

SALIVARY AND SERUM MODIFICATIONS OF THE BIOCHEMICAL PARAMETERS IN PREGNANT WOMEN WITH TOOTH DISORDERS

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Abstract: Introduction: Saliva plays an important part in the maintenance of oral health as it contains many innate and acquired factors with a protective role on the oral tissue.(1) Changes in salivary composition during pregnancy induce an increased response of the gingival tissues to local factors, such as plaque, tartar and hard tissues.(2) Purpose: The aim of the study was to investigate the concentrations of salivary and serum concentrations of calcium, phosphate, urea, total proteins and ALT (alanine aminotransferase), AST (aspartate aminotransferase), ALP (alkaline phosphatase) and to assess whether the activities of the enzymes can serve as a tool for the diagnostic of tooth disorders in pregnant women. Materials and methods: The studied batch was divided in the following groups: (1) pregnant women with dental caries n=12 (2) pregnant women with gingivitis n=15 (3) non-pregnant women n =15. The results were compared to a control group matched with the study groups in terms of age, BMI, blood pressure. All determinations were done on HITACHI 912 Roche Diagnostics. Results and discussions: Pregnant women with dental caries had a higher salivary AST 7.23 ± 6.12 U/L than non-pregnant women 2.87 ± 1.9 ($p = 0.03$). We obtained significant variations between serum and saliva for some parameters (see table no. 4). For other parameters, no important differences between serum and saliva ($p > 0.05$) were noticed. Conclusions: An increased salivary enzyme reflects the biological activity in the gingival tissue. Saliva can be used as diagnostic tests in pregnant women with tooth disorders.

Cuvinte cheie: gingivită, carii dentare, gravidă, ser salivă

Rezumat: Introducere: Saliva joacă un rol important în menținerea sănătății orale datorită numeroșilor factori innăscuți și dobândiți având un rol protector asupra țesuturilor orale. Modificările compoziției salivare din timpul sarcinii induc răspuns din partea țesuturilor gingivale la factorii locali cu apariția plăcii și tartrului dentar. Scop: Scopul acestui studiu a fost de a investiga concentrațiile de calciu, fosfat, uree, proteine totale și AST (aspartat aminotransferază), ALT (alanin aminotransferază), ALP (fosfatază alcalină) din ser și salivă și de a evalua măsura în care dozarea enzimelor poate servi ca un instrument în diagnosticarea afecțiunilor dentare la femeile gravide. Material și metodă: Lotul studiat a fost împărțit în următoarele grupe: (1) femeile gravide cu carii dentare n = 12 (2), femeile gravide cu gingivită n = 15 (3) femei negravidă n = 15. Rezultatele au fost comparate cu un grup de control asemănător cu grupurile de studiu privind vârsta, IMC (index de masă corporală), tensiunea arterială. Toate determinările biochimice au fost făcute pe 912 HITACHI Roche Diagnostics. Rezultate: Femeile însărcinate cu carii dentare au avut o concentrație salivară mai mare a AST 7.23 ± 6.12 U/L comparativ cu lotul martor 2.87 ± 1.9 U/L ($p = 0,03$). Am obținut diferențe semnificative între ser și salivă pentru unii parametri Pentru alți parametri nu s-au observat diferențe semnificative între ser și salivă ($p > 0.05$) (vezi tabelul nr. 3). Concluzii: Creșterea enzimelor salivare reflectă o activitate biologică în țesutul gingival. Saliva poate fi utilizată ca modalitate de diagnostic la femeile gravide cu afecțiuni dentare.

INTRODUCTION

Saliva plays an important part in the maintenance of oral health, as it contains many innate and acquired factors with a protective role on the oral tissue. The diffusion of the salivary ions such as calcium and phosphor preserves the teeth enamel which consists essentially of hydroxiapatite, a basic calcium phosphate salt. Many studies have indicated that hormones influence the composition of women's saliva. Saliva contains water, organic and inorganic molecules which are exposed to hormonal changes in females. So, pregnancy, menstruation, and hormone replacement therapy can have a direct effect on the entire body including the metabolism of the periodontal tissues.(1)

Changes in salivary composition during pregnancy induce an increased response of the hard tissues and gingival tissues to local factors such as plaque and tartar. In the physiological state of pregnancy, decreased salivary pH and flow rate associated with changes of salivary electrolyte levels have been demonstrated.(2,3)

