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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 columnbased total RNA extraction, spectrophotometric quality control, and first-strand cDNA synthesis. Relative gene expression of RANKL and OPG was quantified by RT-qPCR using beta-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. center dot A complete clinical workflow using non-invasive saliva collection for column-based RNA extraction and RT-qPCR amplification following MIQE guidelines. center dot Optimised pre-analytical and analytical steps. center dot A prospective three-group design enables isolation of vector-specific gene expression changes during fixed appliance mechanics.

Keywords

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

Keywords Plus: WHOLE SALIVA

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Data availability statement

Data will be made available on request.

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