Actuality of Studying the Steroid Profile of Saliva in the Planning of Dental Implantation

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Abstract

The aim of this review is to analyze the value of determining saliva steroid profile for diagnosis of various conditions and diseases. Steroid hormones, due to their high lipophilicity, quite easily pass from the blood to the saliva. Saliva steroids can reflect the concentration in plasma of a free fraction of hormones, that is, non-conjugated with proteins.

During a systematic review of the literature, publications were considered in the electronic databases Google Scholar and PubMed, the main concept of which was to study the steroid profile of saliva and its diagnostic significance.

56 publications were initially studied, of which 39 were included in the review after analysis by exclusion criteria.

The study of saliva steroid profile is an appropriate technique, which can confirm some diagnoses, such as Cushing's syndrome. At the same time, it is necessary to keep in mind the limitations imposed by the saliva itself as a biomatrix due to the variability of its composition and cross sensitivity of components to certain reagents.

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Introduction

The human body is a dynamic open system, and, in its regulation, the endocrine system takes not the last place, which performs its work through the secretion of certain hormones. In various pathophysiological conditions and diseases disorders of organ activity is possible due to excessive/insufficient release of certain hormones.

The group of steroid hormones, including sex hormones, glucocorticoids, mineralocorticoids and cardiotonic steroids, is widespread in terms of the number of target organs and their effects[1-4]. These substances, in particular, their quantity, are measured in clinical practice for the purpose of diagnosing pathologies and planning the further structure of the endocrine diseases treatment. Laboratory analysis can be subjected to completely different in composition and characteristics biological substrates: plasma and blood serum, urine and others[6,7,8].

Now, saliva is a promising matrix for calculating concentrations of steroids and some other biologically active substances in terms of laboratory diagnostics[7-11]. The technique of saliva sampling is not particularly difficult, it is non-invasive and short in time, allows to get several samples in a short period of time, which facilitates the process of obtaining a sample for the patient, since the procedure is painless and does not lead to excessive excitement, stress. Also, the procedure does not require special training of both medical personnel and the patient, as well as equipment[9-12].

Salivary steroids can reflect the levels of free circulating in blood steroids, that are not bound to some types of proteins. These nonconjugated hormones make up about 2 to 10% of their total content. During the analysis for the saliva steroid profile it is possible to suspect or confirm the presence of kidney pathologies, for example, chronic renal failure, adrenal glands, and genital glands[7,12,13].

The purpose of this review article is to determine the relevance of steroid hormones

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concentration in saliva tests for the diagnosis of various diseases, in comparison with standard matrices (blood, urine).

Materials and methods

The analysis of literature sources in the electronic databases Google Scholar and PubMed, as well as references in found articles, was performed to collect up-to-date information for writing the presented review article (Table 1). Search terms included: "saliva steroids", "saliva hormones", "steroid analysis in saliva", "Cushing's syndrome and cortisol".

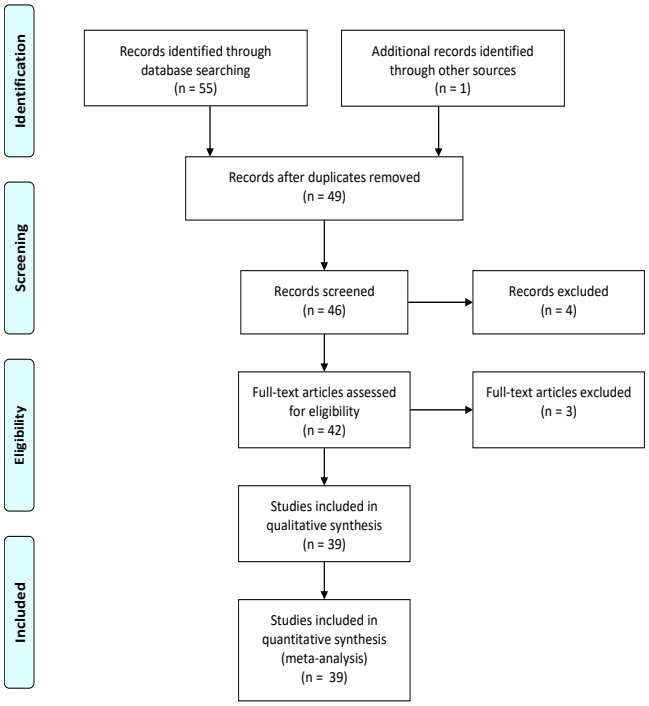

Table 1. Article selection process.

