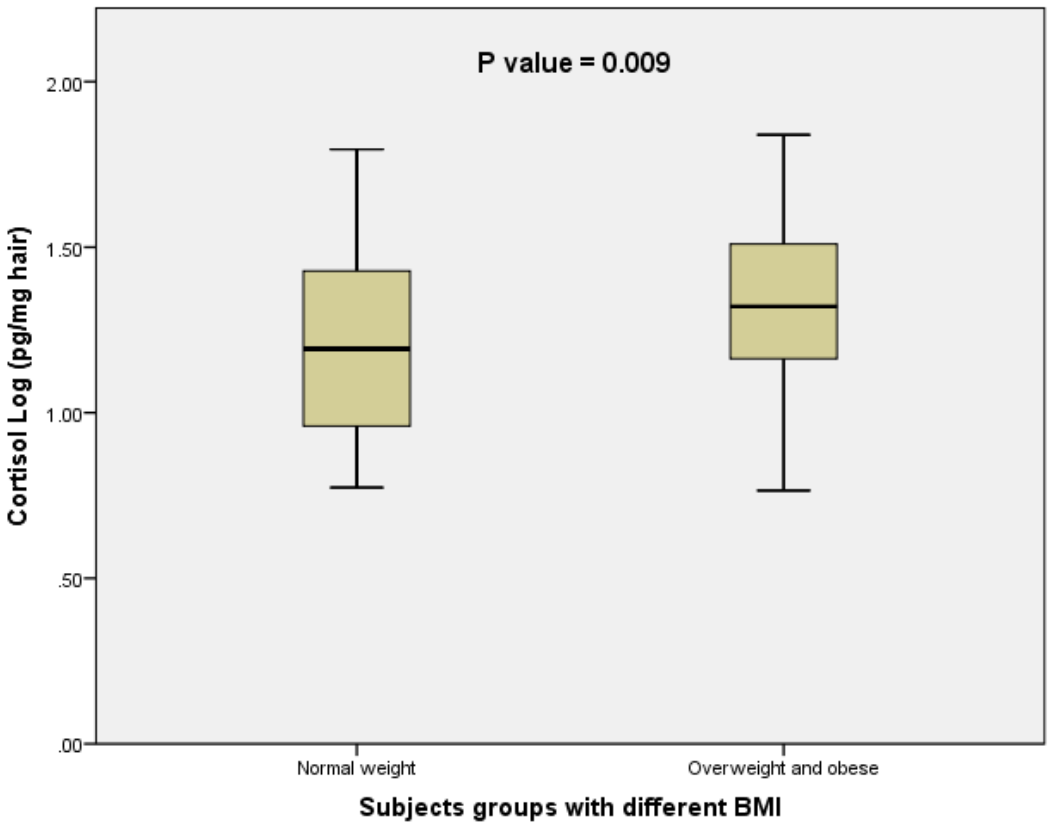

Fig 3-8. Comparison of hair cortisol levels (Hair cortisol log), between the normal weight subjects and overweight/obese subjects.

3.15 Comparison of hair cortisol in proximal and distal hair segments

Comparison of hair segments reports higher hair cortisol level in the proximal segments compared to the distal segments of the same study subjects (P = 0.013), as shown in Figure 3-9.

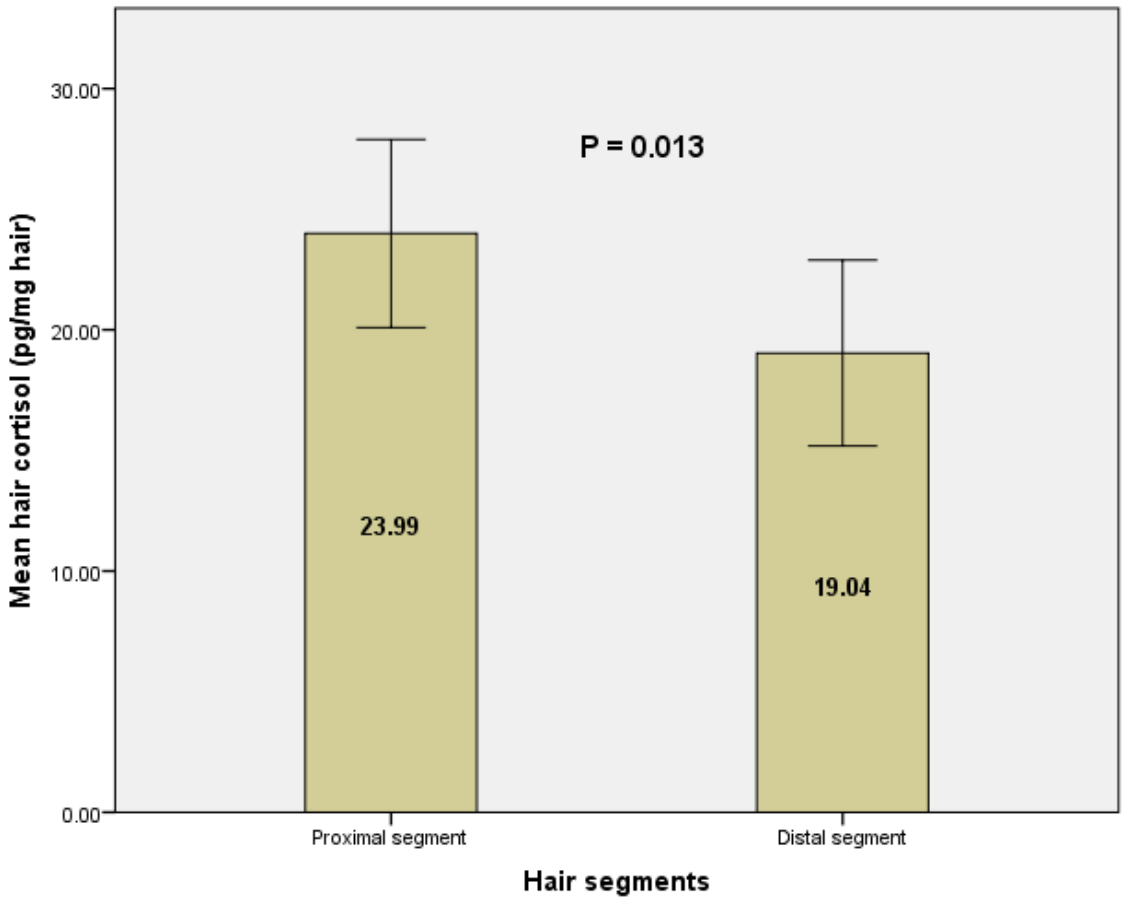

Fig 3-9. Comparison of mean hair cortisol level between proximal and distal hair segments.

