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Original Article

Hard tissue barrier detection in permanent mandibular molars that have undergone direct pulp capping and partial pulpotomy: A retrospective cohort study

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Abstract *Background/purpose:* The presence of a hard tissue barrier (HTB) is commonly considered as a reliable indicator of a successful vital pulp therapy (VPT). This study therefore aimed to evaluate the probability of HTB detection over time, identify factors affecting HTB detection, and compare the mean time of HTB detection across subgroups.
Materials and methods: This retrospective cohort study included permanent mandibular molars that had undergone VPT. The examiners detected the HTB by comparing the follow-up with the immediate postoperative bitewing radiographs. A flexible parametric survival analysis was conducted to assess the probability of HTB detection over time and its associated factors. The restricted mean survival time was used to compare the mean time of HTB detection across subgroups.
Results: This study included 149 radiographs of 70 teeth from 67 patients. The mean follow-up was 20.7 ± 14.7 months. The probability of HTB detection increased over time, reaching 0.75 (95 % confidence interval: 0.61–0.87) at 48 months, and was significantly greater in teeth

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treated with partial pulpotomy (PP) or with pre-operative periapical lesions. The mean time of HTB detection was significantly earlier after PP than direct pulp capping (20.03 vs. 36.50 months, $P = 0.003$), for teeth with than without periapical lesions (17.84 vs. 28.89 months, $P = 0.021$), and for teeth with incomplete than complete root formation (22.34 vs. 31.45 months, $P = 0.031$).

Conclusion: The probability of HTB detection increased over time and was influenced by VPT type, periapical lesion status, and root formation status.

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Introduction

Vital pulp therapy (VPT) is designed to preserve and maintain the health of pulp tissue by promoting a defensive response to harmful stimuli in pulp tissue by stimulating its healing and facilitating the formation of a hard tissue barrier (HTB) underneath the injury site.[1,2] HTB formation is among the many indicators that are often considered a sign of successful VPT.[3—7] The HTB has also been identified as a marker of healing after VPT as it signifies that the pulp tissue remains vital,[4,7—11] enabling the production of growth factors and cytokines such as transforming growth factor (TGF)-β1, which are known to promote mineralization and regenerative processes.[12]

Many pulp dressing materials used in VPT have the potential to induce HTB formation. In the past, calcium hydroxide had been considered the gold standard and recommended to be the material of choice, but recent studies showed that the HTB induced by calcium hydroxide is variable and unpredictable, with more tunnel defects leading to bacterial microleakage when compared to those induced by hydraulic calcium silicate cements such as ProRoot® MTA (Dentsply, Tulsa Dental Specialties, Tulsa, OK, USA), RetroMTA® (BioMTA, Seoul, Republic of Korea), and Biodentine™ (Septodont, Saint-Maurdes-Fosses, France).[13] However, one disadvantage of Biodentine™ is its radiopacity, which is close to that of natural dentin but lower than that of mineral trioxide aggregate (MTA) and gradually decreases over time, making it difficult to be differentiate from dentin on radiographs.[14]

While previous studies have reported a wide range of HTB formation, from 0 to 100 %, in teeth that have undergone VPT,[4,7,10,11,15—19] HTB detection was often evaluated as a secondary outcome, with assessments that were subjective and cross-sectional in design. Additionally, no studies have specifically investigated the factors affecting HTB formation or the time frame during which the HTB can be radiographically detected. Therefore, this study aimed to evaluate the probability of the HTB detection over time, identify factors affecting HTB detection, and compare the mean time that the HTB can be radiographically detected across subgroups.

Materials and methods

Study design

This retrospective cohort study was approved by the Human Experimentation Committee of the Faculty of Dentistry at Chiang Mai University, Chiang Mai, Thailand (registration number: 39/2017). It was reported according to the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines.[20]

Study participants

This study retrospectively examined the permanent mandibular molars of patients aged 6—18 years that had undergone VPT at the Pediatric Dentistry Clinic, Faculty of Dentistry, Chiang Mai University, Chiang Mai, Thailand, between September 2012 and June 2023.

