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ORIGINAL ARTICLE

Assessment of hair cortisol in euthyroid, hypothyroid, and subclinical hypothyroid subjects

Darya Saeed Abdulateef[1] · Taha Othman Mahwi[2]

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Abstract

Purpose Hypothyroidism is associated with an increase in serum cortisol level while the long-term activity of hypothalamic-pituitary-adrenal (HPA) axis in hypothyroid, and subclinical hypothyroid (SCH) subjects has not been studied. This study aimed to assess the hair cortisol levels as a long-term activity of HPA axis in hypothyroid, SCH and a group of healthy adult subjects. Also, it aimed to examine the correlation of hair cortisol levels with hypothalamic-pituitary-thyroid (HPT) axis and anthropometric measures.

Methods We prospectively evaluated a group of normal, SCH and hypothyroid subjects. Serum TSH, FT4, and FT3 were measured as a component of the HPT axis. Hair samples were collected, prepared, followed by extraction of hair cortisol and measurement in pg/mg of hair. Hair cortisol levels were compared in normal, SCH and hypothyroid groups and correlated with HPT axis and anthropometric data.

Results A total of 65 healthy volunteers were analyzed, and the mean hair cortisol level was reported to be 17.38 pg/mg of hair. Hair cortisol level was slightly higher in the SCH subjects, 18.19 pg/mg of hair; however the difference was not significant. Compared to the euthyroid subject, a significantly higher hair cortisol level was recorded in the hypothyroid subjects, 24.17 pg/mg hair, $p < .05$. Hair cortisol was significantly and positively associated with each of the serum TSH, age, weight and BMI ($p < .05$).

Conclusions Overt hypothyroidism but not SCH is significantly associated with higher hair cortisol levels compared to normal subjects, and a significant relation between hair cortisol with HPT axis was found. Also, weight and BMI were positively correlated with hair cortisol level.

Keywords Hair cortisol · Hypothyroid · Subclinical hypothyroidism · Euthyroid · TSH · BMI

Introduction

In response to physiological or physical stress, cortisol is produced and released by adrenal glands through activation of the hypothalamic-pituitary-adrenal (HPA) axis. Cortisol affects several organs and body tissues. Studies reported that hyperactivity of HPA axis can result in several disease conditions [1–5].

The HPA-axis functionally related to hypothalamic-pituitary-thyroid (HPT) axis in animals and humans [6–13], especially in cases with hypothyroidism [12] or hypercortisolism [10, 11]. For example a study reported that the HPT axis was inhibited at the level of hypothalamus and pituitary [7] and decreased peripheral conversion of thyroxine (T4) to triiodothyronine (T3) in subjects with increased cortisol level due to stress induction [8].

Both hyper- and hypothyroidism have been found to be associated with increased cortisol levels [6, 9, 12]. The increase in cortisol levels in hyperthyroidism was related to increased activity of HPA axis [6], whereas hypothyroidism was related to decrease clearance, decreased effectiveness of the negative feedback of cortisol on the HPA axis and metabolic stress effect. These factors led to an increase in the release of cortisol (the stress hormone) [12, 13].

Subclinical hypothyroidism (SCH) is a condition manifested by a mild decrease in thyroid function in which

✉ Darya Saeed Abdulateef
darya.abdulateef@univsul.edu.iq
daryaslatif@yahoo.com

[1] Physiology Department, College of Medicine, University of Sulaimani, Sulaymaniyah, Kurdistan region, Iraq

[2] Department of Medicine, College of Medicine, University of Sulaimani, Sulaymaniyah, Kurdistan region, Iraq

serum thyroid stimulating hormone (TSH) is increased without a decrease in the TH levels [14]. The positive TSH cortisol association found in hypothyroidism [12] was detected in SCH subjects or even in euthyroid with high normal TSH range [13]. However, this is reported to be a physiological relation [13].

Previous reports measured the systemic cortisol levels to study the relationship of cortisol with TH [12, 13, 15], which does not represent long-term cortisol exposure. The systemic cortisol (the blood, urine, or saliva) is also affected by diurnal variations, and circadian and pulsatile rhythm [13, 16]. Besides these tests are also affected by patient's method of collection [9], renal problem in case of urine cortisol [17], stress-related increase in cortisol such as in serum cortisol due to venipuncture [9, 18]. Furthermore, blood cortisol suffers from the shortcoming that it reflects both free and bound cortisol. Also, these tests need to be repeated several times to attain chronic cortisol concentration [19].