**p* value evaluated using Paired Samples T Test.

3.16 Correlation of hair cortisol with socio-demographic and thyroid functions.

Correlation of hair cortisol level with other variables was performed using Pearson's correlation as shown in Table 3-17. A significantly positive correlation was found between hair cortisol and each of the age, weight, BMI and serum TSH ($P < 0.05$). Figure 3-10 illustrates the relation of hair cortisol (cortisol log) with serum TSH.

There was significant correlation between hair cortisol level of proximal and distal hair segment ($r = 0.517$, $p = 0.012$) And significant negative association between distal hair segment and mean sleep duration per 24 hour were found ($r = - 0.478$, $P = 0.021$).

Table 3-17. Correlations between hair cortisol and other variables among main study sample.

Parameters		**Age**	**Wt**	**BMI**	**Waist**	**TSH** [e] **(mIU/L)**	**FT4 (ng/dl)**	**FT3**	**TPO titer**
Hair cortisol	Pearson's Correlation	**.215***	**.203***	**.206***	.106	**.192***	-.129	-.182	.092
	P value	.021	.031	.029	.322	.039	.171	.171	.357

Pearson correlation was used for correlation between hair cortisol log and all normally distributed variables. [e] Spearman's correlation is used for correlation between original hair cortisol and non-parametric variable, TSH. *P value <0.05

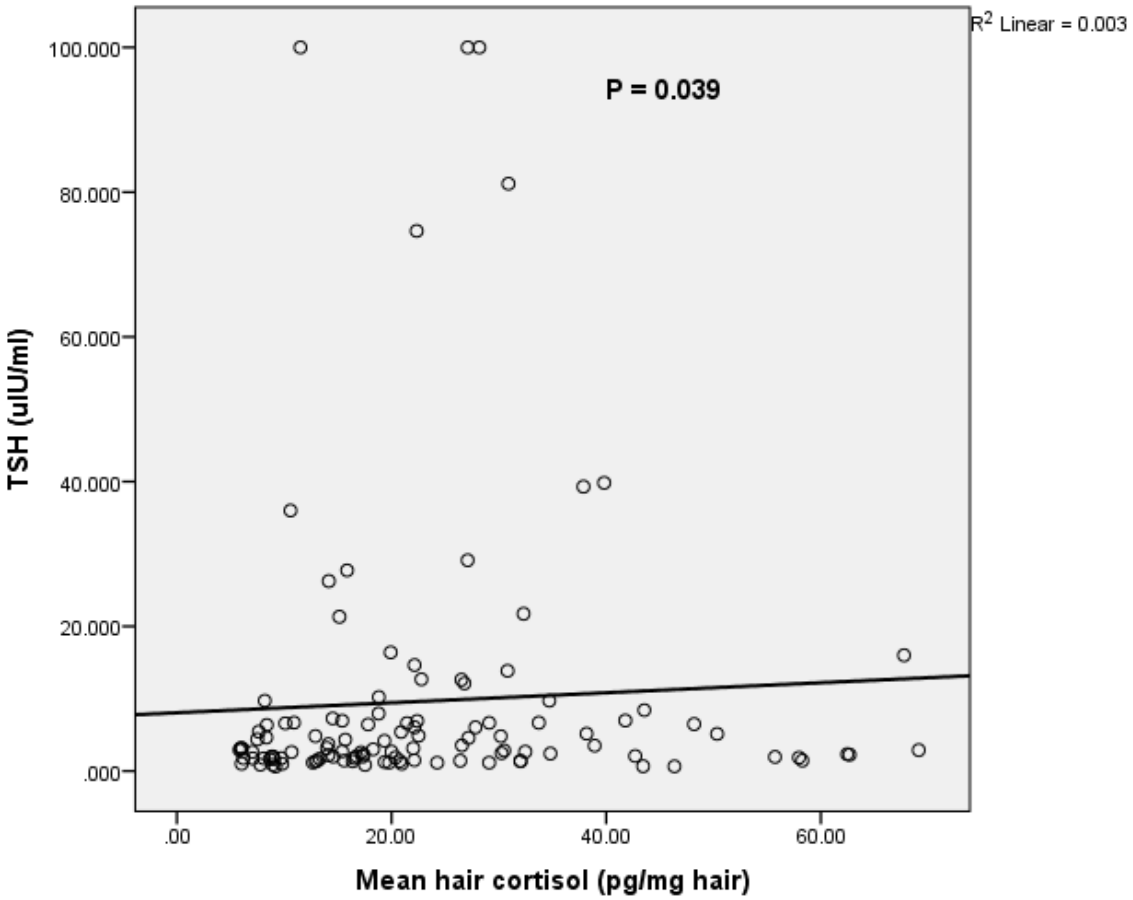

Fig 3-10. The correlation between hair cortisol level and serum TSH of the main study sample.

Discussion

Part 1

Clinical score and anti-TPO assessment in SCH compared with euthyroid with different TSH levels.

4.1 Comparison of biochemical parameters between SCH and euthyroid subjects

According to the results of the present study, a higher mean anti-TPO titer was found in SCH than that in euthyroid subjects with a similarly higher rate of TPO positivity among the SCH group. The mean anti-TPO value among TPO-positive subjects was 190.88 IU/ml (±170.59). This study reported 10.2% TPO positivity among the euthyroid group, this is in accordance with the study by Roos et al (148) who evaluated 2394 euthyroid subjects for TPO positivity, of which, 8.4% of those subjects were TPO-positive (148). The higher anti-TPO in SCH could be explained by decreased TH production in the presence of anti-TPO antibody and thus higher TSH level in these subjects.

The frequency of hyperprolactinemia in the SCH group of the present study is 27%, others found 20.4% (149). In a study in India, 34.93% of SCH subjects had hyperprolactinemia. However, when they evaluated the hyperprolactinemia among the SCH groups with higher TSH (> 7.5), the percentage of hyperprolactinemia was raised to 49% (97). Other studies

reported lower rates of hyperprolactinemia (19%, and 8%) (13,150). The current study found a comparable result of hyperprolactinemia between SCH and euthyroid subjects, which is inconsistent with other studies in which a higher rate of hyperprolactinemia was reported in SCH patients compared to euthyroid subjects. The difference in the study subjects and TPO-positive subjects in various studies might be responsible for these variances, and the presence of a higher record of hyperprolactinemia in previous studies might also be due to their inclusion of those with higher TSH values in their studies. A small percentage of this study's participants has TSH values more than 7.0 mIU/L. As stated by another study, hyperprolactinemia is higher at higher TSH levels (97).

