

Original Article

The Pulpal Response to Posterior Bite Raisers: A Prospective Study of Material and Height Effect

Azheen J. Ali^{*1} , Darwn S. Abdulateef² , Lawand Barzinji³ 

Abstract

Objective: Posterior bite raisers (PBRs) induce temporary posterior disocclusion during fixed orthodontic treatment but may affect pulp vitality under prolonged occlusal load. **Objective:** To compare pulpal responses to injectable nanohybrid composite PBRs and resin-modified glass ionomer cement (RMGIC) PBRs at two heights (1–2 mm; >2–4 mm) in relation to gender and age.

Methods: Eighty patients (16–40 years old, with a mean age of 22.9 ± 5.7 years and an equal male-to-female ratio) were assigned to four groups according to the material and height of PBRs, with twenty patients in each group. Cold testing and electric pulp testing (EPT) were documented at baseline, 1 week, 1 month, 2 months, and 3 months. The outcomes were divided into two groups: sensibility (positive or negative), and the differences among the four groups were compared in relation to gender and age.

Results: At 3 months, negative pulpal responses were observed only in the composite groups: >2–4 mm (15%) and 1–2 mm (5%). Material choice was the strongest independent predictor of EPT threshold change ($P=0.008$), with the composite associated with significantly decreased responsiveness. The interaction between material and height was highly significant ($p=0.012$), indicating a fivefold higher risk of negative sensibility responses with high-volume composite than with RMGIC. Younger patients showed greater susceptibility to pulpal changes ($p=0.03$).

Conclusions: Composite PBRs, particularly at heights exceeding 2 mm, are associated with increased pulpal stress, whereas RMGIC demonstrates superior pulpal stability and is recommended for initial orthodontic phases to minimize adverse biological effects.

Keywords: Nanohybrid Composite, Posterior Bite Raisers, Pulp Sensibility, Resin-Modified Glass Ionomer Cement.

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Introduction

Posterior bite raisers (PBRs) are widely used during fixed orthodontic therapy to achieve posterior disocclusion, reduce occlusal interferences, and facilitate tooth movement. However, concentrating occlusal forces on a limited number of teeth may affect pulpal microcirculation, vascular integrity, and neural response¹. Previous studies have demonstrated that orthodontic forces alone can alter pulpal blood flow, provoke reversible inflammatory responses, and modify sensitivity thresholds^{2,3}.

Furthermore, pulpal hemodynamics may become more vulnerable when orthodontic loading is combined with direct occlusal forces applied through bite-raising appliances⁴. The biological response of the pulp to functional or orthodontic stress is influenced not only by the magnitude and duration of force but also by the properties of the material through which the force is transmitted. Composite resin, commonly used for PBR fabrication, exhibits a relatively high elastic modulus, enabling occlusal loads to be transmitted more directly to underlying tooth structures⁵.

Rigid materials such as composites have been associated with increased pulpal strain under functional or orthodontic loading^{6,7}. In contrast, resin-modified glass ionomer cement (RMGIC) demonstrates a lower modulus of elasticity and superior stress-absorbing characteristics due to its polyalkenoate matrix reinforced with light-activated resin components. This hybrid composition has been shown to reduce load concentration and attenuate stress transmitted to dentin-pulp tissues when used in clinical settings⁸.

Riser height also represents a critical mechanical and biological variable. Increasing the vertical dimension concentrates occlusal force distribution and can lead to greater pulpal strain. Higher bite blocks have been associated with altered sensitivity, discomfort, and temporary pulpal changes in orthodontic patients⁹. In addition, patient-related factors, particularly age and gender, may influence pulpal reactivity. Younger individuals generally exhibit larger pulp chambers, greater vascularity, and heightened metabolic activity, making their pulpal tissues more responsive to mechanical stimuli¹⁰⁻¹⁴.

Previous research using laser Doppler flowmetry has confirmed that younger pulps exhibit greater reductions in pulpal blood flow following orthodontic force application than in adults¹⁵. Although gender-related differences in pain perception and neural threshold have been reported, their clinical relevance in orthodontic bite-raising procedures remains unclear^{15,16}. Diagnostic tools such as cold testing and electric pulp testing (EPT) remain essential for evaluating pulpal status during orthodontic treatment.

