

# The use of hormones indicators in human saliva in diagnosing parodontitis in pregnant women

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**AIMS:** The purpose of this work– was to study the dynamics of biochemical parameters of human saliva and analyze the features of the chemical composition of the saliva of women with abnormal pregnancy and in periodontitis against pregnancy.

**MATERIALS AND METHODS:** The study included four groups of women: a control group of nonpregnant women of childbearing age (10), pregnant women with physiological pregnancy (24-28 weeks) without any signs of periodontal disease (10), pregnant with a generalized periodontitis I–II degrees in remission (10), women with pathological pregnancy with no signs of periodontal inflammation (10). In each of the groups over two samples of saliva were collected, the first collection of saliva in the morning on an empty stomach. Then mouthwash 0.9% sodium chloride solution was assigned and after 30 minutes the second portion of saliva. By enzyme immunoassay in samples of saliva of control groups of nonpregnant and pregnant women, as well as women with signs of a pathological course of pregnancy, the content of estriol, testosterone, and dehydroepiandrosterone sulfate was determined.

**STATISTICAL ANALYSIS USED:** Statistical data analysis was performed by the standard technique using Student's t-test.

**RESULTS:** The results of biochemical analysis of saliva samples collected before rinsing the mouth with saline in groups of healthy nonpregnant and pregnant women were compared. It was established that during pregnancy the concentration of salivary estriol increases, but in pregnant women with periodontitis, the amount of this hormone in the saliva was significantly reduced. The highest content of testosterone in saliva samples, observed in healthy pregnant women, was significantly higher than nonpregnant women.

In pregnant women with periodontitis concentration of testosterone in saliva is reduced, while remaining significantly higher than its level in the saliva of nonpregnant women.

The highest concentration of testosterone is observed in the saliva of healthy pregnant women with periodontitis, but the smallest concentration of testosterone is found in the saliva of nonpregnant women. Also the nonpregnant group has the lowest levels of DHEA in pregnancy, and its content increases almost threefold when periodontal disease further grows.

**CONCLUSIONS:** It was established that periodontitis against pregnancy is characterized by higher levels of salivary DHEA sulfate and lower estriol, compared with a control group of pregnant women.

**Key words:** Man, pregnancy, parodontitis, saliva

## Introduction

Relevance of the study of the pathogenesis and course of periodontitis,<sup>[1]</sup> together with the development of new methods of its early diagnosis<sup>[2]</sup> is due, firstly, to the prevalence of this disease.<sup>[3]</sup> Second, the data that chronic periodontitis increases the risk to the health and lives in groups of patients with certain systemic diseases<sup>[4,5]</sup> violate the physiological course of pregnancy.<sup>[6]</sup> Introduction of methods for early detection of disease based on biochemical analysis of saliva contributes to the minimization of risks.<sup>[2]</sup> Prospects for development of laboratory analysis of saliva is due to the improvement of noninvasive monitoring in practical medicine, including endocrinology<sup>[7]</sup> and pharmacology<sup>[8]</sup> and toxicology.<sup>[9]</sup> In normal human levels of some organic<sup>[10]</sup> and mineral<sup>[11]</sup> the components of saliva have fairly constant values, which greatly increase their diagnostic value.<sup>[12]</sup>

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Thus, revealing of patterns of qualitative and quantitative changes in the biochemical composition of human saliva in health and disease is of great practical importance.<sup>[13]</sup> According to the results of clinical observations, noninvasive monitoring of pregnancy, based on laboratory analysis of saliva, deserves wider application in obstetric practice.<sup>[14]</sup>

The purpose of this work was to study the dynamics of biochemical parameters of human saliva and analysis features of the chemical composition of the saliva of women with abnormal pregnancy and in periodontitis against pregnancy.

### Materials and Methods

This paper presents the results of four groups of women, including healthy pregnant women of reproductive age (20-35 years, 10), as well as pregnant women with physiological pregnancy (24-28 weeks) without any signs of periodontal disease (10) and with a generalized periodontitis I-II degrees in remission (10). In addition, the surveyed women with abnormal pregnancy without any signs of periodontal inflammation (10) were also included.

Violations of the physiological course of pregnancy were detected by measuring blood pressure, levels of human chorionic gonadotropin in serum, and ultrasound data. In each of the examined groups of patients and healthy subjects saliva samples were collected. Within 24 hours prior to the collection of saliva, in accordance with the published literature with guidance,<sup>[15]</sup> patients were recommended a diet that reduces nitrite load of the body and prevents entry of excess salt and animal proteins into the body.

