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ORIGINAL RESEARCH

Assessment of permanent teeth development in children with multiple persistent primary teeth

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Abstract

Background: Persistent primary teeth (PPT) may occur due to various local factors, or it may develop due to general factors such as systemic diseases and syndromes. Since eruption and dental development are two different processes, it is important to investigate both processes to determine the actual cause of delayed tooth eruption. The study aimed to evaluate the dental development of a group of Turkish children with multiple PPT using the Willems dental age estimation method. Study Design: Digital panoramic radiographs of children and adolescents aged between 9 and 15 years were retrieved, assessed and categorized. A total of 80 radiographs of patients with more than one PPT were selected and matched with children without PPT. Dental age was calculated using the method of Willems *et al*. All analyses were conducted using the SPSS statistical software. The statistical significance was set at 0.05. Results: The permanent tooth development of children with multiple PPT could be delayed by 0.5–4 years compared to healthy ones. A strong positive correlation was found between the number of PPT and deviation for both females and males ($p < 0.001$). Conclusion: In conclusion, we found that permanent tooth development of children with multiple PPT could be delayed compared to healthy ones. In addition, as the number of PPT increased, the difference between chronological age and dental age also increased, especially in males.

Keywords

Persistent primary teeth; Retained tooth; Chronological age; Dental age; Forensic odontology; Willems method

1. Introduction

Tooth eruption is defined as the movement of a tooth in an axial and occlusal direction from its developmental position within the jaw to its final functional position in the occlusal plane [1]. The continuous process of tooth eruption and shedding replaces the exfoliated deciduous teeth with succedaneous teeth. The eruption pattern of primary and permanent teeth is usually extensive and occurs at different chronological age levels [2]. Racial, sexual, environmental and individual factors can influence tooth eruption and are usually considered when determining the standards of normal eruption. Comparatively, impaired tooth eruption manifests as either delayed or complete absence of eruption [3].

In some cases, when a primary tooth is retained beyond its normal exfoliation period, this extended lifespan of that tooth is termed "persistence". A persistent primary tooth (PPT) can be defined as a primary tooth that is still in position even though the eruption moment of the permanent successor tooth has expired for more than one year [4]. PPT may occur due to various local factors such as congenital agenesis of the permanent tooth, impaction or intraosseous migration of the permanent successor, the existence of pathology (odontoma, cysts, tumors), or it may develop due to general factors such as systemic diseases and syndromes [4, 5].

The development of a tooth and its emergence into the oral cavity is a complicated process, and the underlying mechanisms are not yet fully understood. Dental development and eruption in the oral cavity occur *via* a complex process involving bone remodeling on opposite sides of the developing tooth [6]. In addition to bony changes, the eruption of permanent teeth can cause resorption and exfoliation of the primary teeth. Both delayed tooth emergence and PPT are correlated with the complex mechanism of tooth eruption [7]. Although the eruption of succedaneous permanent teeth into the oral cavity is affected by the presence of primary teeth, the intraosseous development of permanent teeth is affected by systemic and local factors. Since eruption and dental development are two different processes, it is important to investigate both processes to determine the actual cause of delayed tooth eruption [8].

Chronologic age (CA) is usually used to describe delayed tooth eruption due to its practicality, although it may not necessarily represent biological age and dental development [9]. Several methods have been proposed for assessing dental development, referred to as dental aging [3, 10, 11]. The estimation of age through the study of the calcification of permanent teeth has been shown to provide reliable and accurate methods. However, the results of several dental age (DA) estimation methods varied among different populations. One of the dental age methods, the Willems method, a modification of the Demirjian method, was found to be highly applicable in the Turkish population [10–14].

The study aimed to evaluate the dental development of a group of Turkish children with multiple PPT using the Willems dental age estimation method.

2. Materials and methods

2.1 Sample size and setting

For estimating the required population size, based on the desired confidence interval of 95%, a margin of error of 5% and a 4.5% expected frequency of PPT, it was calculated that at least 67 children were needed to reach the study endpoint, as previously described [15]. Thus, by increasing the sample size by 20%, we included 80 children in each group.

Digital panoramic radiographs of children and adolescents aged between 9 and 15 years were retrieved, assessed and categorized. They were grouped by sex (42 females and 38 males) and seven age groups. The distribution of the age groups were 9–9.9 (11 children), 10–10.99 (13 children), 11–11.99 (11 children), 12–12.99 (12 children), 13–13.99 (17 children) and 14–14.99 (16 children).