These methods have demonstrated high reliability and diagnostic validity in assessing pulpal vitality and differentiating transient pulpal alterations from irreversible changes^{1,17}. Using a combination of sensibility tests is recommended, particularly when occlusal loading may influence pulpal responsiveness¹⁸⁻²⁰.

While the impact of orthodontic forces on pulpal blood flow is documented, there is limited clinical data comparing the biological response of the pulp to different PBR materials under varying vertical dimensions. Specifically, the interaction between the high elastic modulus of composite resins and the stress-dampening properties of RMGIC at different clinical heights remains poorly understood. Furthermore, the influence of patient-related factors such as age and gender on these specific mechanical challenges has not been fully elucidated. The aim of this prospective clinical study was to compare pulpal responses to posterior bite risers (PBRs) made from composite resin and resin-modified glass ionomer cement (RMGIC) at two different vertical heights (1–2 mm and >2–4 mm) using cold testing and electric pulp testing (EPT). A secondary aim was to evaluate the influence of age and gender on vitality outcomes following the application of PBRs during fixed orthodontic treatment.

The null hypothesis stated that there is no significant difference in pulpal vitality outcomes between teeth treated with composite PBRs and those treated with RMGIC PBRs. Vertical height of the PBR (1–2 mm vs. >2–4 mm) has no effect on pulpal vitality or EPT threshold changes. Furthermore, age and gender have no influence on pulpal response following the application of PBRs.

Patients and Methods

This prospective stratified randomized clinical study included 80 participants (16–40) years old with a mean age of 22.9 ± 5.7 years and an equal male-to-female ratio from 126 screened patients (IOTN 2 and 3) requiring posterior bite risers (PBRs) as part of fixed orthodontic treatment as shown in the Consort flow diagram (Figure 1). The study was conducted in a private dental center in Sulaimani, between July 2025 and December 2025." Universal injectable nanohybrid composite (GC G-aenial (GC, Japan)) and RMGIC (GC Fuji Ortho Band, Japan) were used for the fabrication of PBRs on mandibular 1st molars (Fig. 2). Patients were divided into four equal groups (n = 20 each): 1. Composite PBR, 1–2 mm 2. Composite PBR, >2–4 mm 3. RMGIC PBR, 1–2 mm 4. RMGIC PBR, >2–4 mm. Randomization was carried out using a computer-generated sequence, and allocation was concealed in sealed envelopes to minimize selection bias. All PBRs were placed on the mandibular first molars following standard bonding protocols. A periodontal probe was used to verify riser

height, and adjustments were made to achieve the desired disocclusion.

Randomization

Following the screening process, participants were enrolled and assigned to groups via stratified randomization to balance groups by gender and riser height. This approach resulted in equal numbers of males and females in each group (1:1 ratio), minimizing potential confounding effects of gender on pulpal response outcomes. Participants were assigned to riser height groups based on their pre-existing clinical needs to (1-2 mm) or (>2-4 mm) subgroups, and only the material was randomized. The material (Composite vs RMGIC) was assigned using a computer-generated sequence, and allocation was concealed in sealed envelopes to minimize selection bias. The envelopes were opened only by a dental assistant during the procedure to assign participants to their respective groups. While the operators were not blinded due to material characteristics, the primary outcome was assessed using objective instrumental measurements (Electric Pulp Test). The data analyst was successfully blinded to the group allocation. The primary data were coded (e.g., Group 1, 2, 3, 4) by the principal investigator before being transferred to the statistician for analysis. The group identities were revealed only after the statistical modelling was completed.