The increased prolactin level in hypothyroidism and SCH explained by that TRH will also stimulate prolactin secretion, not only TSH when decreased TH negatively feeds back on hypothalamus (75). While small decrease in TH within the normal range accompanied with slight elevation in TSH (especially in SCH of TSH < 10) probably has no effect on the prolactin secretion.

4.2 Comparison of Zulewski's score and other clinical parameters between SCH and euthyroid subjects

In this study, SCH subjects were reported to have one-point higher Zulewski's clinical scores than euthyroid subjects. A good correlation between the Zulewski's clinical score and TSH was observed as well. This finding is supported by previous studies including studied carried out in Colorado (81) and Swiss (12), and studies by Reuter (151) and Cooper (152), in which the SCH group more frequently presented with hypothyroid

symptoms than euthyroid subjects. The Colorado study also revealed a clear correlation between a number of symptoms and elevated TSH (81). Zulewski found an obvious correlation between the score and FT4 and TSH in SCH subjects (12). However, a study by Goel showed no significant difference in clinical presentation between SCH and the euthyroid group (150), but they didn't explain the cause.

In this study, fatigue, muscle cramp, and cold intolerance were the frequent symptoms in SCH and significantly higher than euthyroid subjects. In a study of SCH women by Kong et al (153), the most frequent symptom was fatigue (83%). Although the percentage of some clinical symptoms, especially fatigue, was also high in euthyroid subjects in the present study, these clinical features were significantly higher in SCH than in euthyroid subjects. The presence of some clinical features in euthyroid subject is due to that most of these features are non-specific, but the higher clinical sign and symptoms in SCH compared to euthyroid group might be explained by that even this slight decrease in TH (even within the reference range) on general body metabolism and organ functions.

4.3 Comparison of biochemical data between euthyroid subjects with LNT and HNT levels.

In the present study, higher titers of anti-TPO and lower serum FT4 level were detected in euthyroid subjects with HNT compared to those with LNT. This might suggest that the presence of higher anti-TPO titer is in part responsible for the decrease in FT4 and its concomitant increase in serum TSH, even if the TSH is within the reference normal limit. This could be supported by the finding of a positive correlation between Anti-TPO titer

and TSH level demonstrated in the present study, which is in line with previous studies (148,154). The Roos et al's (148) study documented a higher prevalence of anti-TPO positivity among the higher quartile for TSH in the euthyroid range (148). In a study by Li et al, a significantly higher TSH value was reported in patients who were positive for both Anti-TPO and thyroglobulin antibody (Tg-Ab) than in populations with negative antibodies or only one positive antibody (155).

4.4 Correlations between socio-demographic, biochemical and clinical parameters in all studied group

When the relation between hormones and age was evaluated in this study, a significant negative correlation between FT3, prolactin, and age were found, which might suggests slightly lower hormone secretion with advancing age and low thyroid hormone secretion leads to a compensatory increase in TSH release from the pituitary. This supports a higher TSH reference range in elderly. Although studies reported different effect of aging on hormone (98,156), a complex changes occur in endocrine system of old people (156).

No significant gender differences were found between TPO-positive and TPO-negative groups. However, a study by Roos et al reported higher TPO positivity in females (148). The difference in the number of male and female participated in the study might have effect on this differences. In the present study the numbers of males who participated in the study were significantly lower than females.

In agreement with a study done in Iran (157), significant correlations between TPO titer and TSH were revealed. No significant correlation between prolactin and TSH was found in present study. This is inconsistent with studies by Goel et al and Sharma et al, who showed a positive correlation between TSH and prolactin levels (97,150). The absence of correlation between prolactin and TSH level in the present study might be due to lower TSH level in SCH group that explained previously, suggestive of no association if TSH slightly elevated.

Part 2

Effect of summer and winter seasonal climate on thyroid functions

The present study was performed in Sulaymaniyah city, situated 884.8m above sea level having latitude 35° 34' 0.7104" North and longitude 45° 24' 57.9852" East (102). This place, with a significant difference between the climatic conditions in summer and winter season, is the best region for studying the effect of climatic variations on the hormone level.

4.5 Comparison between thyroid function during summer and winter season in euthyroid and SCH subjects.

In this study, a steady TSH and TH values in euthyroid and SCH subjects during summer and winter were observed, except for a small but

significantly increase in FT3 in euthyroid individuals (P <0.001) and small decrease in FT4 in SCH individuals (P<0.05) during winter.

The effect of duration of outdoor stay was only observed during winter in the FT3 level of euthyroid individuals; apart from which no appreciable change in pituitary-thyroid axis (PTA) function was observed in both the groups. It found that the variations in the climatic components were associated with the level of TH in euthyroid subjects, with no effect on SCH subjects. This might be due to the same explanation of defective normal response of hypothalamus and to temperature variation in hypothyroid subjects.

The small increase in FT3 in euthyroid subjects during winter was in agreement with previous studies carried out in temperate climates (29,158). In another study performed on 13 healthy male and 13 healthy female subjects in Belgium, the total T3 showed a lower value during spring and summer while TSH showed higher value during winter– spring (28). A similar data was obtained in a study involving euthyroid and SCH subjects in Korea (17) where the serum TSH increased during winter and spring season leading to transition to SCH.

In a study conducted for six year in Japan, another country with a temperate climate, reported a decreased TSH concentration during summer that got reversed in winter; there existed a negative correlation between the daily temperatures and TSH, and FT3 concentrations (106). But these two studies (17,106) had a shortcoming; the entire study, i.e., the follow-up was not carried out on the same subjects. The subjects who developed thyroid disease or were on thyroid treatment throughout the study could not be excluded because the studies were carried out retrospectively. In the present

study, no significant change in TSH was found indicating that the seasonal variations in the selected region had no appreciable effect on the diagnosis of SCH subjects; also neither in their monitoring or treatment.

Previous studies have shown that there can be more than one reason behind the response of hormone levels to seasonal variations. Increase in thyroidal secretion cannot be the only reason for the seasonal variation in TH level. In such a case, there should be an increase in both T3 and T4, but in most studies T3 increased with no change or slight decrease in T4 in winter as mentioned earlier. It can be a primary response of the PTA axis to changing the light and ambient temperature (21), or it is due to small decrease in either the volume of distribution or serum T4 (17,21). It is possible that seasonal changes in TH level are due to change in their metabolism (159) or the combination of all factors mentioned above.