All patients provided informed consent and permission for data usage from their parents. The study was conducted in compliance with the principles of the Declaration of Helsinki. Their radiographs were obtained from the archive of the Department of Pediatric Dentistry, based on the patients' first dental examination between March 2019 and December 2021. The same panoramic radiography device (Morita Veraview Pacs®, Kyoto, Japan) was used for the radiographs of all patients.

2.2 Determination of PPT

The panoramic radiographs of each patient were assessed by a single examiner, an experienced pediatric dentist (B.G.T.). PPT was defined as a primary tooth that did not exfoliate and the eruption time of the permanent successor tooth had expired for more than one year, as previously described [4, 16–18]. The eruption time of the permanent successor tooth was examined based on the chronological scale determined by considering the genders of the Turkish children [19].

A total of 80 radiographs with more than one PPT, having complete records, panoramic radiographs of adequate quality and similar ethnic origin were included in the study. Patients with syndromes, craniofacial anomalies, systemic diseases such as endocrinal disorders, malnutrition, or hematologic abnormalities that could have affected teeth development were excluded. Patients with severe orthodontic crowding (>7 mm) and tooth impactions or transpositions were also excluded. PPT cases associated with any pathological conditions such as cysts, odontoma, tumor, ankylosis, tooth agenesis of the successor, supernumerary teeth or root anomalies were not included in this study. Primary teeth with more than one-third of the root resorption were excluded (Fig. 1). To compare the dental development, a total of 80 radiographs of healthy children without PPT were obtained from the same archives, and their age and sex were matched with the PPT group. A flow diagram of the study is shown in Fig. 2.

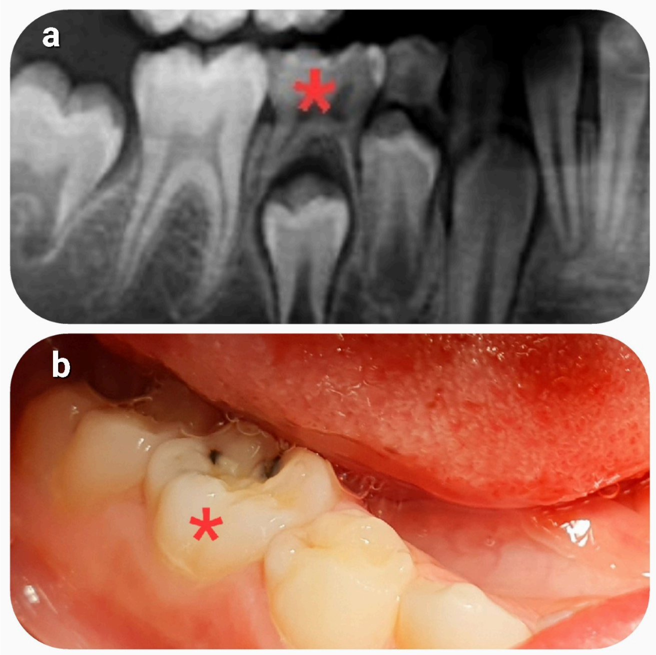

FIGURE 1. An image of a patient with PPT (chronological age:13.8) (a) Radiographic image of a mandibular primary second molar which was accepted as persisted. (b) Clinical image of a mandibular primary second molar which was accepted as persisted.

2.3 Dental age estimation by Willems method

First, the CA of the children was calculated by subtracting their date of birth from the date of the panoramic radiographs, and the results were converted to decimal ages.

Panoramic radiographs were examined for DA, and each tooth in the left lower quadrant (except the third molar) was scored according to Demirjian's criteria for radiomorphological development [14]. Corresponding Demirjian stages were derived from an evaluation of eight mineralization stages, alphabetically marked from A to H. The first stage A represents the beginning of calcification, seen at the superior level of the dental crypt, without fusion of this calcification, while the last stage H represents the finished calcification of the tooth with apical ends of the roots completely closed. For each stage, Demirjian [14] presented a specific self-weighted score, and the summed score on all seven teeth represented a dental maturity score that could be converted to dental age. DA was calculated according to Demirjian's criteria, but the developmental score was obtained from the sex-specific tables for each tooth defined by Willems *et al.* [11] using a weighted Analysis of Variance (ANOVA) (Fig. 3).