More recently measuring hair cortisol is regarded as a representative of long-term activity of the HPA axis [17, 19, 20]. Moreover, this test has been supported by several animal and human researches [9, 17, 19–28]. Free cortisol can be incorporated in hair matrix as it is a lipophilic substance [29] 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 [30]. Thus, 3 cm length of hair sample represents cortisol exposure of the three preceding months.

Collection of the scalp hair is an easy procedure and can be performed in outpatients [31]. The hair sample can easily be stored, with no need for special storage procedure. The sample, when stored in a foil in a dark and dry place at room temperature, remains stable for several months [17, 32–34]. Several studies measured the hair cortisol in normal subjects and found that its levels were different according to the population studied, method of hair preparation and measurement assay used. A hair cortisol of 9.9–204 pg/mg hair [25, 26, 35], with the mean ranging from 7.7 to 46 pg/mg in different studies was recorded [5, 26, 27, 34, 36, 37]; however, in most studies of healthy adult subjects, the mean was within 20 to 30 pg/mg of hair [27, 38].

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 [24, 25] and proximal hair cortisol correlated with the early biochemical data in this disease [28]. Hair cortisol is a marker of chronic stress [39] and risk of a cardiovascular event [40, 41]; it might be abnormal in psychiatric abnormalities such as depression and other [42]; it is related to obesity [16] and metabolic syndrome [43].

To the best of our knowledge, 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. No study measured hair cortisol level in the Sulaymaniyah city or in any other part of Iraq.

The present study measured the average hair cortisol levels among a sample of healthy adult subjects, SCH and hypothyroid subjects in the Sulaymaniyah city, Iraq to compare hair cortisol levels between these subjects, and to find the relation between hair cortisol as a long-term activity of HPA axis with HPT axis.

Methods

The study was held prospectively at the Shar hospital in the Sulaymaniyah city on 152 participants with 83 healthy volunteers who included healthy hospital staff, friends, relatives, and patient accompanies, 37 SCH and 32 hypothyroid subjects. After exclusion of subjects who had at least one of the exclusion criteria, 124 subjects remained: 69 euthyroid, 28 SCH and 27 hypothyroid subjects. The normal (euthyroid) and SCH subjects were separated according to their TSH levels. The participants were further divided according to BMI into two groups, the first group was normal weight subjects and the second group included overweight and obese subjects.

Hypothyroid cases include subject with diagnosed hypothyroidism that did not receive treatment for at least 3 months before the study or their thyroid function tests were still abnormal; the presence of a combination of high TSH and low FT4.

Exclusion criteria included those who had serum TSH < 0.5 mIU/L or TSH > 10 mIU/L and those with abnormal FT4 or FT3 for SCH subjects, those with HPA axis abnormality or previously diagnosed thyroid problem other than hypothyroidism for hypothyroid subjects, the presence of a thyroid nodule, those on any treatment for thyroid disease or treatment affecting HPA axis, inpatients and those with any chronic disease (e.g., diabetes) or those with acute illnesses, and those who had psychological problem.

A questionnaire with the following detail was filled for each participant. The questionnaire contained socio-demographic status (age, height, weight, waist circumference, and lifestyle, detail of their work, sleep duration per 24 h), history of systemic diseases, thyroid disease, adrenal insufficiency or hyper-function, drug history, surgical history and history of stress or psychological abnormality.

Laboratory measurement and reference ranges

The participants were asked to fast overnight on the day of investigation and to come in the morning between 9:00 and

11:00 a.m. for blood drawing. The blood samples were centrifuged, and the serum was separated; the sera were analyzed for FT3, FT4, TSH, and TPO-Ab. All biochemical tests were analyzed by the modern immunoassay method: 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 by running of quality control.

The FT3, FT4, and TSH had a reference range of 2.0–4.4 pg/mL, 0.93–1.70 ng/dL, and 0.27–4.2 mIU/L, respectively, in the lab they were analyzed in.

