Comparison of Salivary Cortisol, Serum Cortisol, Plasma ACTH and Urinary Free Cortisol Levels in Thyrotoxic and Hypothyroid Patients

Tirotoksikozlu ve Hipotiroidili Hastalarda Tükürük Kortizol, Serum Kortizol, Plazma ACTH ve İdrar Serbest Kortizol Düzeylerinin Karşılaştırılması

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ABSTRACT Objective: Hypothalamo-pituitary-adrenal (HPA) axis is affected by thyroid hormones. The present study was designed to compare the level of salivary cortisol, serum cortisol, plasma adrenocorticotropic hormone (ACTH) and urinary free cortisol (UFC) levels in patients with subclinical and overt thyrotoxicosis and hypothyroidism. **Material and Methods:** We analyzed the salivary cortisol, serum cortisol, plasma ACTH and UFC levels in 123 patients with thyroid dysfunction. The patients were classified into four groups; overt thyrotoxicosis (n= 32), subclinical thyrotoxicosis (n= 29), overt hypothyroidism (n= 28) and subclinical hypothyroidism (n= 34). **Results:** There were no significant differences in terms of salivary cortisol, serum cortisol, plasma ACTH and UFC levels in patients with subclinical and overt thyrotoxicosis ($p > 0.05$). Similarly, no significant differences could be detected in terms of salivary cortisol, serum cortisol, plasma ACTH and UFC levels in patients with subclinical and overt hypothyroidism ($p > 0.05$). The comparison of patients with hypothyroidism and thyrotoxicosis also did not yield any significant difference in terms of salivary cortisol, serum cortisol, plasma ACTH and UFC levels ($p > 0.05$). **Conclusion:** Similar salivary cortisol, serum cortisol, plasma ACTH and UFC levels were detected in patients with hypothyroidism and thyrotoxicosis. Thus, we may suggest that thyroid hormone status does not play a role in the HPA axis. The major limitation of this study was the absence of a healthy control group. Further studies with large numbers of patients are required to clarify the association between thyroid hormone dysfunction and glucocorticoid levels.

Key Words: Thyrotoxicosis, hypothyroidism; cortisol; ACTH

ÖZET Amaç: Hipotalamo-hipofizer-adrenal (HPA) aks, tiroit hormonları tarafından etkilenir. Bu çalışma, klinik ve subklinik tirotoksik hastalarda ve hipotiroidi olgularında serum ve üriner serbest kortizol (UFC) ve plazma ACTH düzeylerinin yanı sıra, tükürük kortizol düzeylerini karşılaştırmak amacıyla tasarlandı. **Gereç ve Yöntemler:** Tiroit disfonksiyonu olan 123 hastada tükürük kortizol, serum kortizol, plazma ACTH ve idrar serbest kortizol düzeyleri ölçüldü. Bu hastalar dört grupta sınıflandı; klinik tirotoksikozlu grup 32 hastadan, subklinik tirotoksikozlu grup 29 hastadan, klinik hipotiroidi grubu 28 hastadan ve subklinik hipotiroidi grubu da 34 hastadan oluşmaktaydı. **Bulgular:** Klinik tirotoksikozlu ve subklinik tirotoksikozlu hastalarda tükürük kortizol, serum kortizol, plazma ACTH ve idrar serbest kortizol düzeyleri arasında anlamlı fark tespit edilmedi ($p > 0,05$). Klinik hipotiroidili ve subklinik hipotiroidili hastaların tükürük kortizol, serum kortizol, plazma ACTH ve idrar serbest kortizol düzeyleri de birbirinden anlamlı ölçüde farklı bulunmadı ($p > 0,05$). Benzer şekilde, tirotoksikozlu ve hipotiroidili hastalar arasında yapılan karşılaştırma, tükürük kortizol, serum kortizol, serum ACTH ve idrar serbest kortizol düzeyleri arasında anlamlı bir fark olmadığını ortaya koydu ($p > 0,05$). **Sonuç:** Klinik ve subklinik hipotiroidili, klinik ve subklinik tirotoksikozlu hastalarda benzer serum kortizol, plazma ACTH, idrar serbest kortizol ve tükürük kortizol düzeyleri saptandı. Çalışmamızdaki bu sonuçlar, değişik düzeylerdeki tiroit disfonksiyonunun hipotalamo-hipofizer adrenal aks üzerinde etkisi olmadığını göstermektedir. Sağlıklı kontrol grubunun olmaması çalışmamızın en önemli kısıtlayıcı faktörüydü. Tiroit disfonksiyonu ve glukokortikoit düzeyleri arasındaki ilişkiyi ortaya koymak için daha geniş çaplı yeni çalışmalara ihtiyaç vardır.