Each publication was evaluated for inclusion criteria during the review period, such as: dated 2006 and later, figuring out in the subject and discussion of articles the evaluation of composition saliva and significance of the hormones analysis, including steroid ones, for diagnosis.

The articles were selected and included in the analysis in several stages. At the first stage, the exclusion criterion was dating earlier than 2006. Then the title and short content of the publications were evaluated. At the last stage, a complete reading and analysis of the content and full-text versions of selected articles was carried out.

For the included studies, the risk of systematic error was assessed using the two-component Cochrane Collaboration tool, and the risks were put forward at each selection stage of the publications [34]. The levels of systematic error were classified according to the following: low risk- all criteria were met; moderate risk - only one criterion was missing ; high risk- two or more criteria were missing; unclear risk- too few details to make a decision on a particular risk assessment.

Results

During the systematic review of data, 56 publications were reviewed (12 - PubMed database, 43 - Google Scholar and 1 article link). After analyzing each article for inclusion and exclusion criteria, the total number of publications have become 39. In included in the discussion studies the composition of saliva, ways of penetration of different substances into it, as well as the possibility of saliva use as a biomatrix for analyzing the steroid profile of the body in the presence of various pathologies were described.

Discussion

Mixed saliva has a complex composition. It is a conglomerate of major (parotid, sublingual) and minor salivary glands secretion, gingival fluid, desquamated oral epithelium, microorganisms and their products of vital activity, food residues[9,12,13]. It, in addition to the aqueous phase, which represents up to 99% of the total volume, contains inorganic components and more than 200 different biologically active substances involved in the homeostasis of the oral cavity and the entire body [13,23]. Daily secretion ranges from 500 to 1500 ml, with basal flow velocity averaging about 0.5 ml/min[17,18]. The primary saliva, located within the acinus, has the same ionic composition as plasma, that it is isotonic. The final saliva modified by ductal cells by transferring various substances using specific transporters and ion channels and entering the oral cavity through the ducts of the glands is hypotonic due to the increased content of bicarbonate buffer components and low concentrations of chlorides and sodium[18,20].

Based on the chemical composition, it can be said that saliva is a plasma ultrafiltrate, which is why it is able to reflect the concentration of various substances in the blood with sufficient

accuracy. Saliva is a matrix into which substances are either transported from blood vessels, or independently produced by the glands and can be incorporated into its composition in various ways. The most frequent pathway is ultrafiltration, which allows relatively small lipophilic molecules, including hormones of a steroid structure, with a mass about 1900 Da[17] to enter the oral fluid through intercellular slits. Also, the phenomenon of simple diffusion along the concentration gradient occurs quite often. Lipophilic molecules, such as steroids, are transported through cellular barriers faster than hydrophilic ones, such as peptides. Active transport requires the expenditure of a certain energy amount and is directly dependent on the rate of salivation, pH, and chemical structure of the transferred substance. Active, energy-dependent transport is associated with some difficulties in interpreting the concentrations of certain saliva molecules, in particular, a number of peptide-nature hormones, since the concentrations of salivary peptides correlate with plasma content at a low level, while the concentration of steroids in saliva reflects a biologically active «free» fraction of the blood hormonal profile that is not conjugated with plasma proteins[7,16,19].