4.6 Correlation between climate parameters and thyroid functions in studied participants.

The comparison of the climate components with the hormone levels of euthyroid subjects exhibited significant correlation with the thyroid hormone, FT3 and the FT3/FT4 ratio. This data suggests that the increase in FT3 and FT3/FT4 in winter is partly due to decreased temperature and sunshine and increased humidity, cloud cover and atmospheric pressure, especially a day before sample collection. On the other hand, a decrease in TSH and FT4 was observed due to increased cloud cover and humidity respectively. The correlations of climatic weather with FT3 and FT3/FT4 ratio might be suggestive of effect of the weather on metabolism and activity

of deiodinase 2 (D2), especially effect of climate by one day duration is too short to have effect on TSH or TH secretion.

Few studies correlate the weather components with thyroid functions (28,29), the study in Italy (29) focused only on the evaluation of the correlation between climatic components and TSH and found no significant correlation between them in euthyroid subjects while the study conducted in Belgium (28), focused on the association between climatic components and T3, and showed an inverse relationship. The negative correlation between temperature and FT3 level is in contrast to studies carried out in arctic or subarctic regions (19) which may partly be due to the difference in the latitude and partly due to the sample size; lesser participation when study was conducted in subarctic region. Altitude of the region plays an important role in the outcome of the study.

So far, the data obtained from both cold and temperate climates have not been very conclusive as most of the studies have been done at high altitudes, where along with the temperature, factors such as hypobaric condition, changes in oxygen partial pressure, increased ultraviolet radiation might affect the secretion of hormones. A study conducted in a hypobaric chamber demonstrated that a simulated altitude of 3810 m affects the TH level (160). The studies are also influenced by the sleep pattern, dietary changes and plasma volume shifts (161).

There are no reports regarding a comparative study between climatic components and thyroid hormone level in SCH individuals. Occurrence of SCH has been reported in 4% to 18% of the adult population (116). The basal TSH was found to be negatively correlated with the seasonal alterations in ambient temperature in a small number of hypothyroid patients

on thyroxine treatment (17). In addition, in primary hypothyroid patients, a small but significantly lower level of TH was found in winter suggesting that the dose required for replacement of thyroid hormone in patients with hypothyroidism may be higher in winter than in summer (116).

4.7 Correlation between some socio-demographic characteristics and thyroid functions in SCH subjects.

Subclinical hypothyroidism has been known to be influenced by age, sex, BMI, dietary iodine intake, ethnicity, smoking status, latent autoimmune thyroid diseases and TSH secretion (31). Reportedly, people with SCH and TSH levels exhibited higher measures of BMI and waist-to-hip ratio than subjects with normal or lower TSH levels (162). Similarly, we also observed an increased TSH level in the SCH population who exhibited a higher BMI and waist circumference compared to the euthyroid population.

Studies on the BAT (117), skeletal muscle and thermogenesis (111)suggest the increase in D2 activity by cold exposure; this is in concordance with the present study. Based on the correlations found in the present study, we can suggest that the slight increase of FT3 and FT3/FT4 and decrease of FT4 in winter is partly due to the effect of climate, especially one day before the sample collection, on the peripheral conversion of FT4 to FT3 and thus on D2 activity. Thus, it can be concluded indirectly that lower temperature and sunshine duration, higher humidity, cloud cover and atmospheric pressure present during winter month can slightly increase

the activity of D2 in peripheral tissue. Increased sunshine duration and temperature during summer decrease the activity of this enzyme slightly.

Part 3

Assessment of Hair cortisol in Euthyroid, hypothyroid and SCH subjects.

In the present study, we examined hair cortisol in overt hypothyroidism and a group of SCH subjects to reveal if the mild TSH increase in SCH has an effect on hair cortisol and to study if there is any relationship of HPT axis with hair cortisol. To the best of our knowledge, till date, no study has studied the correlations between the long-term HPA axis activation (hair cortisol) and the HPT axis or thyroid function tests. Also, no study has assessed the hair cortisol level in SCH and overt hypothyroid subjects.

4.8 The mean and median hair cortisol levels in euthyroid subjects.

The mean and median hair cortisol in this study sample of the healthy adult subjects in the Sulaymaniyah city were 17.22 pg/mg hair and 16.74 (range: 5.82-69.12) pg/mg of hair, respectively.

Approximately the same results of mean and/or range of hair cortisol levels were recorded in most of the previous studies (56,58,68,163). In Germany in a study by Dettenborn et al. in 2012, healthy subjects of different ages were studied, the mean of hair cortisol was 21.82 pg/mg among healthy adult subjects of 18 to 49 years old (58). In a study conducted

in Netherland; Manenschein et al. compared a group of adult healthy subjects to a group of cyclic Cushing's syndrome. They found a range of 9.9 to 75.9 pg/mg of hair cortisol in the healthy adult group with the mean of 27.3 (CI: 24.6-30.4) (56). In a study by Raul et al. (163) a mean hair cortisol concentration of 18 pg/mg, ranging from 5 to 91 pg/mg was recorded (163). In another study in Netherland, hair cortisol level was 28.18 pg/mg in healthy subjects, compared to those with bipolar disorder (68).

The present study is inconsistent with some studies in which the hair cortisol showed significantly higher levels than the present study. In a Canadian study, a median hair cortisol of 46.1 (17.7 - 153.2) pg/mg (57) and among Caucasian healthy adults a mean hair cortisol level of even 113 ± 54 were recorded (128). However, in these two studies, the sample size was small and the ages of participants were differing.

Different method of hair preparation and various immunoassay techniques may be the cause of variability found between different studies. In this study, ECLIA was used in contrast to most studies in whom ELISA kit was used (57,68,128), and in more recent studies CLIA (58,66,163,164) and LC/MS (127) were used. The slightly lower hair cortisol results recorded in this study might be due to the use of ECLIA as an immunoassay technique to measure hair cortisol in comparison to studies using ELISA and CLIA. In general, even relatively lower mean cortisol level (7.7 ±8.12 pg/mg) was recorded in a study using LC/MS than other studies (127).

4.9 Comparison of hair cortisol levels in euthyroid, SCH and hypothyroid subjects.

The results of the present study suggests that no significant differences exists in hair cortisol levels between SCH and euthyroid subjects, however, hair cortisol were significantly higher among hypothyroid compared to euthyroid subjects.