The radiographs were evaluated by a trained and a calibrated pediatric dentistry research assistant (A.T.), blinded to the CA, group, name and sex of the participants. To assess intra-

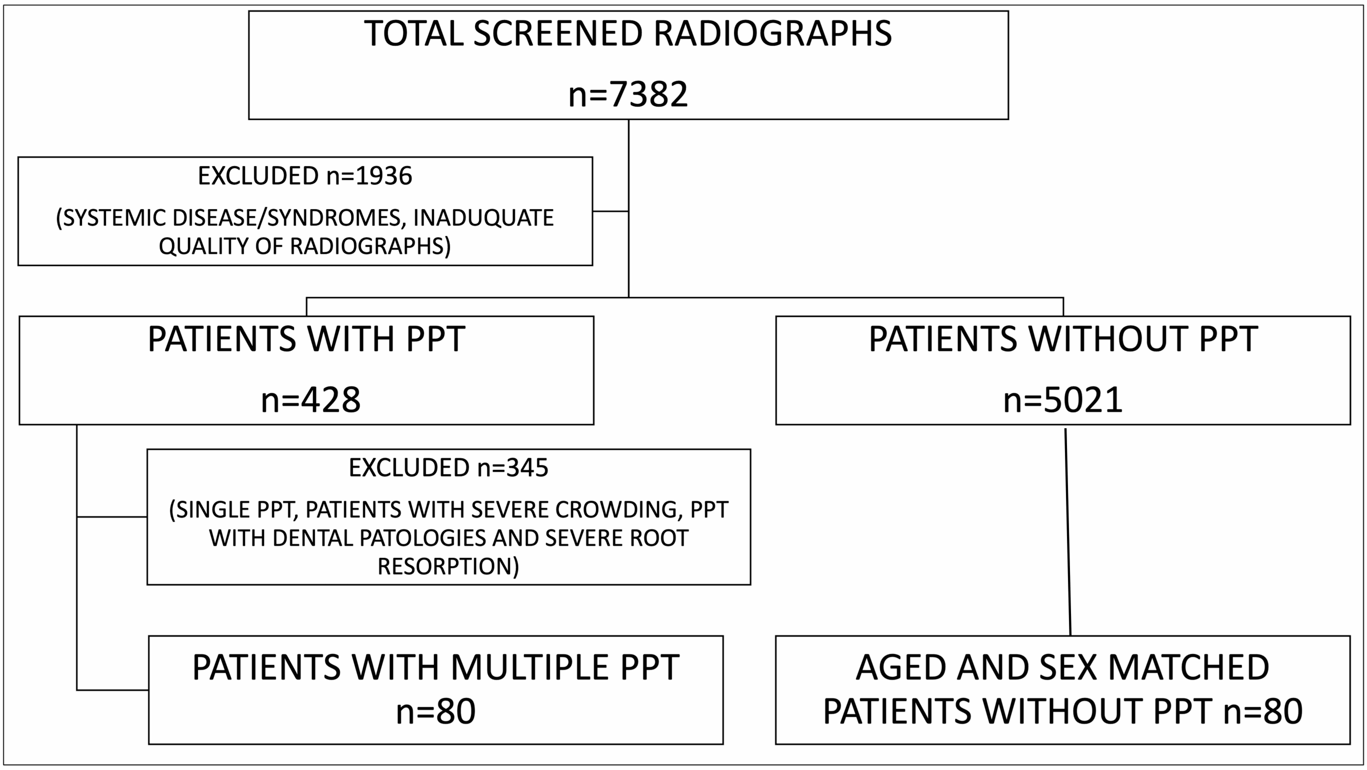

FIGURE 2. A flow diagram of the study. PPT: Persistent primary teeth.

examiner reproducibility, 20 radiographs were re-examined by the observer following a two-week interval and their intra-class correlation coefficient (ICC) was calculated.

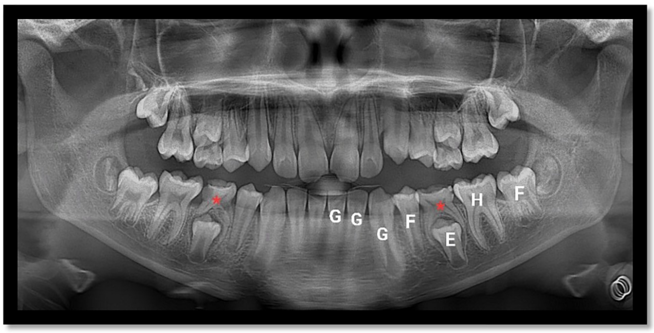

FIGURE 3. A panoramic radiograph of a female patient with PPT. The chronological age of female patient was 13.06, dental age was 11.95, dental age and chronological age difference was 1.11, teeth included as persistent primary teeth were mandibular primary second molars, maxillary primary second molars were not included due to severe root resorption (>1/3 of the root length).

