Original Research Article

SALIVARY TEC AND NESPRIN-2 LEVELS IN POST-ORTHODONTIC PATIENTS

ABSTRACT

Introduction: Tyrosine-protein kinase protein (Tec) is known in activating calcium signalling, which is significant in bone remodelling, while nuclear envelope spectrin repeat (Nesprin) 2, is an outer nuclear membrane protein that provides cells with mechanosensory functions, including in osteocytes. Osteocytes, in turn, take role in promoting bone resorption.

Aim: To quantify the levels of salivary Tec protein and Nesprin-2 among control and post-orthodontic patients, using an enzyme-linked immunosorbent assay (ELISA).

Study design: A quasi-experimental study

Place and Duration of Study: Centre for Paediatric Dentistry and Orthodontics Studies, Faculty of Dentistry, Universiti Teknologi MARA (UiTM), Sungai Buloh Campus, Sungai Buloh, Selangor, Malaysia, between September 2022 and September 2023.

Methodology: Collection of a 5 ml unstimulated whole saliva samples from each subject: 10 healthy individuals as the control group, and 10 post-orthodontic patients at the immediate debond stage. Concentrations of Tec protein and Nesprin-2 were determined using commercially available ELISA kits. An independent-sample t-test was conducted to compare the scores between the two groups.

Results: The mean salivary Tec protein level was significantly lower in the saliva of post orthodontic subjects compared to the healthy control group ($P<0.05$). Nesprin-2 level was slightly lower at immediate debond, but the difference was small without statistical significance.

Conclusion: This study highlighted reduced activity of bone remodelling at the immediate debond stage, by decreased level of salivary Tec protein and Nesprin-2 at the immediate debond stage. These two proteins may be useful in orthodontic retention monitoring.

Keywords: Orthodontics, salivary proteins, retention, ELISA, saliva.

1.INTRODUCTION

Long-term orthodontic stability studies indicate a complex interaction among treatment modalities, retention protocols, and individual patient factors. Recent studies highlight the significance of retention strategies, and the diversity of outcomes associated with various orthodontic methods [1–3]. These factors have been demonstrated to lack reliability as predictors in long-term stability. Relapse can be defined as any unwanted change in tooth position that deviates from a corrected malocclusion following orthodontic treatment [4]. The relapse of lower incisor irregularity is a challenge in orthodontics, resulting in crowding. Research demonstrates that fixed retainers can reduce this issue; however, a certain level of relapse remains evident.

A study indicated that the Little Irregularity Index (LII) showed improvement post-treatment; however, it increased to a medium degree after two years, suggesting relapse [5]. Factors leading to this relapse include the detachment of retainers, changes in intercanine width, and the buildup of biofilm [5,6]. Some studies indicate that the relationship between treatment modalities and relapse is complex, suggesting that biological factors may significantly influence the stability of incisor alignment [7]

In past centuries, orthodontic relapse studies have centred on the reorganization of gingival and periodontal tissues following treatment, which could affect stability [8]. Nowadays, research on orthodontic biomarkers continues to advance, emphasizing their potential clinical application. Recent studies indicate that localized administration of osteoprotegerin (OPG) can markedly minimize relapse rates by suppressing osteoclast activity, thereby improving tooth stability following orthodontic treatment [9,10]. Moreover, alkaline phosphatase (ALP) levels in gingival crevicular fluid can be evaluated to monitor bone turnover during the retention phase; however, research suggested no direct correlation between ALP levels and relapse distance [11].

These biomarkers, present in saliva and gingival crevicular fluid, can offer new perspectives on bone remodelling and treatment-related complications, thereby emphasizing the potential of biomarkers in predicting relapse. Effective biomarkers must be determined in readily accessible bodily fluids, including urine, serum, blood, gingival crevicular fluid (GCF), saliva, and cerebrospinal fluid (CSF). Saliva has become recognized as an important medium owing to its non-invasive collection, affordability, and capacity to indicate systemic changes in the body [12]. It functions as a diagnostic instrument for various medical issues, including cancers and infectious diseases, by identifying biomarkers such as RNA, protein, and DNA. In orthodontic therapy, salivary biomarkers can signify alterations active orthodontic tooth movement, thereby providing a non-invasive diagnostic instrument [13]. Saliva encompasses biomarkers indicative of bone deposition and resorption, facilitating pain management and treatment efficacy [14].