An important part among the saliva components are the various enzymes. The response of the organism to the periodontal infection includes the production of several enzyme families which are released from stromal, epithelial, inflammatory or bacterial cells. The analysis of these enzymes in the salivary secretion, can contribute to clarify the

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CLINICAL ASPECTS

pathogenesis and to make a prompt diagnosis of the periodontal disease.(4)

The intracellular enzymes are increasingly released from the damaged cells of the periodontal tissues into the gingival crevicular fluid and saliva, as well as in the surrounding fluids. Some enzymes, such as aminotransferases, alkaline phosphatase, lactate dehydrogenase, gamma-glutamyl transferase appear to be useful in identifying the periodontal diseases or in measuring the effectiveness of the periodontal therapy.(5,6,7,8) Saliva is considered nowadays an important biological material for new diagnostic tests. It has some distinctive advantages: can be collected non-invasively from persons with limited training and without special equipment.(6) The oral pathology is reflected in the changes of salivary biochemical parameters.

PURPOSE

The aim of the study was to assess the salivary and serum concentrations of calcium, phosphate, urea, uric acid, total proteins and AST (aspartate aminotransferase), ALT (alanine aminotransferase), ALP (alkaline phosphatase) of the unstimulated whole saliva in pregnant women with dental caries and gingivitis compared to a control group. The second objective was to investigate whether the activities of the enzymes can serve as a diagnostic indicator in gingivitis and dental caries during pregnancy.

METHODS

The study was carried out on 27 normal pregnant women, 18 - 39 years old, between the fifth and the ninth month of pregnancy, selected among the patients who are attending the Obstetrics and Gynecology Clinics of Oradea. All subjects signed an informed consent form prior to the enrolment. The study was approved by the institutional ethical committee.

The patients were divided in the following groups:

- (1) pregnant women with dental caries n=12
- (2) pregnant women with gingivitis n=15
- (3) non-pregnant women n=15, representing the control group, matched with the studied groups in terms of age, body mass index (BMI), blood pressure.

Diagnostic criteria for dental caries: destruction of the hard tooth tissue, followed by coronal or root cavitation and for the gingivitis - gingival edema, a reddish violet colour of the gingival margin, gum bleeding, spontaneous or induced by teeth brushing or mastication.

Maternal venous samples were collected after overnight fasting. The blood was collected in vacuum tubes. Specimens were transported to the laboratory immediately after collection, centrifuged at 1500g for 10 min to separate serum.

Unstimulated whole saliva samples were collected under resting conditions between 7.30 and 10.30 a.m. The patients were asked to sit passively and spit into sterile plastic containers for 5 min as the saliva accumulated in the floor of the mouth. Saliva samples were centrifuged (centrifugal force: 1,000g) for 10 min. to remove bacteria and other extraneous material. The resulting clarified fluid was used for the biochemical assays.

All biochemical parameters were measured on Hitachi 912 instrument, Roche Diagnostics using Diasys reagents. Urea was determined with urease – GLDH: enzymatic UV test.(9,10) Uric acid was measured with an enzymatic photometric test using TBHBA (2, 4, 6-tribromo-3-hydroxybenzoic acid) (Cat. No. 1 3021 99 10 704).(9,10) The evaluation of total calcium was performed by a photometric test using cresolphthalein complexone (Cat. No.1 1121 99 10 704).(9,10) Inorganic

phosphate was measured using the phosphomolybdate/ UV method (Cat. No.11489348216).(9,10)

For ALP, we used a kinetic photometric test according to IFCC (cat No 1 044199 10 704).(9,10,11) Optimized UV-test according to IFCC (International Federation of Clinical Chemistry and Laboratory Medicine) modified was used for the measurement of AST (Cat. No.1 2701 99 10 704) and ALT (Cat. No.1 2601 99 10 70).(9,10,12) For total proteins we used the photometric test according to the Biuret method (Cat. No. 1 2311 99 10 704).(9,10)

Data are expressed as mean \pm SD (standard deviation). Determination of significant differences was performed by the Student's test. A p value < 0.05 was considered statistically significant.(13)

RESULTS

The biochemical parameters are presented in table no. 1. There was no significant difference in age, body weight, body height, blood pressure between pregnant women and non-pregnant controls at the time of the recruitment (table no. 2).

