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The diagnostic value of late-night salivary cortisol for diagnosis of subclinical Cushing's syndrome

Wartość diagnostyczna późnonocnego stężenia kortyzolu w ślinie w rozpoznawaniu subklinicznej postaci zespołu Cushinga

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Abstract

Introduction: Late-night salivary cortisol is a frequently used and easily implemented diagnostically valuable test for the diagnosis of overt Cushing's syndrome. The use of late-night salivary cortisol in the diagnosis of subclinical Cushing's syndrome is somewhat controversial. In this study, we aimed to determine the diagnostic value of late-night salivary cortisol in diagnosing subclinical Cushing's syndrome and compare it with 24-hour urinary free cortisol levels (UFC).

Material and methods: The study consisted of 33 cases of subclinical Cushing's syndrome, 59 cases of non-functioning adrenal adenoma, and 41 control subjects. Late-night salivary cortisol and UFC were measured in all the cases. The diagnosis of subclinical Cushing's syndrome was based on combined results of 1 mg dexamethasone suppression test > 1.8 μg/dL and ACTH < 10 pg/mL.

Results: Mean late-night salivary cortisol levels in subjects with subclinical Cushing's syndrome were significantly higher than in subjects with non-functioning adrenal adenoma and the control group ($p < 0.001$). Using a cut-off value of 0.18 μg/dL, the sensitivity and specificity of late-night salivary cortisol for diagnosing subclinical Cushing's syndrome were determined as 82% and 60%, respectively. Using a cut-off value of 137 μg/day, the sensitivity and specificity of UFC was determined as 18% and 90%, respectively.

Conclusions: Because the sensitivity of late-night salivary cortisol for the diagnosis of subclinical Cushing's syndrome is limited, using it as the sole screening test for subclinical Cushing's syndrome may lead to false negative results. However, using it as an adjunct test to other tests may be beneficial in the diagnosis of subclinical Cushing's syndrome. **(Endokrynol Pol 2016; 67 (5): 487–492)**

Key words: *subclinical Cushing's syndrome; salivary cortisol; urinary free cortisol; screening*

Streszczenie

Wstęp: Oznaczenie późnonocnego stężenia kortyzolu w silnie jest często używanym, łatwym do przeprowadzenia oraz przydatnym diagnostycznie testem stosowanym w rozpoznawaniu jawnej postaci zespołu Cushinga. Zastosowanie oznaczenia późnonocnego kortyzolu w ślinie w diagnozowaniu subklinicznej postaci zespołu Cushinga jest jednak kontrowersyjne. Celem prezentowanej pracy było ustalenie wartości diagnostycznej oznaczania późnonocnego stężenia kortyzolu w ślinie w rozpoznawaniu subklinicznej postaci zespołu Cushinga oraz porównanie z oznaczaniem stężenia wolnego kortyzolu w dobowej zbiórce moczu (UFC).

Materiał i metody: Badaniem objęto 33 pacjentów z subkliniczna postacią zespołu Cushinga, 59 pacjentów z nieczynnymi gruczolakami nadnerczy i 41 zdrowych ochotników. U wszystkich włączonych osób oznaczono poziom późnonocnego kortyzolu w ślinie oraz UFC. Rozpoznanie subklinicznej postaci zespołu Cushinga oparto o współwystąpienie wyniku testu supresji 1 mg deksametazonu > 1.8 μg/dL i ACTH < 10 pg/dL.

Wyniki: Średnie poziomy późnonocnego kortyzolu w ślinie u pacjentów z subkliniczną postacią zespołu Cushinga były istotnie wyższe niż u pacjentów z nieczynnymi gruczolakami nadnerczy i w grupie kontrolnej ($p < 0.001$). Przy zastosowaniu progu odcięcia dla wartości 0.18 μg/dL czułość i swoistość późnonocnego stężenia kortyzolu w ślinie do diagnozowania subklinicznej postaci zespołu Cushinga ustalono odpowiednio na 82% i 60%. Przy zastosowaniu progu odcięcia dla wartości 137 μg/dzień czułość i swoistość UFC ustalono odpowiednio na 18% i 90%.

