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Mandibular Lower Incisors: Alternative Approach for Ligature-Induced Periodontitis Model in Rat, Clinical and Bone Serum Biomarker Assessments

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Authors' contributions

This work was carried out in collaboration among all authors. Author MHZ designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author MFHH managed the analyses of the study. Author FHAB managed the literature searches. All authors read and approved the final manuscript.

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Short Research Article

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ABSTRACT

Aims: The ligature-induced periodontitis model is one of the standard approaches through ligating the second maxillary molar tooth in rat models, however the procedure is technique sensitive, thus we proposed the technique of ligation of the lower incisor.
Place and Duration of Study: Animal Laboratory, Faculty of Dentistry, Universiti Teknologi MARA, Sungai Buloh Campus, Selangor, Malaysia.
Methodology: 16 rats were used in the model to simulate periodontal tissue destruction by a two-week ligature placement around the mandibular incisor. The rats were analyzed for clinical attachment loss and radiographical finding to evaluate presence inflammatory bone loss, as well as changes in bone serum biomarkers.
Results: Two weeks after ligature placement gingival inflammation was significantly induced as well as increase depth of gingival sulcus accompanied by increased plaque level at local site. Mean probing depth increased from 0.41 ± 0.02 mm to 1.35 ± 0.04 mm as well radiographical changes increase from 6.41 ± 0.02 mm to 7.36 ± 0.04 mm after 14 days of ligature which are both

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statistically significant (P < 0.05). Bone serum biomarker assessment also shows significant difference for of OPG from 574.06 ± 30.76 to 508.70 ± 18.30, DKK1 at T0 was 1163.29 ± 56.87and 1154.86 ± 63.99, SOST from 1598.06 ± 172.77 to 1425.35 ± 225.75 and FGF23 from 590.27 ± 13.87 to 624.00 ± 18.34.

Conclusion: This proposed technique is likely to facilitate the use of the rat ligature-induced periodontitis model and thus add to a better understanding of the immunopathological mechanisms of periodontitis.

Keywords: Ligature; rat model; oral infection; periodontitis; animal study; methodology; periodontal disease; biomarkers.

1. INTRODUCTION

Chronic periodontitis by definition is "an inflammatory disease of the supportive tissues of the teeth caused by specific microorganisms or groups of specific microorganisms, resulting in progressive destruction of the periodontal ligaments and alveolar bones with increased probing depth formation, recession, or both" [1].

Animal models have contributed to the development of novel understanding in the biological sciences as well as periodontology. The use of animal models has provided a vast collection of data, where it is sometimes difficult to determine whether the findings are relevant to humans. Rats are regularly used in experimental periodontitis models as periodontal anatomy in the molar region shares specific similarities with humans [2].

Numerous literatures indicate that ligature-induced periodontitis model has been used regularly in mice rather than in larger animals, as for example in rats, dogs, or primates. This was a common choice because it was the most convenient, low-cost, and versatile model. One of the significant advantages of the ligature-induced periodontitis model is that the disease model can be initiated at any known time along with a predictable sequence of events leading to alveolar bone loss in days, e.g. in the mouse and rats, or several weeks in the case of dogs and non-human primates [2].

The practical procedure involves placing ligatures usually made of silk or cotton around the tooth of the animal. These ligatures are believed to facilitate local accumulation of dental biofilm, thereby enhancing bacteria-mediated destruction and alveolar bone loss. The present method of molar ligation however requires special equipment as well as a complex procedure. This method is also tending to cause operative trauma of the subjects [3]. Thus, emphasizing the aim of the current study, to assess alternative means in the induction of periodontitis in rats through ligature of the lower mandibular central incisors, evaluated clinically, radiographically and through assessment of bone serum biomarkers.

2. MATERIALS AND METHODS

2.1 Animal

A number of 16 male rats of Sprague-Dawley species is used with weight of 100- 150 mg generally obtained from LAFAM (Laboratory Animal Facility and Management) UiTM. The rats were screen to look for signs of any abnormality, infection or diseased. Following this they are then were placed in UiTM Sg Buloh animal holding for three days for acclimatization.

2.2 Experimental Ligature Induced Periodontitis Procedure

All procedures for the rats were performed under intraperitoneal administration of Zoletil 100 (250 mg tiletamine, 250 mg zolazepam) 50 mg/Kg. Experimental periodontitis model was induced with the placement of 3.0 silk ligature in a figure of "8" around mandibular central incisors and secured on buccal surface (Fig. 1). Suture was applied and tied gently to prevent damage to the periodontal tissue. Daily check of the ligature was made after the application and in case of loss of ligatures, fresh ligatures were placed. The ligatures remained in place in all rat throughout the experimental period.

Fig. 1. Mandibular incisor ligation

2.3 Clinical Data

Records were taken in relation to the incisal tip till the deepest probable periodontal pocket using Williams probe per millimetre marking and clinical stent. Attachment level changes were presented in mm and values indicated attachment loss is assessed by the change of measurement from day 1 to day 14. An interrater reliability analysis using the Kappa statistic was 0.817 with $p < 0.001$.