The protocol for the VPT and assessment has been previously described.[21—23] Immediate postoperative radiographs were taken. Follow-up appointments were scheduled approximately every six months, following the practice recommendations in the Caries-Risk Assessment and Management for Infants, Children, and Adolescents guidelines of the American Academy of Pediatric Dentistry and for the postoperative assessment of VPT by the European Society of Endodontology (ESE).[24,25]

Eligibility criteria and data collection

To be included in this study, the permanent mandibular molars must (i) be treated with direct pulp capping (DPC) or partial pulpotomy (PP) using ProRoot®MTA or RetroMTA® as the pulp dressing material, (ii) have immediate postoperative and at least one follow-up bitewing radiograph, and (iii) have complete data on all independent variables. This study excluded teeth treated with Biodentine™ due to its density resembling that of dentin, complicating the identification and demarcation of the HTB border.

The independent variables were extracted from patients' charts, including age group (6—10, >10—18 years), sex (male, female), VPT type (DPC, PP), pre-operative

pulpal diagnosis (normal pulp, reversible pulpitis, irreversible pulpitis), pre-operative periapical lesion status (absence, presence), root formation status (complete, incomplete), and VPT outcome (success, failure). The immediate postoperative and follow-up bitewing radiographs were collected and saved as JPEG files with unique study codes. Bitewing radiographs were excluded if they (i) were of low quality, (ii) contained restorations or devices that interfered with the radiographic evaluation of the pulp chamber, and (iii) were taken >48 months postoperative, as this study focused on outcomes within 48 months, following the practice recommendations for postoperative assessment of VPT by the ESE.[25]

Outcome parameters

In this study, HTB was defined as a radiopaque layer forming across the surface of the exposed pulp beneath the pulp dressing materials.[1,26] Radiographic detection of the HTB was determined by comparing the follow-up with immediate postoperative bitewing radiographs and classified as either "presence" or "absence." The presence of the HTB was considered an event of interest. The time to HTB detection was recorded in months, measured from the treatment date to its first radiographic detection.

Before the evaluation, two postgraduate dental students underwent a training and calibration session with the gold standard examiner, using a set of 56 bitewing radiographs. To assess intra- and inter-examiner reliability, the two calibrated examiners independently evaluated another set of 30 bitewing radiographs twice, two weeks apart. All examiners were blinded to all independent variables.

Statistical analysis

Cohen's kappa (accepted value at ≥0.8) was calculated to quantify the level of inter-examiner agreement in HTB detection.[27] A flexible parametric survival analysis was conducted to assess the probability of HTB detection over time and its associated factors with 95 % confidence intervals (CIs). The restricted mean survival time (RMST) was used to compare the mean time to HTB detection between age groups, sexes, VPT types, pre-operative diagnoses, pre-operative periapical lesion status, and root formation status.[28] The software used for all these analyses was Stata 18.0 software (College Station, TX, USA). A $P < 0.05$ was considered statistically significant.

Results

As shown in Fig. 1, 714 teeth from 541 patients were initially screened, of which 89 permanent mandibular