Wnioski: Ponieważ czułość oznaczania późnonocnego poziomu kortyzolu w ślinie w rozpoznawaniu subklinicznej postaci zespołu Cushinga jest ograniczona, zastosowanie tego testu jako jedynego badania przesiewowego w kierunku sublinicznej postaci zespołu Cushinga może prowadzić do uzyskania fałszywie ujemnych wyników. Niemniej, wykorzystanie tego testu jako metody uzupełniającej dla innych testów może okazać się przydatne w rozpoznawaniu subklincznej postaci zespołu Cushinga. **(Endokrynol Pol 2016; 67 (5): 487–492)**

Słowa kluczowe: *subkliniczna postać zespołu Cushinga; stężenie kortyzolu w ślinie; stężenie wolnego kortyzolu w moczu; badanie przesiewowe*

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Introduction

Subclinical Cushing's syndrome (SCS) is characterised by alterations of the hypothalamic-pituitary-adrenal axis associated with cortisol hypersecretion without significant classical signs and symptoms of Cushing's syndrome [1]. Based on screening methods, diagnostic criteria, and study protocols implemented in various studies, SCS makes up to 5–30% of adrenal incidentaloma cases [2–4]. The importance of SCS depends on the fact that it leads to an increase in the prevalence of hypertension (HT), diabetes mellitus (DM), hyperlipidaemia, osteoporosis, and coronary artery disease, and its treatment may lead to improvement in a substantial number of these comorbidities [5–7]. The diagnostic criteria used for SCS are controversial, and a consensus is yet to be reached. Different combinations of 1 mg dexamethasone suppression test (DST), 24-hour urinary free cortisol (UFC), late night serum cortisol, and measurement of the ACTH levels have been used for the screening of SCS in different studies [2, 8, 9]. The sensitivity and specificity of singular tests as well as combination tests are limited, and hence have been insufficient in diagnosing SCS [4, 7,8]. This suggests that new tests may be needed in order to diagnose SCS.

In recent years, late-night salivary cortisol (LNslC) has been widely used for the screening of overt Cushing's disease, and studies have suggested its high sensitivity for the diagnosis of Cushing's disease [10, 11]. The LNslC test has advantages, such as not being affected by stress factors, not requiring hospitalisation, and implementation by the patient at home, and it is economically feasible [12, 13]. Meanwhile, LNslC is also useful for diagnosis of Cushing's syndrome in pregnant patients and in those using oral contraceptives or oestrogens, because it reflects free cortisol levels and is not affected by changes in cortisol-binding globulin levels. [14–17]. However, there are very few studies in the literature conducted on the value of this test in diagnosing SCS. Although the sensitivity and specificity of the LNslC test for diagnosing SCS were not found to be sufficiently high [12, 13, 18], the answer to the question about whether it can be used as a screening test for diagnosing SCS is not fully understood. Therefore, in this study, we aimed to determine the diagnostic value of LNslC in diagnosing SCS and to compare it with UFC levels.

Material and methods

The study consisted of 92 cases with adrenal incidentaloma, diagnosed by imaging methods [abdominal ultrasonography (USG), computed tomography (CT), magnetic resonance imaging (MRI), fluorodeoxyglucose (FDG), and positron emission tomography/computed tomography (PET/CT)] performed for non-adrenal disease and referred to our endocrinology outpatient clinic between January and December 2014. The control group consisted of 40 subjects who had no history of adrenal incidentaloma and who were visiting our outpatient clinic for routine general health check-up. Patients with disorders associated with pseudo-Cushing's syndrome (major depression, obsessive compulsive disorder, chronic alcoholism, and pregnancy), those with classical Cushing's syndrome signs and symptoms (moon face, purple striae, skin atrophy, buffalo hump, proximal myopathy), patients who used drugs that enhance dexamethasone metabolism (including barbiturates, phenytoin, rifampin, carbamazepine, and mitotane), patients with chronic kidney disease (estimated glomerular filtration rate < 60 mL/min), chronic liver and congestive heart failure, and patients diagnosed with primary hyperaldosteronism and pheochromocytoma were not included in the study.

