

The Dynamics of Salivary Parameters in Patients Undergoing Orthodontic Treatment

ROXANA BUZATU¹, SMARANDA LAURA GOTIA^{2*}, ANCA S. VALCEANU¹, IOANA SCROBOTA³, DOINA ONISEI⁴, CAMELIA SZUHANEK⁵

¹ University of Medicine and Pharmacy Victor Babes Timisoara, Faculty of Dental Medicine, Dental Aesthetics, 2 Eftimie Murgu Sq, 300041, Timisoara, Romania

² University of Medicine and Pharmacy Victor Babes Timisoara, Faculty of Medicine, Department of Physiology, 2 Eftimie Murgu Sq, 300041, Timisoara, Romania

³ University of Oradea, Faculty of Medicine and Pharmacy, Department of Dental Medicine, 10, 1 Decembrie Sq, 410073, Oradea Romania

⁴ University of Medicine and Pharmacy Victor Babes Timisoara, Faculty of Dental Medicine, Periodontology, 2 Eftimie Murgu Sq, 300041, Timisoara, Romania

⁵ University of Medicine and Pharmacy Victor Babes Timisoara, Faculty of Dental Medicine, Department of Orthodontics, 2 Eftimie Murgu Sq, 300041, Timisoara, Romania

From assisting in digestion and lubricating soft tissues to neutralizing plaque and promoting tooth remineralization, saliva is crucial to the oral environment. Our objective was to analyze the dynamics of salivary parameters in patients with orthodontic treatment. Subjects were divided into two age-related groups. Results showed an increase in the number of epithelial cells and leukocytes, as well as various changes in glutathione and C-reactive protein levels. Another significant finding was that young people were more prone to developing oral candidiasis after receiving a fixed orthodontic appliance.

Keywords : orthodontic treatment, leukocytes, glutathione (GSH) , C-reactive protein (CRP)

It is well known that saliva, besides the role it plays in digestion, is of utmost importance when it comes to maintaining oral health. First of all, it serves a lubricative function, coating the mucosal surfaces of the oral cavity and preventing desiccation. Second, salivary enzymes help protect teeth from bacterial decay. Orthodontic treatment, while greatly benefiting dental health in the long run, can pose some risks: alterations in oral hygiene habits, gingival response due to proximity or mechanical procedures, plaque accumulation. The introduction of fixed orthodontic appliances into the mouth increases the number of retention areas, and thus the progression of a gingival reaction might result in increased periodontal involvement and damage [1]. Gingivitis, with subsequent evolution towards the more aggressive periodontal disease, can convey its signs to salivary composition. This study was conducted as a means to detect and measure changes in the properties of saliva after one week of orthodontic treatment (it should be noted that only metallic, non-allergenic brackets were used). Variables taken into account were age, salivary flow rate, salivary content, viability of salivary cells, presence of glutathione and C-reactive protein (CRP).

Experimental part

This clinical trial involved 18 patients, all females, divided into 2 groups. The first one comprised of 10 patients within the 12-22 age range (17.7 ± 3.67 years), while the second comprised of 8 patients within the 25-35 age range (30.75 ± 5.72). Illness, medication, previous periodontal treatments and smoking habits were deemed excluding factors in the selection. Each patient was subjected to thorough explanations regarding the nature of this study and went on to sign a written consent. Patients were instructed to avoid teeth brushing and any food or liquid intake 90 min prior to saliva harvesting. Another precaution

concerned forcibly stimulating the salivary glands during the sampling process (patients were advised to refrain from coughing or clearing their throats). The unstimulated saliva was collected for 10 minutes and the salivary gland flow rate was expressed as the volume of saliva (in ml) secreted per min [2]. There was no need to freeze the saliva specimens due to the fact that they were submitted for analysis shortly after collection. Harvesting was performed at the beginning of the study and a week after inserting the fixed orthodontic appliances. Samples were centrifuged at 3000 rpm for 15 min. Salivary sediment was used to determine salivary cells: epithelial cells, leukocytes, and their viability were quantified. GSH and CRP levels were determined from the supernatant liquid. All samples were prepared in duplicate, and the average of the duplicates was used in the statistical analyses. Intra- and inter-assay coefficients of variation were less than 10 and 15% respectively [3].