In some cases within the parenchyma of the salivary glands, a cascade of enzymatic reactions is possible, for example, under the action of 11β -hydroxysteroid dehydrogenase II, cortisol passes into cortisone, which in turn leads to the probability of false-positive results and discrepancies in plasma and saliva cortisol concentrations due to insufficient selectivity of analytical systems. This dictates a more accurate attitude to the study of reagents used in analytical kits cross-reactivity[7,26,28].

It must be that peptides are the most difficult to analyze by determining the concentration of hormones through the saliva biomatrix, so their detection is more often interpreted as markers for specific oral diseases. In contrast, the analysis of saliva steroid profile (concentrations of such hormones as cortisol, progesterone, androgens, estrogens - in free form, describing up to 10% of their total number) can qualitatively reflect their concentration in plasma, including in long-term observations. Moreover, conjugated steroids, such as, for example, dehydroepiandrosterone[1,5], are present in saliva in reduced concentrations and account for less than 1% of the unbound plasma concentration [7].

The determination of the steroid saliva profile is practiced not only in endocrinology, but also in pediatrics, pharmacology, toxicology, and sports medicine for a fairly long period of time. Due to the availability and non-invasiveness of this analysis, rapid diagnosis and confirmation of the disease presence is possible.

Certain help in promoting saliva use as biomatrix for determining the concentration of sex steroids became the following fact, observed by the overwhelming majority of the authors: in contrast to blood, saliva does not contain sex hormone–binding globulin (SHBG), and similarly, the absence of corticosteroid-binding globulin was observed [35,36]. Plasma SHBG, produced primarily by the liver, is the main plasma transport protein for biologically active androgens and estrogens, mediating their breadth of distribution and access to target tissues[37]. Non-conjugated hormones (e.g., testosterone, estradiol, dihydrotestosterone), which penetrate in various ways in oral fluid, will not be bind with a specific protein due to its absence and will correctly reflect the quantitative characteristics of the required "free" hormone (5-10% of the total content in the blood) mediating the functional activity on target cells. However, some authors have raised concerns about the possibility of transferring the discussed globulins to the salivary glands, and then to the saliva secreted by them, which can distort to one extent or another the test results[11]. This statement dictates a more sensitive attitude to checking the purity of the studied samples.

Steroid hormones are responsible for many metabolic and physiological processes in the body, which is why it is worth to consider the possibility of an excess or lack secretion leading to a particular pathology, based on data of their content in saliva in correlation with plasma levels.

CORTISOL

Cortisol (glucocorticoid synthesized by the zona fasciculata of the adrenal cortex) is a hormone that properly reflects the functional activity of the hypothalamic-pituitary-adrenal axis, which determines the significance of variations in its concentrations in the pathogenesis of certain diseases[6,13,21]. Tracking the trend of total cortisol-to-salivary cortisol ratio is a complex issue due to the presence of corticosteroid-binding globulin, which binds up to 500-600 nmol/l of cortisol,

which appears to be a barrier to its free penetration by ultrafiltration or passive diffusion into saliva. According to research data, saliva cortisol correlates well with its free fraction in the blood plasma, which exerts its biological functions. The levels of cortisol in saliva correlate with those in plasma even after ACTH stimulation and physical exertion under stress in clinico-diagnostic practice[12,21,26,33].

Various methods of saliva collecting can be used: collection of unstimulated and stimulated saliva by chewing a cotton or polyester roller, the use of various commercially available devices, aspiration, and simple spitting into a sterile container [21]. It is necessary to make sure that the samples are not contaminated with residues of orally taken medications, especially of a hormonal nature, blood to prevent false-positive results.

Various authors have suggested the clinical significance of determining the cortisol concentration in saliva in the diagnosis of Cushing's and Addison diseases, secondary adrenal insufficiency and hypercorticism, congenital adrenal hyperplasia[6,20,32,33]. Also, presumably, the level of salivary cortisol can be a valuable marker for assessing the degree of an individual adaptation to environmental conditions, as well as evaluating the presence of mental pathologies, such as chronic fatigue syndrome, enduring\lasting stress and depressive disorder[22,23,32].

