UNIVERSITY OF MEDICINE AND PHARMACY "VICTOR BABEŞ"
TIMIŞOARA FACULTY OF DENTAL MEDICINE

DEPARTMENT II

BUZATU ROXANA

PhD Thesis

Modification of gingival and periodontal parameters in dental medicine

Scientific Coordinator

PROF. UNIV. DR. ONISEI DOINA

T i m i ş o a r a

2 0 1 7

SUMMARY

The **scientific objective** of the present thesis is to evaluate the possible changes in periodontal health and of its clinical indexes caused by periodontal inflammation at orthodontic patients.

The general part of the thesis starts with a chapter which analyse the gingival components of smile, describing them followed by an thorough full description of the esthetical area periodontal structures especially form an esthetical point of view.

The next chapter analysis the saliva parameters followed by a short description of the periodontal inflammation clinical parameters. After that the chlorhexidine is briefly presented and the chapter is concluded with a review of gingival recession and their classification.

The first study of the special part of PhD thesis was conceived as a prospective clinical trial. Twenty-six patients aged between 20 and 30 years receiving fixed appliance orthodontic treatment in a private practice, were selected to take part in this study. The nature of this trial was explained and understood by each patient before signing a written consent. Ethics Committee approval was obtained for conducting this study.

Only clinical healthy patients were included. Subjects with medication or previous periodontal treatment were excluded, as well as smokers. The patients were undergoing either upper or both upper and lower fixed appliance with a 018 standard edgewise system with brackets. They were undergoing treatment for at least 6 months. One of the mandatory conditions for the subjects to take part in this study was to have at least one site with signs of active gingival inflammation on the basis of the following criteria: bleeding on probing (BOP) at least 30% and a gingival index GI (Lŏe and Silness) greater than 0.5.

The patients were split in two groups. Each subject was clinically examined and plaque index (PI), simplified oral hygiene index (OHI-S), bleeding on probing (BOP), gingival index (GI) and probing depth (PD) were assesed.

The first group (3 males and 10 females) received after scaling, a subgingival application of 10 ml 0.2% chlorhexidine gluconate gel (Glucosite, Cerkamed). Subjects in the second group (4 males and 9 females) received after scaling, a subgingival application of 10 ml 0.1% chlorhexidine digluconate gel (RxPerioflush, Dental Life Sciences). Patients in both groups were then instructed for correct oral hygiene, and further applications of the chlorhexidine gels for the next 2 weeks. The gels were applied twice daily, after tooth brushing and mouthwash, 10 ml each time for 1 minute. Instructions regarding oral hygiene and how to use the study products were explained by an individual who wasn`t involved in the examination procedure. On day 14, subjects were clinically examined again and PI, OHI-S, BOP, GI and PD were assessed by the same periodontist.

T-test and Mann-Whitney U test for 2 samples were used to compare differences between the two groups and sessions. A $p<0.001$ was considered as statistically significant. The software program used for statistical analysis was SPSS version 2.0.

No statistically significant differences were found for baseline parameters between groups. At the 4 weeks' examination, there was a significant decrease in BOP, GI and PD in both groups, compared to baseline ($p<0.001$). However, there was only a slight decrease, not statistically significant, for PI and OHI-S in the experimental group where the 0.2% chlorhexidine gel was applied, in comparison to the group using the 0.1% chlorhexidine gel, which showed significant decreases ($p=0.000$). A notable statistically significant difference ($p=0.000$) was found for GI, between groups, after 4 weeks. Subjects that used the 0.2% chlorhexidine gluconate gel had a major decrease in GI values. Even though, BOP might not have had such a spectacular evolution, a decrease in GI values might mean that a higher concentration of chlorhexidine has a stronger effect on gingival inflammation.

In the management of periodontal disease, a core element of therapy is effective tooth brushing. In some circumstances, however, chlorhexidine may be used as an adjunctive treatment. A possibility for those orthodontic patients who are undergoing a long orthodontic treatment is the local application of chlorhexidine gels.

The results of the present study seem to agree with the findings of previous studies where chlorhexidine gluconate was used in a similar population.

