



SEX HORMONES IN THE SALIVA AND PERIODONTAL HEALTH OF CHILDREN IN PUBERTY

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ABSTRACT

Introduction: Children at the age of sexual maturation are suitable subjects for studying the influence of sex hormones on periodontal health, due to increased hormone levels as well as the increased frequency of gingival conditions at that age.

Aim: To study the quantity of sex hormones in the saliva and the periodontal health of children in puberty.

Material and methods: 60 children aged 10-14 years subjected to monitoring- 30 without gingivitis (up to 25% PBI) and good oral hygiene and 30 children with plaque-induced gingivitis (over 50% PBI). For the study of sex hormones (estradiol, progesterone and testosterone), samples of unstimulated saliva were taken fasting in the morning, which were tested by using marked immunological analysis and liquid chromatography with mass spectrometry (LC-MS (MS(QQQ))).

Results: The results of this study show that from the ages of 10 to 14, there is no significant difference in the levels of the sex hormones in the saliva ($P>0.05$). In children with gingivitis in relation to healthy children, only estradiol exhibits plausibly higher values ($P<0.05$). Progesterone and testosterone show no variation based on periodontal status ($P>0.05$).

Conclusion: Saliva is an appropriate, non-invasive environment for studying the quantities of sex hormones and their link to oral pathology.

Keywords: sex hormones, saliva, periodontal diseases

INTRODUCTION

Sex hormones have a proven affinity towards the gingival tissue and the alveolar bone. They also affect the formation of the subgingival microflora. Children at the age of sexual maturation are suitable subjects for studying the influence of sex hormones on periodontal health, due to increased hormone levels as well as the increased frequency of gingival conditions at that age [1, 2].

The effects of these hormones on gingival inflammations have not been fully studied yet. While it is undisputed that the biofilm plays a key role in the occur-

rence of a gingival inflammatory response, it is not yet clear whether the sex hormones participate in the potentiation of said response [3, 4].

The development of the “oral-based diagnostics” field – the use of saliva as a non-invasive diagnostic environment – gives the opportunity for the influence of various factors on the periodontal health of children and young adults to be studied, among which factors are also the sex hormones [5, 6, 7]. It is considered that the concentration of sex hormones in the saliva and the blood serum is in a directly proportional correlation [8]. This type of study requires a precise assessment of the gingival status of the children, so that the physiological states of the periodontium can be differentiated: during eruption, states of a healthy, but still unstable periodontium, with nascent inflammatory gingival changes present, with clinically diagnosable processes of gingival hyperplasia present and others [7, 8, 9].

Aim

To study the quantity of sex hormones in the saliva and the periodontal health of children in puberty.

OBJECTIVES

1. To give a description of the periodontal status of children during the period of sexual maturation.
2. To give a quantitative analysis of the sex hormones in the saliva of children with and without plaque-induced gingivitis, during the period of sexual maturation (10 – 14 years of age).

MATERIALS AND METHODS

Subjects of the study were 60 children between 10 and 14 years of age undergoing the processes of sexual maturation and formation of the permanent dentition, who did not suffer from any systemic diseases and had not any antibiotic intake three months prior. The children were divided into two groups:

- 30 children without gingivitis (up to 25% PBI) and with good oral hygiene;
- 30 children with plaque induced gingivitis (over 50% PBI).

The periodontal status of all children was registered through the use of a purpose-made medical card. The following indices were assessed: Oral Hygiene Index of Green Vermillion (OHI-GV), PBI (Papilla Bleeding Index Saxer & Mulhean) – average values (severity) and percentage of bleeding papillae (spread).

Methodology for the study of sex hormones in the saliva

For the study of sex hormones in the saliva (estradiol, progesterone and testosterone) samples of unstimulated saliva were taken in the morning, with the subjects being fasted prior to sampling. The saliva was collected in sterile, plastic containers and the frozen (- 20° C).

- Quantitative analysis of steroid hormones in the saliva

The sex hormones in the saliva of the subjects were studied through a marked immunological analysis and liquid chromatography-mass spectroscopy (LC-MS (MS(QQQ))).

The analyses were carried out with a Q Exactive

(ThermoScientific, Germany) mass spectrometer equipped with a Transcend ThermoScientific, Germany) autosampler, TurboFlow (ThermoScientific, Germany) ultra-effective chromatographic system and a source for chemical ionization – APCI (ThermoScientific, Germany). Registering, storing and processing the data gathered from the analyses were carried out with the Xcalibur 4.1 (Thermo Scientific, USA) software package.

The study was carried out in the “Analysis and Synthesis of Biologically Active Substances” laboratory of the “Medical Chemistry and Biochemistry” department, MU-Sofia, with Associate Professor Valentin Lozanov as lead researcher.

RESULTS

Objective one

To give a description of the periodontal status of children during the period of sexual maturation.

1. Oral hygiene status of the subjects

The results from the OHI-GV assessment of the oral hygiene of the subjects are presented in the tables below.

