

UNIVERSITI TEKNOLOGI MARA

**ELUCIDATING SALIVARY
BIOMARKERS IN POST-
ORTHODONTIC PATIENTS USING
ENZYME-LINKED
IMMUNOSORBENT ASSAY (ELISA)**

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Dissertation submitted in partial fulfilment
of the requirements for the degree of
Doctor in Orthodontics

Faculty of Dentistry

July 2025

ACKNOWLEDGEMENT

First and foremost, I would like to express my deepest gratitude to Allah, the Most Powerful and Most Merciful, for granting me the opportunity to embark on and complete this journey in the Doctor in Orthodontics course.

This journey would not have been possible without the guidance, support and encouragement of many individuals. My deep appreciation and utmost respect go to my main supervisor, Professor Dr Aida Nur Ashikin Abd Rahman, and my co-supervisors and co-researchers, Associate Professor Dr Saied Reza Doustjalali, Professor Dr Rohana Ahmad, Associate Professor Ts Khor Goot Heah, and Associate Professor Dr Negar-Shafiei Sabet. Their expertise, mentorship and patience have been instrumental in shaping the direction and quality of my research.

I would also like to extend my sincere thanks to Mr Yusri Idorus and Mr Ong Chin Chui for their invaluable assistance with the laboratory work, which greatly contributed to the successful completion of this study.

Special thanks to my clinical supervisors, senior colleagues and my beloved batchmates, the Orthobots, for their support and camaraderie, especially during the clinical phase. I am sincerely grateful to all the patients who participated in this study for their time, trust and commitment.

I am also sincerely grateful to the Ministry of Health Malaysia, my employer, for granting me the opportunity to pursue this postgraduate study and for supporting it through a scholarship.

I would also like to acknowledge the financial support received from the UiTM Postgraduate Grant and the Selangor UiTM Grant, which contributed significantly to the completion of this research.

To my beloved mother and the rest of my family, thank you for your unwavering love, patience and prayers. Your strength and encouragement have been a pillar of support throughout this journey.

To my dearest husband, Mr. Mohd Khairul Fatasha, and our wonderful children, this dissertation is dedicated to you. Your steadfast support, sacrifices and love have been the bedrock of my journey, and I am forever grateful to have you by my side.

Lastly, with all my heart, I wish to honour the memory of my late father, Mr. Omar Ibrahim, whose love continues to live within me. Though he is no longer here to witness this milestone, I carry his hopes, his values and his quiet strength in everything I do. This accomplishment is deeply rooted in the faith he had in me and in the dreams we once shared. This dissertation is, in many ways, also his.

ABSTRACT

Tyrosine protein kinase Tec (Tec) is known to activate calcium signalling, which plays a crucial role in bone remodelling. Nuclear envelope spectrin repeat protein 2 (Nesprin-2) is an outer nuclear membrane protein that contributes to cellular mechanosensation, particularly in osteocytes, which influence bone resorption. Zinc finger proteins ZNF729 and ZNF648 are involved in various biological functions and are believed to maintain the dynamic balance of the “pressure tension theory” during orthodontic tooth movement. This study aimed to elucidate the expression patterns of these proteins during the retention phase using the enzyme linked immunosorbent assay (ELISA). Unstimulated whole saliva (5 ml) was collected via passive drooling from 10 post orthodontic patients and 10 healthy controls. Samples from the treated group were collected at debonding (T1), six months post debonding (T2), and one year post debonding (T3). Salivary concentrations of Tec, Nesprin-2, ZNF648, and ZNF729 were analysed using commercially available ELISA kits. Clinical parameters, namely incisor irregularity, intercanine width, and intermolar width, were recorded at each timepoint in the treated group. Independent sample t-tests were used to compare T1 protein levels with controls, while repeated measures ANOVA assessed changes over time. Spearman’s correlation analysis explored associations between protein levels and clinical measurements. All four proteins were active during the post orthodontic retention phase. At T1, Tec, ZNF729, and ZNF648 levels were significantly lower than those in the control group. Nesprin-2 also showed reduced levels at T1, but without statistical significance. Across timepoints, all protein levels increased. Tec exhibited a gradual rise from T1 to T3; Nesprin-2 and ZNF729 showed marked increases between T2 and T3; and ZNF648 rose sharply from T1 to T2. By T3, all protein concentrations had either approached or surpassed control levels. In terms of clinical changes, maxillary parameters including incisor irregularity, intercanine width, and intermolar width remained stable over time. In the mandible, although the irregularity index showed a significant reduction, it was considered clinically acceptable and did not reflect true relapse. Minimal changes were observed in mandibular intercanine and intermolar widths. Since no subject exhibited a relapse greater than 3 mm, a relapse group could not be formed, and comparisons between relapse and non relapse groups were not feasible. Correlations between protein levels and clinical data at T1 were not included in the main analysis, as T1 served as the baseline for both protein expression and clinical parameters. From T2 to T3, some variations in correlations were observed, potentially due to different cellular mechanisms, protein expression dynamics, and local inflammatory responses. The strongest and most significant correlations were observed one year into the retention phase between Tec and ZNF729 with the upper and lower incisors, as well as the intermolar width of the maxilla. This suggests that protein expressions were consistent with changes in tooth movement at T3. The findings of this study indicate that Tec, Nesprin-2, ZNF729, and ZNF648 have potential as biomarkers for monitoring the outcomes and stability of completed orthodontic treatment.

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CHAPTER 1

INTRODUCTION

1.1 Research Background

Orthodontics is a specialised field of dentistry focused on positioning teeth to ensure proper function and stability while also achieving aesthetic appeal. These functional and aesthetic aspects are closely related and must be considered throughout orthodontic treatment. Orthodontics is generally divided into two phases: the active treatment phase and the retention phase (Jelić, 2024; Pezza Joseph, 1973).

During the first, active phase, teeth are moved into their ideal alignment, ensuring that the maxillary and mandibular apical bases are properly aligned. However, this phase does not complete the treatment or stabilise the new occlusions; it only marks the transition to the next phase, retention.

The retention phase is passive and aims to maintain the teeth's positions achieved during the active treatment, ensuring that this alignment remains stable afterwards. This phase is crucial for preventing relapse, where the teeth may return to their original misaligned state. Relapse can compromise the long-term success of orthodontic treatment and reduce patient satisfaction, making it a key focus in both orthodontic research and clinical practice (Jelić, 2024).

Post-treatment stability remains one of many significant concerns among orthodontists. Even after successfully establishing ideal occlusion, the arduous work of maintaining the teeth in their proper alignment continues. On the other hand, patients frequently have different expectations since they anticipate their treatment outcomes to last many years, if not for life, due to their investment of time, effort, and money in orthodontic treatment.

According to Maltha et al. (2017), there are three or four probable causes of post-treatment changes; namely, reorganisation of the supporting tissues of the teeth in their new position; neuromuscular imbalance due to the new potentially unstable situation; continued facial growth and ageing after orthodontic treatment; and lasting unwanted habits.

The majority of orthodontic stability studies were conducted over a lengthy