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Correlation analysis of cortisol concentration in hair versus concentrations in serum, saliva, and urine

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Abstract

Introduction: Cortisol concentration is measured in blood, urine, and saliva samples. It has been recently proven that cortisol could also be detected in hair samples. Cortisol measurements in different samples have their own individual characteristics and clinical utility. We aimed to investigate the correlation between hair cortisol concentration and standard cortisol measurements used in clinical practice.

Material and methods: Fifty adult volunteers with a negative history of endocrine disorders were enrolled in the study. Morning serum cortisol (MSC), evening serum cortisol (ESC), evening free salivary cortisol (EFSC), urine free cortisol (UFC), and hair cortisol concentration (HCC) were analysed in all participants. Eventually, 41 volunteers were included into the study, whose cortisol concentration in the 1 mg overnight dexamethasone suppression test (1 mg ONDST) were < 50 nmol/L, and cortisol levels in serum, saliva, and urine were within reference ranges. Hair cortisol concentration test was performed for 20 mg of hair strands of the proximal 1 cm hair segments.

Results: Hair cortisol concentration ranged from 0.3036 to 2.65 nmol/mg, and the average value was 0.8125 ± 0.4834 nmol/mg. No significant correlations were found between HCC and MSC (rho = 0.04419, p = 0.7838), HCC and ESC (rho = –0.2071, p = 0.1938), HCC and EFSC (rho = 0.1005, p = 0.532), or HCC and UFC (rho = 0.1793, p = 0.262).

Conclusions: This work is another step in the discussion on the application of HCC determinations in clinical practice. Our results have showed no correlations between HCC and single point cortisol assessment in blood, saliva, and urine in patients with reference cortisol levels. (**Endokrynol Pol 2020; 71 (6): 539–544)**

Key words: *hair cortisol; serum cortisol; salivary cortisol; urine cortisol; cortisol assessment; cortisol correlation*

Introduction

Evaluation of disorders of the hypothalamic-pituitary-adrenal axis (HPA) is often challenging in endocrine diagnostics. The most common pathologies of HPA include Cushing's syndrome/disease (overt, subclinical, cyclic, and iatrogenic), primary/secondary adrenal insufficiency, and assessment of possible hormonal adjustment in patients receiving glucocorticosteroids (GCS) substitution doses [1, 2]. In addition, pseudo-Cushing's syndrome in the course of e.g. depression, alcoholism, type 2 diabetes, abdominal obesity, or polycystic ovary syndrome is also an important diagnostic challenge [3, 4]. Cortisol concentration is routinely measured in blood, urine, and saliva samples. It has been recently shown that cortisol could be also detected in hair samples. Cortisol measurements in different samples have their own individual characteristics and clinical utility.

The determination of serum cortisol mostly refers to its total fraction (serum total cortisol, STC), including cortisol bound to plasma proteins like transcortin (cortisol binding globulin, CBG), but also to sex hormone binding globulin (SHBG) and albumin [5, 6]. Hence, non-primary HPA-related factors may influence the laboratory measurements. Oral contraception, pregnancy, or hyperthyroidism increase STC level (false positive results), while significant hypoalbuminaemia < 25 g/dL lowers STC concentration (false negative results) [5–8]. Serum free cortisol (SFC) measurement is not routinely performed. It is assumed that SFC constitutes about 3–5% of the total cortisol fraction [7]. Serum cortisol levels are tested in the morning, evening, and night hours, in order to assess its circadian rhythm of secretion. The absence of circadian cortisol rhythm is one of the first manifestations of endogenous hypercortisolaemia. Therefore, circadian cortisol measurement is one of the first-line tests in the assessment of HPA disorders. However, determination of cortisol concentrations at night might be difficult for outpatient departments due to the working hours of laboratories. Serum cortisol is also assessed in dexamethasone suppression test, Synacthen stimulation test, corticotrophin releases hormone (CRH), or vasopressin stimulation tests used in the diagnosis and differentiation of cortisol level disorders [8, 9].