In overt hypothyroidism study, Iranmanesh et al. (39) suggested that decreased TH production in hypothyroidism affects the adrenal axis by elevating cortisol levels in the serum. However, in the diseased state of cortisol deficiency, TSH production is stimulated (165). These observations are suggestive of a feedback loop between HPT and HPA axis that decreased TH cause an increase in the TSH and cortisol production. At the same time, when the cortisol levels are high, the TSH is suppressed and TH is decreased (40).

4.10 Correlation of hair cortisol with socio-demographic and thyroid functions.

A significant relationship was found between hair cortisol and TSH, age, weight and BMI.

In a comparison of hair cortisol levels between genders, a higher hair cortisol among men was detected than in women in some studies (58,70). On the other hand, no gender differences were observed in the present and some other studies (55,68,128,163). In the present study, the number of males who participated in the study was few, owing to either baldness or insufficient hair in the posterior vertex area in those with a newer model of haircut among younger males.

In the present study, in agreement with the study performed by Dettenborn et al. (58) upon analyses conducted across the whole age range of healthy adult, a positive relationship between age and hair cortisol levels was found. This is in contrast with other studies (68,70) performed on healthy adult groups, in which no significant correlation between hair cortisol levels and age was observed. The suggested mechanism of increased hair cortisol with age is decreased hair growth with advancing age, and lower hair growth rate might cause the possibility of more prolonged hair exposure to the cortisol level from blood.

4.11 Comparison of hair cortisol levels between normal weight subjects and overweight/obese subjects.

Consistent with other studies (47,143), the current study revealed hair cortisol level to be significantly higher in overweight and obese subjects in comparison to normal weight subjects. And in contrast to the study by Manenschijn et al. (69) in 2013 and in line with previous studies (55,67,73,127,164), a significant positive relationship of hair cortisol with weight, and BMI was found among study participants. The higher hair cortisol in subjects with greater BMI could be due to higher stress in these individuals which associated with increase in both cortisol and BMI. As stress may play a major role in development and maintenance of obesity (166).

4.12 Comparison of hair cortisol levels between in proximal and distal hair segments.

In accordance with some studies (58,69,167), the comparison between proximal and distal hair segment revealed significantly lower hair cortisol

level in the distal hair segment in the hair sample of the same subjects (intra-individual difference), the possible mechanism is washing out of cortisol from distal segment due to more prolonged environmental exposure in comparison to the proximal segments. Although no significant differences between the proximal and distal hair segments were found in other studies (55,128). The length and level of segments that assessed to compare hair cortisol level between proximal and distal segment suggestive of the difference in between studies.

4.13 Limitations

Because the study was performed prospectively, the number of study subjects was small; a larger sample size can give more accurate results.

Another shortcoming of this study is that the subjects were did not checked for vitamin D deficiency, as vitamin D deficiency is common in this country (Iraq) (168,169), and most of the hypothyroid symptoms are nonspecific; fatigue and muscle cramping are also common complaints of those with vitamin D deficiency.

One of the limitations of this study for hair cortisol assessments is that; most of the SCH subjects were diagnosed few weeks before or during recruitment to the study to have SCH. The duration of elevated TSH in SCH should be taken into consideration because subjects who recently developed SCH might still have no effect on HPA axis, especially for hair cortisol which represents a retrospective measure of cortisol levels. Thus the 3 cm length of hair cortisol measured represents cortisol levels of approximately 3 months before the study.

Another limitation of hair cortisol measurement in general is variation in the growth rate of hair in different subjects as this decrease with age and some diseases such as hypothyroidism or change in cortisol levels due to the local metabolism of hair (170).

Conclusions and Recommendations

Conclusions

1. This study concludes that general symptoms (non-specific) of hypothyroidism, especially fatigue, muscle pain and cold intolerance are present in a large fraction of SCH subjects when compared with euthyroid subjects. The Zulewski's hypothyroid score correlated well with TSH level and SCH subjects presented with a higher score than euthyroid subjects.
2. The frequency of anti-TPO positivity is high (43.8%) in SCH subjects and presence of anti-TPO significantly associated with an increase in TSH level. Additionally, euthyroid subjects with high-normal TSH levels have a higher level of anti-TPO titer than euthyroid subjects with the low-normal of TSH, with no difference in the clinical parameters between the two groups.
3. Regarding seasonal change in thyroid hormone level, it concluded that each climatic component such as humidity, sunshine duration, temperature, cloud cover and atmospheric pressure has its share on the slight variance of hormone levels found in different seasons; the effect being mainly on FT3 and FT3/FT4 ratio. The effect of climate was mostly on peripheral metabolism and most probably on the conversion of FT4 to FT3 rather than central causes (PTA). Duration of outdoor exposure slightly affects hormone level, but no significant correlation exists between them.
4. Since SCH had no significant difference in serum TSH apart from slightly higher FT4 during the summer season, it was concluded that

seasonal variations in this region climate have no effect on the diagnosis of SCH cases.

5. Regarding hair cortisol assessment, the authors reach to the conclusion that a hypothyroidism is associated with higher hair cortisol level, compared to euthyroid and a significant relationship exists between hair cortisol an index of long-term HPA activity and TSH, a component of HPT axis.
6. It also concluded that the SCH subjects are not associated with significantly higher hair cortisol levels, unlike overt hypothyroid subjects. This could be explained by the absence of a relation between the two axes when both axes function normally and their levels are within the normal physiological range.

Recommendations

1. This study recommends the use of the Zulewski's clinical score along with biochemical tests for severity assessment of SCH and treatment decisions.
2. It also recommends testing and follow-up of subjects with TSH within the high-normal level for anti-TPO. This is especially important for early diagnosis of SCH, as the treatment in some cases of SCH is mandatory and prevents the risk of progression to overt hypothyroidism and its clinical consequences. Especially, treatment should occur in those who have cardiovascular risk factors and those who have unexplained menstrual disturbances. Treatment is also important in women who want to become pregnant who might go undiagnosed because of non-specific thyroid symptoms, this is to decrease pregnancy outcome.
3. A larger study is needed in the future with follow up of the euthyroid subject with high-normal TSH level or those with positive anti-TPO to know the frequency of progression to SCH among this group. And to assess SCH who received treatment with Zulewski's score to find whether the score reduced with treatment, or affected by the biochemical changes during follow up.
4. For assessment of seasonal change in hormone level, measurement of urinary T3 is recommended to get a clear idea regarding change in the rate of T3 clearance.