If we talk about Cushing's disease, the most informative saliva samples are those that were collected at night between 23.00 and 24.00, since peak cortisol concentration during these hours is observed, according to most publications[6,21,25,32]. Late measurements of salivary cortisol are useful for monitoring patient's remission and/or relapse after pituitary surgery for Cushing's disease. 92-100% specificity and 91-100% sensitivity of the saliva test was reported, which is similar to a late-night serum cortisol measurement cut-off value exceeding 240 nmol/L[12,25]. It is recommended to refrain from interpreting the converted values of salivary cortisol below 0.5 nmol/L and above 60 nmol/L (according to LC-MS/MS data) [28]. Cortisol analysis by the urine matrix is quite difficult for the patient, since in some cases it requires 24-hour collection, also the procedure of blood sampling can cause stress for patients, which will also affect the results of the test.

Due to the fact that saliva is an ultrafiltrate of plasma, salivary cortisol measurement can also be performed during the test with ACTH stimulation in patients with an increased concentration of corticosteroid-binding protein in the blood plasma due, for example, to excessive estrogen stimulation[21].

Determination of cortisol in saliva is also indicated for the detection of functional hypercorticism and its control in dexamethasone treatment. The authors suggested that the salivary cortisol test should be used in the diagnosis of adrenal insufficiency[6,21,32]. However, in the course of reasoning and detailed consideration of the circadian rhythm of the HGH axis, it was noted that measurements of basal morning salivary cortisol levels are not able to provide reliable data for the diagnosis of primary or secondary adrenal insufficiency, because of the test specificity and sensitivity were at the level of only 35% when evaluating a sample collected at 8.00 am[21].

According to the experiment, non-stimulated and stimulated total saliva had similar concentrations of cortisol as well as testosterone and androstenedione, while cortisone in stimulated saliva showed the 16% less values, which is probably connected with the limitation of the reaction rate of cortisol and cortisone conversion under the action of 11β-hydroxysteroid dehydrogenase II[33].

Most identifications of cortisol in saliva are performed using immunoassays. This study is based on binding of the analyzed substances to antibodies that exhibit different specificity for the molecules to be detected. A limitation in the use of saliva as a biomatrix for determining the concentration of cortisol is the presence of a certain cross-reactivity of cortisol and its metabolite cortisone, which is formed in the salivary glands under the action, as previously indicated, of 11β -hydroxysteroid dehydrogenase type II. The cross-reactivity of cortisone is particularly important, given that its concentration is up to 280% compared to cortisol, due to its long half-life. This fact leads to an increase ratio of cortisone compared to cortisol, with a decrease in the concentration of cortisol in the final values[28,32].

It is necessary to take into account that the hypothalamic-pituitary-adrenal system is very sensitive to stress states, undoubtedly this statement dictates to take any sample in calm

conditions. Saliva, in relation to causeing fear in the patient, is one of the best methods of evaluating the hormonal profile. However, we should not exclude the study of total cortisol serum levels, since this technique is quite accurately able to reflect the concentration of the hormone and its individual fractions, as well as evaluate the functional activity of the hypothalamic-pituitary-adrenal system.

ANDROGENS.

Saliva testosterone is used for diagnosis and confirmation of male hypogonadism and eugonadism. In one of the studies the authors obtained data that the concentration of salivary testosterone does not depend on the rate of salivation and that, preferably, choose morning saliva samples from 7.00 to 9.00 for a more accurate analysis. Just morning samples were selected by the researchers for the differential diagnosis of eugonadism and hypogonadism. Clear cut - off values evaluated for salivary, as well as serum free and bioactive testosterone showed 100% sensitivity and specificity for excluding hypogonadism. The concentration of testosterone in a healthy man in the morning is 369 pmol/L on average, which is statistically different from the level of the presented hormone in patients with androgen deficiency (51-249 pmol/L)[24,29]. Unlike testosterone, dehydroepiandrosterone-sulfate is not detected in saliva under normal conditions, since its polar group disrupts intermembrane transport of the molecule, and its penetration into saliva is associated with injury of the oral mucosa and bleeding[32].