Term definition

Euthyroid was defined as a subject with TSH and FT4 within the reference range. 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 that had to be 2 weeks apart). Hypothyroid was defined as subjects who had a combination of elevated TSH and low FT4 (TSH > 4.2 and FT4 < 0.93 ng/ml). Thyroid peroxidase positivity was defined as having TPO-Ab above 34 IU/mL. Normal weight: any subject with BMI between 18 and 25 Kg/m^2. Overweight: any subject with BMI between 25 and 30 kg/m^2. Obese: any subject with BMI above 30 kg/m^2.

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 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. [26] 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 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 °C for 16 h. After incubation, the test tubes were centrifuged at 5000 rpm for 10 min. The supernatant was then separated into another glass test tube; methanol was evaporated at 60 °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 for 30 s. The vortexing was repeated twice, and then hair cortisol was analyzed by electrochemiluminescence assay Roche Diagnostic Cobas e-411, with Roche diagnostic cortisol kit, as described by Iglesias et al. [44]. The measured hair cortisol was recorded and the amount of cortisol within each mg of hair was calculated in pg/mg. In 24 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.

Statistical method

The data were entered and analyzed statistically using SPSS 22. All demographic and biochemical data were represented as mean ± SD or median (range) for non-parametric data as hair cortisol and serum TSH levels, and frequency (%) for categorical data. 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 Kruskal–Wallis *H* test and Mann–Whitney *U* test was used to compare the median hair cortisol and TSH levels between the groups. The mean of each data was compared between euthyroid, SCH and hypothyroid subjects using ANCOVA, body weight, BMI and waist circumference used as a covariate. Mean hair cortisol levels were compared between subjects with different BMI, using independent sample *t*-test and mean of hair cortisol level between proximal and distal segment were compared using Paired samples *T* test. Correlation between hair cortisol levels with other variables was evaluated using Pearson's correlation with *p*-value of ≤0.05 regarded as significant.

Results

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

Table 1 lists the biochemical and socio-demographic characteristics of the study participants.

The comparison between euthyroid, SCH and hypothyroid subjects in demographic and biochemical parameters are presented in Table 2. Hair cortisol was slightly higher in the SCH group, but the difference was not

Table 1 Characteristics of the study participants

Variables	Mean (SD) Number (%)
Total number	118
Age	34.24(9.88)
Sex	
Male	12(9.8%)
Female	110(90.2%)
Marital status	
Married	71(59.6)
Single	48(40.4)
Work	
Student	15(12.6)
Employee	39(32.9)
No work	46(38.6)
Free work	11(15.9)
Smoker	
Yes	7(5.7)
No	115(94.3)
Exercise	
Rarely	62(53)
Sometime	43(36.8)
Regular	12(10.3)
Weight	66.224(13.02)
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 h (hours)	6.74(1.89)
TSH (uIU/ml)[a]	3.14 [99.36]
FT4 (ng/dl)	1.102(.278)
FT3 (pg/ml)	3.187(.526)
TPO titer (IU/ml)	91.82(161.05)
Cortisol (pg/mg hair)[a]	20.94 [63.18]
Cortisol Log	1.279(.271)
Cortisol (range)[b]	19.01 (5.75-69.18)

[a]Median [range] of original data

[b]Mean and range of cortisol anti-log of log transformed data

statistically significant ($p = 0.783$), however it was significantly higher in hypothyroid subjects compared to euthyroid subjects, $p = .047$, Fig. 1

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 Fig. 2.

There was significant correlation between hair cortisol level of proximal and distal hair segment ($r = .517$, $p = .012$) And significant negative association between distal hair segment and mean sleep duration per 24 h were found ($r = -.478$, $p = .021$). Comparison of hair segments reports higher hair cortisol level in the proximal segments compared to the distal segments of the same study subjects ($p = .013$), as shown in Fig. 3.

Correlation of hair cortisol level with other variables was performed using Pearson's correlation as shown in Table 3. A significantly positive correlation was found between hair cortisol and each of the age, weight, BMI and serum TSH ($p < 0.05$). Figure 4 illustrates the relation of hair cortisol (cortisol log) with serum TSH.