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The determination of cortisol in saliva reflects its free fraction (free salivary cortisol, FSC) because its presence in saliva is the result of passive diffusion of SFC from the blood into saliva. The same amount of cortisol is converted to cortisone in the salivary glands by 11b-hydroxysteroid dehydrogenase type 2 (11bHSD2). Therefore, FSC concentrations are lower than serum cortisol. It is assumed that FSC constitutes about 50–70% of SFC [10]. A saliva sample collection is a stress free non-invasive procedure, and it does not require the involvement of medical personnel; hence, it is cost effective and minimises the impact of stress-induced hypercortisolaemia related to hospitalisation and invasive derivation of blood samples. Furthermore, cortisol in saliva is stable at room temperature for up to seven days, which allows a sample to be taken at any time of the day of night. This in turn allows easy analysis of the circadian rhythm of cortisol. All this makes the assessment of cortisol in saliva a very good screening test for cortisol level deviation [11, 12].

In the past, urine free cortisol (UFC) was recognised as the main test (gold standard) in cortisol secretion disorder assessment. Currently it is used as a complementary assay. Similar to salivary cortisol, UFC concentrations are also significantly reduced due to the renal activity of 11bHSD2 [13]. Difficulties in assessing UFC include frequent interference of determinations with drugs or GCS metabolites, such as prednisolone, methylprednisolone, spironolactone, dexamethasone, cortisone, 17-hydroxyprogesterone, or carbamazepine. In addition, patients' self-urine collection is not always properly performed, which in turn might affect UFC results. In patients with renal failure (glomerular filtration rate [GFR] < 30 mL/min), it is possible to obtain false negative results. Due to these disadvantages, instead of UFC assessment it has been suggested to measure the cortisol:creatinine ratio in a single urine sample [14].

Hair cortisol concentration (HCC) assessment is not routinely performed due to a number of pre-laboratory impairing factors. The first determinations of HCC in humans were described in 2004 [15]. Considering the average hair growth of 1 cm per month, a hair sample, depending on its length, might reflect cortisol levels over the last days, weeks, or months [16]. An additional benefit of the HCC assessment is the non-invasive sample collection, and the ability to store it at room temperature for an extended period of time without any preparation [17]. The presence of cortisol in hair, like other endogenous substances and xenobiotics, is the result of the passive diffusion of free cortisol from the blood to the hair matrix during its formation in the bulb [18]. In addition, a certain amount of cortisol may come from sweat and sebum. Therefore, HCC may depend on additional factors, such as gender (stimulation of sweat and sebum production by androgens), age (slower hair growth with age), dyeing hair (possible leaching of cortisol by chemical agents, interference in the assay), physical activity, hygiene habits (frequency of hair washing), or exposure to sunlight [19–25]. One study demonstrated the possibility of cortisol production by hair bulb cells [26]. HCC determinations are performed by liquid chromatography tandem-mass spectrometry (LC-MS/MA) or immunoenzymatic assays [27, 28]. Assessment of HCC in healthy volunteers versus standard blood cortisol measurement methods was presented in our previous work [29]. Here we present analysis of HCC correlation with cortisol concentrations in blood, saliva, and urine in individuals with no HPA disorders.

Material and methods

All participants gave written informed consent. The study was approved by the Bioethics Committee at the Medical University of Gdansk.

Fifty adult volunteers with a negative history of endocrine disorders were enrolled into the study. Subjects treated with GCS over the past year prior to the enrolment were excluded from the study. Whole blood (3 mL) was collected at 8.00 a.m. and 8:00 p.m. on the same day to assess serum total cortisol concentration in the morning (morning serum cortisol, MSC) and evening (evening serum cortisol, ESC), respectively. In order to assess the daily excretion of UFC, 24 h urine collection under medical supervision was done, and 5 ml of urine sample was immediately sent for analysis. Saliva collection was carried out using a dedicated kit at 8 p.m. (evening free salivary cortisol, EFSC). The obtained material was frozen at –32°C and stored for further analysis. After collecting samples for MSC, ESC, UFC, and EFSC, 1 mg overnight dexamethasone suppression test (1mg ONDST) was performed. Eventually, 41 volunteers were included in the study, whose cortisol concentration in the 1 mg ONDST were < 50 nmol/L, and cortisol levels in serum, saliva, and urine were within reference ranges (Tab. 1).