Conclusions

Within the limits of this study, we showed that usage of chlorhexidine gels in patients undergoing orthodontic treatment reduce PI, GI and BOP and PD, but no significant difference exists, except for the initial phase of the inflammatory process of the gingival tissue. Thus, this study showed that additional chlorhexidine usage can reduce gingival inflammation and dental plaque, but this effect is slightly depended upon the concentration used.

The second study of the thesis was a clinical trial that involved 18 patients, all females, split in two 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 minutes 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 minute. There was no need to freeze the saliva specimen's 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 mins. 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.

The cellular content of saliva was determined using cytometry with Burker Turck camera. Epithelial cells and leukocytes were quantified. The spectrophotometric method for glutathione (GSH) involves oxidation of GSH by the sulfhydryl reagent 5,5′-dithio-bis(2-nitrobenzoic acid) (DTNB) to form the yellow derivative 5′-thio-2-nitrobenzoic acid (TNB), measurable at 412 nm. All samples were assayed for CRP using a commercially available immunoassay without modification to the manufacturer's recommended protocol. The test volume was 15 µL, 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. 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, 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.

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.

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.

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 = -0858) 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 was 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 orthodontic treatment, showed signs of reinforcement and manifested widely among the youth group by the time second assessments were made. 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.

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.

The thesis special part third study its called Investigation of gingival esthetical modifications in orthodontic treatment and their relevance towards gingival inflammation. Its aim was to evaluate the association between the orthodontic treatment and the possibilities of esthetical gingival modifications occurrence. The study included 38 patients which were clinical investigated from a periodontal, dental and dental esthetical point of view before and after orthodontic treatment. The orthodontic systems used were metallic brackets from American Orthodontics and ORMCO. The brackets had a 0.022" Roth slot and the initial strings were NiTi made. The patients were all in good health, with an average age of 26.24± 3.642 years, 31 were female and 7 males. The following clinical parameters were registered: API, BOP, GI, OHI-s, PD, REC. The gingival recessions were recorded as present or absent without taking into consideration there Classes. From an esthetical point of view it was analysed the gingival biotype of each patient, its gingival height, GAL line and GAL angle of each dental arch.

The statistical analysis was performed with Mann-Whitney test for statistical semnificative differences of the clinical parameters distribution

between the two moments of clinical examination. The signifiante level was $p<0.05$. Wilcoxon test was also used to compared the parameters values. Also Chi square test and Student T test were used to calculate the statistical significative differences between the esthetic parameters. ($p<0.05$).

The following conclusions resulted from the study: The orthodontic treatment with fix devices lead to significant changes in gingiva esthetical parameters. Those changes can be quantified and have a big impact on dental aesthetics. Because of this it is important for every treatment plan to take into consideration at start the dental esthetical modifications. Our study concluded that the gingival height distribution through classes has remain unchanged after the completion of the orthodontic treatment. The number of gingival recessions have significant increased after the completion of the orthodontic treatment. There is no correlation between patient sex and the gingival recessions. Our study has shown a significant reduction of GAL Classes II, III and IV.

At the end the thesis is concluded by the description of the personal contributions: Considering the increased number of esthetic dental treatments and the increasing need for achieving better aesthetic results, I consider that the PhD thesis deals with a modern subject of high scientific relevance, which is the evaluation of the changes in the health status of the periodontium and its esthetic parameters, caused by periodontal inflammation in patients receiving orthodontic treatment; The originality of this paper can be found in the analysis of the changes of the esthetic parameters of the gingiva of orthodontic treated patients realized in a clinical trial setup. Esthetic changes have been analyzed not only from the orthodontist point of view, but also from the periodontology specialist and patient perspective; One of the concepts proposed by this study is to modify the orthodontic treatment plan of the patient taking into account from the beginning of its developing the possible changes in gingival esthetics., Also, the studies performed showed esthetic changes due to periodontal inflammation occurring in patients receiving orthodontic therapy and also underline the necessity of enrolling a periodontology specialist in order to prevent and fight it.