Discussion

The HPT axis plays an important role in growth and metabolism via secretion of thyroid hormones. Alterations in thyroid hormones have been linked to various psychiatric diseases [45, 46]. The receptors for thyroid hormones are distributed widely within the central nervous system [47] that significantly influences physiology and behavior [48].

The HPA and HPT axes are two endocrine systems that respond to stress; thus studies to explore communication between the HPA and HPT axes have been performed. The activity of the HPA axis regularly evaluated by measuring cortisol the end product of HPA axis activation [48, 49]. Previous data have revealed that glucocorticoids can inhibit the HPT axis at the level of the hypothalamus and pituitary [7]. Also, the peripheral de-iodination of T4 to T3 can be inhibited by glucocorticoids. As a result, serum T3 decreased in response to stress [8]. In a study on a rat, repeated exposure of the rat to stress caused a significant decrease in serum levels of T3 and T4. The study revealed a significant correlation between plasma corticosterone levels and HPT axis, including FT4 level [48]. TSH can be suppressed by increased corticosteroids, both endogenously, such as by stress, or exogenously, such as in case of Cushing's syndrome [10, 11]. Conversely, products of the HPT axis can influence the HPA axis [48].

In overt hypothyroidism study, Iranmanesh et al. [12] 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 [50].

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 [13].

In a study on healthy young adult subjects Walter et al, investigated the correlation between serum TSH levels in the range of 2.5–10 uIU/L and serum cortisol. A positive correlation was found between serum TSH and serum

Table 2 Comparison between euthyroid, SCH and hypothyroid subjects in demographic and biochemical data

Variables	Euthyroid ($n = 65$)	p^a	SCH ($n = 26$)	p^b	Hypothyroid ($n = 27$)	p^c
Age (years)	33.12(10.38)	.329	36.85(13.44)	.948	37.97(10.91)	.134
Weight (Kg)	64.51(13.94)	.073	70.47(9.33)	.928	72.49(11.71)	**.027**
BMI (Kg/m^2)	26.36(5.74)	.198	28.25(4.36)	.888	29.32(4.53)	.062
Waist circumference (cm)	82.24(12.62)	**.048**	89.9(15.24)	.917	92.82(11.03)	**.009**
TSH (uIUml)[d]	1.95 [.82]	**<0.001**	6.41 [.65]	**<0.001**	16.4 [98.64]	**<0.001**
FT4 (ng/dl)	1.25(.169)	.219	1.17(.15)	**<0.001**	0.829(.34)	**<0.001**
FT3 pg/ml	3.29(.42)	.210	3.49(.46)	**<0.001**	2.56(.73)	**<0.001**
TPO titer (IU/ml)	23.59(73.86)	.124	74.0(137.22)	**<0.001**	228.27(192.26)	**<0.001**
Cortisol (pg/mg hair)						
Median[d]	16.74 [63.3]	.417	19.86 [42.84]	.115	26.79 [57.65]	**.010**
Cortisol Log	1.236(.299)	.783	1.277(.249)	.322	1.383(.193)	**.047**
Mean (antilog)	17.22		18.92		24.17	
Range	5.75-69.18		7.41-50.12		10-67.61	

All variance were compared between the groups using ANCOVA. p-value < .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

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

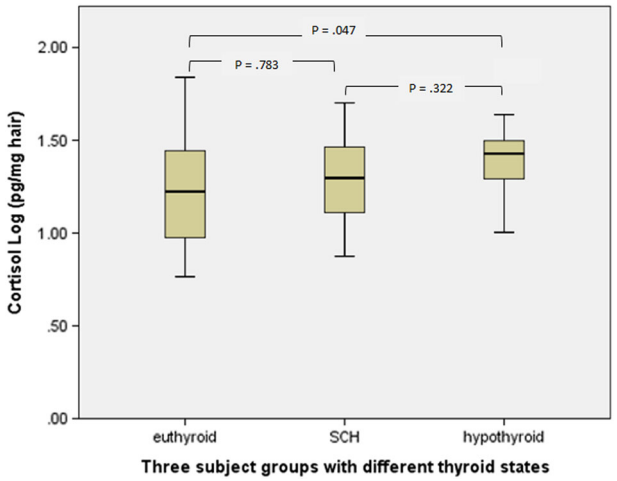

Fig. 1 Mean hair cortisol level (hair cortisol log) in the euthyroid, SCH and hypothyroid subjects

