Salivary RANKL and OPG gene expression quantification during intermaxillary elastic traction in orthodontic patients

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Abstract

This protocol describes a non-invasive workflow for quantifying salivary RANKL and OPG gene expression in orthodontic patients undergoing intermaxillary elastic traction. Unstimulated whole saliva was collected by passive drooling at three predefined time points: baseline before elastic initiation (T0), 24 h (T1), and 7 days (T2), from 30 female orthodontic patients allocated to Class I fixed appliance-only, Class II elastics, and Class III elastics treatment groups. Salivary pellets obtained by sequential centrifugation and physiological saline washing were subjected to column-based total RNA extraction, spectrophotometric quality control, and first-strand cDNA synthesis. Relative gene expression of RANKL and OPG was quantified by RT-qPCR using β-actin as the internal reference gene. Fold-change expression was calculated relative to individual patient baseline values. Group and temporal comparisons were performed using linear mixed models with Bonferroni-

corrected pairwise contrasts. This protocol provides sufficient procedural detail for direct replication in prospective cohort investigations of mechanically induced molecular events in orthodontic treatment.•A complete clinical workflow using non-invasive saliva collection for column-based RNA extraction and RT-qPCR amplification following MIQE guidelines.•Optimised pre-analytical and analytical steps.•A prospective three-group design enables isolation of vector-specific gene expression changes during fixed appliance mechanics.

Author keywords

Clinical orthodontics; Orthodontic elastic; Orthodontic tooth movement; OTM biomarkers; RT-qPCR

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