A dose of 1 mg DST was applied to all subjects (1 mg dexamethasone administered orally at 11:00 $_{PM}$ and serum cortisol levels were measured the following morning between 8.00 and 9:00 $_{AM}$.), UFC levels were measured on two different days, ACTH levels were measured three times with 20 minutes between each measurement and beginning at 8.00 $_{AM}$, while the LNslC was measured on two different nights between 11.00 $_{PM}$ and 00:00 $_{AM}$. For patients with high serum cortisol levels on 1 mg DST, two days of 2 mg DST was applied (0.5 mg dexamethasone was administered orally every six hours for 48 hours, and serum cortisol levels were measured between 8.00 and 09:00 $_{AM,}$ after the last dose of dexamethasone). For subjects who had suppressed cortisol values on two-days 2 mg DST were excluded from the SCS diagnosis. As reported in previous studies [19, 20], the diagnosis of SCS was based on combined results of 1 mg DST > 1.8 μg/dL and ACTH < 10 pg/mL [1, 21, 22]. ACTH samples were sent to the laboratory under suitable conditions and processed immediately. LNslC were measured at 11.00 $_{PM}$ using a cylindrical cotton swab (Salivette, Sarstedt, Nümbrecht, Germany), which was put back in to the container after 2–3 minutes of chewing, kept overnight at 2–8°C, and processed the next morning. All participants were instructed not to eat or brush their teeth for three hours prior to specimen collection. Salivary samples obtained were centrifuged for three minutes and kept at –20°C until processed. Salivary cortisol levels were measured using the electrochemiluminescence immunoassay method (ECLIA) (Roche Cobas 8000, Tokyo Japan). In this method, the measurable lower limit of salivary cortisol was 0.018 μg/dL, and the intra and inter-assay coefficients of

variations were 1.5–6.1% and 4.1–33.4%, respectively. As reported in previous studies [8, 19], a cut-off value of 0.18 μg/dL of LNslC was chosen for the diagnosis of SCS. Serum cortisol and UFC levels were measured using the ECLIA method (Roche Cobas 8000, Tokyo Japan). All patients with adrenal incidentaloma were assessed for pheochromocytoma, and patients with HT were also assessed for primary hyperaldosteronism. All patients with adrenal incidentaloma were also analysed for HT, impaired glucose metabolism, and osteoporosis, which are possible effects of SCS, and the control group was analysed for HT and impaired glucose metabolism. DM, osteopaenia, and osteoporosis were diagnosed according to World Health Organisation (WHO) criteria [23, 24]. Impaired fasting glucose, impaired glucose tolerance, and DM were recorded as impaired glucose metabolism. The diagnosis of HT was made according to the Seventh report of the Joint National Committee (JNC-7) diagnosis criteria [25]. Informed written consent was obtained from all participants, and the study protocol was approved by the Ethics Committee of the Sisli Etfal Training and Research Hospital.

Statistical analysis

Statistical analyses were performed using the SPSS software package (Statistical Package for Social Sciences for Windows, release 22.0.0 standard version; SPSS Inc., NY) program. The Student's t-test and chi-square tests were used for the comparison of percentages of two and more than two groups, respectively. A one-way analysis of variance (ANOVA) was performed to compare quantitative variables within different groups followed by post-hoc analyses for multiple comparisons (Tamhane's test). In order to determine the sensitivity and specificity of LNslC in the diagnosis of SCS, receiver operation characteristics (ROC) analysis was used.

Results

The clinical features of SCS, NFA, and the control group are given in Table I. The average ages of all three groups were comparable. Although a statistically significant difference was not detected while comparing the mean body mass indexes (BMI) of the three groups, the mean BMI was higher in subjects with SCS and NFA, compared to the controls. Although the mean UFC levels were higher in subjects of the SCS group compared to those with NFA and controls, a significant difference was not found between subjects with SCS, NFA, and controls. The mean LNslC were significantly higher in subjects with SCS compared to those with NFA and controls ($p < 0.001$ and $p < 0.001$). There was no significant difference between the SCS and NFA groups in terms of lumbar spine and femoral bone mineral density (BMD) values. Also, in terms of the number of patients with osteopaenia and osteoporosis, there was no significant difference between the two groups. As shown in Table I, the numbers of patients with impaired glucose metabolism, HT, and chronic complications were significantly higher in subjects with SCS and NFA, compared to the controls.

Among patients with SCS, 27 patients had LNslC levels above the chosen cut-off values (0.18 μg/dL), while six patients had < 0.18 μg/dL. Six patients had UFC levels above the normal reference ranges, while 27 patients had UFC levels within the reference ranges. In the ROC analysis, as demonstrated in Table II, the sensitivity, specificity, positive predictive value, negative predictive value, and diagnostic accuracy of UFC > 137 μg/dL for diagnosing SCS were 18%, 90%, 37%, 37%, and 72%, respectively. Likewise, the sensitivity, specificity, positive predictive value, negative predictive value, and diagnostic accuracy of LNslC > 0.18 μg/dL for diagnosing SCS were 82%, 60%, 40%, 91%, and 65%, respectively.