Pulp Sensibility Testing

Two diagnostic tests were used to evaluate pulpal response: first, the cold test, performed with ethyl chloride spray. An absence of response after repeated stimuli suggested loss of sensibility. Second test: Electric Pulp Testing (EPT) with a digital electric pulp tester (Digitest II, Parkell Inc., USA) used to measure sensibility thresholds. The lowest numerical reading at which the patient responded was recorded as the EPT value (10-13). The diagnostic value of combining cold test and EPT has been well established, improving accuracy in distinguishing vital from non-vital pulps^{21,22}. Testing intervals included: Baseline, 1 week, 1 month, 2 months, and 3 months.

Pulpal status was assessed using standardized and validated sensibility tests, consisting of cold testing with ethyl chloride and electric pulp testing (EPT). For the cold test, a cotton pellet saturated with refrigerant spray (Hygenic Endo-Ice) was applied to the mid-buccal surface for up to 5 seconds. A positive response was recorded when the patient reported a sharp, transient sensation that was resolved immediately upon removal. A negative response was defined as the absence of

sensation after two consecutive applications separated by 1 minute. For the EPT assessment, the electrode was placed on the mid-buccal surface, gingival to the buccal tube, with toothpaste as a conducting medium. The numerical value at which the patient first perceived a tingling sensation was recorded as the EPT threshold. All readings were documented duplicate to ensure reliability, and the lower value was used for analysis.

Diagnosis of non-vital pulp required both no response to the cold test, confirmed by repeating the test twice; and no response at maximum output on EPT (80 reading), repeated twice to exclude false-negative results due to anxiety, thick enamel, or temporary neural suppression. To avoid misdiagnosis, teeth that initially showed no response were retested after 1 week, as orthodontic forces and occlusal loading may cause temporary sensory suppression. Only teeth that showed a consistent lack of response in both tests and at two separate appointments were classified as non-vital.

Bite raiser height measurement

Patients were first categorized into two strata (1–2 mm or >2–4 mm) based on the vertical clearance required to unlock the malocclusion or prevent bracket interference. Then, the incremental application of the chosen material (Nanohybrid Composite or RMGIC) was applied to the mid-occlusal surface of the mandibular first molars. The material was built up incrementally to reach the target height. A calibrated periodontal probe was used to measure the vertical height of the material from the deepest part of the central fossa to the highest point of the riser.

To ensure the height was functionally accurate, articulating paper and calibrated shim stock were used to confirm bilateral simultaneous contact. This ensured that the vertical dimension was uniform and that the patient did not have an unstable or deflected path of closure.

Inclusion and Exclusion Criteria

To minimize confounding variables, the following criteria were applied:

Inclusion criteria

- Patients with orthodontic treatment need (IOTN 2 and 3)
- Permanent mandibular first molars
- Healthy teeth with no restorations or ongoing pulpal pathology

Exclusion criteria

- Age below 16 years
- Extensive restorations
- Systemic diseases affecting pulpal physiology
- Trauma history
- Pre-existing non-vital teeth
- Patients with 'short-face' morphology (brachyfacial) and those with existing deep bites were excluded to prevent molar intrusion.

Ethical Approval

Ethical approval was obtained from the College of Dentistry, University of Sulaimani on 11/08/2025 (no. 320/25). The study was conducted in strict accordance with the Declaration of Helsinki and local ethical guidelines. Informed consent was collected from all participants.

Statistical Analysis

All analyses were performed using SPSS version 26.0, with significance set at $P < 0.05$. Descriptive statistics summarized the data. Fisher's Exact test was used to evaluate differences in positive and negative outcomes among groups. To evaluate changes in pulpal sensibility thresholds over the five-time intervals (Baseline, 1 week, 1 month, 2 months, and 3 months), a Repeated Measures ANOVA, Multivariable Linear Regression for factors influencing 3-month EPT change, and a Generalized Linear Mixed Model for factors influencing pulpal sensibility were conducted.

Sample size calculation

Sample size was determined by power analysis using G*Power version 3.1.9.7 ($\alpha = 0.05$, power = 80%), based on Abtahi et al. (2016), we targeted a large effect size ($f=0.40$), assuming a 10-unit difference in EPT Digitest II scores and a standard deviation of 12²³. This yielded 18 participants per subgroup; increased to 20 to account for 10% attrition, giving a total of 80 participants ($n=80$, 20 per group).