Each subject collected two saliva samples: The collection of the first portion of saliva was performed on an empty stomach at 9.00 at rest in a sitting position. Then the subjects rinsed the mouth three times with 0.9% sodium chloride solution and 30 minutes after the mouthwash the second portion of saliva was collected. The saline solution was prepared in distilled water using chemically pure sodium chloride; the quantity of saline osmolality was 270 mOsm/kg H<sub>2</sub>O. In saliva samples collected after centrifugation for 15 at 3000 rpm using the enzyme immunoassay with standard test systems

for *in vitro* diagnostic tests in saliva the content of the following hormones was determined: Estriol and testosterone (diagnostic kits, produced by Human, Germany), dehydroepiandrosterone sulfate (diagnostic kit manufactured by DRG, USA). Statistical data analysis was performed by the standard technique using Student's *t*-test.

### Results

The results of biochemical analysis of saliva samples collected before rinsing the mouth with saline in groups of healthy nonpregnant and pregnant women were compared. The results of the analysis of hormones in saliva to rinse a 0.9% NaCl solution are shown in Table 1. It was established that during pregnancy the concentration of salivary estriol increases, but in pregnant women with periodontitis, the amount of this hormone in the saliva was significantly reduced. The highest content of testosterone in saliva samples, observed in healthy pregnant women, was significantly higher than nonpregnant women. In pregnant women with periodontitis concentration of testosterone in saliva is reduced, while remaining significantly higher than its level in the saliva of nonpregnant women. Dynamics of DHEA in subjects is as follows: Its smallest concentration is noted in nonpregnant women; during pregnancy, its concentration in saliva increases; and in pregnant women with periodontitis, content of DHEA in saliva reaches the

**Table 1: Contents of hormones in saliva of women with pathological course of pregnancy, pregnant with parodontitis, and healthy pregnant before rinsing of the mouth with salt solution (M±m)**

| Studied indices                         | Nonpregnant women<br>n=10 | Healthy pregnant<br>n=10            | Pregnant with parodontitis<br>n=10                          |
|---|---------------------------|-------------------------------------|---|
| Estriol, ng/ml                          | 0.183±0.016               | 3.766±0.321<br>p <sub>1</sub> <0.01 | 2.948±0.252<br>p <sub>1</sub> <0.01<br>p <sub>2</sub> <0.05 |
| Testosterone, nmol/l                    | 0.140±0.013               | 0.240±0.022<br>p <sub>1</sub> <0.01 | 0.298±0.018<br>p <sub>1</sub> <0.01<br>p <sub>2</sub> <0.05 |
| Dehydroepiandrosterone -sulfate, mkg/ml | 0.024±0.002               | 0.042±0.003<br>p <sub>1</sub> <0.01 | 0.056±0.005<br>p <sub>1</sub> <0.01<br>p <sub>2</sub> <0.05 |

n=Number of observations. p<sub>1</sub>=Index of significant differences within a group of nonpregnant women; p<sub>2</sub>=Index significant differences within a group of healthy pregnant women

highest value. Further studies of samples of saliva were carried out after rinsing the mouth of women of all these groups with a solution of sodium chloride. The fact that the group of pregnant women with periodontitis shows the highest values of the rate of formation of saliva attracts more attention. After rinsing the mouth with 0.9% saline [Table 2], the dynamics of hormone levels in saliva has been tested in all of these groups and it keeps with the trends presented above. The concentration of estriol in saliva of healthy pregnant women is several times higher than that in nonpregnant women, but in periodontitis hormone levels significantly decreased, while remaining higher than in nonpregnant women.

The highest concentration of testosterone is observed in the saliva of healthy pregnant women with periodontitis, but the smallest concentration of testosterone is found in the saliva of nonpregnant women.

Also the nonpregnant group has the lowest levels of DHEA in pregnancy, and its content increases almost threefold when periodontal disease further grows.

## Discussion

In our opinion, as one of the most promising areas of research, the dynamics of steroid hormones in the saliva of pregnant women with periodontitis can be chosen to study while one is closer to solving the problems

outlined. This conclusion is based on data from the literature that the physiological course of pregnancy is accompanied by increased production of estriol, dehydroepiandrosterone (DHEA), and other steroid hormones, synthesized by feto-placental complex and maternal adrenal glands.<sup>[16]</sup>

Amplification during pregnancy produces these hormones; most authors regard this as one of the main factors that create the conditions necessary for the adaptation of the female body.<sup>[17-20]</sup> It was also reported that estrogens<sup>[21]</sup> and DHEA<sup>[22,23]</sup> have distinct cytoprotective effects, reduce the damaging effects of inflammatory mediators in the tissue, and stimulate the protective reaction of the body. Not less important, in our opinion, are estrogens that have an important role in the regulation of metabolic and reparative processes of the epithelium lining the oral cavity of human.<sup>[24]</sup> In addition, clinical observations indicate that DHEA may be involved in protective and reparative processes of periodontium in periodontitis.<sup>[22]</sup>

Estriol - the main estrogen of pregnancy - was chosen as a marker of physiological pregnancy. DHEA - one of corticosteroids during pregnancy - plays a key role in the biosynthesis of estrogens feto-placental complex,<sup>[25]</sup> as evidenced by the high intensity of its absorption sincytotrophoblasts.<sup>[26]</sup> According to some authors, DHEA during pregnancy not only serves as an important substrate for the biosynthesis of estrogen, but also has its own regulatory effects required for successful gestation.<sup>[26,27]</sup>

The analysis showed that samples of the saliva control group of pregnant women have a distinct increase in the concentration of estriol in comparison with nonpregnant against the backdrop of increasing concentrations of DHEA. Although periodontitis causes a moderate decrease in the concentration of estriol in saliva, compared with a control group of pregnant women, while the content of DHEA in the saliva of pregnant women with periodontitis increased.