Anahtar Kelimeler: Tirotoksikoz; hipertiroidizm; kortizol; ACTH

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Although, previous studies have examined the effects of altered thyroid function on the secretion and metabolism of adrenocortical hormones, there are still conflicting results with regard to cortisol levels in thyrotoxicosis and hypothyroid patients.[1-11]

There are limited studies regarding the association between thyrotoxicosis and hypothalamo-pituitary-adrenal (HPA) axis. Circulating cortisol levels were higher,[1,2] lower[3,4] or normal[5,7] in patients with thyrotoxicosis compared to healthy controls.

Another thyroid dysfunction, hypothyroidism, may alter cortisol levels.[6-11] In one report, hypothyroidism has no effect on cortisol levels.[7] In others, hypothyroidism may either decrease[8,9] or increase serum cortisol levels.[10,11]

These results may also be related with increase in cortisol binding globulin and albumin serum levels that increase total serum cortisol levels.[12] Salivary cortisol appears to be a valuable and convenient alternative method for free serum cortisol determination.[13-16]

In the literature, we did not find any study evaluating salivary cortisol, serum cortisol, plasma adrenocorticotrophic hormone (ACTH) and urinary free cortisol (UFC) levels in hypothyroid and thyrotoxicosis patients. In this study, we aimed to investigate these parameters in patients with hypothyroidism and thyrotoxicosis.

MATERIAL AND METHODS

This study was conducted in the Selçuk University, Meram Medical Faculty, Division of Endocrinology and Metabolism, Konya, Turkey, between January 2008 and April 2008. One hundred and twenty three patients (32 overt thyrotoxicosis, 29 subclinical thyrotoxicosis, 28 overt hypothyroidism, and 34 subclinical hypothyroidism; 18 males and 105 females) were enrolled in the study. None had received any antithyroid or thyroid hormone replacement medication. Patients with renal failure, liver and heart failure, psychiatric disorders, pregnancy, diabetes, malignancy, alcohol abuse; using medications such as steroid, phenytoine, barbiturate or oral contraceptive pills, those with erythrocyte sedimentation rate over 30 mm/h and current smokers were excluded from the study. At least 30 minutes before the saliva sampling, patients were advised to avoid eating, drinking, and gum chewing, and brushing teeth.

The diagnosis of thyrotoxicosis and hypothyroidism were based on serum thyroid stimulating hormone (TSH), free triiodothyronine (T3) and free tetraiodothyronine (T4) levels. Overt thyrotoxicosis was defined as low serum TSH level with high free T4 and/or free T3 levels, and subclinical thyrotoxicosis with high TSH level and normal free T4 and free T3 levels. Overt hypothyroidism was defined as high TSH level with low free T4 and/or free T3 levels and subclinical hypothyroidism with high TSH level and normal free T4 and free T3 levels.

Twenty-four-hour urinary samples were collected to measure UFC. After an overnight fast, blood samples were drawn in the morning for plasma ACTH and serum cortisol analyses. Saliva secretion was stimulated with parafilm and samples were collected using special saliva sampling device (Salivette; Sarstedt cat.51.1534). Saliva samples were collected at least 30 minutes after wake-up and between 7:00 and 8:00 am. Then, the saliva samples were centrifuged at 2500g to remove particles and were stored at -80°C until analysis.