2.4 Statistical analysis

All analyses were conducted using the SPSS statistical software (Version 26, IBM Inc., Chicago, IL, USA). Statistical analysis included descriptive statistics and the Kolmogorov-Smirnov test to verify the normality of the data distribution. Comparison of the parameters between children with and without PPT was performed using independent samples Mann-Whitney U test. The chronological and dental age were compared using the Wilcoxon test dependent samples. The Spearman correlation coefficient test was used to analyze the number of PPT and deviations. The level of statistical significance was set at 0.05.

3. Results

The intra-examiner reliability was 0.94 and 0.97 for females and 0.97 and 0.99 for males with and without PPT, respectively.

The mean CA of children with and without PPT included in the study was 12.15 (1.74) and 12.21 (1.74). Based on the participants' sex, the mean CA of females with and without PPT was 12.17 (1.75) and 12.09 (1.78) years, and for males, it was 12.25 (1.76) and 12.21 (1.71) years with and without PPT, respectively. The difference between the chronological age of children with and without PPT was not statistically significant ($p > 0.05$).

Children with PPT exhibited a delay in dental development compared to those without PPT. The median overall deviation (Δ: DA-CA) between the DA and CA was -0.1 and 0.1 years for females and males without PPT and -0.9 and -1.0 years for females and males with PPT ($p < 0.001$). The comparison of the CA, DA, deviation and absolute deviation between children with and without PPT is shown in Table 1.

According to the number of PPT, 37 children had 2 PPT, 15 children had 3 PPT, 17 children had 4 PPT, and 11 children had more than 5 PPT (Fig. 4). A strong positive correlation was found between the number of PPT and deviation for both sex (female, r = 0.625; $p < 0.001$; male, r = 0.675; $p < 0.001$). We also observed that as the number of PPT increased, the difference between chronological age and dental age also

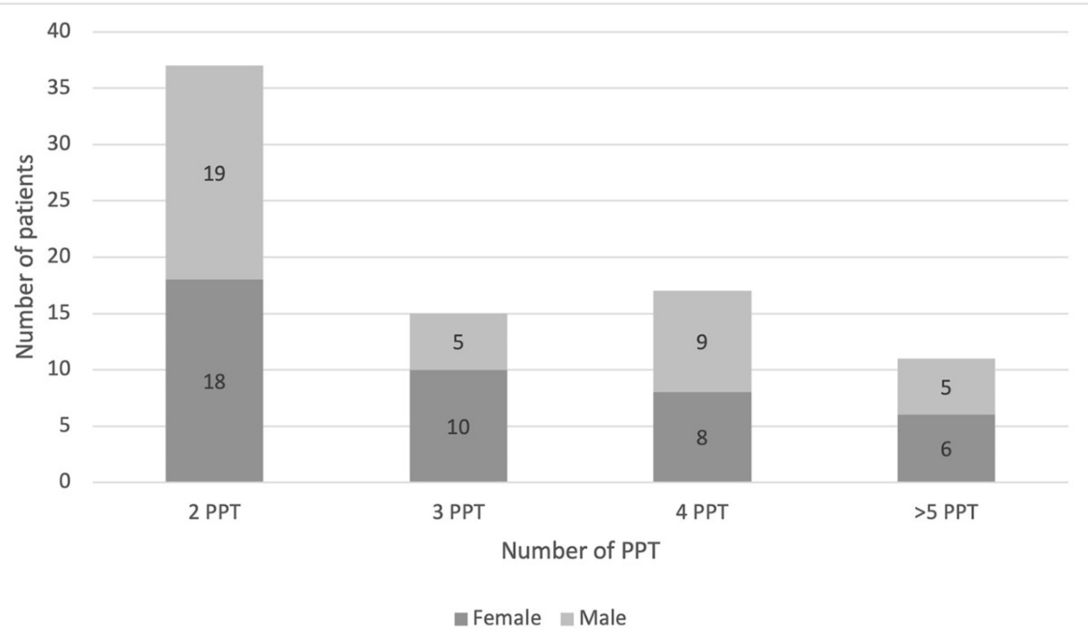

FIGURE 4. The distribution of children with PPT according to the number of PPT and sex.