According to the literature DHEA is the universal precursor of sex hormones, both male and female.<sup>[28]</sup> Therefore, it is permissible to consider increased synthesis and secretion of DHEA during pregnancy, amid falling clearance of sex hormones, including androgens,<sup>[29]</sup> as the main reasons for the increase of testosterone in the saliva of pregnant women.

**Table 2: Contents of hormones in saliva samples collected after rinsing of the mouth with a salt solution in the group of women with pathological during pregnancy, pregnant with parodontitis, and healthy pregnant (M±m)**

| Studied indices                        | Nonpregnant women<br>n=10 | Healthy pregnant<br>n=10            | Pregnant with parodontitis<br>n=10                          |
|--|---------------------------|-------------------------------------|---|
| Estriol, ng/ml                         | 0.116±0.009               | 4.167±0.362<br>p <sub>1</sub> <0.01 | 2.936±0.261<br>p <sub>1</sub> <0.01<br>p <sub>2</sub> <0.01 |
| Testosterone, nmol/l                   | 0.165±0.015               | 0.280±0.026<br>p <sub>1</sub> <0.01 | 0.215±0.027<br>p <sub>1</sub> <0.05<br>p <sub>2</sub> <0.05 |
| Dehydroepiandrosterone sulfate, mkg/ml | 0.015±0.001               | 0.044±0.004<br>p <sub>1</sub> <0.01 | 0.090±0.008<br>p <sub>1</sub> <0.01<br>p <sub>2</sub> <0.01 |

n=Number of observations. p<sub>1</sub>=Index of significant differences within a group of nonpregnant women; p<sub>2</sub>=Index of significant differences within a group of healthy pregnant women

The results of recent research show that DHEA is not only a substrate for the synthesis of sex hormones, but also has its own regulatory effects.<sup>[30,31]</sup> It is proved that DHEA is involved in regulation of energy metabolism,<sup>[32]</sup> controls the activity of the hypothalamic-pituitary level that manages the endocrine status of the organism,<sup>[31]</sup> and develops atrial natriuretic peptide - a major humoral regulators volemic homeostasis.<sup>[33]</sup>

In addition, studies, *in vitro*, found that DHEA can modulate the output of the synthesis of nitric oxide endothelium of blood vessels<sup>[34]</sup> and angiogenesis.<sup>[35]</sup>

At present, it is found that DHEA synthesizes the net area of the cortex of adrenal glands and, during pregnancy, feto-placental complex, influenced by sulfotransferase converted into DHEA sulfate - the major transport form of the hormone, with its subsequent conversion back sulfatase in target tissues.<sup>[30,36]</sup>

It is reported that the intensity of the absorption cell molecule DHEA sulfate is regulated by specific carrier proteins of organic anions and depends on the gradient of sodium and pH, as well as the level of activity of the sodium/potassium ATPase.<sup>[26]</sup> DHEA has the ability to inhibit the pathogenic mechanisms induced by mediators of inflammation.<sup>[28]</sup>

It is revealed that proinflammatory cytokines inhibit the activity of sulphotransferase and reduce their rate of biosynthesis.<sup>[30]</sup> Currently, the study of the pathway of steroid hormones in the tissues of the oral cavity is not enough, so we do not allocate ourselves the task of comparative analysis of levels of investigated hormones in blood plasma and saliva of pregnant women, because their metabolism in tissues, including organs of the oral cavity, can be characterized by a specific regional characteristics. Mechanisms that induce increased concentration of DHEA sulfate in samples of saliva of pregnant women with periodontitis, in our opinion, may be subject to more in-depth, independent research. In addition, attention is drawn to increase the hormone in the saliva of pregnant women with periodontitis under the influence of saline. Perhaps the increase in sodium chloride concentration in saliva during mouthwash brine affects the intensity of the sodium-dependent transport of the hormone in mouth. Indirect confirmation of this assumption is to reduce the concentration of osmotic active substances in the saliva after rinsing the mouth with saline.

Given the antiinflammatory<sup>[28]</sup> and antioxidant<sup>[23]</sup> action of DHEA it may be assumed that the higher hormone levels in saliva samples collected in the group of pregnant women with periodontitis, before and after rinsing the mouth with saline, reflect a protective response in the body in response to the inflammation of periodontal tissues.

## Conclusions

It is established that periodontitis against pregnancy is characterized by higher levels of salivary DHEA sulfate and lower estriol, compared with a control group of pregnant women.

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