The hair sampling technique consisted of cutting off approximately 100 hair strands from the posterior vertex area of the head with a sterile scalpel close to the scalp. Next, 1 cm hair fragments were cut away close to their follicles, and after weighing 20 mg samples they were sealed in paper envelopes until further analysis. In HCC assessment, the hair samples were placed into a 10 ml plastic tube and flushed with methanol. Next, they were transferred into 5 ml tubes and incubated at 50°C in methanol for 24 hours. The extract was transferred into a 3 mL tube for methanol evaporation. Subsequently, the residue was dissolved in 250 μL of phosphate buffered saline (PBS) by incubation for 1 hour and sent for cortisol

Table 1. *Reference ranges of the laboratory assay*

Assay	Reference ranges
1 mg ONDST	< 50 nmol/L
Morning serum cortisol	138–690 nmol/L
Evening serum cortisol	55–331 nmol/L
Evening free salivary cortisol	1.10–11.32 nmol/L
Urine free cortisol	138–524 nmol/24 hr
Hair cortisol concentration	Not validated

1 mg ONDST — 1 mg overnight dexamethasone suppression test

Table 2. *Characteristics of the population. Data presented as mean ± standard deviation (SD) and median ± interquartile range (IQR)*

Variable	Total (n = 41)	Female (n = 29)	Male (n = 12)	p value
Age (years)	62.97 ± 10.92	63.65 ± 9.78	61.33 ± 13.64	0.56
1 mg ONDST [nmol/L]	34.49 ± 6.32 (28 ± 8)	33.41 ± 6.94 (30 ± 9)	30.25 ± 3.88 (28 ± 3)	0.07
Morning serum cortisol [nmol/L])	329.5 ± 108.7	318.1 ± 109.6	357.1 ± 106	0.3
Evening serum cortisol [nmol/L]	110.5 ± 48.68	114.9 ± 48.78	99.67 ± 48.78	0.37
Urine free cortisol [nmol/24 hr]	207.7 ± 130.1	213.1 ± 130.4	194.7 ± 134.1	0.69
Evening free salivary cortisol [nmol/L]	2.09 ± 1.18 (1.78 ± 0.99)	2.07 ± 1.21 (1.83 ± 0.91)	2.16 ± 1.18 (1.71 ± 1.55)	0.99
Hair cortisol concentration [nmol/mg]	0.81 ± 0.48 (0.65 ± 0.3)	0.70 ± 0.28 (0.65 ± 0.26)	1.08 ± 0.73 (0.78 ± 0.75)	0.19

1 mg ONDST — 1 mg overnight dexamethasone suppression test

measurement. Finally, HCC obtained in the samples was calculated for 1 mg hair specimens (nmol/mg).

All cortisol assessments were performed in the Central Clinical Laboratory of the Medical University of Gdansk, Poland. Commercial ELISA kit (IBL International GmbH, Hamburg, Germany, catalogue number RE 52611) was used for the MSC, ESC, EFSC, and HCC measurements. According to the manufacturer's data, cross-reactivity was determined to be 30% for prednisolone, 7% for 11-desoxycortisol, 4.2% for cortisone, 2.5% for prednisone, 1.4% for corticosterone, and < 1% for other test substances. The detection threshold was set at 0.138 nmol/L, with functional sensitivity set at 0.828 nmol/L. The intra and interassay coefficient of variation was determined at 7.3% for concentrations 7.452 nmol/L and 8.8% for concentrations 14.904 nmol/L (saliva), and 9.9% for concentrations 49.68 nmol/L and 20% for concentrations 41.4 nmol/L (serum). Urine free cortisol determinations were made with the set from the same manufacturer (IBL International GmbH, Hamburg, Germany, catalogue number RE52241). In UFC measurements, cross-reactivity was determined at 18.7% for 11-alpha-desoxycortisol, 10.8% for cortisone, 2.4% for corticosterone, and < 0.1% for the other substances. The declared intra assay variation was 7%, and the inter assay variation was < 9%. Cortisol measurements in saliva and hair were done twice, and arithmetic average values were used in statistical analysis.