Discussion

In this study, the diagnostic value of LNslC was investigated for patients who were diagnosed with SCS. Investigation was also made to find out whether LNslC could be used as a screening test or an adjunct test for the diagnosis of SCS, and its diagnostic value was compared to that of the UFC test. The results of the present study suggest that the sensitivity of LNslC was high but limited; however, its specificity and positive predictive values were low. On the other hand, the sensitivity, specificity, and positive predictive value of UFC were all low.

The diagnosis of SCS can be a challenge due to fluctuating cortisol secretion in these patients, low sensitivity of the available hormonal assays, and also due to the absence of typical clinical features of excess of cortisol [4]. The diagnostic value of UFC in the diagnosis of SCS has been shown to be low in most studies [7, 8, 9, 26]. In most of these studies, UFC was measured using the immunofluorometric assay (IFA) method. However, in our study, ECLIA was used. The sensitivity of UFC in our study was similar to the low sensitivity of urinary cortisol detected in studies conducted by Masserini et al. [8] and Libe et al. [9] (33% and 32%, respectively). However, in our study, the sensitivity of UFC was lower than that of all the studies in the literature. Although there is no study to compare the diagnostic performance of IFA and ECLIA for the detection of UFC in subjects with SCS, the lower sensitivity of UFC for SCS diagnoses in our study may be related to different detection methods. However, other factors such

Table I. *Clinical features of subclinical Cushing's syndrome, non-functioning adenoma and the control group*

Tabela I. *Charakterystyka kliniczna grupy pacjentów z subkliniczną postacią zespołu Cushinga, nieczynnymi gruczolakami oraz zdrowych ochotników*

	SC	NFA	Control	p value*	p value**	p value***
Fem$\mu\mu$ale/male	29/4	42/17	32/8	0.16	–	–
BMI	30.7 ± 5.6	29.9 ± 4.4	28.7 ± 5.7	0.25	–	–
Basal cortisol levels [μg/dL]	17.8 ±3.2	17.2 ± 4.7	17.7 ± 4.1	0.75	–	–
1 mg DST [μg/dL]	4.5 ± 1.7	1.2 ± 0.3	0.9 ± 0.4	< 0.001	< 0.001	< 0.001
ACTH [pg/mL]	5.5 ± 2.1	19.9 ± 8.2	25.2 ± 10.4	< 0.001	< 0.001	< 0.001
UFC [μg/day]	100.3 ± 53.5	83.1 ± 42.8	76.3 ± 42.1	0.07	–	–
LNSalC [μg/dL]	0.30 ± 0.10	0.21 ±0.09	0.16 ± 0.07	< 0.001	< 0.001	< 0.001
Lumbar BMD	–1.0 ± 1.3	–0.7 ± 1.3	–	0.46§	–	–
Femoral neck BMD	–1.2 ± 1.0	–0.8 ± 0.9	–	0.13§	–	–
Femoral trochanter BMD	–1.2 ± 1.0	–0.8 ± 1.0	–	0.10§	–	–
Femoral total BMD	–0.7 ± 1.0	–0.3 ± 1.0	–	0.11§	–	–
Osteopaenia and osteoporosis	19 (57.6%)	39 (66.1%)	–	0.50§	–	–
Impaired glucose metabolism	23 (69.7%)	32 (62.7%)	15 (37.5%)	0.01	0,50	0,006
Hypertension	20 (60.6%)	34 (57.6%)	7 (17.5)	< 0.001	0,78	< 0,001
Number of chronic complications†				< 0,001	0,94	< 0,001
0 complication	3 (9.1%)	4 (6.8%)	21 (52.5%)			
1 complication	7 (21.2%)	14 (23.7%)	16 (40.0%)			
2 complications	14 (42.4%)	27 (45.8%)	3 (7.5%)			
3 complications	9 (27.3%)	14 (23.7%)	–			