TABLE 1. The comparison of chronological age, dental age, and deviation between children with and without PPT.

PPT	CA Med (Min–Max)	DA Med (Min–Max)	Δ age Med (Min–Max)	*p* value
Sex				
Female				
Without PPT	12.2 (9.1–14.8)	11.9 (8.8–14.8)	−0.1 (−1.4–1.5)	0.048*
With PPT	12.2 (9.0–14.9)	11.2 (7.8–14.1)	−0.9 (−2.9–1.7)	
Male				
Without PPT	12.7 (9.0–14.4)	12.3(8.3–14.8)	0.1 (−1.8–1.8)	0.002*
With PPT	12.3 (9.1–14.9)	11.5 (8.1–14.3)	−1.0 (−4.7–0.5)	

*PPT: Persisted Primary Teeth; Med: Median; Min: Minimum; Max: Maximum; CA: Chronologic age; DA: dental age; Δ: The deviation of age (DA-CA); *$p < 0.05$.*

increased. In particular, the median DA was 4 years less than the CA of males with more than 5 PPT (Table 2).

The comparison of DA and deviation according to age groups of children without PPT is shown in Table 3. The difference between the median CA and DA was significant only in the age groups of 10–10.99 for males ($p < 0.05$).

The comparison of DA and deviation according to the age groups of children with PPT is shown in Table 4. The results showed that the difference between the median CA and DA was not significant in the age groups of 9–9.99 and 10–10.99 in males and females ($p > 0.05$). Additionally, underestimation was observed in the age groups of 12–14 years for both sexes, with the corresponding medians ranging between −0.6 and −2.0 for females and −0.8 and −1.9 for males ($p < 0.05$).

4. Discussion

Permanent tooth eruption is a complex and tightly regulated process that can be delayed by many local and systemic factors [3, 9, 20]. Both delayed tooth emergence and persistent primary teeth are correlated with the complex mechanism of tooth eruption. The influence of genetics on tooth eruption has been described in several studies [7]. Differential gene expressions by dental follicles needed for osteoclastogenesis, osteogenesis and pressure from underlying successor teeth are responsible for the timely exfoliation of primary teeth and the eruption process of permanent teeth [9]. It was previously reported that the rs9594738 polymorphism in RANKL was associated with permanently delayed tooth eruption, while RANKL expression and RANKL/OPG ratio were associated with PPT [21]. Although delayed eruption is commonly associated with PPT, it can also occur in the absence of PPT. The occurrence of PPT is considered a consequence rather than a cause of delayed eruption [9, 22]. On the other hand, a strong correlation was reported between eruption time and dental maturity [23]. Tooth development and eruption are complex processes involving alveolar bone resorption and eruption pathway formation [24]. Considering the association between chronological age and dental age would be inconsistent in case of delayed eruption [9] and disturbances in dental development could lead to PPT and delayed tooth eruption, we found that there was a need to determine whether delayed eruption in children was due to PPT

TABLE 2. The comparison of chronological age, dental age, and deviation according to number of PPT.

Sex	CA Med (Min–Max)	DA Med (Min–Max)	Δ age Med (Min–Max)	*p* value
Number of PPT				
Without PPT				
Female (n = 42)	12.2 (9.1–14.8)	11.9 (8.8–14.8)	−0.1 (−1.4–1.5)	NS
Male (n = 38)	12.7 (9.0–14.4)	12.3 (8.3–14.8)	0.1 (−1.8–1.8)	NS
2 PPT				
Female (n = 18)	11.5 (10.0–13.4)	11.1 (9.5–13.1)	−0.4 (−1.3–1.1)	0.003*
Male (n = 19)	11.4 (9.2–14.9)	11.4 (8.1–14.3)	−0.5 (−1.8–0.5)	0.002*
3 PPT				
Female (n = 10)	13.3 (9.0–14.9)	11.5 (8.3–14.1)	−1.2 (−2.8–1.7)	0.028*
Male (n = 5)	12.3 (9.5–14.3)	10.2 (8.2–12.2)	−1.9 (−2.6–0.6)	0.043*
4 PPT				
Female (n = 8)	12.7 (9.0–14.4)	10.7 (7.8–12.2)	−1.3 (−2.9–−0.3)	0.012*
Male (n = 9)	13.4 (9.1–14.3)	12.2 (8.9–13.1)	−1.2 (−2.4–−0.2)	0.008*
>5 PPT				
Female (n = 6)	14.5 (11–14.9)	12.0 (10.7–12.9)	−2.1 (−2.7–−0.2)	0.027*
Male (n = 5)	14.0 (10.9–14.9)	9.9 (8.1–12.9)	−4.0 (−4.7–−2.0)	0.043*