2.4 Radiographical Data

Intra oral periapical radiograph measurement was made from incisal tip to the base of the alveolar bone with the sample held in standardized acrylic position indicator for standardization. Periodontal alveolar bone changes were presented in mm and values by digital radiographic software (Easy Dent V4 Viewer, Software version 4.1.4.5), indicated bone loss is assessed by the change of measurement from day 1 to day 14.

2.5 Biochemical Analysis

While under anesthesia, blood sample were taken from the rat's tail intra venous method. Blood were collected in a capillary tube; an amount of 0.2 ml was needed for testing by Miliplex (ELISA) for serum levels of Osteoprotegerin (OPG), Dickkopf WNT signaling pathway inhibitor 1 (DKK1), Sclerostin (SOST) and Fibroblast growth factor 23 (FGF23). Blood sample were taken on day 1 and day 14.

2.6 Statistical Analysis

The data collected was entered into a Microsoft Excel data sheet and analyzed using IBM SPSS Statistics version 25 for Windows.

3. RESULTS

3.1 Clinical Data

Data was normally distributed between two sample groups observed by the differences of probable incisal length alterations at ending inspection except. The total attachment level measurement is taken from total probable incisal length of day 1 minus total probable incisal length day 14. It was found that mean probing depth initially was 0.41 ± 0.02 mm, and after 14 day was 1.35 ± 0.04 mm (Table 1). Kruskal-Wallis H test was done showing a *P* value of <0.05 concluding that there is statistical significance difference between groups.

Table 1. Periodontal attachment level changes

Variables	T0	T14	P Value	X^2 Statistic (df) a
Attachment level (millimeters)				
Mean SD	0.41 ± 0.02	1.35 ± 0.04		
Median	0.36	1.36	0.001	28.12 (1)
Variance	0.01	1.35		
Kruskal-Wallis H Test				
SD Standard Deviation				
Significant Level set at p value < 0.05				

3.2 Radiographical Data

Data was normally distributed between two sample groups. The total alveolar bone level changes found that mean probing depth initially was 6.41 ± 0.02 mm, and after 14 day was 7.36 ± 0.04 mm. Kruskal-Wallis H test was done showing a *P* value of <0.05 concluding that there is statistical significance difference between groups (Table 2).

3.3 Bone Serum Biomarker Assessments

Mean total serum concentration of OPG at T0 was 574.06 ± 30.76 and 508.70 ± 18.30 for T14. Kruskal- Wallis H test was done showing a *P* value of >0.05 concluding that there is no statistical significance difference between groups as a whole (Table 3).

Mean total serum concentration of DKK1 at T0 was 1163.29 ± 56.87and 1154.86 ± 63.99 for T1 group. Kruskal- Wallis H test was done showing a *P* value of <0.05 concluding that there is statistical significance difference between groups as a whole (Table 4).

Mean total serum concentration of SOST at T0 was 1598.06 ± 172.77 and 1425.35 ± 225.75 for T1 group. Kruskal- Wallis H test was done showing a *P* value of <0.05 concluding that there is statistical significance difference between groups as a whole (Table 5).

Mean total serum concentration of FGF23 at T0 was 590.27 ± 13.87 and 624.00 ± 18.34 for T1 group. Kruskal- Wallis H test was done showing a *P* value of >0.05 concluding that there is no statistical significance difference between groups as a whole (Table 6).

Table 1. Radiographic bone level changes

Variables	T0	T14	P Value	X^2 Statistic (df) a
Attachment level (millimeters) Mean SD	**6.41 ± 0.02**	**7.35 ± 0.04**		
Median	**6.40**	**7.35**	**0.001**	**28.13 (1)**
Variance	**0.01**	**0.28**		
Kruskal-Wallis H Test				
SD Standard Deviation				
Significant Level set at p value < 0.05				

Table 2. Serum OPG levels

Variables	T0	T14	P Value	X^2 Statistic (df) a
OPG (Pg/ml)				
Mean SD	**574.06 ± 30.76**	**508.70 ± 18.30**		
Median	**632.60**	**494.36**	**0.130**	**-1.52 (2)**
Variance	**22073.35**	**8033.02**		
Kruskal-Wallis H Test				
SD Standard Deviation				
Significant Level set at p value < 0.05				

Table 3. Serum DKK1 levels

Variables	T0	T14	P Value	X^2 Statistic (df) a
DKK1 (Pg/ml)				
Mean SD	1163.29 ± 56.87	1154.86 ± 63.99		
Median	1135.21	1702.01	0.001	-4.51 (2)
Variance	77607.21	98387.32		
Kruskal-Wallis H Test				
SD Standard Deviation				
Significant Level set at p value < 0.05				

Table 4. Serum SOST levels

Variables	T0	T14	P Value	X^2 Statistic (df) a
SOST (Pg/ml)				
Mean SD	1598.06 ± 172.77	1425.35 ± 225.75		
Median	1582.34	1191.46	0.004	-2.87 (2)
Variance	238806.68	407697.77		
Kruskal-Wallis H Test				
SD Standard Deviation				
Significant Level set at p value < 0.05				