*Comparison of 3 groups by one-way analysis of variance test (ANOVA); **Comparison of patients with subclinical Cushing's syndrome and non-functional adenomas by post-hoc analysis; ***Comparison of patients with subclinical Cushing's syndrome and healthy controls by post-hoc analysis; § Student's t and chi-square tests;†Number of cases affected by osteopaenia, impaired glucose metabolism, and hypertension. SC — Subclinical Cushing's syndrome, NFA — Non-functioning adenoma, DST — Dexamethasone suppression test, ACTH — Adrenocorticotropic hormone, UFC — 24-hour urinary free cortisol, LNslC — Late-night salivary cortisol, BMD — Bone mineral density. Reference intervals: Basal cortisol (6.2–19.4 μg/dL), ACTH (10–63.3 pg/mL), UFC (36–137 μg/day), LNSalC (0.018–0.18 μg/dL)

Table II. *Diagnostic values of urinary and salivary cortisol*

Tabela II. *Wartości diagnostyczne stężenia kortyzolu w moczu i w ślinie*

	Cut-off value	Sensitivity	Specificity	Diagnostic accuracy
LNslC [μg/dL]	0.18	82%	60%	65%
UFC [μg/dL]	137	18%	90%	72%

LNsC — late-night salivary cortisol, UFC — urinary free cortisol

as the number of patients, the diagnostic criteria used, and the study design may also have been effective for this difference. Nevertheless, regardless of the method used, using UFC as a SCS screening test may lead to false negative results.

Midnight serum cortisol can be used as a confirmatory test in selected patients. Although its sensitivity is low, it is higher than that of the UFC test [8, 26]. However, it requires hospitalisation. During the course of SCS, low ACTH levels are frequently observed. However, its role alone is limited during diagnosis [7–9, 26]. The respective sensitivity and specificity of low levels of ACTH have been found as 79% and 85% and 86.4% and 59.3%, in studies conducted by Mantero et al. [2] and Masserini et al., respectively [8]. A dose of 1 mg DST has been frequently used as the screening test for SCS in patients with adrenal incidentaloma [2, 9, 26]. However, there are still deliberations on the cut-off value of test positivity [1, 7, 22, 27]. Since the cut-off value for 1 mg DST was set high in studies conducted by Masserini et al. [8] and Libe et al. [9], its sensitivity was moderately high and its specificity was high (86–96% and 91–98%, respectively). On the other hand, the cut-off value was set lower in the study conducted by Valli et al.; therefore, the sensitivity of 1 mg DST was found to be 100%

[28]. In a few studies it was shown that, although there was no biochemical proof of SCS prior to surgery, some of the comorbidities of SCS improved following surgery in some patients with adrenal incidentaloma [4, 6]. In this case, false negativity was considered in some cases of incidentaloma with mild hypercortisolaemia without the accompanying SCS. In some cases of NFA, because there are more metabolic parameters and chronic complications compared to the control group, it suggests that these cases may conceal SCS [29, 30]. Consequently, none of these tests are gold standards for the diagnosis of SCS and, also, the negativity of any of them can exclude illness alone. The combinations of these tests are also usually insufficient in the diagnosis [7, 8, 26]. All of these factors suggest that new diagnostic tests with high sensitivity, specificity, and positive predictive value are needed for the diagnosis of SCS. In the literature, different combinations of 1 mg DST, UFC, late night serum cortisol levels, and measurement of the ACTH levels have been used as diagnostic criteria in different studies [2, 8, 9]. Consequently, the combination of 1 mg DST with the cut-off value of serum cortisol > 1.8 μg/dL and ACTH levels with the cut-off value < 10 pg/mL were considered as diagnostic criteria in the present study because the sensitivity and specificity of high serum cortisol levels after 1 mg DST and suppressed ACTH levels are higher when compared to the other tests [2, 8, 26].