Examiner Calibration and Reliability Testing

To ensure consistency and repeatability of measurements, the examiner performing all pulp sensibility tests underwent a calibration process prior to data collection. Calibration consisted of performing cold and EPT tests on 10 orthodontic patients not included in the study sample, followed by comparison of repeated readings taken 1 week apart. Intra-examiner reliability was assessed using the intra-class correlation coefficient (ICC) for EPT threshold values and Cohen's Kappa for categorical cold-test outcomes (vital vs. non-vital). The ICC for EPT readings was 0.91, indicating excellent

reliability, while the Kappa coefficient for cold-test responses was 0.88, demonstrating strong agreement. During the study period, 10% of randomly selected participants were re-examined at the same appointment to verify consistency, and no significant differences were noted between the first and second recordings. Regarding PBR height measurements, to ensure intra-operator reliability, the primary investigator performed duplicate measurements for the first 10 participants, achieving an Intraclass Correlation Coefficient (ICC) of 0.92, indicating excellent consistency.

Results

Intra-examiner reliability was assessed using the intra-class correlation coefficient (ICC) for EPT threshold values and Cohen's Kappa for categorical cold-test outcomes (vital vs. non-vital). The ICCs for EPT readings and PBR measurements were 0.91 and 0.92, respectively, indicating excellent reliability, while the Kappa coefficient for cold-test responses was 0.88, demonstrating strong agreement.

Demographic characteristics of the study groups are summarized in Table 1. At the 3-month evaluation, negative responses to cold tests were observed in the composite PBR groups: composite 1–2 mm, 1/20 (5%); composite >2–4 mm, 3/20 (15%), as illustrated in Table 2. In contrast, no cases of negative responses were recorded in either RMGIC group. The Fisher's exact test showed no statistical differences between groups ($P>0.05$).

The repeated measures ANOVA revealed significant changes in pulpal sensibility thresholds over the 3-month study period (Table 3). The composite PBR (1–2 mm) showed a statistically significant change over time ($P=0.012$). A significant drop occurred at 2 months, followed by a slight recovery by the third month.

The composite PBR (>2–4 mm) group showed a significant longitudinal change ($p < 0.00$). EPT values decreased progressively from the baseline to their lowest point in 2 months. Although a minor increase was noted in 3 months, the overall decline from baseline remained substantial.

RMGIC PBR (1–2 mm) group demonstrated the highest level of pulpal stability ($p = 0.584$) with no statistically significant deviation.

RMGIC PBR >2–4 mm group showed a minor but statistically significant change over time ($P=0.042$). The thresholds remained relatively stable compared to the composite groups, though a gradual decline was noted from baseline to 3 months.

A multivariable linear regression analysis was performed to identify independent predictors of change in the 3-month EPT threshold while controlling for clinical and demographic covariates (Table 4). Material choice was identified as the strongest independent predictor, with the use of composite associated with a significant decrease in pulpal responsiveness compared to RMGIC ($P=0.008$). Furthermore, riser height significantly influenced the outcome ($P = 0.012$), confirming that vertical disarticulation exceeding 2 mm independently contributes to reduced pulpal responses. Among the demographic factors, Age was a significant covariate ($P=0.03$), indicating that older participants exhibited slightly higher baseline resistance to electrical stimulation, whereas gender did not significantly influence the sensory outcome ($P=0.16$).

Based on the Generalized Linear Mixed Model in Table 5, the results demonstrate that pulpal sensibility is significantly influenced by the PBR material ($P=0.032$). While the independent effect of riser height was not statistically significant on its own ($P = 0.108$), the material-by-height interaction was highly significant ($P = 0.012$), indicating that the risk of a negative sensibility response is over five times higher when high-volume composite is used compared to RMGIC. Furthermore, the significant interaction between material and time ($P=0.015$) confirms that this divergent response between materials becomes progressively more pronounced as orthodontic treatment continues.

Table 1: Demographic characteristics by groups.