The cellular content of saliva was determined using cytometry with Burkert Turck camera. Epithelial cells and leukocytes were quantified. The spectrophotometric method for glutathione (GSH) involves oxidation of GSH by the sulfhydryl reagent 5,5,2'-dithio-bis(2-nitrobenzoic acid) (DTNB) to form the yellow derivative 5,2'-thio-2-nitrobenzoic acid (TNB), measurable at 412 nm [4]. All samples were assayed for CRP using a commercially available immunoassay without modification to the manufacturer's recommended protocol. The test volume was 15 μ L, with a range of standards from 93.75 to 3000 pg/mL, and the assay had a lower limit of sensitivity of 10 pg/mL [5]. Another important step was the assessment of cell viability. The dye exclusion test is used to determine the number of viable cells present in a cell suspension. It is based on the principle that live cells possess intact cell membranes that exclude certain dyes, such as Trypan blue, Eosin, or propidium, whereas dead cells do not. In this test,

* email: lauragotia@yahoo.com; Phone: +40745865003

Table 1

VARIATION OF SALIVARY PARAMETERS (M ± SD) BEFORE AND AFTER 1 WEEK OF INTRA-ORAL FIXED ORTHODONTIC APPLIANCE, TO YOUNG PEOPLE

Parameters	Salivary flow rate (mL/min)	Epithelial cells (nr/ μ L)	Leukocyte (nr/ μ L)	Cells viability (%)	GSH (μ mol/L)	Positive CRP (%)
Before	0.608 ± 0.12	1600 ± 916.51	568 ± 312.12	79.4 ± 9.85	36.764 ± 24.36	60
After	0.744 ± 0.178	2120 ± 449.19	900 ± 556.77	78.4 ± 5.75	70.902 ± 47.002	80
Variation vs initial	↑ 22%	↑ 32.5%	↑ 58%	↓ 1.3%	↑ 92%	↑ 33

Table 2

VARIATION OF SALIVARY PARAMETERS (M ± SD) BEFORE AND AFTER 1 WEEK OF INTRA-ORAL FIXED ORTHODONTIC APPLIANCE, TO ADULTS

Parameters	Salivary flow rate (mL/min)	Epithelial cells (nr/ μ L)	Leukocyte (nr/ μ L)	Cells viability (%)	GSH (μ mol/L)	Positive CRP (%)
Before	0.6 ± 0.075	700 ± 226.77	237.5 ± 138.22	90.625 ± 1.76	73.2 ± 35.78	75
After	0.63 ± 0.11	1950 ± 936.55	1200 ± 523.72	94.25 ± 1.38	52.52 ± 28.03	25
Variation vs initial	↑ 5%	↑ 178%	↑ 405%	↑ 4%	↓ 28.25%	↓ 33.3

a cell suspension is simply mixed with Trypan blue and then visually examined to determine whether cells take up or exclude dye. In the protocol presented here, a viable cell will have a clear cytoplasm whereas a nonviable cell will have a blue cytoplasm [6-9].

Mean ±, standard deviation, parameter variation with respect to baseline assessments and the r Pearson correlation index between salivary parameters were calculated for both groups.

Results and discussions

7 days after inserting the fixed orthodontic appliance, salivary flow rate increased by 22% in the first group and only by 5% in the second group. The first group manifested an increase in epithelial cells (by 32.5%), leukocytes (by 58%), CRP (by 33%) and GSH (by 92%) compared to baseline measurements (table 1). After setting the orthodontic appliance, the second group displayed an increase in epithelial cells (by 178%), leukocytes (by 405%), a decrease in GSH (by 28.25%) and CRP (by 33.3%) compared to initial assessments (table 2). The increase in salivary epithelial cells and leukocyte count was considered to represent a defence reaction to the insertion of a foreign body in the oral cavity. Exfoliated epithelial cells in the saliva that exceed normal turnover can reduce the integrity of the oral mucosa, with decreased skin barrier function and risk of local inflammation. The difficulty in maintaining oral hygiene in the presence of an orthodontic

appliance can maintain local inflammatory processes. A rise in cell numbers (exfoliated epithelial cells and leukocytes), CRP and GSH can be traced back to local inflammation. In younger people, the r Pearson test showed positive high age/salivary flow rate ($r = 0.793$) and epithelial cells/GSH ($r = 0.573$) correlations. A negative high correlation was found between epithelial cells and cell viability ($r = -0.932$). These negative correlations can support the idea of a weaker barrier function of the oral epithelium and decreased local defence capacity of salivary leukocytes, the possibility of GSH being released by damaged cells and the presence of certain mediators responsible for the onset and maintenance of local inflammation. Therefore, we recommend a rigorous local hygiene. Baseline investigations in the second group showed negative high age/salivary flow rate ($r = -0.848$), age/epithelial cells ($r = -0.858$) and age/leukocyte count ($r = -0.672$) correlations. Negative age/salivary flow rate correlations associated with negative age/cell viability correlations and the reduced levels of salivary GSH suggest a decrease in the local defence capacity. The large increase of salivary leukocytes in adults after initiating orthodontic treatment (fig. 2) was considered to be a local response designed at stimulating the defence system of the oral mucosa. At one week, the r Pearson test shows negative high age/salivary flow rate ($r = -0.672$), age/epithelial cells ($r = -0.705$) age/salivary leukocytes ($r = -0.914$) and salivary flow rate/cell viability ($r = -0.948$) correlations in adults. Candidiasis, albeit present in some cases before