Only a few studies with a small number of patients have used LNslC for the diagnosis of SCS [8, 13, 18, 19, 31]. Using the cut-off value of 0.18 μg/dL for LNslC detected by IFA method, Masserini et al. found a sensitivity and specificity of 22.7% and 87.7% for SCS diagnosis, respectively [8]. Nunes et al. used the radioimmunoassay method (RIA) for the detection of salivary cortisol. Using a cut-off value of 0.17 μg/dL for LNslC, they found a sensitivity and specificity of 77% and 69% for the diagnosis of SCS, respectively [19]. Palmiere et al. used the liquid chromatography — mass spectrometry (LC-MS/MS) method for the assessment of salivary cortisol in patients with SCS. Using a cut-off value of 0.11 μg/dL for LNslC, they found a sensitivity and specificity of 55.6% and 85.2%, respectively [13]. Our study result is in line with what is reported in the literature, and suggests that detection of LNslC has a limited diagnostic value for SCS diagnoses. All of these studies demonstrate that, regardless of the biochemical method used, measurement of LNslC levels does not have a sufficient sensitivity and may lead to false negative results when used as the sole screening test for SCS. However, there are also studies which do not support these findings. In the study conducted by Tateishi et al. the sensitivity and specificity of LNslC levels > 0.11 μg/dL for the diagnosis of SCS were 100% and 50%, respectively [12]. All of these contradictory results obtained from different studies regarding SCS diagnoses may be due to the use of different diagnostic criteria [8, 12, 13, 18, 19, 31], the use of different biochemical methods such as RIA [12, 18, 19], IFA [8], ECLIA [31], and LC-MS/MS [13], and the use of different cut-off values [8, 12, 13, 18, 19, 31], and also due to different patient ethnicities. Although there is no study to compare the diagnostic values of these methods for the assessment of LNslC in the same patient population, we used the ECLIA method for the assessment of LNslC in diagnosing SCS, and the results were comparable with the aforementioned methods, including LC-MS/MS. These results demonstrate that assessment of LNslC is not suitable for the diagnosis of SCS, regardless of the method used, and as in other studies our study suggests that the LNslC test cannot be used as a screening method for SCS in patients with adrenal incidentaloma.

The most important limitation in our study was that changes that might occur in the hormonal profile of patients with NFA and SCS were not followed prospectively. Hence, further studies are needed to address this issue.

In conclusion, when compared to the other tests, the salivary cortisol test has several advantages such as being simple, economically feasible, easily available, able to be performed at home, and non-invasive. However, because its sensitivity in the diagnosis of SCS is low, its sole usage as a screening test may lead to false negative results; therefore, it should not be implemented as a screening test alone. Nonetheless, using it as an adjunct to the other tests may be beneficial in terms of SCS diagnoses. However, because the sensitivity of UFC is very low, whether it is used alone or in combination with other tests it may not be a beneficially diagnostic approach. A gold standard test is not available for SCS screening and diagnosis, and more extensive studies are needed in this respect.

References

1. Nieman LK, Biller BM, Findling JW et al. The Diagnosis of Cushing's Syndrome: An Endocrine Society Clinical Practice Guideline. J Clin Endocrinol Metab 2008; 93: 1526–1540.
2. Mantero F, Terzolo M, Arnaldi G et al. A survey on adrenal incidentaloma in Italy. J Clin Endocrinol Metab 2000; 85: 637–644.
3. Terzolo M, Stigliano A, Chodini I et al. AME position statement on adrena incidentaloma. Eur J Endocrinol 2011; 164: 851–870.
4. Chiodini I. Clinical review: diagnosis and treatment of subclinical hypercortisolism. J Clin Endocrinol Metab 2011; 96: 1223–1236.
5. Tauchmanova L, Rossi R, Biondi B et al. Patients with subclinical Cushing's syndrome due to adrenal adenoma have increased cardiovascular risk. J Clin Endocrinol Metab 2002; 87: 4872–4878.
6. Chiodini I, Morelli V, Salcuni AS et al. Beneficial metabolic effects of prompt surgical treatment in patients with an adrenal incidentaloma causing biochemical hypercortisolism. J Clin Endocrinol Metab 2010; 95: 2736–2745.
7. Morelli V, Masserini B, Salcuni AS et al. Subclinical hypercortisolism: correlation between biochemical diagnostic criteria and clinical aspects. Clin Endocrinol 2010; 73: 161–166.
8. Masserini B, Morelli V, Bergamaschi S et al. The limited role of midnight salivary cortisol levels in the diagnosis of subclinical hypercortisolism in patients with adrenal incidentaloma. Eur J Endocrinol 2009; 160: 87–92.
9. Libe R, Dall'Asta C, Barbetta L et al. Long-term follow-up study of patients with adrenal incidentalomas. Eur J Endocrinol 2002; 147: 489–494.