Tyrosine-protein kinase (Tec) is a tyrosine kinase encoded by the TEC gene. Tec comprises five domains: the N-terminal pleckstrin homolog (PH) domain, the Tec homology (TH) domain, the Src homology (SH3) domain, the Src homology (SH2) domain, and the C-terminal protein tyrosine kinase (PTK) domain. Tec participates in the intracellular signalling pathways of cytokine receptors, lymphocyte surface antigen heterotrimeric G-protein-coupled receptors, and integrin molecules. Tec is a crucial regulator of the immune system [15,16]. Tec has demonstrated involvement in RANKL-induced osteoclastogenesis. [17] demonstrated that osteoclasts, rather than osteoblasts, exhibit the highest expression levels of Tec mRNAs. This result was validated through quantitative polymerase chain reaction (qPCR) and immunoblot analysis. The group proposed that Tec is the protein molecule that links the RANK and

immunoreceptor tyrosine-based activation motif (ITAM) pathways, along with the Btk molecule, to initiate calcium signalling, which is crucial for bone remodelling (Figure 1).

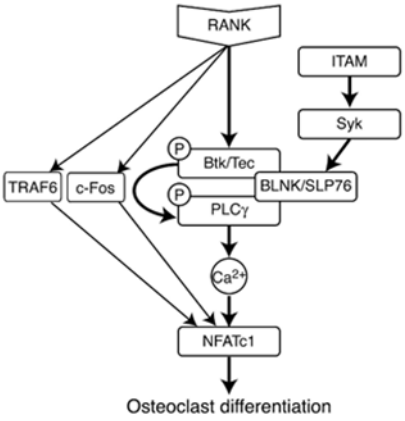

Figure 1: Integration of the RANK and ITAM Signals by Tec Kinases

Adapted from [17]

Nesprin-2 is a protein which is encoded by the SYNE2 gene in humans. Nesprins are structured proteins that feature a central extended spectrin-repeat (SR) rod domain and a C-terminal Klarsicht/ANC-1/Syne homology (KASH) transmembrane domain that functions as a NE-targeting motif. The internal integrity of the nucleus is maintained by the binding of Nesprin-2 (Nesp2) to cytoplasmic F-actin, which anchors the nucleus to the cytoskeleton. 'Mechanosensory function' is the primary function of Nesprin-2. Through actin filaments, the protein establishes a connection between the nuclear envelope cytoskeletons. The connection enables the nucleus of the cell to detect and respond to mechanical difficulties during cellular stresses, as well as to maintain the cell nucleus's position [18].In the event of orthodontic tooth movement and relapse, osteoclasts are among the cells observed in the alveolar bone. Osteocytes are widely recognized as the mechanosensing cells of the bone [19,20], by playing role to facilitate bone resorption by secreting RANKL and engaging in apoptosis.

The potential of Tec and Nesprin as biological markers of stability has been emphasized by [21] who have utilized saliva samples from post-orthodontic patients. To date, no research has measured the amount of Tec and Nesprin2 based on the degree of incisor irregularities during the retention phase. It is challenging to identify the precise predictor of relapse, as it is influenced by a variety of factors, as previously mentioned. Therefore, the objective of the investigation was to quantify the stability and relapse of Tec and Nesprin2 in post-orthodontic patients by employing an enzyme-linked immunosorbent assay (ELISA).

2.MATERIAL AND METHODS

2.1 Sample Characterization

The sample size was determined based on a prior study [22]. The calculation was performed using G* Power 3.1.9.7, with a significance level of 0.05, statistical power of 0.8, and an effect size of 1.34. A sample size of 10 patients was necessary for each group. It follows that a total sample size of 24 patients seeking treatment at the Centre of Paediatric and Orthodontic Studies, Faculty of Dentistry, Universiti Teknologi MARA (UiTM) was recruited for this study. Prior to the study, informed consent was acquired. The protocol received approval from the UiTM Research Ethics Committee (REC/08/2022 (PG/MR/197).