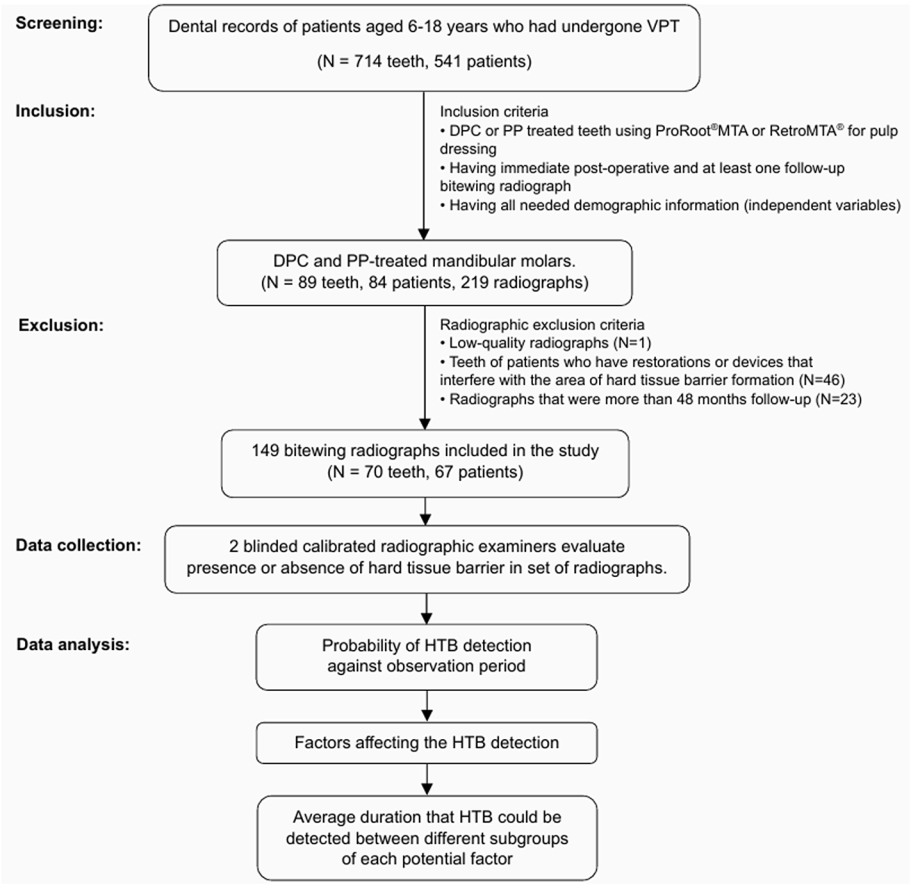

Figure 1 Flowchart of the study. DPC, direct pulp capping; HTB, hard tissue barrier; PP, partial pulpotomy; VPT, vital pulp therapy.

molars from 84 patients met the inclusion criteria. Of the 219 bitewing radiographs available, 70 were excluded. Therefore, this study included 149 bitewing radiographs of 70 teeth from 67 patients, with at different follow-up times up to 48 months postoperative.

The inter-examiner reliability between the two calibrated examiners and the gold standard examiner was 0.84 and 1.00, while the inter-examiner reliability between the calibrated examiners was 0.84. The intra-examiner reliability of both examiners was 0.83 and 1.00.

Table 1 Radiographic detection of hard tissue barrier in vital pulp therapy-treated mandibular permanent molars: comparison of baseline demographic and clinical characteristics.

Demographic/Clinical Variables	Total	Radiographic detection of hard tissue barrier, n (%)		*P* value
		Yes	No	
Number of teeth	70 (100)	42 (60.0)	28 (40.0)	—
Age group (year)				
6—10 years	30 (42.86)	18 (42.86)	12 (42.86)	1.000[a]
>10—18 years	40 (57.14)	24 (57.14)	16 (57.14)	
Sex				
Male	27 (38.57)	19 (45.24)	8 (28.57)	0.212[a]
Female	43 (61.43)	23 (54.76)	20 (71.43)	
Vital pulp therapy type				
Direct pulp capping	30 (42.86)	8 (19.05)	22 (78.57)	0.000[a]
Partial pulpotomy	40 (57.14)	34 (80.95)	6 (21.43)	
Pre-operative pulpal diagnosis				
Normal pulp and reversible pulpitis	40 (57.14)	16 (38.10)	24 (85.71)	0.000[a]
Irreversible pulpitis	30 (42.86)	26 (61.90)	4 (14.29)	
Pre-operative periapical lesion status				
Presence	12 (17.14)	11 (26.19)	1 (3.57)	0.021[a]
Absence	58 (82.86)	31 (73.81)	27 (96.43)	
Root formation status				
Complete	35 (50.00)	21 (50.00)	14 (50.00)	1.000[a]
Incomplete	35 (50.00)	21 (50.00)	14 (50.00)	
Vital pulp therapy outcome				
Success	68 (97.14)	45 (66.18)	23 (33.82)	1.000[a]
Failure	2 (2.86)	1 (50.00)	1 (50.00)	

IQR, interquartile range; SD, standard error.
[a] Fisher's exact test.

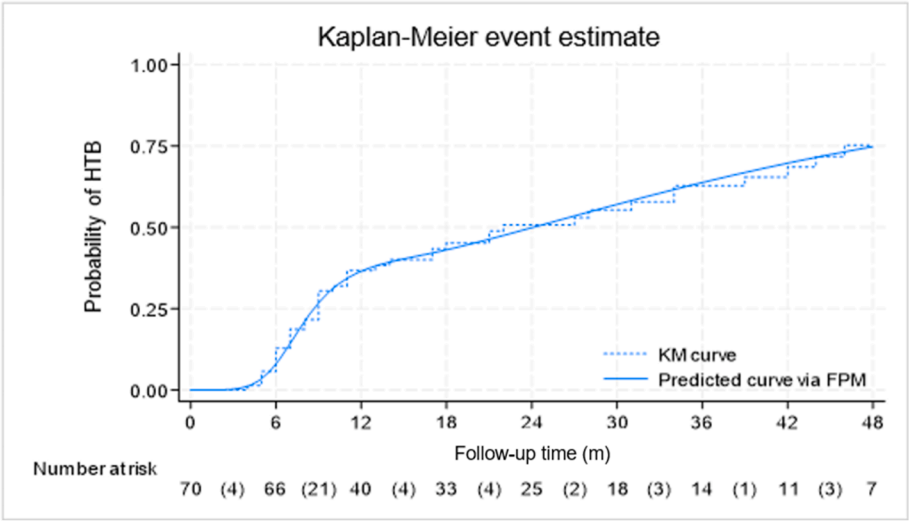

Figure 2 Probability of hard tissue barrier detection over time. FPM, flexible parametric survival models; HTB, hard tissue barrier; KM, kaplan-meier.