5. The author would like to recommend a mid-latitude study on the effect of season and correlation of climatic components on the activity of D2 and UCP in BAT and skeletal muscle to check if decreased temperature and sunshine duration during winter causes an increase in activity of extrathyroidal D2 or BAT in humans.
6. Estimation of thyroid hormone deposition in hair matrix in euthyroid, SCH and hypothyroid subjects to relate with their serum thyroid hormone levels.
7. Estimation of heterophile antibody that may interact with the assay method and affect the TSH level.
8. In future work, Leptin and Irisin hormones can be included to be measured in patients with SCH and hypothyroidism.

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Addendum

Publications

The following articles abstracted from the thesis content have been published in international Journal with impact factors according to Clarivate Analytics (Thomson Reuters):

1. Abdulateef DS and Mahwi TO. Assessment of hair cortisol in euthyroid, hypothyroid, and subclinical hypothyroid subjects. Endocrine. Sep 2018.

Publisher: Springer, Journal impact factor: 3.17

2. Mahwi TO and Abdulateef DS. Relation of different components of climate with human Pituitary-Thyroid axis and FT3/FT4 ratio: A study on Euthyroid and SCH subjects in two different seasons. International Journal of endocrinology. Jan 2019.

Publisher: Hindawi, Journal impact factor: 2.34

Appendix A

Questionnaire

Code (ID): **Date**: / / **Resident:**

Name: **Phone**:

Gender: 1. Male 2. female **Age**: …….. …y

Ht: ……. cm **Wt**: ……...Kg Waist Circumference: …. cm

Menopause: 1. No 2.Yes

Life style:

Smoker: 1.No 2. Yes …… cigarette/day

Alcoholic: 1.No 2. Yes **coffee**: 1.mild 2.mod 3.heavy

Exercise: 1. Rarely 2. Sometime 3. Daily

Sleep time: …… hr/day

Education: 1. Illiterate 2. Primary 3. Secondary 4. University

Work: 1. Employee 2. Free work 3. Student 4. House wife 5. No work 6. Retired 7.Worker

Working time: 1: morning 2: afternoon 3: evening 4: night 5: both day & night 6: morning & afternoon

Exposure to environmental temp.(outside): …… min./day

Marital status: 1. married 2. Single 3. Divorce

Children: 1. No 2. Yes child no.: … **G**: …….. **P**:…….. **A**:…… **D**:…..

Mens. Dist.: 1.no 2. Slight 3.heavy 4.irregular 5. Short int 6.long int 7.menopause

Complain (chief complain): 1.No 2.Yes ……

Thyroid symptom: 1.No 2.Yes (if yes go to Page 3)

Infertility: 1.No 2.Yes (if yes go to Page 4)

Hx of thyroid disease:

Hx of syst. Dis.: New **Inf.**, **trauma** or **infl**:

Treatment hx: **Fever:**

1. No 2. Thyroid Rx 3. Infertility Rx 4.Other

Type of Rx: **recent hx of Stressful event:** 1. No 2.Yes

Name of Drugs:

Previous Ix: Neck US & Ix of other hormones

Biochemical Assessment

Lab test results:

Previous test	**Ix**	First time	Summer	Winter
	FT4 ng/dl			
	TSH mIU/L			
	TPO IU/ml			
	Prolactin ng/ml			
	Hair cortisol pg/mg hair			

Appendix B

Clinical assessment questionnaire

Zulewski's clinical score:

On the basis of		New score	
		Present	Absent
Symptoms			
Diminished sweating	Sweating in the warm room or a hot summer day	1	0
Hoarseness	Speaking voice, singing voice	1	0
Paraesthesia	Subjective sensation	1	0
Dry skin	Dryness of skin, noticed spontaneously, requiring treatment	1	0
Constipation	Bowel habit, use of laxative	1	0
Impairment of hearing	Progressive impairment of hearing	1	0
Weight increase	Recorded weight increase, tightness of clothes	1	0
Physical signs			
Slow movements	Observe patient removing his clothes	1	0
Delayed ankle reflex	Observe the relaxation of the reflex	1	0
Coarse Skin	Examine hands, forearms, elbows for roughness and thickening of skin	1	0
Periorbital puffiness	This should obscure the curve of the malar bone	1	0
Cold skin	Compare temperature of hands with examiner's	1	0
Sum of all symptoms and signs present		12	0

Sum of the score: ☐

Other clinical parameters:

Goiter:	Grade 0	Grade 1	Grade 2
Decreased Appetite:	No	Yes	
Fatigue:	No	Yes	
Muscle cramp:	No	Yes	
Memory loss:	No	Yes	
Cold intolerance:	No	Yes	
Hirsutism:	No	Yes	
Alopecia:	No	Yes	
Galactorrhea:	No	Yes	

Appendix C

Informed consent form

من كه له خوارەوه واژۆم كردووه، رازيم ئەم پشكنينه (پشكنينى غوددە، فحص غدة الدرقية) ئەنجام بدەم، لەگەل هەر پشكنينێک كه پەيوەندى پێوەى هەيه لەگەڵ وەرگرتنى زانيارى پێويست لێم لەو بارەيەوه بۆ مەبەستى ئەو توێژينەوەيه:

I signed below and agree to do this test (thyroid function test) and all other tests, examination done for this purpose and any information related to this research

	ناو (name)	واژۆ(signature)	بەروار(date)
١			
٢			
٣			
٤			
٥			
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١٧			

پەیوەندی کەش و هەوا و ئاستی هۆرمۆنی کۆرتیزۆڵ لە قژدا لەگەڵ فرمانی ڕژێنی پەریزادەدا

لێکۆڵینەوە یەکە، پێشکەش بە ئە نجوومەنی کۆلێجی پزیشکی زانکۆی سلێمانی کراوە ، بۆ تەواوکردنی بەشێك لە پێویستیەکانی پلەی دکتۆرا لە زانستی کارئەندام زانی پزیشکی

لەلایەن

دەریا سعید عبداللطیف

ماستەر لە کارئەندام زانی دا

بە سەرپەرشتی

د. طه عثمان محوي

پرۆفیسۆر لە پزیشکی هەناوی و دڵ و کوێرە ڕژێندا

ربيع الثاني 1440 بەفرانبار 2718 Jan 2019

پەیوەندی کەش و هەوا و ئاستی هۆرمۆنی کۆرتیزۆڵ لە قژدا لەگەڵ فرمانی ڕژێنی پەریزادەدا