2.2 Selection Criteria

The post-orthodontic patients were selected from individuals nearing the debonding phase. They underwent orthodontic treatment involving the extraction of four premolars, utilizing MBT prescription 0.022 x 0.028-inch slot pre-adjusted edgewise fixed orthodontic appliances (Victory SeriesTM, 3M Unitek, Germany). All patients were generally healthy and exhibited good periodontal status. Individuals with unsatisfactory oral hygiene, those who smoke, pregnant individuals, and those with bonded retainers were excluded from. Ten healthy non-orthodontic individuals were chosen as the control group.

2.3 Saliva Sampling Protocol

A thorough scaling was performed one week ahead to ensure optimal oral health before saliva collection. Patients were instructed to refrain from any oral activities, specifically abstaining from food consumption for a minimum of 1.5 hours prior to the procedure [23].

Saliva samples were consistently collected within a standardized time frame, specifically between 10 and 11 am, to mitigate circadian variation. Saliva samples were obtained from each patient immediately following the removal of fixed appliances. Patients were told to sit upright and instructed to rinse their mouths with distilled water, followed by a 5-minute rest prior to saliva collection. Five millilitres of unstimulated whole saliva was collected by passive drooling into a 50 ml sterile centrifuge tube [24] .Patients were instructed to refrain from speaking or moving their tongues during the collection process. The head was inclined downward to allow the saliva to collect in the mouth. Saliva samples were obtained over a duration of 7 minutes. All samples were maintained on ice throughout the procedure. Saliva samples were subsequently centrifuged at 10,000 rpm and 4° C for 10 minutes to

eliminate insoluble materials, cells, and debris. The supernatant was obtained and aliquoted into 10 mL centrifuge tubes, each containing a volume of 100 µL, to ascertain protein concentration. The pellets were discarded. Each sample was preserved at -80° C until subsequent analysis to maintain protein biomarkers. The identical saliva collection procedures were implemented for the control group.

2.4 ELISA Protocol

The ELISA was conducted following the protocol provided by an ELISA kit from BlueGene Biotech (China). A standard curve was established by plotting the logarithm of Tec and Nesprin-2 concentrations against the logarithm of the mean absorbance for each standard, with the optimal fit line determined through regression analysis (Microsoft Excel 2024). The Tec concentration in each sample was ascertained by comparing the optical density (OD) of the samples to a standard curve established for each Tec analysis. The sensitivity of the ELISA kit was 1.0 ng/mL.

2.5 Statistical Analysis

All data were analysed utilizing SPSS version 22.0. The Cronbach's Alpha for intra-examiner agreement regarding incisor irregularities was assessed. The normality of the data was determined using the Shapiro-Wilk test. Subsequently, the data were analysed using an independent samples t-test to ascertain the statistical differences between the mean protein levels of the control group and the immediate debond stage. Differences were deemed significant when $P<0.05$.

3.RESULTS AND DISCUSSION

A total of 20 patients aged between 18 and 33 years were recruited. Among these, 60% were female and 40% were male. The number of female patients exceeded that of male patients. Studies demonstrate that females pursue orthodontic treatment more frequently than males, influenced by diverse psychosocial and aesthetic considerations. A study involving 126 patients revealed that 82.5% of patients seeking orthodontic treatment were female, motivated by factors such as social harassment and a desire for enhanced appearance [25]. A survey of secondary school students in Bangkok indicated that 78.7% of females sought orthodontic treatment, in contrast to 66.1% of males [26]. Moreover, socioeconomic status and geographical context significantly affect treatment attitudes, with females exhibiting a greater propensity to pursue orthodontic care [27]

The average concentration of Tec and Nesprin-2 is presented in Table 1.