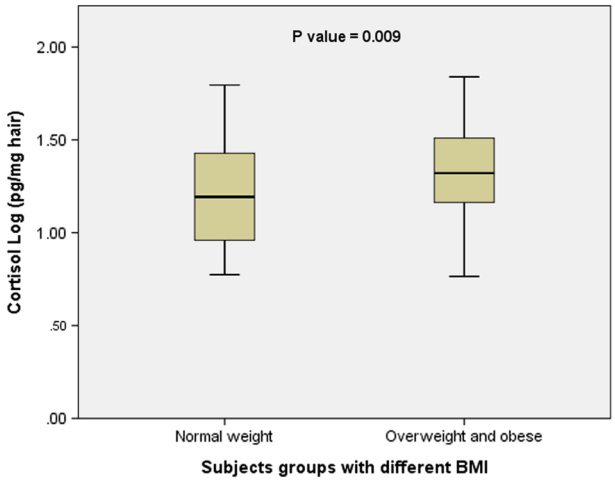

Fig. 2 Hair cortisol levels (Hair cortisol log), between the normal weight subjects and overweight/obese subjects

cortisol in both healthy subjects and SCH subjects [13]. It was known that overt hypothyroidism results in an increase in serum cortisol due to decreased clearance and decreased responsiveness of the HPA axis to cortisol negative feedback [12].

Over several years cortisol measured in plasma, urine and saliva to monitor the activity of HPA axis. The level of plasma and salivary cortisol are under the effect of circadian rhythm and environmental factor, and urinary cortisol evaluates HPA activity for 24 h, only. Thus, multiple sampling is needed to roughly provide cortisol level over time, even though; none of these routes can provide the true index of chronic HPA axis activity [49]. Because cortisol is steroid and steroid substances are lipophilic, they incorporated into the growing hair shaft mainly from blood supply to the follicular cells that the hair shaft generated from. The deposition of cortisol in the growing shaft of scalp hair in human is constant, thus a single scalp hair cortisol sample can be used as an index of long-term HPA axis activity over several weeks and months in diseased and healthy population. Because the hair grows in an average rate of about 1 cm/month [49], the most proximal 6 cm length of hair sample can give the cortisol secretion over the preceding 6 months.

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

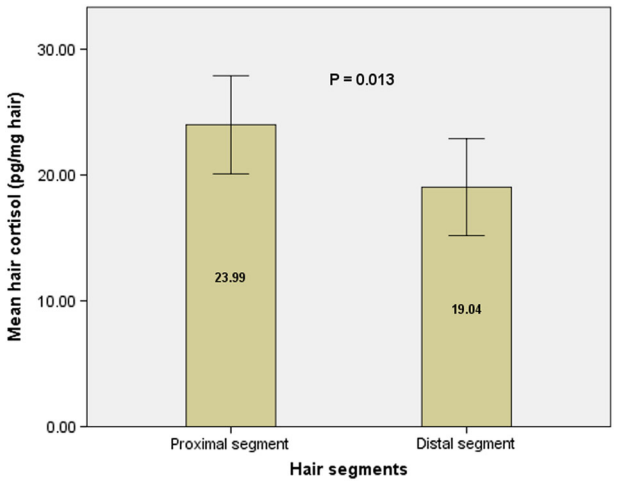

Fig. 3 Mean hair cortisol level in the proximal and distal hair segment. *p value evaluated using paired samples T test

level in SCH and overt hypothyroid 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. The results suggest that no significant difference exists in hair cortisol levels between SCH and euthyroid subjects, however, hair cortisol were significantly higher among hypothyroid compared to euthyroid subjects. A significant relationship was found between hair cortisol and TSH, age, weight and BMI.

In accordance with some studies [27, 28, 39], 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 decline in the hair cortisol level from proximal to distal hair segment reported by the present and some previous studies [27, 28, 39] may be due to the effect of hair washing on the hair cortisol content [39]. In a study by Dettenborn et al, 2012 [39] hair cortisol concentration were negatively associated with the frequency of hair washing in the most distal hair segments but not proximal segments. Although no significant differences between the proximal and distal hair segments were found in other studies [24, 35]. Although no significant differences between the proximal and distal hair segments were found in other studies [24, 35].