Table no. 1. Values of the biochemical parameters in pregnant women with dental caries and gingivitis

	AST U/L	ALT U/L	Urea g/l	Calcium mg/dl	Phosphate mg/dl	ALP U/l	Uric acid g/L	Total Proteins g/L
Group 1 serum	16.45 \pm 5.05	10.76 \pm 3.56	0.152 \pm 0.04	8.78 \pm 0.49	3.49 \pm 0.55	-	3.13 \pm 0.50	-
Group 1 Saliva	7.23 \pm 6.12	3.58 \pm 2.53	0.24 \pm 0.17	4.05 \pm 3.04	12.32 \pm 4.74	12.16 \pm 10.25	2.81 \pm 3.33	1.58 \pm 1.03
Group 2 serum	15.17 \pm 1.82	10.52 \pm 2.35	0.11 \pm 0.04	8.78 \pm 0.8	3.89 \pm 0.48	-	3.12 \pm 0.96	-
Group 2 saliva	5.45 \pm 3.38	3.02 \pm 2.47	0.26 \pm 0.14	3.82 \pm 1.22	15.03 \pm 4.96	10.26 \pm 8.41	3.14 \pm 1.62	1.12 \pm 0.71
Control saliva	2.87 \pm 1.90	1.72 \pm 0.91	-	-	-	4.94 \pm 1.50	2.63 \pm 1.44	-

Table no. 2. Values of clinical parameters of the studied groups

	Medium	Age (years)	Body weight (kg)	Body height (m)	Systolic blood pressure (mmHg)	Diastolic blood pressure (mmHg)	Age of pregnancy (weeks)
Group 1 n=12	41.66% U 58.33% R	30.1 \pm 4.95	69.5 \pm 14.69	1.65 \pm 0.06	115.6 \pm 13.25	70.8 \pm 9.70	33.85 \pm 7.60
Group 2 n=15	40% U 60% R	27.08 \pm 4.67	74.08 \pm 12.67	1.67 \pm 0.03	112.08 \pm 11.12	71.91 \pm 10.09	25.95 \pm 8.81
Control Group (n= 15)	70% U 30% R	26.09 \pm 3.74	67.02 \pm 9.80	1.65 \pm 0.05	116.90 \pm 9.53	70.55 \pm 8.31	-

Pregnant women with dental caries had a higher salivary AST than non-pregnant women (p =0.03) (table no. 3). No remarkable differences of the parameters included in table no. 3 were noticed regarding the two groups, neither in serum nor in saliva (p>0.05).

We obtained significant variations between serum and saliva of all studied parameters, except urea in group 1 and uric acid in both groups (table no. 3).

CLINICAL ASPECTS

Table no. 3. The p value of the studied groups

	Group 1 Serum Group 2 Serum	Group 1 Saliva Group 2 Saliva	Group 1 Serum Group 2 Serum	Group 1 Saliva Group 2 Saliva	Group 1 Control Saliva	Group 2 Control Saliva
	p	p	p	p	p	p
AST	>0.05	0.24	0.0001	0.0001	0.03	0.04
ALT	>0.05	0.68	0.0001	0.0001	0.03	> 0.05
Urea	>0.05	0.62	0.21	0.004	-	-
Uric acid	>0.05	0.75	0.78	0.98	> 0.05	> 0.05
Calcium	>0.05	0.81	0.0003	0.0001	-	-
Phosphate	>0.05	0.22	0.0001	0.0001	-	-
ALP	-	0.66	-	-	0.03	0.05
Total proteins.	-	0.24	-	-	-	-

DISCUSSIONS

Estrogen and progesterone receptors are found in the periodontal tissues. In pregnancy, there is a progressive increase of the levels of these steroid hormones which induce a response of the tissues. The extracellular matrix, gingival vessels and fibroblasts are all affected. Estrogen regulates the cellular proliferation, differentiation and keratinisation and thus, estrogen seems to stimulate matrix synthesis and along with progesterone, it enhances the localized production of inflammatory mediators, especially prostaglandin E₂ (PGE₂), a potent stimulant of the osteoclastic activity.(14)

Our research was done on unstimulated whole saliva because this type of saliva predominates during the most part of the day, maintains the oral health reflecting the physiological status of the oral cavity and of the entire body. Saliva contains many enzymes. The enzymes AST, ALT are found in all cells, and their levels vary in different cell types. These are intracellular enzymes included in the metabolic processes of cells and they are mostly present in the cells of the soft tissues.