Results

The assessment of variable distribution was performed by using the quantile-quantile plots. The t-test and the Mann-Whitney U test were used for comparisons between subgroups. Correlations were analysed by the Spearman test. The general linear model was fitted to perform multivariate analysis. Some variables were transformed before inclusion into the linear model. Values of $p < 0.05$ were considered to be statistically significant. R Studio software (RStudio Inc., Boston, USA, ver. 1.2.1335) was employed for the statistical analysis.

The values had a normal distribution for: age ($p = 0.5999$), MSC ($p = 0.3008$), ESC ($p = 0.3725$), and UFC ($p = 0.6905$). A non-normal distribution was detected for: 1mgONDST ($p = 0.2741$), EFSC ($p = 0.9886$), and HCC ($p = 0.1873$). HCC ranged from 0.3036 to 2.65 nmol/mg, and the average value was 0.8125 ± 0.4834 nmol/mg. The characteristics of the studied group are presented in Table 2.

No significant correlations were found between HCC and EFSC (rho = 0.1005, $p = 0.532$), HCC and MSC (rho = 0.04419, $p = 0.7838$), HCC and ESC (rho = –0.2071, $p = 0.1938$), and HCC and UFC (rho = 0.1793, $p = 0.262$) (Fig. 1).

The general linear model was fitted to perform multivariate analysis. The dependent variable was the concentration of cortisol in the hair. Independent variables for the model were selected by the step method based on the AIC (Akaike Information Criterion) values. However, a statistically significant linear regression model could not be fitted.

Discussion

Several publications have indicated a strong relationship between serum cortisol and salivary cortisol [6, 30–34]. It is a result of the simultaneous cortisol secretion into the blood by the adrenal glands and subsequent cortisol diffusion from blood into saliva. Hence, salivary cortisol correlates with cortisol concentrations in blood at a given time point. On the other hand, correlations of HCC with cortisol concentrations in blood, urine, or saliva have been assessed as moderate or weak [35]. Our results have shown no correlation between HCC and cortisol concentrations in saliva, urine, and blood. The passive diffusion of free cortisol from the blood into the hair bulb is the main mechanism of the presence of cortisol in hair. Thus, HCC reflects the average concentration of serum cortisol over a given period of time depending on the length of hair used for analysis. Indeed, statistically significant correlations between HCC and multi-point measurements of urine and salivary cortisol have been reported [35–38]. Our