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 done elsewhere [25, 27, 38, 51]. 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 [27]. 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 9.9–75.9 pg/mg of hair cortisol in the healthy adult group with the mean of 27.3 (CI: 24.6–30.4) [25]. In a study by Raul et al. [51] a mean hair cortisol concentration of 18 pg/mg, ranging from 5 to 91 pg/mg was recorded [51]. In another study in Netherland, hair cortisol level was 28.18 pg/mg in healthy subjects, compared to those with bipolar disorder [38].

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 [26] and among Caucasian healthy adults a mean hair cortisol level of even 113 ± 54 were recorded [35]. However, in these two studies, the sample size was small. Different method of hair preparation and various immunoassay techniques may be the cause of variability found between different studies. In our study, ECLIA was used in contrast to most studies in whom ELISA kit was used [26, 35, 38], and in more recent studies CLIA [27, 36, 52, 53] and LC/MS [43] 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 [43].

In a comparison of hair cortisol levels between genders, a higher hair cortisol among men was detected than in women in some studies [27, 40]. On the other hand, no gender differences were observed in the present and some other studies [24, 35, 38, 51]. 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. [27] 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 [38, 40] performed on healthy adult groups, in which no significant correlation between hair cortisol levels and age was observed.

Consistent with other studies [16, 54] 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. [39] in 2013 and in line with previous studies [24, 37, 43, 52, 55], a significant positive relationship of hair cortisol with weight, and BMI was found among study participants.

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

Parameters		Age	Weight	BMI	Waist circumference	TSH(uIU/ml)[a]	FT4(ng/dl)	FT3	TPO titer
Hair cortisol	Pearson correlation	.215*	.203*	.206*	.106	.192*	−.129	−.182	.092
	Sig. (2-tailed)	.021	.031	.029	.322	.039	.171	.171	.357

Pearson correlation was used for correlation between hair cortisol log and all normally distributed variables

[a]Spearman's correlation is used for correlation between original hair cortisol and non-parametric variable, TSH

*p value < 0.05

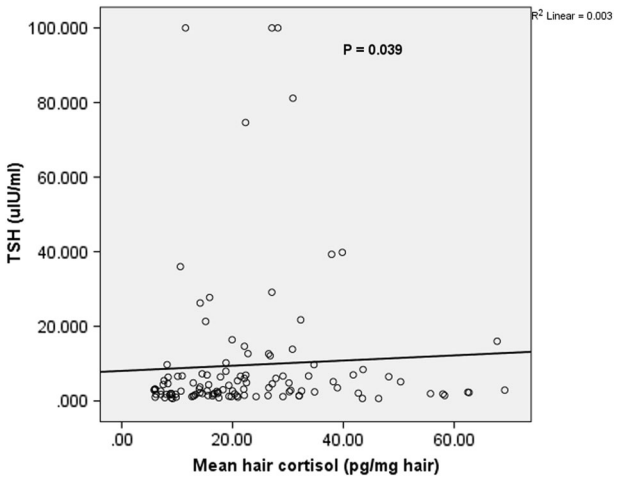

Fig. 4 The association between hair cortisol level and serum TSH of the main study sample

Conclusion

In conclusion, 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. We also conclude 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 significant relation between the two axes when both axes function normally and their levels are within the normal physiological range.

Limitations and recommendations

The present study had some limitations. First, the study sample that included SCH and overt hypothyroid subjects was small, larger sample sizes are recommended. Another limitation of this study 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. One of the limitations 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 [56]. Comparison between different immunoassay techniques (ELISA, CLIA, ECLIA, and LC/MS) to measure hair cortisol levels is recommended. Also, the mean and reference range should be defined according to each technique.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval The study was approved by the ethics committee of the College of Medicine-University of Sulaimani under meeting number 62, and all procedures in the study have been performed in accordance with the ethical standards of the 1964 Declaration of Helsinki.

Informed consent Written informed consent was obtained from all individual participants included in the study.

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