سەرەتا: باری کەم ڕشتنی هۆرمۆنی پەریزادەی کلینیکی نادیار (SCH) ڕەنگە بگۆڕدرێت و ببێتەوە بە پەریزادەی ئاسایی(Euthyroid) بەبێ وەرگرتنی هیچ چارەسەرێك, لە هەمان کاتدا SCH لەوانەیە چەندین سکاڵای سستی پەریزادە (Hypothyroid)یان هەبێت. چەند گۆڕانکاری یەك لەئاستی هۆرمۆنەکانی پەریزادە (TH) و هۆرمۆنە هەژێنەکانی پەریزادەدا (TSH) تێبینی کراوە لە وەرزە جیاوازەکاندا. هەروەها دەرکەوتووە کە پەیوەندی هەیە لە نێوان سستی پەریزادە و ئاستی بەرزی کۆرتیزۆڵ لە خوێندا, بەڵام پەیوەندی نێوان دەردانی ماوە درێژی کۆرتیزۆڵ بەهۆی چالاکی درێژخایەنی تەوەری هایپۆسەلەمەس- ژێرمێشکە ڕژێن- سەرگورچیلە ڕژێن HPA لەو کەسانەی Euthyroid و ئەوانەش کە SCH هەیەلێکۆڵینەوەی بۆ نەکراوە.

مەبەستەکان: مەبەستی ئەم لێکۆڵنەوەیە هەڵسەنگاندنی کلینیکی و کیمیاژیانی یە (TSH, FT3, FT4, TPO, Prolactin) بۆ ئەو کەسانەی SCH یان هەیە بەبەراورد بەو کەسانەی Euthyroid یان هەیە و لە دوو ئاستی TSH ی جیاوازدان. هەروەها بۆ دۆزینەوەی کاریگەری کەش و هەوا لەسەر جیاوازی وەرزی هۆرمۆنی TSH و TH. لە هەمان کاتدا مەبەستمان دۆزینەوەی ئاستی کۆرتیزۆڵ ە لە مووی سەردا (قژدا) وەك هەڵسەنگەرێك بۆ چالاکی درێژخایەنی تەوەری HPA دا. لە هەمان کاتدا مەبەستمان دۆزنەوەی پەیوەندی نێوان پێکهاتە جیاوازەکانی کەش و هەواو ئاستی کۆرتیزۆڵە لەسەر تەوەری HPT .

ڕێگای کارکردن: ئەم توێژنەوەیە ئە نجام دراوە بۆ 233 کەس, کە 166 لە بەشداربووەکان Euthyroid یان هەیە و 67 یان SCH. ئەوانەی Euthyroid ن جارێکیکە دابەش کراون بۆ دوو گرووپ بە پێی بەرز و نزمی TSHەکانیان, بۆ TSH ی ئاست نزم و ئاست بەرز. لە سەرەتادا هەموو بەشداربووان پشکنینی کیمیای ژیانی و کلینیکی و هەڵسەنگاندنی خاڵەکانی زولێوسکی (Zulewski's score) یان بۆ ئە نجام دراوە. هەروەها نموونەی مووی سەر بۆ پێوانە کردنی ڕێژەی کۆرتیزۆڵ لە 118 کەس وەرگیراوە و ئاستەکەی بەراورد کراوە بە 27 نەخۆشی Hypothyroid. لە هەمان کاتدا ئاستی

کیمیای ژیانی بەراووردکراوە لە هەردوو وەرزی هاوین و زستاندا بۆ 152 نەخۆشی ئاسایی و 25 SCH دا.

پەیوەندی نێوان پێکهاتەکانی کەش و هەوا و ئاستی کۆرتیزۆڵ لەقژدا کراوە لەگەڵ هەریەک لە پێکهاتەکانی تەوەری HPT.

ئە نجامەکان: لەو کەسانەی Euthyroid ن , ئاستی TPO بەرزترە لەو کەسانەی بەرزە ئاستی TSH یان هەیە وەك لەوانەی نزمە ئاستیان هەیە. وە هەریەکە لە ئاستی TSH و خاڵەکانی زولێوسکی پەیوەندی ڕاستەوانەیان هەیە لەگەڵ TPO (P-value < 0.05).لە ناو ئەم بەشداربووانەدا ئاستی FT3 بەڕێژەیەکی کەم زیاد بوونی بەخۆوە دی لە وەرزی زستاندا. ئاستی هەریەك لە FT3, FT3/FT4 ratio پەیوەندی پێچەوانەی هەیە لەگەڵ پلەی گەرماو ماوەی دەرکەوتنی تیشکی خۆر, و پەیوەندی ڕاستەوانەی هەیە لەگەڵ ڕاددەی شێ و پەستانی هەوا. وە هەروەها ئە نجامەکان دەریان خستووە کە ڕێژەی کۆرتیزۆڵ زیاترە لە کەسانی Hypothyroid بە بەراوورد بە Euthyroid.

دەرئە نجامەکان: هەڵسەنگاندنی کلینیکی بە پێی زولێوسکی سوودی هەیە بۆ ئەو کەسانەی SCH ن, وە پەیوەندی بەرچاو هەیە لە نێوان خاڵەکانی زولێوسکی و دژەتەنی TPO.

پێکهاتەی کەش و هەوا تا ڕاددەیەك پەیوەندی هەیە بە جیاوازی هۆرمۆنەکانی سایرۆید لە وەرزە جیاوازەکاندا وە جیاوازی یەکە زیاتر خۆی ئەبینێتەوە لە گۆڕینی FT4 بۆ FT3 لە خانە و ئەندامەکانی دەرەوەی تەوەری HPT کە ئەبێتە هۆی کەمێك جیاوازی لە FT3 دا.ئەو کەسانەی Hypothyroid ن ڕێژەی کۆرتیزۆڵ یان لە قژدا زیاترە لەوانەی Euthyroid ن. بەڵام ئەم جیاوازی یە بەرچاو نی یە لەوانەی SCH یان هەیە, وە ئەم لێکۆڵینەوەیە پیشانی داوە کە پەیوەندی ڕاستەوانەی بەرچاو هەیە لە نێوان کۆرتیزۆڵ ی قژ و تەوەری HPT.