Table 2 Probability over time of radiographic hard tissue barrier detection.

Follow-up time (m)	Number of vital pulp therapy-treated teeth	Radiographic hard tissue barrier detection		
		Number of teeth	Probability	(95 % confidence interval)
0	70	0	–	–
6	66	9	0.13	(0.07–0.23)
12	40	16	0.37	(0.26–0.49)
18	33	5	0.45	(0.34–0.58)
24	25	3	0.51	(0.39–0.64)
30	20	2	0.55	(0.43–0.68)
36	17	3	0.63	(0.49–0.76)
42	11	2	0.69	(0.55–0.81)
48	7	2	0.75	(0.61–0.87)

Probability of hard tissue barrier detection over time

The mean follow-up duration of the 70 included mandibular molars was 20.7 ± 14.7 (range: 4–48) months, with a median (interquartile range) of 15.5 (9–31) months. The patients' baseline demographic and clinical characteristics, including age group, sex, VPT type, pre-operative diagnosis, pre-operative periapical lesion status, root formation status, and VPT outcome, are presented in Table 1. Among the included teeth, HTB was detected significantly more frequently in those treated with PP, diagnosed with irreversible pulpitis, or with a pre-operative periapical lesion. HTB was detected in 60 % (42/70) of the treated teeth. The probability of HTB detection increased over time, from 0.13 (95 % CI: 0.07–0.23) at six months to 0.75 (95 % CI: 0.61–0.87) at 48 months (Fig. 2 and Table 2).

Factors affecting hard tissue barrier detection

Due to the small number of failures, VPT outcome (success or failure) was excluded from the analysis. A univariable model indicated that a pre-operative pulpal diagnosis was significantly associated with the radiographic detection of an HTB. The probability of HTB detection was significantly greater for teeth with a pre-operative diagnosis of irreversible pulpitis than for teeth with normal pulp or reversible pulpitis (unadjusted hazard ratio [HR]: 2.48, 95 % CI; 1.29–4.79, $P = 0.007$). However, this factor was not significant in a multivariable model (Table 3).

VPT type and pre-operative periapical lesion status were significantly associated with radiographic HTB detection in both univariable and multivariable models (Table 3). The probability of HTB detection was significantly greater for teeth with PP than with DPC (unadjusted HR: 6.20, 95 % CI: 2.36–16.32, $P = 0.000$; adjusted HR: 4.96, 95 % CI: 1.53–16.12, $P = 0.008$) and for teeth with than without pre-operative periapical lesions (unadjusted HR: 3.00, 95 % CI: 1.33–6.73, $P = 0.008$; adjusted HR: 2.90, 95 % CI:

Table 3 Data distribution of the 70 permanent mandibular molars that have undergone vital pulp therapy according to factors affecting the radiographic detention of hard tissue barrier.

Factors	Total n (%)	Univariable model			Multivariable model		
		HR[a]	95 % CI	*P* value	HR[b]	95 % CI	*P* value
Age group (year)							
6 to 10	30 (42.86)	1.00	Reference		1.00	Reference	
>10 to 18	40 (57.14)	1.26	(0.68–2.33)	0.460	2.34	(0.91–6.02)	0.078
Sex							
Male	27 (38.57)	1.00	Reference		1.00	Reference	
Female	43 (61.43)	0.66	(0.36–1.19)	0.164	0.63	(0.31–1.27)	0.197
Vital pulp therapy type							
Direct pulp capping	30 (42.86)	1.00	Reference		1.00	Reference	
Partial pulpotomy	40 (57.14)	6.20	(2.36–16.32)	0.000*	4.36	(1.69–11.30)	0.002*
Pre-operative pulpal diagnosis							
Normal pulp and reversible pulpitis	40 (57.14)	1.00	Reference		1.00	Reference	
Irreversible pulpitis	30 (42.86)	2.48	(1.29–4.79)	0.007*	1.42	(0.64–3.17)	0.391
Pre-operative periapical lesion status							
Absence	12 (17.14)	1.00	Reference		1.00	Reference	
Presence	58 (82.86)	3.00	(1.33–6.73)	0.008*	2.99	(1.45–6.16)	0.003*
Root formation status							
Complete	35 (50.00)	1.00	Reference		1.00	Reference	
Incomplete	35 (50.00)	0.96	(0.53–1.75)	0.906	2.50	(0.95–6.55)	0.062

Hazard ratio from [a]uivariable and [b]multivariable flexible parametric survival regression analyses.
*P value < 0.05.