Group	N(%)	Age (mean $\pm$ SD)
Composite 1-2 mm	20 (25%)	21.4 $\pm$ 4.1
Composite >2–4 mm	20 (25%)	23.1 $\pm$ 6.2
RMGIC 1-2 mm	20 (25%)	22.8 $\pm$ 3.9
RMGIC >2–4 mm	20 (25%)	24.2 $\pm$ 7.5
Total	80 (100%)	22.9 $\pm$ 5.7

Table 2: Cold test response at 3 months by group (Positive / Negative; n, %) using Fisher's Exact test.

Group	Positive n (%)	Negative n (%)	Total n (%)	P-value
Composite 1-2 mm	19 (95%)	1 (5%)	20 (100%)	P=0.115
Composite >2–4 mm	17 (85%)	3 (15%)	20 (100%)	
RMGIC 1-2 mm	20 (100%)	0 (0%)	20 (100%)	
RMGIC >2–4 mm	20 (100%)	0 (0%)	20 (100%)	
Total	76 (95%)	4 (5%)	80 (100%)	

Table 3: Electric pulp test (EPT) thresholds (mean $\pm$ SD) with Repeated Measure ANOVA.

Group	Baseline	1 week	1 month	2 months	3 months	P-value
Composite1-2 mm	44.1 $\pm$ 3.1	44.7 $\pm$ 2.8	42.8 $\pm$ 6.3	36.0 $\pm$ 15.4	39.4 $\pm$ 14.7	0.012
Composite >2–4 mm	43.8 $\pm$ 2.9	44.1 $\pm$ 3.2	41.6 $\pm$ 7.3	31.2 $\pm$ 17.9	34.2 $\pm$ 16.3	<0.001
RMGIC 1-2 mm	44.5 $\pm$ 2.7	44.6 $\pm$ 2.4	43.9 $\pm$ 4.3	43.9 $\pm$ 4.8	43.1 $\pm$ 3.1	0.584
RMGIC >2–4 mm	45.1 $\pm$ 2.1	44.7 $\pm$ 1.9	44.4 $\pm$ 2.6	43.3 $\pm$ 5.1	40.6 $\pm$ 5.7	0.042

Table 4: Multivariable Linear Regression for factors influencing 3-month EPT change (n=80).

Variable	β coefficient	95% CI Lower	95% CI Upper	p-value
Material (Composite, RMGIC)	-6.2	-10.1	-2.3	0.008
Height (1–2 mm, 2–4 mm)	-4.8	-8.5	-1.1	0.012
Age (Year)	0.3	0.03	0.57	0.03
Gender (Male, Female)	-0.25	-6.1	1.1	0.16

Table 5: Generalized Linear Mixed Model for factors influencing pulpal sensibility.

Variable	Odds Ratio	95% CI Lower	95% CI Upper	p-value
Material (Composite, RMGIC)	4.35	1.14	16.38	0.032
Height (1–2 mm, 2–4 mm)	2.95	0.81	10.72	0.108
Time (month)	1.9	0.9	3.95	0.09
Material $\times$ Height	5.16	1.44	18.5	0.012
Material $\times$ Time	3.2	1.2	8.2	0.015
Height $\times$ Time	2.12	0.85	5.3	0.11