Table 1. Average OHI-GV values in all children by age

Age	healthy(1)		gingivitis(2)		Ind T-test
	N	Mean ± SD	N	Mean ± SD	
10 y.	6	0.45 ± 0.27	6	1.67 ± 0.21	t _{1,2} =-8.822 p=0.000
11 y.	6	0.28 ± 0.13	6	1.97 ± 0.45	t _{1,2} =-8.788 p=0.000
12 y.	6	0.42 ± 0.38	6	2.38 ± 0.73	t _{1,2} =-5.780 p=0.000
13 y.	6	0.67 ± 0.65	6	2.32 ± 0.43	t _{1,2} =-5.184 p=0.000
14 y.	6	0.99 ± 0.34	6	2.41 ± 0.56	t _{1,2} =-5.291 p=0.000
Total	30	0.56 ± 0.44	30	2.15 ± 0.56	t _{1,2} =-12.235 p=0.000

The data points that that a plausible difference in the level of oral hygiene between the children with plaque-induced gingivitis and their healthy peers is present (p<0.05). No plausible differences can be found between the age groups (p>0.05).

- Oral hygiene status of the subjects, grouped by sex

The results are systematized in the following table.

Table 2. Average OHI-GV values of the subjects, grouped by sex

age	boys(1)		girls(2)		Total		Ind T-test
	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD	
healthy	15	0.58 ± 0.46	15	0.55 ± 0.44	30	0.56 ± 0.44	t= 0.206 p=0.838
gingivitis	15	2.16 ± 0.65	15	2.14 ± 0.47	30	2.15 ± 0.56	t= 0.095 p=0.925
	t=5.280p=0.000		t=5.050 p=0.000				

No plausible differences in the average values of the OHI-GV index can be found between boys and girls (P>0.05).

2. Assessment of the gingival status of the subjects through PBI

- PBI-severity (average value)

The results from the PBI-severity assessment of the subjects are presented in the table below.

Table 3. PBI – severity by age

Age	healthy		gingivitis		Ind T-test
	N	Mean ± SD	N	Mean ± SD	
10 years	6	0.17 ± 0.21	6	0.78 ± 0.09	t _{1,2} =-6.458 p=0.000
11 years	6	0.11 ± 0.12	6	0.99 ± 0.34	t _{1,2} =-5.882 p=0.000
12 years	6	0.19 ± 0.31	6	1.25 ± 0.48	t _{1,2} =-4.546 p=0.000
13 years	6	0.12 ± 0.13	6	1.03 ± 0.38	t _{1,2} =-5.570 p=0.000
14 years	6	0.44 ± 0.33	6	0.86 ± 0.10	t _{1,2} =-2.995 p=0.000
Total	30	0.21 ± 0.25	30	0.98 ± 0.33	t _{1,2} =-10.030 p=0.000
Ind T –test	t _{11,14} =-2.290 p=0.045 t _{13,14} =-2.237 p=0.049 in the remaining cases p>0.005		t _{10,12} =-2.391 p=0.038 in the remaining cases p>0.005		

The data points that the average values of gingival bleeding (PBI-severity) are plausibly higher in children with plaque-induced gingivitis in relation to those of healthy children in all age groups. Higher values can be seen in the ages of 11 and 12 (from 0.99 to 1.25 ± 0.48) in the children with plaque-induced gingivitis, with no plausible differences, present between the age groups.

Objective two

To give a quantitative analysis of the sex hormones in the saliva of children, during the period of sexual maturation (10 – 14 years of age).

The table below presents a comparison between the quantities of the isolated sex hormones (estradiol, progesterone and testosterone) in the saliva. The children are grouped by sex.

Table 4. Average values of sex hormones – a comparison between girls and boys

	Estradiol(ng/ml)		Testosterone (ng/ml)		Progesterone (ng/ml)	
	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD
Girls	30	1.090 ± 0.54	30	0.118 ± 0.07	30	0.186 ± 0.19
Boys	30	0.887 ± 0.53	30	0.116 ± 0.06	30	0.121 ± 0.15
Total	60	0.957 ± 0.53	60	0.117 ± 0.05	60	0.152 ± 0.15
Ind T –test	t _{1,2} =0.271 p=0.787		t _{1,2} =0.059 p=0.953		t _{1,2} =0.197 p=0.849	

The data shows that no plausible differences in hormone levels can be found between girls and boys (p>0.05). samples of unstimulated saliva of children, grouped by age.