Table 5. Serum FGF23 levels

Variables	T0	T14	P Value	X^2 Statistic (df) a
FGF 23 (Pg/ml)				
Mean SD	580.27 ± 13.87	624.00 ± 18.34		
Median	592.89	603.56	0.369	-0.897 (2)
Variance	4616.40	8070.38		
Kruskal-Wallis H Test				
SD Standard Deviation				
Significant Level set at p value < 0.05				

4. DISCUSSION

The current study opts for the mandibular incisor ligation. This is based on a study with the practical placement of thread around the cervical region of the lower incisors, the ligature placement encouraged gingival inflammation and the initial sign and symptoms of periodontitis were noted as early as the third day of experiment [3]. The method also noted that there is a significant alveolar bone loss confirmed via histopathological analysis done after 14 days. While other researches on the same procedural technique also noted presence of alveolar bone loss, however at different point of time, as some occurs in the seventh day of induction [3].

After 14 days, inflammatory modifications detected clinically for instance plaque accumulation around the ligated silk thread and the dentogingival junction, increased tooth mobility, gingival tissue bleeding, and changes in probing pockets depth as well as biological modifications trough changes in OPG concentration showing significance findings. Kruskal- Wallis H test was done showing a p value of <0.05 concluding that there is statistical significance difference between groups (p 0.01). Analysis however shows no statistically significant finding was noted between groups.

The present study shown that there is statistical significance difference between groups as a whole in comparison serum levels of DKK1 post ligature induction. Other studies have revealed that the mRNA and protein levels of Dkk1 were elevated in cases of chronic periodontitis compared to the non-periodontitis group [4]. Wnt proteins and the antagonist of Wnt, Dkk1 played important roles in bone remodeling. The research noted that the expressions of Wnt3a and Dkk1 by ELISA and immunohistochemistry, showing Wnt3a was significantly reduced in rats with periodontitis, this reduction follows a time-dependent manner, while Dkk1 was significantly elevated as similar manner. Studies have revealed that the mRNA and protein levels of Dkk1 were elevated in cases of chronic

periodontitis compared to the non-periodontitis group. Wnt proteins and the antagonist of Wnt, Dkk1 played important roles in bone remodeling. The research noted that the expressions of Wnt3a and Dkk1 by ELISA and immunohistochemistry, showing Wnt3a was significantly reduced in rats with periodontitis, this reduction follows a time-dependent manner, while Dkk1 was significantly elevated as similar manner [5].

Sclerostin (SOST) and DKK1 both are known inhibitors of bone formation, while it was noted that their tissue distribution is dissimilar. Osteocytes or mature osteoblasts are the main tissue expressing Sclerostin while tissue distribution of DKK1 is wider. In clinical studies it has been seen that the GCF level of sclerostin in patients with chronic periodontitis was significantly elevated than that in healthy individuals. The current study shows that a significant change is noted higher in post induction of ligature induce periodontitis. This has been also in agreement with a similar study in which the sclerostin amount and the ratio RANKL to OPG in GCF of periodontal diseases were examined; the GCF amount of sclerostin may be more consistent than the RANKL/OPG ratio as a prognostic and diagnostic marker of periodontal disease as well as treatment outcome. Sclerostin may be related with periodontal disease and is possibly a possible candidate for bone defense and an effective therapeutic objective for management of periodontal diseases [6].

FGF23 is responsible for metabolism of both phosphate and vitamin D. The main role of FGF23 is the plasma regulation of phosphate concentration. FGF23 is secreted via osteocytes in response to increase level of calcitriol. In relation to the oral cavity, severe dental disease including abscesses, periodontal disease manifestation of systemic disease and malocclusion has been observed in phosphate deficient patients in relation to irregular FGF23 expression, Loss of dental attachment can result from defects in cementum, periodontal ligament and/or alveolar bone [7]. With respect to the function of FGF23; no statistical difference was noted in comparison before and after induction. Although further experiments are needed, assumption can be made that serum phosphorus levels might not have influence in periodontal disease as supported by other research [8] that serum phosphorus levels in patients having untreated form of chronic periodontitis have been shown to be similar in comparison to patients that was treated.

The experimental procedure of mandibular incisor ligation in this study delivered the expected results, as the silk thread acting as a bacterial plaque retentive factor it contributes to periodontitis seen in the changes in clinical findings. In the end of the experiment at day 14 significant clinical changes was noted in regards to changes in gingival bleeding, tooth mobility, and clinical attachment level. This has suggested in previous research that bacterial accumulation is the inducer that leads to a host response, which in then promotes infiltration of inflammatory cell, osteoclast formation and differentiation, alveolar bone loss and the loss of tooth attachment.

5. CONCLUSION

The rationale of usage of this procedure is that the simplification of surgical intervention by decreasing the operative trauma of the subjects as in molar ligation [3].

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined. Ethics for animal was obtained from the UiTM Animal Ethics Committee (ref- 600 FF.PS.17/2/1).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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