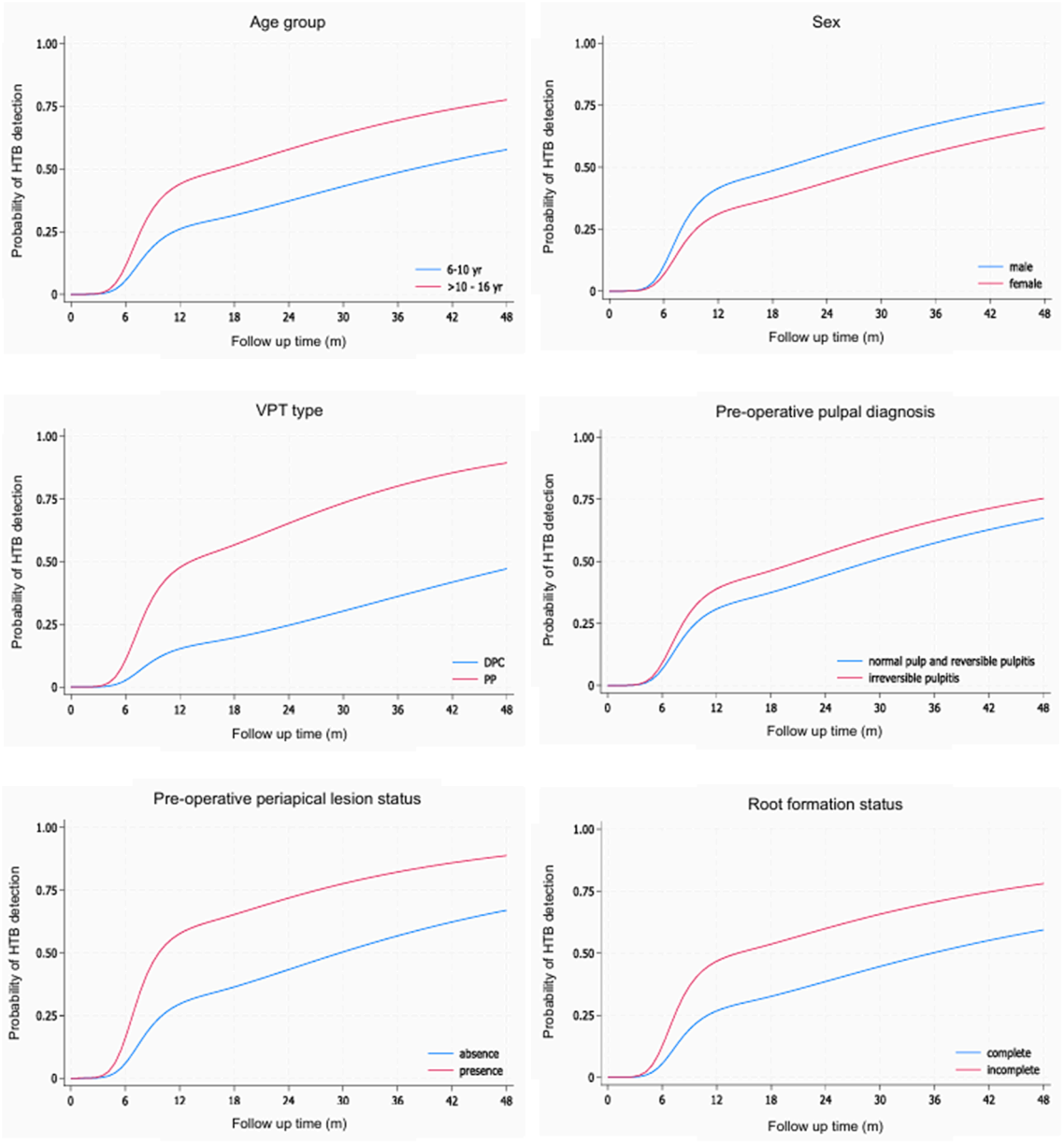

Figure 3 Probability of radiographic detection of hard tissue barrier over 48-month follow-up time: flexible parametric survival analysis by factors affecting HTB detection, including (a) age group, (b) gender, (c) type of vital pulp therapy, (d) pre-operative pulpal diagnosis, (e) pre-operative periapical lesion, (f) root formation. DPC, direct pulp capping; HTB, hard tissue barrier; PP, partial pulpotomy; VPT, vital pulp therapy.