Table 1: Descriptive analysis of protein concentration

Proteins	Mean (SD)(ng/mL)	
	Control group	Debond goup
Tec	5.35(±1.83)	3.92(±1.18)
Nesprin-2	3.04 (±1.96)	2.61 (±2.34)

Table 2: Independent-sample t-test of Tec and Nesprin-2 in control and debond group.

Note *Significant (*P*<0.05), n=10.

Proteins	Mean differences (SD) (ng/mL)	95% Confidence Interval (CI)	t value (df)	*p*-value
Tec	-1.42 (±0.69) *	-0.026, 2.875	2.06	0.05
Nesprin-2	-0.43 (±0.97)	-1.60	2.46	0.66

The results indicated that both protein levels in the control group were higher than those in the immediate debond group. A mean concentration of Tec level nearly twice as high was observed in the control group. The average salivary Tec protein concentration was substantially lower in the saliva of post-orthodontic individuals compared to the healthy control group *(P*<0.05). The Nesprin-2 level was marginally reduced at immediate debond, yet the difference was minimal and lacked statistical significance.

No studies have yet examined the levels of Tec and Nesprin-2 in the saliva of post-orthodontic patients utilizing the ELISA technique. Prior research examined the concentrations of diverse biomarkers in the gingival crevicular fluid. OPG and bone alkaline phosphatase (BALP) have the potential to detect alveolar bone formation, while receptor activator of nuclear factor kappa-B ligand (RANKL) serves as a marker for resorption [28]. Meanwhile, the administration of raloxifene has recently been shown to diminish relapse, although this research was conducted using a rodent relapse model [29].

Preventing relapse following active orthodontic tooth movements presents a clinical challenge. The alveolar bone undergoes ongoing remodelling after orthodontic tooth movement, resulting in the formation of a new compression zone in the direction opposite to the tooth movement. Nevertheless, 73% of relapses occurred merely one day post-appliance removal [30] while 98% of relapses were noted two weeks following retention [29] .This corresponds with the current study, in which the Tec level was markedly reduced at immediate post-debond *(P*<0.05). The present research postulated that as saliva samples were collected immediately post-debonding, the biological alterations in the alveolar bone may not have commenced. It is hypothesized that an extended retention period will lead to an increased concentration of Tec as the alveolar bone and periodontal fibres restore their structural integrity, eventually resulting in relapse.

The limited presence of Nesprin-2 in the debond group correlates with the postponed expression of bone mineralization markers (osteopontin and osteocalcin), which are typically expressed in the later phases of anabolic bone remodelling [31] Future research investigating the correlation of Nesprin-2 levels should persist in longitudinal orthodontic stability studies.

This study is the first to identify the novelty of salivary Tec and Nesprin-2 during the orthodontic retention phase. An increasing number of studies have indicated the potential of various biological markers, specifically BALP, OPG, RANKL, and OPN [28] . However, these studies utilized gingival crevicular fluid samples to assess alveolar bone remodelling in relation to the efficacy of orthodontic treatment. Comparing these studies with ours is challenging due to the differing methodologies employed in qualitative and quantitative measurements.

Given the constraints of the saliva sampling timeframe, the application of these results in a clinical context necessitates additional research over an extended observational period. Our data may provide a foundation for the future quantification of Tec and Nesprin-2. The current study presents opportunities

for additional efforts to identify suitable candidate proteins for future monitoring kits aimed at detecting teeth at risk of relapse, to be utilized clinically, independent of solely relying on clinical findings. A longitudinal study is recommended to monitor the patterns of Tec and Nesprin-2 in relation to changes in relapse severity.

4.CONCLUSION

This study emphasized diminished bone remodelling activity at the immediate debond stage, evidenced by reduced levels of salivary Tec protein and Nesprin-2 during this phase.

ETHICAL APPROVAL AND CONSENT

This study was approved by the UiTM Research Ethics Committee (REC) REC/08/2022 (PG/MR/197)and follows the Helsinki Declaration. All the patients who agreed to participate in this study signed the consent form.

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