According to the authors of one of the studies, if the concentration of testosterone is more than 25 pmol/L, the question of hypogonadismin presence in patient is not raised, that is, we should look for the causes of these pathologies in another ways[32].

Salivary androstenedione correlates well with its free plasma concentrations and can be used in studies along with DHEA and testosterone[12,30]. We should not forget about the possibility of cross-reaction of untreated samples, because of it is necessary to confirm the data by chromatographic studies[12]. There may also be some data distortion while using different methods for collecting saliva. Concentrations of testosterone and androstenedione decreased when using synthetic Salivettes (58% and 41% respectively) and higher when using cotton Salivettes (217% and 46% respectively)[33].

Patients with polycystic ovarian syndrome have elevated salivary levels of the testosterone/androstenedione ratio, which correlates with their disturbed plasma concentrations and can be considered as adverse metabolic indicators when planning treatment [27].

ESTROGENS AND PROGESTERONE

Determination of estriol and progesterone concentrations, including in saliva, as well as their mutual ratio can be valuable for predicting complicated and early labor in the period up to 34 weeks. A lower concentration of progesterone and a high ratio estriol to progesterone in saliva samples from women who gave birth before 34 weeks, confirm the authors' hypothesis that an imbalance between these hormones may be associated with preterm birth, but, of course, it is not the only pathogenetic factor in the initiation of this process[19].

The levels of 17B-estradiol at a high-level correlate with the plasma concentration of the discussed hormone. The test for the presence of 17B-estradiol in saliva can be used as one of many diagnostic matrices to confirm/deny the patient's infertility[32].

Saliva progesterone is sometimes used as an ovulation test, as there is a good correlation between this hormone in blood plasma and saliva. Unfortunately, the authors noticed that there was insufficient sensitivity and specificity of the test when comparing the follicular and luteal phases (78% and 77% respectively). Joint determination of progesterone and estradiol in saliva can be useful for monitoring fertility[12].

Conclusion

Determining the steroid profile of saliva is a fairly common diagnostic system in various areas of medicine. Due to the noninvasiveness, cheapness and ease of the procedure, an increasing number of diseases caused by an imbalance of steroids can be detected at an early stage.

Unfortunately, saliva is not the gold standard and cannot fully replace and displace other widely used biomaterials, such as blood and urine. In particular, the changeability of saliva matrix variables and their sensitivity to certain reagents, the stability of steroid hormones in various conditions, the presence of binding proteins and anomalies in the reference range can affect the quality of the obtained results to a greater or lesser extent.

Declaration of Interest

The authors report no conflict of interest.