1.44—5.85, $P = 0.003$). The probability of HTB detection by follow-up time for each potential factor is shown in Fig. 3.

Comparison of the mean time of hard tissue barrier detection across subgroups

The RMST over the 48 months of follow-up represents the mean time at which an HTB was radiographically detected in each subgroup (Table 4). Among the factors examined, PP, presence of pre-operative periapical lesions, and incomplete root formation significantly shortened the time to HTB detection. Within the 48-month observation period, an HTB was detected at 36 months on average in teeth after DPC, compared to only 20 months after PP, indicating that an HTB was detected approximately 16 months earlier in teeth treated with PP than DPC. In addition, an HTB appeared approximately 11 months earlier in teeth with than without pre-operative periapical lesions. Similarly, HTB appeared approximately nine months earlier in teeth with incomplete than with complete root formation. Fig. 4 shows radiographic examples of the average time to HTB detection between the subgroups for each potential factor.

Table 4 Comparison of the average duration for hard tissue barrier detection between different subgroups of each potential factor at 48 months follow-up.

Factors Reference vs. comparator groups	Reference	Comparator	Difference between groups		
	RMST (95 % CI)	RMST (95 % CI)	RMST Difference	95 % CI	*P* value
Age group (year)					
6 to 10 vs. >10 to 18	31.52 (24.67−38.37)	23.32 (16.81−29.83)	−8.20	(− 18.30 to 1.91)	0.112
Sex					
Male vs. female	24.25 (18.38−30.13)	28.74 (22.74−34.74)	4.49	(− 2.75 to 11.73)	0.224
Vital pulp therapy type					
Direct pulp capping vs. partial pulpotomy	36.50 (29.14−43.86)	20.03 (13.62−26.43)	−16.47	(− 27.31 to − 5.62)	0.003*
Pre-operative pulpal diagnosis					
Normal pulp and reversible pulpitis vs. irreversible pulpitis	28.60 (21.66−35.54)	24.88 (18.25−31.52)	−3.71	(− 13.60 to 6.17)	0.461
Pre-operative periapical lesion status					
Absence vs. presence	28.89 (23.72−34.05)	17.84 (6.69−20.93)	−11.05	(− 20.44 to − 1.67)	0.021*
Root formation status					
Complete vs. incomplete	31.45 (25.10−37.80)	22.34 (16.52−28.16)	−9.11	(− 17.38 to − 0.84)	0.031*

RMST, restricted mean survival time.
*P value < 0.05.

Discussion

From this study, HTB was radiographically detected in 60 % of teeth treated with DPC or PP using MTA. The probability of detecting HTB increased over time, from 0.13 at 6 months to 0.75 at 48 months. The increase in HTB detection may be due to increasing HTB thickness over time. Similarly, one study reported an increase in HTB detection over time in both teeth treated with DPC using MTA (from 76 % at 6 months to 86 % at 12 months) and Biodentine™ (from 71 % to 95 % at 6 and 12 months).[17] While the percentage of teeth with a detected HTB differs between studies, their trends both show that time is a crucial factor in HTB detection. Histological studies have found that HTB can be detected as early as two weeks after VPT.[29,30] However, the histological approach is not applicable in clinical settings. Therefore, our study detected HTBs from routine bitewing radiographs. However, the HTB must be sufficiently thick to be detectable on radiographs. In their quantitative histopathologic analysis, De Rossi et al. reported that HTB thinner than 0.5 mm could not be detected on radiographs.[31]

Our results also revealed that the probability of HTB detection over time was significantly greater for teeth exhibiting advanced caries progression, such as those with irreversible pulpitis, pre-operative periapical lesions, and treated with PP, than for those with normal pulp or reversible pulpitis, those without pre-operative periapical lesions, and those treated with DPC. However, a pre-operative pulpal diagnosis was only significant in the univariable model. Rapid caries development triggers increased cellular and molecular interactions, leading to a more intense immune and inflammatory response.[32−34] A large carious lesion that exposes the pulp will be infected with bacteria that produce bacterial products and mediators throughout the root apex and evoke an inflammatory response in the periapical tissue, causing a periapical lesion.[35] The severe damage to the teeth may trigger the activation of pulp tissue healing mechanisms, resulting in higher detection of HTBs following VPT. Since several studies have now included teeth with periapical lesions in the indications for VPT,[13,22,23,36,37] clinicians should keep in mind that HTB may be more commonly detected in these teeth.