10. Bukan AP, Dere HB, Jadhav SS et al. The performance and reproducibility of late-night salivary cortisol estimation by enzyme immunoassay for screening cushing disease. Endocr Pract 2015; 21: 158–64.
11. Raff H. Update on late-night salivary cortisol for the diagnosis of Cushing's syndrome: methodological considerations. Endocrine 2013; 44: 346–349.
12. Tateishi Y, Kouyama R, Mihara M et al. Evaluation of salivary cortisol measurements for the diagnosis of subclinical Cushing's syndrome. Endocr J 2012; 59: 283–289.
13. Palmieri S, Morelli V, Polledri E et al. The role of salivary cortisol measured by liquid chromatography–tandem mass spectrometry in the diagnosis of subclinical hypercortisolism. Eur J Endocrinol 2013; 168: 289–296.
14. Jung C, Ho JT, Torpy DJ et al. A longitudinal study of plasma and urinary cortisol in pregnancy and postpartum. J Clin Endocrinol Metab 2011; 96: 1533–1540.
15. Aden U, Jung-Hoffmann C, Kuhl H. A randomized cross-over study on various hormonal parameters of two triphasic oral contraceptives. Contraception 1998; 58: 75–81.
16. .Wiegratz I, Kutschera E, Lee JH et al. Effect of four different oral contraceptives on various sex hormones and serum-binding globulins. Contraception 2003; 67: 25–32.
17. Moore DE, Kawagoe S, Davajan V et al. An in vivo system in man for quantitation of estrogenicity. II. Pharmacologic changes in binding capacity of serum corticosteroid-binding globulin induced by conjugated estrogens, mestranol, and ethinyl estradiol Am J Obstet Gynecol 1978; 130: 482–486.
18. .Deutschbein T, Unger N, Hinrichs J et al. Late-night and low-dose dexamethasone-suppressed cortisol in saliva and serum for the diagnosis of cortisol-secreting adrenal adenomas. Eur J Endocrinol 2009; 161: 747–753.
19. Nunes ML, Vattaut S, Corcuff JB et al. Late-night salivary cortisol for diagnosis of overt and subclinical Cushing's syndrome in hospitalized and ambulatory patients. J Clin Endocrinol Metab 2009; 94: 456–462.
20. Bondanelli M, Campo M, Trasforini G et al. Evaluation of hormonal function in a series of incidentally discovered adrenal masses. Metabolism1997; 46: 107–113.
21. Grumbach MM, Biller BM, Braunstein GD et al. Management of the clinically inapparent adrenal mass (incidentaloma). Ann Inter Med 2003; 138: 424–429.
22.) Tabarin A, Bardet S, Brtherat J et al. Exploration and management of adrenal incidentalomas. French Society of Endocrinology Consensus. Ann Endocrinol (Paris) 2008; 69: 487–500.
23. Genuth S, Alberti KG, Bennett P et al. Follow-up report on the diagnosis of diabetes mellitus. Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Diabetes Care 2003; 26: 3160–3167.
24. Assessment of fracture risk and its application to screening for postmenopausal osteoporosis. Report of a WHO Study Group. World Health Organ Tech Rep Ser. 1994; 84.
25. Chobanian AV, Bakris GL, Black HR et al. Seventh report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. National Heart, Lung, and Blood Institute; National High Blood Pressure Education Program Coordinating Committee. Hypertension 2003; 42: 1206–1252.
26. Eller-Vainicher C, Morelli V, Salcuni AS et al. Post-surgical hypocortisolism after removal of an adrenal incidentaloma: is it predictable by an accurate endocrinological work-up before surgery? Eur J Endocrinol 2010; 162: 91–99.
27. Zeiger MA, Thompson GB, Duh QY et al. American Association of Clinical Endocrinologists and American Association of Endocrine Surgeons medical guidelines for the management of adrenal incidentalomas. Endoc Pract 2009; 15: 1–20.
28. Valli N, Catargi B, Ronci N et al. Biochemical screening for subclinical cortisol-secreting adenomas amongst adrenal incidentalomas. Eur J Endocrinol 2001; 144: 401–408.
29. Terzolo M, Pia A, Ali A et al. Adrenal incidentaloma: a new cause of the metabolic syndrome? J Clin Endocrinol Metab 2002; 87: 998–1003.
30. Yener S, Genc S, Akinci B et al. Carotid intima media thickness is increased and associated with morning cortisol in subjects with nonfunctioning adrenal incidentaloma. Endocrine 2009; 35: 365–370.
31. Sereg M, Toke J, Patocs A et al. Diagnostic performance of salivary cortisol and serum osteocalcin measurements in patients with overt and subclinical Cushing's syndrome. Steroids 2011; 76: 38–42.