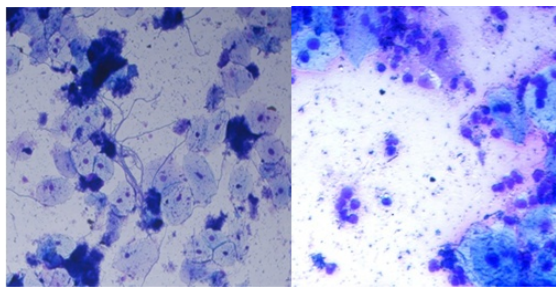


Fig. 1

Fig. 2

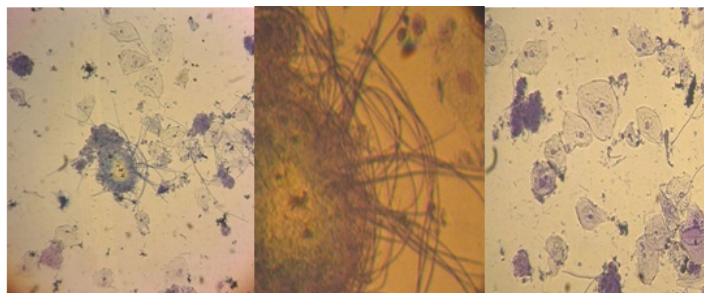


Fig. 3

Fig. 4

Fig. 5

orthodontic treatment, showed signs of reinforcement and manifested widely among the youth group by the time second assessments were made (fig. 1, fig. 3, fig. 4, fig. 5). This tendency was mainly attributed to inadequate hygiene. In adults, orthodontic appliances were also responsible for an insignificant increase in salivary bi nucleated epithelial cells (fig. 1, fig. 5).

Salivary smear (MGG stain): *Candida* filaments, bi nucleated epithelial cell (fig. 1), numerous leukocytes (fig. 2).

Salivary smear (vital Trypan blue coloration): bundle of *Candida* filaments (fig. 3, fig. 4), bi nucleated epithelial cell, sparse *Candida* filaments (fig. 5).

Conclusions

The insertion of a stainless-steel orthodontic appliance triggers almost immediate changes in cellularity and the salivary antioxidant capacity (expressed by GSH), changes that can be noticed and quantified a week after commencing orthodontic treatment. The salivary response in both groups, manifested by an increase in epithelial cells, leukocytes, GSH and CRP, depicts the natural response of the mucosa to the insertion of a foreign body in the oral cavity. Poor hygiene associated with orthodontic appliances may increase the risk of developing oral candidiasis in young people.

References

1. ZACHRISSON, S., ZACHRISSON, B. U., Gingival condition associated with orthodontic treatment, no. 1, vol. 42. Zachrisson and Zachrisson, JUNE 1972, p. 26-34

2. NAGLER, R. M., SALAMEH, F., REZNICK, A. Z., LIVSHITS, V. and NAHIR A.M., Salivary gland involvement in rheumatoid arthritis and its relationship to induced oxidative stress, July 2003, vol. 42, p. 1234-1241
3. DOROTHEE, O., ROSALIE, J. H., GRANGER, D.A., GAYLE, G.P., STEPHANIE, J. W., Assessing salivary C-reactive protein: Longitudinal association with systemic inflammation and cardiovascular disease risk in women exposed to intimate partner violence, vol. 26, no. 4., 2012 May, Brain Behav Immun. p.543-551
4. IRFAN, R., ARUNA, K., SAIBAL, K. B., Assay for quantitative determination of glutathione and glutathione disulfide levels using enzymatic recycling method, January 2007, Nature protocols, p. 3159-3165
5. OWENS, C.W.I., BELCHER, R.V., A colorimetric micro- method for the determination of glutathione, vol 94, 1965, Biochim J., p. 705-711
6. JOHNSON, S., NGUYEN, V., CODER, D., Assessment of cell viability, Curr Protoc Cytom 2013, chapter 9: unit 9.2
7. STÖBER, W., Trypan Blue Exclusion Test of cell viability, 2001, Current protocols in immunology, 21:3B:A.3B.1-A.3B.2
8. OANCEA, R., BONTE, D., PODARIU, A.C., SAVA ROSIANU R., TAMPA, M., MATEI, C., Evaluation of salivary biochemical parameters in caries free and caries active children, Rev. Chim. (Bucharest), **65**, no. 10, 2014, p. 1200
9. SZUHANEK, C., JIANU, R., CIRCIUMARU, L., NEGRUTIU, M., SINESCU, C., CLONDA, C.S., SCHILLER, E., POPA, A., GRIGORE, A., Microstructural changes in orthodontic arch-wires after alternative bending techniques, Rev. Chim (Bucharest), **67**, no. 11, 2016, p. 2363-2364

Manuscript received: 26.11.2016