HTB was detected more often in teeth treated with PP than with DPC, an explanation for this finding may be the greater contact area of pulp tissue with the pulp dressing material in PP-treated teeth. The increased contact area allows the pulp dressing material to stimulate cell adhesion and proliferation, induce hard tissue deposition, and promote mineralization.[38,39] Notably, one previous study found no HTB formed after PP with MTA and calcium hydroxide on teeth with irreversible pulpitis at 6, 12, and 24 months postoperatively.[19] Based on our findings, the mean time to radiographic detection of an HTB after PP was 20 months, ranging from 13 to 26 months postoperatively. Therefore, the lack of HTB detection in the previous study may be due to its relatively short follow-up period.

In this study, HTB was detected 16 months earlier in teeth treated with PP than in teeth treated with DPC. Due to the wide contact area between the pulp dressing material and pulpal tissue during the PP procedure, Bogen et al. also revealed that teeth with larger multiple pulpal exposures above 2 mm were more likely to demonstrate a

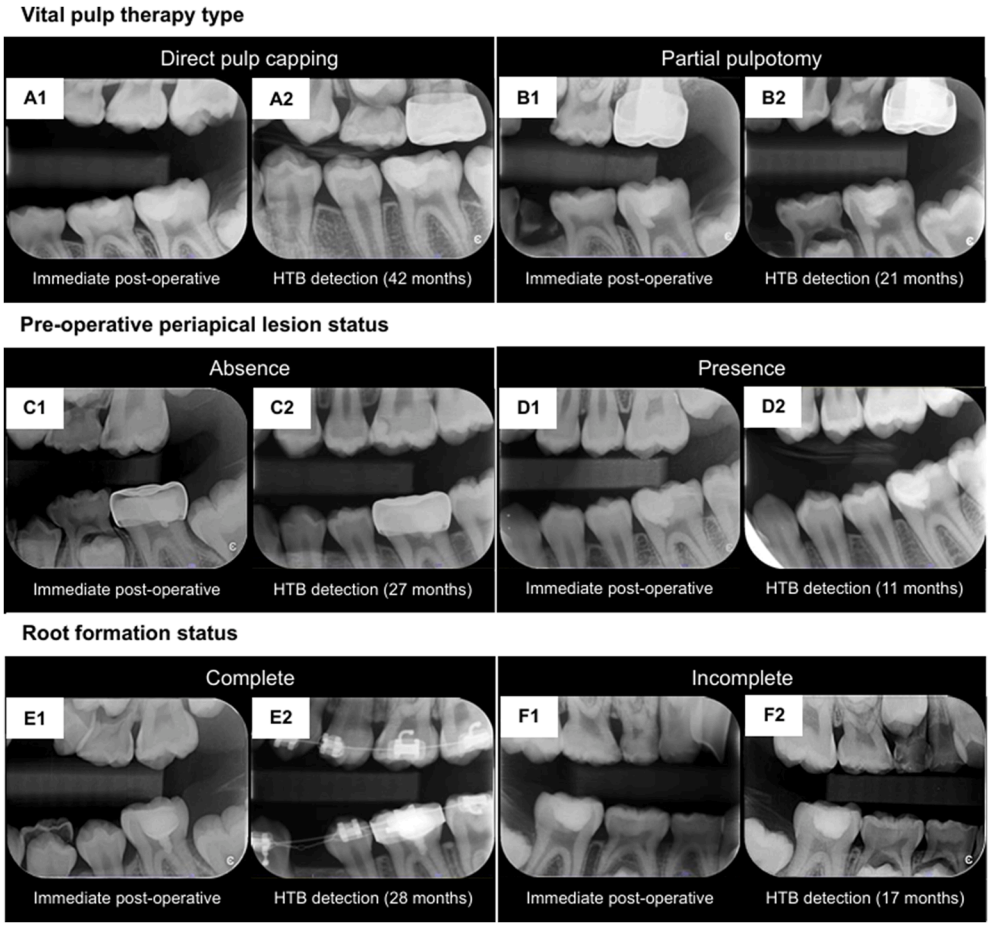

Figure 4 Radiographic examples of average duration for HTB detection across different subgroups: A1) immidiate post-operative radiograph of tooth 36 treated with direct pulp capping; A2) HTB detection on radiograph of tooth 36 at 42 months; B1) immidiate post-operative radiograph of tooth 36 treated with partial pulpotomy; B2) HTB detection on radiograph of tooth 36 at 21 months; C1) immidiate post-operative radiograph of tooth 36 without pre-operative periapical lesion; C2) HTB detection of radiograph of tooth 36 at 27 months; D1) immidiate post-operative radiograph of tooth 36 with pre-operativeperiapical lesion; D2) HTB detection of radiograph of tooth 36 at 11 months; E1) immidiate post-operative radiograph of tooth 36 with complete root formation; E2) HTB detection of radiograph of tooth 36 at 28 months; F1) immidiate post-operative radiograph of tooth 46 with incomplete root formation; F2) HTB detection of radiograph of tooth 46 at 17 months; HTB, hard tissue barrier.

higher degree of aggressive HTB formation.[16] Moreover, our study detected an HTB approximately 11 months earlier in teeth with than without pre-operative periapical lesions, possibly because a vigorous healing process is initiated due to the vital pulp defense mechanism's response to severe injury in teeth with periapical lesions.[33] Additionally, our study detected an HTB approximately nine months earlier in teeth with incomplete than with complete root formation, possibly due to the greater healing potential of younger pulp tissue and the rich blood supply to the pulp in young patients.[40] The information on the mean time to HTB detection across subgroups will greatly benefit clinicians because they can be used as a reference for when to expect a radiographic detection of HTB after VPT.