Serum TSH (reference range 0.4-4 μIU/mL), free T4 (reference range 0.8-1.9 ng/dL), cortisol (reference range a.m. 5-25 μg/dL) and plasma ACTH [reference range 10-46 pg/mL (morning)] levels were measured by chemiluminescent method (Immulite® 2000, Diagnostic Products Corp., Los Angeles, CA). Twenty-four-hour UFC levels (reference range 42-218 μg/day) were measured by a solid-phase competitive chemiluminescent enzyme immunoassay method (Siemens Immulate 2000, Germany). The concentration of cortisol in saliva (reference range 0.12-1.47 μg/dL) was determined by using an enzyme immune assay kit (DRG Diagnostics, Germany). The intra- and inter-assay coefficients of variation for salivary cortisol were 1.47-4.52% and 5.82-7.47%, respectively.

The study protocol was approved by the local research ethics committee, in accordance with the Helsinki Declaration and written informed consent was obtained from all participants.

STATISTICAL ANALYSIS

Clinical and laboratory data were expressed as "mean ± standard deviation (SD)". Distributions of the variables were assessed using Shapiro-Wilk test and Kolmogorov-Simirnov test. Comparisons of continuous variables between groups were performed using ANOVA and Kruskal-Wallis tests when appropriate. All data were normally distributed and continuous variables between groups were compared with the Student's t test. Nominal variables were compared via chi-square test with standardized residuals. All statistical analyses were performed through a PC compatible statistics program (SPSS® v.17, Chicago, IL, USA) and p values less than 0.05 were considered statistically significant.

RESULTS

Mean age, body mass index (BMI), and gender distribution of all groups were presented in Table 1. There was no statistically significant difference between groups for age and BMI.

The levels of plasma ACTH, serum cortisol, 24-h UFC and salivary cortisol for all groups were summarized in the Table 2. Plasma ACTH, serum cortisol, 24-h UFC and salivary cortisol levels were not statistically different between overt thyrotoxicosis (n= 32), subclinical thyrotoxicosis (n= 29), overt hypothyroidism (n= 28) and subclinical hypothyroidism (n= 34) groups ($p > 0.05$). Salivary cortisol, serum cortisol, plasma ACTH and 24-h UFC levels did not show significant difference between hypothyroidism and thyrotoxicosis (Table 3, $p > 0.05$).

DISCUSSION

To the best of our knowledge, this is the first study evaluating the salivary cortisol, serum cortisol, plasma ACTH and 24-h UFC levels in patients with overt thyrotoxicosis, subclinical thyrotoxicosis, overt hypothyroidism and subclinical hypothyroidism. The present study has shown that these parameters are similar in a wide spectrum of thyroid dysfunctions ranging from clinical hypothyroidism to clinical thyrotoxicosis.

Thyrotoxicosis is linked with hyperactivity of the HPA axis and associated increases in basal and stimulated ACTH secretions, probably due to a di-

TABLE 1: Demographic and clinical characteristics of the patients.

	Overt Thyrotoxicosis (n= 32)	Subclinical Thyrotoxicosis (n= 29)	Overt Hypothyroidism (n= 28)	Subclinical Hypothyroidism (n= 34)	p-values
Gender (M/F)	9/23	5/24	2/26	2/32	0.04
Age (years)	38.60 ± 14.10	40.62 ± 11.72	37.29 ± 12.41	36.50 ± 11.84	0.51
BMI (kg/m^2)	26.06 ± 4.59	27.76 ± 5.67	29.29 ± 5.58	26.34 ± 6.37	0.105

BMI, body mass index; F, female; M, male; SD, standard deviation. Results are given as "mean ±SD".

TABLE 2: Plasma ACTH, serum cortisol, urinary free cortisol and salivary cortisol levels in overt thyrotoxicosis, subclinical thyrotoxicosis, overt hypothyroidism and subclinical hypothyroidism.