References

1. Ables ET, Drummond-Barbosa D. Steroid Hormones and the Physiological Regulation of Tissue-Resident Stem Cells: Lessons from the Drosophila Ovary. Curr Stem Cell Rep. 2017;3(1):9-18.
2. Shannon Whirledge, John A. Cidlowski. Yen and Jaffe's Reproductive Endocrinology (Eighth Edition). Academic Press; 2019:115-131.
3. Anthony W. Norman, Helen L. Henry. Hormones (Third Edition), Academic Press; 2015:27-53.
4. Boggs AS, Bowden JA, Galligan TM, Guillette LJ Jr, Kucklick JR. Development of a multi-class steroid hormone screening method using Liquid Chromatography/Tandem Mass Spectrometry (LC-MS/MS). Anal Bioanal Chem. 2016 Jun;408(15):4179-90.
5. Cook-Botelho, J. C., Bachmann, L. M., & French, D. Steroid hormones. Mass Spectrometry for the Clinical Laboratory; 2017:205–230.
6. Esteban M. Repetto, Diego Gonzalez, Dario Jacobsen, Fernando Smithuis, Juan Jamardo, Mariel Cano, Claudio Aranda, Adriana Oneto, Gabriela Berg, Bibiana Fabre. Evaluation of an automated chemiluminescent immunoassay for salivary cortisol measurement. Utility in the diagnosis of Cushing's syndrome. Clinical Chemistry and Laboratory Medicine (CCLM); 2016 (55) 3: e65–e68.
7. Michael Gröschl, Current Status of Salivary Hormone Analysis, Clinical Chemistry; 2008 (54) 11: 1759-1769.
8. T. Pfaffe, J. Cooper-White, P. Beyerlein, K. Kostner, C. Punyadeera. Diagnostic potential of saliva: current state and future applications. Clin. Chem; 2011 (57): 675-687.
9. Gröschl M. Saliva: a reliable sample matrix in bioanalytics. Bioanalysis. 2017 Apr;9(8):655-668.
10. Maciejczyk M., Żukowski P., Zalewska A.Salivary Biomarkers in Kidney Diseases. In: Tvarijonaviciute A., Martínez-Subiela S., López-Jornet P., Lamy E. (eds) Saliva in Health and Disease. Springer, Cham.2020.
11. Khurshid Z, Zafar MS, Khan RS, Najeeb S, Slowey PD, Rehman IU. Role of Salivary Biomarkers in Oral Cancer Detection. Adv Clin Chem. 2018;86:23-70
12. Lewis J. G. (2006). Steroid analysis in saliva: an overview. The Clinical biochemist. Reviews; 27(3): 139–146.
13. Farnaud, S.J.C.; Kosti, O.; Getting, S.J.; Renshaw, D. Saliva: Physiology and diagnostic potential in health and disease. Sci. World J. 2010, 10, 434–456.
14. Khurshid Z, Zohaib S, Najeeb S, Zafar MS, Slowey PD, Almas K. Human Saliva Collection Devices for Proteomics: An Update. Int J Mol Sci. 2016 Jun 6;17(6):846.
15. Michael Polet, Laurie De Wilde, Pieter Van Renterghem, Wim Van Gansbeke, Peter Van Eenoo. Potential of saliva steroid profiling for the detection of endogenous steroid abuse: Reference thresholds for oral fluid steroid concentrations and ratios. Analytica Chimica Acta,; 2018 (999):1-12.
16. Proctor GB. The physiology of salivary secretion. Periodontol 2000. 2016 Feb;70(1):11-25.
17. Francesca G. Bellagambi, Tommaso Lomonaco, Pietro Salvo, Federico Vivaldi, Marie Hangouët, Silvia Ghimenti, Denise Biagini, Fabio Di Francesco, Roger Fuoco, Abdelhamid Errachid. Saliva sampling: Methods and devices. An overview. TrAC Trends in Analytical Chemistry 2020(124):100-102.
18. Peter Celec, Ľubomíra Tóthová, Katarína Šebeková, Ľudmila Podracká, Peter Boor. Salivary markers of kidney function — Potentials and limitations. Clinica Chimica Acta. 2016 (453): 28-37.
19. Lachelin GC, McGarrigle HH, Seed PT, Briley A, Shennan AH, Poston L. Low saliva progesterone concentrations are associated with spontaneous early preterm labour (before 34 weeks of gestation) in women at increased risk of preterm delivery. BJOG. 2009 Oct;116(11):1515-9.
20. G.H. Carpenter. The secretion, components, and properties of saliva. Ann. Rev. Food Sci. Technol.; 2013 (4): 267-276.