It has long been recognized that if the exposed dental pulp has formed an HTB, the teeth still have active cells and the capacity to maintain their vitality.[12] However, in their histological examination of teeth with varying degrees of carious lesion extension to explore the evoking of a dentin response to caries and environmental irritation, Ricucci et al. showed the calcified mass formed in response to deep carious lesions or lesions that exposed the pulp does not exhibit the typical features of real dentin, such as a tubular structure.[41] Instead, it seemed to represent a repair process rather than true regeneration. This information contradicts the accepted view that an HTB indicates regeneration and, therefore, is an indicator of VPT success. However, according to the ESE guidelines, the term "tertiary dentin formation" is still used and it is considered to be the aim of VPT.[25]

While our study may be the first to provide insights into HTB detection, it had some limitations. Firstly, due to its retrospective design, the examined bitewing radiographs were taken without strict protocol adherence. Consequently, a significant number did not meet the inclusion criteria and were excluded from this study. Secondly, due

to difficulty interpreting radiographs, only mandibular molars were included in this study, reducing the number of examined teeth. Thirdly, this study did not include all VPT types. Indirect pulp capping was omitted due to the slow rate of newly formed HTBs, complicating the identification of the true border between the HTB and the dentin. Similarly, the newly formed HTB appeared ambiguous after coronal pulpotomy, posing challenges distinguishing it from pulp canal obliteration. Moreover, this study did not include teeth treated with VPT using Biodentine™ as the pulp dressing material because it has a density that is not significantly different from dentin, making it difficult to distinguish them and thus mark the border of the newly formed HTB.[3,7] Although two different types of MTA were used in this study (ProRoot®MTA and RetroMTA®), a randomized controlled trial of various MTA materials (ProRoot®MTA, RetroMTA®, and OrthoMTA® (BioMTA, Seoul, Korea) for partial pulpotomy in permanent teeth did not reveal a statistical difference in calcified barrier formation among three groups.[42] Fourthly, this study did not examine the relationship between age and HTB detection, as it only included children aged 6—18 years. Lastly, this study's design focused on a dichotomous outcome, the presence or absence of an HTB, not the quantity of HTBs. Further questions, such as will the HTB cease or continue to develop over time or can the HTB potentially lead to pulp canal obliteration and pulp necrosis after VPT, can only be answered if the thickness of the HTB is measured and long-term follow-up of VPT-treated teeth is available. Therefore, further studies on this issue are highly recommended.

In conclusion, this study highlighted that HTB detection increased over time. In addition, HTB detection is significantly more likely for teeth treated with PP or with pre-operative periapical lesions. Within the 48-month observation period, HTB was detected earlier in teeth treated with PP, teeth with pre-operative periapical lesions, and teeth with incomplete root formation. These findings can be used as references for postoperative assessment of VPT-treated teeth.

Declaration of competing interest

The authors deny any competing interest related to this study.

Acknowledgments

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