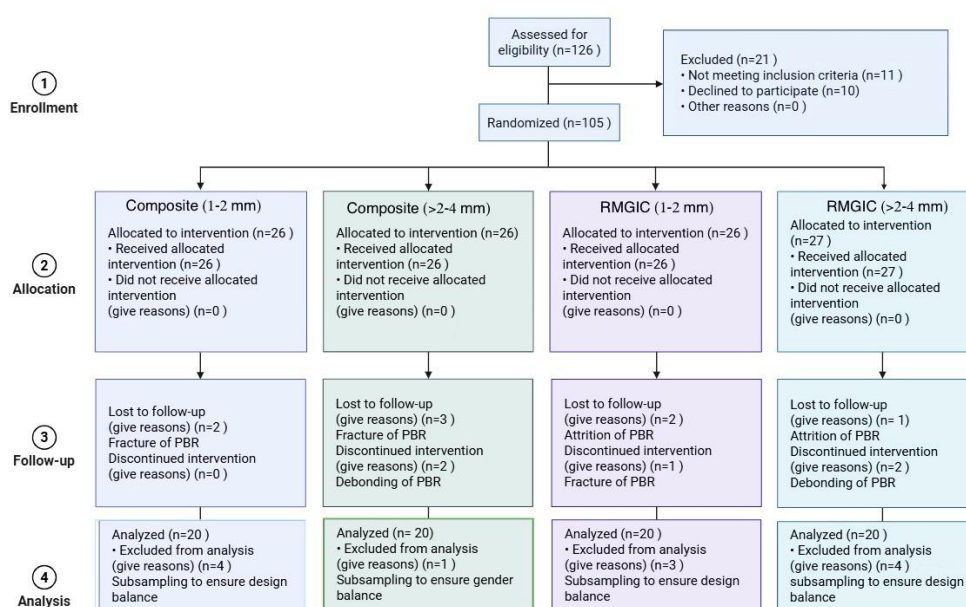


Figure 1: CONSORT Flow Diagram of the study

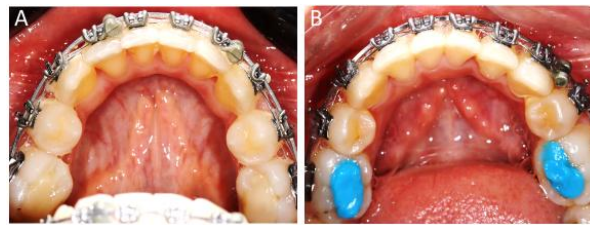


Figure 2: Posterior bite risers (PBRs) A: Composite, B: Resin modified glass ionomer cement.

Discussion

Orthodontic forces naturally influence pulpal blood flow and neural activity, and adding posterior bite risers further concentrates biomechanical loading on individual teeth. In orthodontics, shifts in pulpal sensibility are considered biotracers that reflect tissue adaptation to mechanical stress. Material differences also play a role: composite resin, with its higher stiffness, transmits occlusal forces more directly to enamel and dentin, whereas RMGIC forms a chemical bond with enamel and dissipates stress more effectively. These combined biomechanical and material factors help explain the differing pulpal responses observed in this study²⁴⁻²⁶.

The findings of this study demonstrate that composite posterior bite risers (PBRs) were associated with a higher incidence of negative pulpal responses compared with resin-modified glass ionomer cement (RMGIC). Similar patterns have been reported in studies evaluating occlusal load concentration and pulpal outcomes in orthodontic patients²⁷. This difference was especially pronounced when riser height exceeded 2 mm. These results underscore the importance of material rigidity and vertical elevation in determining the magnitude of occlusal load transmitted to the pulp²⁸. The results of this study support the use of RMGIC over composite resin when designing PBRs, particularly in cases requiring higher elevations.

Composite resin, owing to its relatively high elastic modulus, tends to transmit occlusal forces more directly to the underlying dentin–pulp complex. Under conditions of continuous orthodontic loading, this may increase pulpal strain, leading to reversible or even irreversible changes in pulpal physiology²⁹. In contrast, RMGIC demonstrates a lower elastic modulus and superior capacity for stress absorption. Its hybrid structure, combining polyalkenoate chemistry with resin reinforcement, allows it to dissipate forces more effectively and reduce pulpal stress, which may account for the absence of non-vital responses in the RMGIC groups³⁰⁻³².

The EPT findings further support these biomechanical differences. Composite groups exhibited progressive reductions in EPT thresholds at 2 and 3 months, reflecting altered neural responsiveness under increased load transmission. Conversely, RMGIC groups maintained stable EPT readings, suggesting minimal irritation of pulpal tissues. These results correlate with earlier reports describing reduced pulpal inflammation and neural disruption when stress-dampening materials are used during orthodontic therapy³³.