21. Raff H. Utility of salivary cortisol measurements in Cushing's syndrome and adrenal insufficiency. J Clin Endocrinol Metab. 2009 Oct;94(10):3647-55.
22. Amanda D.L. Roberts, Andrew S. Papadopoulos, Simon Wessely, Trudie Chalder, Anthony J. Cleare. Salivary cortisol output before and after cognitive behavioural therapy for chronic fatigue syndrome. Journal of Affective Disorders, 2009 (115): 280-286.
23. Carla Girardi, Clarice Luz, Karen Cherubini, Maria Antonia Zancanaro de Figueiredo, Maria Lúcia Tiellet Nunes, Fernanda Gonçalves Salum. Salivary cortisol and dehydroepiandrosterone (DHEA) levels, psychological factors in patients with oral lichen planus. Archives of Oral Biology; 2011 (56): 864-868.
24. Arregger AL, Contreras LN, Tumilasci OR, Aquilano DR, Cardoso EM. Salivary testosterone: a reliable approach to the diagnosis of male hypogonadism. Clin Endocrinol (Oxf). 2007 Nov;67(5):656-62.
25. Carroll T, Raff H, Findling JW. Late-night salivary cortisol measurement in the diagnosis of Cushing's syndrome. Nat Clin Pract Endocrinol Metab. 2008 Jun;4(6):344-50.
26. Perogamvros I, Keevil BG, Ray DW, Trainer PJ. Salivary cortisone is a potential biomarker for serum free cortisol. J Clin Endocrinol Metab. 2010 Nov;95(11):4951-8.
27. Münzker, J., Lindheim, L., Adaway, J., Trummer, C., Lerchbaum, E., Pieber, T., Keevil, B. and Obermayer-Pietsch, B. (2017), High salivary testosterone-to-androstenedione ratio and adverse metabolic phenotypes in women with polycystic ovary syndrome. Clin Endocrinol, 86: 567-575.
28. Robert Miller, Franziska Plessow, Manfred Rauh, Michael Gröschl, Clemens Kirschbaum. Comparison of salivary cortisol as measured by different immunoassays and tandem mass spectrometry. Psychoneuroendocrinology; 2013 (38): 50-57.
29. Nikolai Goncharov, Gulinara Katsya, Anna Dobracheva, Alexander Nizhnik, Galina Kolesnikova, Victor Herbst & Jürgen Westermann. Diagnostic significance of free salivary testosterone measurement using a direct luminescence immunoassay in healthy men and in patients with disorders of androgenic status, The Aging Male. 2011(2): 111-122.
30. V. Segeda, L. Izakova, N. Hlavacova, A. Bednarova, D. Jezova. Aldosterone concentrations in saliva reflect the duration and severity of depressive episode in a sex dependent manner, Journal of Psychiatric Research; 2017 (91): 164-168.
31. Eline Jurgens1, Edward J. Knaven1, Eva C.A. Hegeman1, Marjolein W.M. van Gemert1, Judith M.A. Emmen, Ingrid Willemen, Yvonne Mulder, Linda IJsselstijn, Ben M. de Rooij, Theo H.M. Noij. Quantitative Profiling of Seven Steroids in Saliva using LC-MS/MS. Journal of Applied Bioanalysis; 2019(5): 34-45.
32. Vavilova, T. P., Ostrovskaya, I. G., And Medvedev, A. E. (2014). Possibilities and prospects of studying hormones in saliva. Biomedical chemistry, 2014(3): 295-307.
33. Büttler RM, Bagci E, Brand HS, Heijer MD, Blankenstein MA, Heijboer AC. Testosterone, androstenedione, cortisol and cortisone levels in human unstimulated, stimulated and parotid saliva. Steroids. 2018 Oct;138:26-34.
34. Higgins J.P.T., Altman D.G., Gøtzsche P.C., Jüni P., Moher D., Oxman A.D., Savović J., Schulz K.F., Weeks L., Sterne J.A. The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. BMJ. 2011;343:d5928.
35. Natalia Gavrilova, Stacy Tessler Lindau. Salivary Sex Hormone Measurement in a National, Population-Based Study of Older Adults. The Journals of Gerontology: 2009 (64): i94–i105.
36. Keevil BG, Fiers T, Kaufman JM, Macdowall W, Clifton S, Lee D et al. Sex hormone-binding globulin has no effect on salivary testosterone. Annals of Clinical Biochemistry. 2016 Nov 1;53(6):717-720.
37. Geoffrey L. Hammond. Diverse Roles for Sex Hormone-Binding Globulin in Reproduction. Biology of Reproduction; 2011 (3): 431–441.