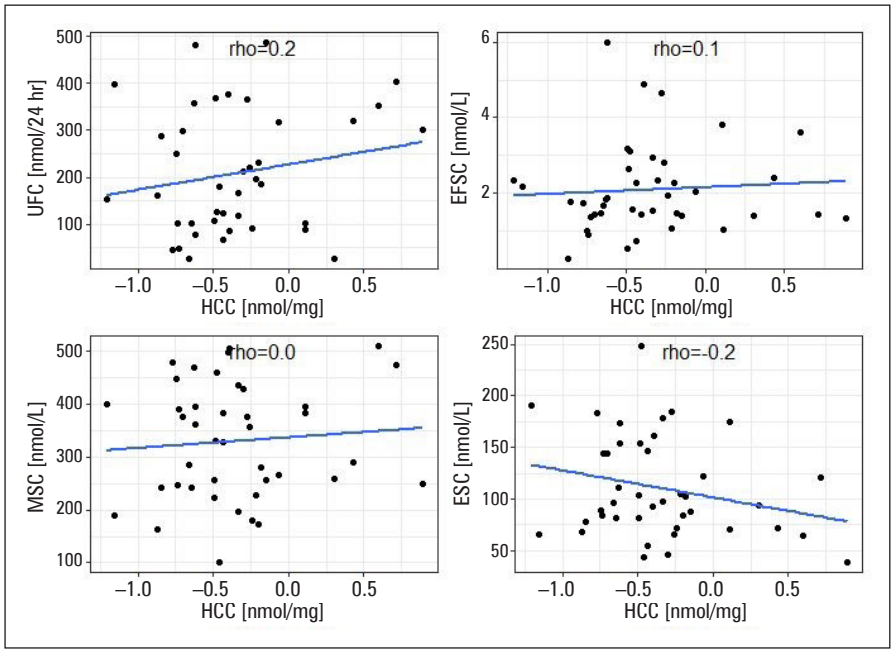

Figure 1. *Correlation analysis between hair cortisol concentration (HCC) vs. urinary free cortisol (UFC), evening free salivary cortisol (EFSC), morning serum cortisol (MSC), and evening serum cortisol (ESC). rho — Spearman's rank correlation coefficient*

results are in turn consistent with a number of studies assessing the relationship between HCC and single point cortisol measurement in saliva and serum where such correlations were weak or absent [36, 38, 39]. In our opinion, despite progress in laboratory diagnostics, including cortisol assays, it is still challenging to transfer complex regulation of cortisol production in vivo to relatively simple statistical models based on laboratory tests. Cortisol is secreted in a pulsating manner that is subject to circadian rhythms, and negative and positive feedback may depend on exogenous factors and numerous genetic variants determining the sensitivity of the cortisol receptor, which in turn, might alter its level and impact on clinical manifestations [6]. This may lead to misinterpretation of laboratory results and incorrect diagnosis [8, 9]. Another problem raised by many other authors is the lack of sufficient validation of studies assessing the correlation of HCC with other biological samples [20, 36, 40]. Additionally, the possibility of pre-analytical errors, especially for more complex determinations such as HCC, should be taken into account.

For instance, the impact of substances used in hair care has not been fully evaluated. Interestingly, we did not see any difference in HCC between males and females despite the fact that the latter population probably use substances such as hair bleach more frequently.

Unlike other studies, our enrolment protocol was based on a preliminary assessment of cortisol levels in routine laboratory determinations of saliva, blood, and urine. Therefore, a number of external factors affecting the HPA axis were eliminated, such as social status, anthropometric parameters, or emotional state [41–44]. The impact of these factors is prominent [22]. Assessed cortisol levels reflected various time intervals: minutes and hours (blood, saliva), hours and days (urine), and days and months (hair). Such a wide time slot of determination of cortisol concentrations may better reflect the variability of its concentrations in analysed individuals. Additionally, we assessed the patients' clinical status during the year preceding enrolment in the study. The studied group was not large; however, it was homogeneous in terms of hormonal status.

The study has one major limitation, which is that a relatively small population was studied. This was due to the protocol of the study, which included strict exclusion criteria. Any potential factors that might have impacted the HPA axis excluded individuals from the study. Hence, we tested only individuals with no HPA axis impairment. The enrolment was performed in a hospital where many patients are given to steroid treatment or suffer from diseases impacting glucocorticosteroids levels. However, the studied population was small, it was very carefully selected, and represented individuals with no disorders of the HPA axis, which is a major advantage of our study. We believe that if the correlation across saliva, blood, and hair cortisol was strong, it would be displayed even in a relatively small population. Indeed, our data are consistent with other studies showing no such correlations.

Conclusion

In summary, this work represents another stage in the discussion on the application of HCC determinations in clinical practice. The studied group was not large, but it was homogeneous in terms of hormonal status (HPA axis disorders were excluded). Our results showed no correlation between HCC and single point cortisol assessment in blood, saliva, and urine in patients with reference cortisol levels. Further analyses of HCC in healthy individuals vs. hyper/hypocortisolaemia patients are needed in order to assess efficient HCC cut-off points for HPA axis disorders.

Competing interests

The authors declare that they have no conflict of interest.

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Authors' contributions

All authors made substantial contributions to the concept and designed of the study, L.C. — collection/assembly of data and writing the article, J.J. — critical revision of the article; PW — data analysis and interpretation; PK — collection of data, S.K. — research concept and design. All authors reviewed the manuscript and approved its final version.

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