	Overt Thyrotoxicosis (n= 32)	Subclinical Thyrotoxicosis (n= 29)	Overt Hypothyroidism (n= 28)	Subclinical Hypothyroidism (n= 34)	p-values
ACTH (pg/mL)(RR: 10-46 pg/mL)	21.87 ± 12.56	19.97 ± 13.06	17.13 ± 7.64	21.27 ± 9.80	0.1
Serum cortisol (µg/dL)(RR: 5-25 µg/dL)	11.96 ± 4.23	11.98 ± 5.85	12.27 ± 5.92	13.51 ± 4.13	0.2
Urinary cortisol (µg/day)RR: 42-218 µg/day	138.48 ± 77.06	127.16 ± 57.27	147.69 ± 125.63	122.02 ± 54.66	0.9
Salivary cortisol (µg/dL)RR: 0.12-1.47 µg/dL	0.66 ± 0.22	0.69 ± 0.21	0.66 ± 0.23	0.76 ± 0.29	0.4

ACTH, adrenocorticotropic hormone; RR, reference range.

TABLE 3: Plasma ACTH, serum cortisol, urinary free cortisol and salivary cortisol levels in thyrotoxicosis and hypothyroidism.

	Thyrotoxicosis (n= 61)	Hypothyroidism (n= 62)	p-values
ACTH (pg/mL) (RR: 10-46 pg/mL)	20.96 ± 12.73	19.4 ± 9.0	0.2
Serum cortisol (µg/dL) (RR: 5-25 µg/dL)	11.96 ± 5.0	12.95 ± 5.0	0.3
Urinary cortisol (µg/day) RR: 42-218 µg/day	133.09 ± 68.3	133.6 ± 93.6	0.9
Salivary cortisol (µg/dL)RR: 0.12-1.47 µg/dL	0.67 ± 0.22	0.71 ± 0.27	0.4

ACTH, adrenocorticotropic hormone; RR, reference range.

rect effect of thyroid hormones on corticotropin-releasing hormone in the hypothalamus and ACTH secretion in the pituitary gland.[1,2]

On the other hand, it is also well recognized that thyroid hormones accelerate glucocorticoid turnover, but its rate of production is also increased, so that circulating levels of cortisol remain low[3,4] or normal[5,7] and may increase glucocorticoid requirements in patients on hydrocortisone replacement.[4] Both production and degradation of cortisol is increased and thyroid hormones stimulate conversion of cortisol to cortisone; thus, serum cortisol levels remain normal.[7,17] This may explain the similar levels of salivary cortisol, serum cortisol, plasma ACTH and 24-h UFC both in hypothyroidism and thyrotoxicosis.

There is substantial evidence that hypothyroidism may alter cortisol levels due to an imbalance between cortisol synthesis and cortisol degradation.[6-11] Therefore, there are contrary reports with respect to cortisol levels in hypothyroid patients. Hypothyroidism may decrease cortisol levels.[8,9] On the other hand, in the hypothyroid state, cortisol degradation is decreased; thus, 24-hour mean serum cortisol levels are higher than in healthy controls.[10] Thyroid hormones have effects on 11β-hydroxysteroid dehydrogenase type 1 activity and cortisol clearance is decreased in hypothyroidism.[11] There is disagreement on the adrenal functional reserve in clinical hypothyroidism; sensitive tests for assessing adrenal functional reserve are required. Therefore, measurement of salivary cortisol levels is recommended as a safe, practical alternative method for the evaluation of HPA axis.[13-16] In our study, plasma ACTH, salivary cortisol and serum cortisol levels were similar in overt and subclinical hypothyroidism. In this study, no difference was found with respect to 24-h UFC levels in all stages of thyroid dysfunction. This finding was in accordance with the results of the study by Hoshiro et al.,[7] that UFC levels were similar in both hypo- and hyperthyroid patients.

CONCLUSION

Similar salivary cortisol, serum cortisol, plasma ACTH and 24-h UFC levels were found in patients with overt and subclinical thyrotoxicosis and hypothyroidism. Our results indicate that thyroid dysfunction status seems to have no effect on HPA axis. Further studies with large number of patients are needed to clarify the association between thyroid hormone dysfunction and glucocorticoid levels.

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