A significant association between age and pulpal outcomes was observed, with younger patients demonstrating greater susceptibility to negative pulpal responses. This pattern conforms with previous studies showing that younger pulps, with larger vascular networks, exhibit greater reactivity to mechanical and orthodontic stimuli¹⁹. RMGIC appears to provide a more favorable biomechanical environment by reducing pulpal stress and minimizing unintended biological consequences. Clinicians should also carefully evaluate patient age and selectively monitor pulpal responses using validated diagnostic tools.

This is consistent with histological evidence indicating that young pulps possess larger vascular networks and heightened metabolic activity, making them more reactive to mechanical stresses³⁴. Such findings highlight the need for conservative design choices such as using RMGIC or minimizing riser height when treating younger orthodontic patients. Although gender differences were not statistically significant, all negative pulpal responses occurred in females. Gender-related differences in pain perception, neural threshold, and vascular responsiveness have been documented in the literature²⁴. While the present findings may partly reflect sampling distribution, they suggest that clinicians should remain aware of potential sex-related variability in pulpal reactivity.

The recovery of vitality in some teeth between the 2-month and 3-month assessments suggests that many

pulpal changes induced by PBRs and orthodontic forces are transient rather than permanent, consistent with the stress-dampening properties of the material³⁵. This highlights the importance of serial pulp testing with cold and EPT tests throughout orthodontic treatment.

To maximize experimental control and standardize occlusal loading, this study isolated the intervention to the mandibular first molars. While distributing posterior bite risers (PBR) to the second molars could theoretically dissipate localized stress, our focused design eliminated the confounding anatomical and mechanical variables associated with multi-tooth loading. Furthermore, limiting the PBR to the first molars served as a preventative measure against the risk of second molar super-eruption, which could occur if these units are left out of active occlusion for extended periods. Although these findings provide high-precision baseline data for the first molar, future research is warranted to determine if these pulpal response patterns remain consistent across different dental units or more complex occlusal configurations where forces are distributed more broadly.

Another limitation of this clinical trial was the inability to achieve true double-blinding. Due to the distinct handling characteristics, curing protocols, and optical properties of Composite versus RMGIC, both the operator and the participants could potentially identify the material used. However, the reliance on objective instrumental data (Electric Pulp Testing) provides an additional layer of protection against the influence of unblinded observations.

Another limitation involves the inherent nature of Electric Pulp Testing (EPT). Although the EPT is an objective instrumental measure of sensory nerve response, it assesses pulpal sensibility (innervation) rather than vitality (blood flow). Consequently, transient changes in EPT thresholds may reflect neurophysiological adaptations to pressure rather than true pathological changes in pulpal health. Future studies incorporating Laser Doppler Flowmetry could provide a more comprehensive assessment by monitoring real-time pulpal microcirculation alongside sensory thresholds.

While the 3-month longitudinal follow-up provided sufficient data to observe the acute and subacute pulpal responses to bite-riser application, it may not capture long-term reparative dentin formation or chronic pulpal changes. Extended observation periods could provide additional insight into the pulp status following the removal of occlusal interference.

Conclusion

The study confirms that both the type of material and the vertical height of posterior bite raisers play a significant role in influencing pulpal sensibility. Composite materials, particularly at greater thicknesses, tend to have a more pronounced effect on pulpal response compared to RMGIC. Additionally, pulpal stress appears to increase progressively over the course of orthodontic treatment. Therefore, the use of RMGIC at a conservative height is advisable during the initial leveling and aligning phase to help minimize potential adverse pulpal effects.

Declarations

Ethics Approval and Informed Consent (Institutional Review Board) Statement: Ethical approval was obtained from the Ethics Committee, College of Dentistry-University of Sulaimani, on August 11, 2025 (no. 320/25). Informed consent was collected from all participants.

Author Contributions: AJA: methodology; DSA: software; AJA: validation; Formal analysis: DSA; investigation: AJA; resources: AJA; data curation: DSA; writing—original draft preparation: AJA; writing review and editing: LB; visualization: LB; supervision: AJA; project administration: AJA; funding acquisition: DSA. All authors have read and approved the published version of the manuscript.

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