Here are the results of the sex hormones tested in

Table 5. Quantity of the sex hormones in children grouped by age

	Estradiol(ng/ml)		Testosterone (ng/ml)		Progesterone (ng/ml)	
	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD
10 years(1)	12	1.044 ± 0.46	12	0.106 ± 0.07	12	0.240 ± 0.29
11 years(2)	12	1.075 ± 0.56	12	0.129 ± 0.05	12	0.205 ± 0.22
12 years(3)	12	0.898 ± 0.47	12	0.102 ± 0.11	12	0.098 ± 0.11
13 years (4)	12	1.187 ± 0.63	12	0.125 ± 0.12	12	0.146 ± 0.16
14 years(5)	12	0.734 ± 0.52	12	0.118 ± 0.04	12	0.095 ± 0.08
Total	60	1.003 ± 0.56	60	0.117 ± 0.07	60	0.153 ± 0.18
Ind T –test	t=0.148÷ 1.327 p>0.005		t=0.036÷ 1.898p>0.005		t=0.036÷ 1.608p>0.005	

The table shows that the average value of estradiol in the subjects is 1.003 ± 0.56 ng/ml, progesterone being at 0.117 ± 0.07 ng/ml and testosterone at 0.153 ± 0.18 ng/ml, with no statistically significant differences between the age groups being present ($P > 0.05$). Thus we can conclude that there are no statistically significant age-based

differences in the quantities of the sex hormones isolated in the saliva ($P > 0.05$).

Average values of the sex hormones that were studied in children with plaque-induced gingivitis and in healthy children are presented in the table below.

Table 6. Average values of sex hormones in children with gingivitis

	Estradiol (ng/ml)		Testosterone (ng/ml)		Progesterone (ng/ml)	
	N	Mean \pm SD	N	Mean \pm SD	N	Mean \pm SD
Healthy	30	0.838 ± 0.53	30	0.105 ± 0.07	30	0.102 ± 0.09
Gingivitis	30	1.252 ± 0.37	30	0.096 ± 0.06	30	0.125 ± 0.06
	$t=2.988$ $p=0.032$		$t=0.598$ $p=0.552$		$t=0.738$ $p=0.469$	

The table shows that only estradiol exhibits higher levels ($P < 0.05$) in children with gingivitis in relation to those without. Progesterone and testosterone exhibit no variation when comparing healthy children to children with gingivitis ($P > 0.05$).

DISCUSSION

Puberty is a temporary physiological state, considered a complex process of sexual maturation, directly linked to increased levels of the steroid sex hormones – estradiol, progesterone and testosterone [2, 3].

The results of this study show that from the ages of 10 to 14, there is no significant difference in the levels of the sex hormones in the saliva ($P > 0.05$). In children with gingivitis in relation to healthy children, only estradiol exhibits plausibly higher values ($P < 0.05$). Progesterone and testosterone show no variation based on periodontal status ($P > 0.05$).

Similar studies by other authors have found out that sex hormone synthesis during puberty increases to a level, which remains constant for the whole reproductive period of the individual [2, 3, 4].

Gingivitis at that age is related to an increase in the quantity of dental plaque. Many research papers show that changes in the hormonal levels are related to the increase of the spread and severity of gingival diseases [4, 7, 10].

The results of this study point to a similar correlation regarding the quantity of estradiol in the saliva of children with gingivitis.

According to other authors, gingival diseases are not necessarily linked to the increase in the quantity of dental plaque [8, 11].

According to Goldie, the gingiva has progesterone and estradiol receptors. Increased plasma levels of estradiol and progesterone cause an accumulation of those hormones in the gingival tissues. Sex hormones have an effect on the microcirculatory system, and they often lead

to noticeable changes in the periodontium. Overall estradiol is behind the changes in the blood vessels, whereas progesterone stimulates the production of inflammatory mediators. Fluctuations in hormonal concentrations cause a dilation of the gingival capillaries and an increase in their permeability and thus lead to an increase in the gingival exudate, edema and accumulation of immune cells and inflammation [12, 13, 14].

The data listed above confirm the well-known position that gingival inflammations may be worsened by physiological conditions, related to increased levels of sex hormones, such as puberty, pregnancy or intake of oral contraceptives. Increased levels of circulating sex hormones are considered to affect the severity of the inflammations as well as the processes of hyperplasia of the gingivitis [15, 16].

It is considered that there is a directly proportional correlation between the concentrations of the sex hormones in the saliva and in the blood serum [17, 18, 19]. This gives a basis to adopt saliva as an appropriate, non-invasive environment for studying the quantity of sex hormones during the period of sexual maturation and their link to oral pathology [8, 12, 13].

CONCLUSIONS:

1. There is no significant change in the levels of the sex hormones in neither girls nor boys from the ages of 10 to 14.
2. Estradiol has some effect on gingival inflammation, while progesterone and testosterone show no effect.
3. Saliva is an appropriate, non-invasive environment for studying the quantities of sex hormones and their link to oral pathology.

Acknowledgements

The authors would like to thank Assoc. Prof. Valentin Lozanov for his excellent technical support of the current project.

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Please cite this article as: Mitova N, Rashkova M. Sex hormones in the saliva and periodontal health of children in puberty. *J of IMAB*. 2019 Oct-Dec;25(4):2817-2821. DOI: <https://doi.org/10.5272/jimab.2019254.2817>

Received: 28/05/2019; Published online: 09/12/2019



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