العلاقة بين المناخ و مستوى هورمون كورتيزول شعرالرأس مع وظائف الغدة الدرقية

اطروحة مقدمة الى مجلس كلية الطب ـ جامعة السليمانية، كجزء من متطلبات نيل شهادة الدكتورا في الفسلجة الطبية

من قبل

دريا سعيد عبد اللطيف

ماجستير فى الفسلجة الطبية

بإشراف

د. طه عثمان محوي

أستاذ في الباطنية والقلبية

ربيع الثاني 1440 **بەفرانبار 2718** **Jan 2019**

العلاقة بين المناخ و مستوى هورمون كورتيزول شعرالرأس مع وظائف الغدة الدرقية

الخلاصة

الخلفية العلمية: حالات قصور الغدة الدرقية التحت السريرية قد تتماثل للشفاء ذاتيا" دون الحاجة الى علاج. هذه الحالات قد تعاني من اعراض قصور الغدة الدرقية. التغيرات المختلفة التي تحدث بمستويات هرمون الغدة الدرقية ومستويات الهرمون المحفز للغدة الدرقية خضعت للمشاهدة في الفصول المختلفة عند الحالات ذوات الوظائف الطبيعية للغدة الدرقية (Euthyroid) والحالات ذوات القصور التحت السريري للغدة الدرقية (SCH) . قصور الغدة الدرقية يرتبط مع ارتفاع مستويات هرمون الكورتيزول بينما لم تتم دراسة التفعيل طويل الامد لمحور ماتحت المهاد البصري-النخامي-الكظري (Hypothalamic Pituitary Adrenal axis-HPA) لحالات قصور الغدة الدرقية السريري (Hypothyroid)وحلات قصور الغدة الدرقية ماتحت السريري(SCH) .

الأهداف: الغرض من هذه الدراسة لتقييم حالات قصور الغدة الدرقية التحت السريري من الناحيتين السريرية والمختبرية ومقارنتها مع الحالات الطبيعية ذات المستويات المختلفة للهرمون المحفز للغدة الدرقية. ولايجاد تأثير تباين درجات الحرارة المناخية في فصول السنة في مدينة السليمانية على مستويات كل من هرمون الغدة الدرقية والهرمون المحفز للغدة الدرقية (TSH) عند الاشخاص ذوو الوظائف الطبيعية للغدة الدرقية واصحاب قصور الغدة الدرقية التحت السريري. كما وتهدف هذه الدراسة لتقييم مستويات هرمون الكورتيزول الموجود في الشعر نتيجة للتفعيل طويل الامد لمحور (HPA)عند هوءلاء الاشخاص. كما وتهدف الى دراسة الى ايجاد العلاقة بين متغيرات المناخ و مستويات هرمون الكوتيزول الموجود في الشعر على محور ماتحت المهاد البصري- النخامي- الدرقي (Hypothalamic-pituitary-thyroid axis- HPT).

الطريقة: تمت دراسة استباقية للحلات المقترنة بحالات ضابطة ل 233 شخص منها 67 حالات SCH مع 166 حالة طبيعية(Euthyroid) . نم تصنيف الحلات الطبيعية وفقا" لمستويات الهرمون المحفز للغدة الدرقية الى: مستويات طبيعية مرتفعة(2.5<) وطبيعية منخفضة(0.5-2.5). في البداية تم فحص جميع الحالات سريريا" وفقا" لمعيير زوليويسكي كما وتم فحصهم مختبريا" لمعرفة مستويات الدم لكل من الهرمون المحفز للغدة الدرقية وFT4, FT3 والهرمون المدر للحليب (البرولاكتين) كجزء من المحور HPT. كما وتم جمع عينات من الشعرالرأس لقياس الكورتيزول في 118 شخص منهم 27 يعانون من قصور الغدة الدرقية ماتحت

السريري. تم جمع ومقارنة جميع النتائج السريرية والمختبرية ل 152شخص حالته طبيعية و 25 من شخص يعانى من قصور الغدة الدرقية قبل السريري في فصلي الشتاء والصيف وتمت مقارنتها مع متغيرات المناخ. تمت مقارنة مستويات الكورتيزول في الشعر بين المجموعتين وتم ربطها مع المحورHPT.

النتائج: تم التوصل الى ان الاشخاص ذوو المستويات الطبيعية المرتفعة للهرمون المحفز للغدة الدرقية كان لديهم مستويات اعلى للأجسام المضادات TPO من الاشخاص ذوو المستويات الطبيعية المنخفضة لنفس الهرمون. معيار زوليويسكي السريري و مستويات الهرمون المحفز للغدة الدرقية كانت ذات علاقة ايجابية مع مستويات الأجسام المضادات TPO. تم ملاحظة زيادة صغيرة لكن ذات اهمية احصائية في الزيادة من مستويات FT4 ونقصان في مستويا ت FT4 خلال فصل الشتاء. كان هنالك ارتباط سلبي كبير بين FT3 و نسبة FT3/FT4 مع درجة الحرارة و مدة شروق الشمس وارتباط ايجابي مع الرطوبة والضغط الجوي. مستويات الكوتيزول بالشعر اعلى بشكل ملحوظ عندحالات قصور الغدة الدرقية التحت السريري, 18.19 على الرغم من ان النتائج لم تكن ذات اهمية احصائيا". مقارنة" مع الحلات الطبيعية تم تسجيل مستويات عالية بشكل ملحوظ من كورتيزول الشعر عند الاشخاص ذوو قصور الغدة الدرقية التحت السريري.

الاستنتاج: المعيار السريري ذو اهمية في معاينة حالات قصور الغدة الدرقية التحت السريري كما وان هنالك علاقة ملحوظة بين معيار زوليويسكي السريري و مستويات الأجسام مضادات TPO. بالاضافة الى ان الحالات الطبيعية ذات المستويات المرتفعة من الهرمون المحفز للغدة الدرقية لها ايضا" مستويات عالية من مضادات TPO لكن المعيار السريري لم يشكل اي زيادة.

عناصر المناخ تشكل تأثير الى حد ما على مستويات الهرمونات في الفصول المختلفة. التأثير كان اكثر وضوحا" على مستويات التحويل المحيطي ل FT4 الىFT3 اكثر منه على المحور النخامي الدرقي موءديا" الى ارتفاع طفيف في مستويات FT3 في فصل الشتاء. التغيرات الفصلية لاتوءثر على تشخيص حالات قصور الغدة الدرقية التحت السريري.

مستويات كورتيزول الشعر مرتفعة بشكل ملحوظ عند حالات قصور الغدة الدرقية الواضح لكن ليس عند حالات قصور الغدة الدرقية التحت السريري مقارنة" مع الحالات الطبيعية كما وتم ايجاد علاقة ايجابية قوية بين مستويات كورتيزول الشعر والمحور ماتحت المهاد البصري-النخامي-الدرقي.