Cells release these enzymes into the extracellular space during periods of tissue necrosis or trauma which are also detectable in the peripheral circulation. If the periodontal tissue is damaged, due to edema or destruction of a cellular membrane, these intracellular enzymes are increasingly released into the gingival fluid and saliva, where their activity can be measured.(5,7) There was found a high level of ALT in the gingival fluid and has been reported as a possible marker of periodontal disease.(15)

Our results showed a high level of AST and ALT in saliva of pregnant women with dental caries and pregnant women with gingivitis compared to the reference group. These results demonstrated the metabolic changes in the inflamed gums. Since the blood level of the transaminases of our patients are in normal range, this indicates that AST present in saliva has as origin the damages of the gingival tissue. The difference between serum and saliva demonstrated that it is not the result of simply filtration from blood into saliva, but this represents an argument for enzyme source in the damaged oral cells.

Alkaline phosphatase is the name given to a group of hydrolytic enzymes, described as orthophosphoric monoester phosphohydrolases which function by liberating inorganic

phosphate from phosphate esters. In healthy adults, the serum ALP is derived from liver, bone and intestine. The enzyme activity increases with the initiation of the osteogenic activity.(16)

In a histological study, ALP was noticed to be present in gingival blood vessels, periostum and periodontal fibres and also in the gingival corium, playing a part in the cell metabolism, deposition of enamel, dentin matrix, keratinisation and calcification.(16) The level of ALP in the normal gums is slight.

A large amount is found in the periodontal membrane which is located on the surface of the cementum and the alveolar bone. We found a high concentration of ALP in saliva of pregnant women with dental caries or gingivitis which indicates a metabolic reaction of enzymes caused by local inflammation. The determination of these enzymes can be used to monitor the pathological changes in the gingival cells and provides valuable diagnostic and prognostic information about the dental affections of the pregnant women.

We did not find any difference in the concentrations of calcium and phosphate in saliva between the pregnant women with dental caries and those with gingivitis groups. Laine, in 2002, also underlined that pregnancy does not induce significant withdrawal of calcium or other minerals from the teeth. Other researchers reported a significant decrease of calcium level between the 21st and the 40th week of gestation in comparison with the controls and a decrease of phosphate at the 21st week.(17) Fetal bone formation requires 25 to 30g of calcium, almost all of it during the second half of pregnancy. Before the major fetal ossification of late pregnancy, intestinal absorption of calcium is enhanced in the mother and her bone mass increases.(18) The decreased concentration of calcium and phosphorus in the last trimester of pregnancy could increase the incidence of caries.

In our study, the difference between salivary and serum urea in the pregnant women with gingivitis is significantly raised (p=0.004). This may be due to free filtration or to a local catabolic process of proteins. The products of this catabolic process are ammonia, aminoacids and urea which can modify the balance of pH. Bacterial colonization takes place through specific, irreversible adhesion between the acquired pellicle receptors and the bacterial molecules known as adhesins. In the end, the mature plaque is formed. Oxygen and nutrients are scarce in its deeper areas and the accumulation of waste products increases. High concentrations of urea in the plaque encourage the deposition of calcium and phosphorus on the plaque. Further processes may be repeated on the calcified plaque. In the group 1 of the pregnant women with dental caries, the difference between salivary and serum urea was not significant (p=0.21). Salivary total proteins did not differ in group 1 compared to the group 2 (p=0.24). For protein contents of saliva and α -amylase activity, an increase in the first part of pregnancy followed by a decrease at the time of delivery was observed.(19)

On the basis of our study we drew the conclusion that the activities of enzymes AST, ALT, ALP were significantly increased in the saliva of pregnant women with dental lesions compared to those healthy. This is probably a consequence of the pathological processes in soft and hard tissues from which these intracellular enzymes are released into saliva. The simplicity of the non-invasive method of saliva collection imposed the salivary tests like a useful tool in the diagnosis and prognosis of tooth disorders in pregnant women.

CLINICAL ASPECTS

CONCLUSIONS

1. In gingivitis and dental caries of pregnant women, salivary activity of ALP, AST, ALT reflects the biological situation of the gingival tissue.
2. The elevated salivary levels of AST found by us in the period of pregnancy are associated with gingival tissue destruction.
3. There are no significant modifications of salivary Ca and phosphorus in our study groups.
4. The salivary enzymes tested by us can be used in the diagnosis and prognosis of tooth disorders in pregnant women.

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