*PPT: Persisted Primary Teeth; Med: Median; Min: Minimum; Max: Maximum; CA: Chronologic age; DA: dental age; Δ: The deviation of age (DA-CA); NS: Non-significant ($p > 0.05$); *$p < 0.05$.*

TABLE 3. The comparison of dental age, and deviation according to age groups of children without PPT.

Sex	CA Med (Min–Max)	DA Med (Min–Max)	Δ age Med (Min–Max)	*p* value
Age group				
9–9.99				
Female (n = 6)	9.3 (9.1–9.9)	9.1 (8.8–9.6)	−0.2 (−0.5–0.1)	NS
Male (n = 5)	9.8 (9.0–9.9)	9.3 (8.3–10.2)	−0.2 (−0.7–0.3)	NS
10–10.99				
Female (n = 7)	10.3 (10.0–10.9)	10.7 (9.0–11.3)	0 (−1.1–1.2)	NS
Male (n = 6)	10.2 (10.0–10.9)	11.1 (10.2–12)	0.6 (0.1–1.8)	0.028*
11–11.99				
Female (n = 7)	11.3 (11.2–11.8)	11.2 (10.1–11.7)	−0.3 (−1.4–0.2)	NS
Male (n = 4)	11.3 (11.2–11.4)	11.8 (10.7–12.8)	0.5 (−0.5–1.4)	NS
12–12.99				
Female (n = 6)	12.6 (12.1–12.8)	12.9 (11.3–13.8)	0.4 (−1.2–1.1)	NS
Male (n = 6)	12.5 (12.0–12.9)	12.3 (10.9–14.3)	0.1 (−1.6–1.4)	NS
13–13.99				
Female (n = 7)	13.3 (13.0–13.8)	13.0 (12.7–13.9)	0.2 (−0.9–0.3)	NS
Male (n = 10)	13.4 (13.2–13.8)	13.6 (12.0–14.3)	−0.1 (−1.3–0.9)	NS
14–14.99				
Female (n = 9)	14.3 (14.0–14.8)	13.8 (12.9–15.8)	−0.2 (−1.2–1.5)	NS
Male (n = 7)	14.3 (14.1–14.4)	13.6 (12.4–15.8)	−0.6 (−1.8–1.7)	NS

*PPT: Persisted Primary Teeth; Med: Median; Min: Minimum; Max: Maximum; CA: Chronologic age; DA: dental age; Δ: The deviation of age (DA-CA); *$p < 0.05$.*

TABLE 4. The comparison of dental age, and deviation according to age groups of children with PPT.

Sex	CA Med (Min–Max)	DA Med (Min–Max)	Δ age Med (Min–Max)	*p* value
Age group				
9–9.99				
Female (n = 6)	9.4 (9.0–10.0)	8.9 (7.8–10.8)	−0.6 (−1.3– 1.7)	NS
Male (n = 5)	9.5 (9.1–10.0)	8.9 (8.1–10.2)	−0.6 (−1.5–0.3)	NS
10–10.99				
Female (n = 7)	10.8 (10.1–11.0)	10.1 (9.5–11.2)	−0.8 (−1.3–1.1)	NS
Male (n = 6)	10.6 (10.1–10.9)	9.6 (8.1–11.2)	−0.8 (−2.8–0.5)	NS
11–11.99				
Female (n = 7)	11.5 (11.0–11.9)	11.1 (10.5–11.7)	−0.2 (−0.6–−0.2)	0.017*
Male (n = 4)	11.3 (11.2–11.4)	11.3 (10.9–11.7)	0 (−0.4–0.3)	NS
12–12.99				
Female (n = 6)	12.5 (12.1–13)	11.6 (10.3–13.1)	−0.6 (−2.2–0.1)	0.046*
Male (n = 6)	12.3 (12.0–12.8)	11.6 (10.2–12)	−0.8 (−2.1–−0.4)	0.028*
13–13.99				
Female (n = 7)	13.4 (13.0–13.5)	12.1 (11.2– 12.8)	−1.2 (−1.9–−0.7)	0.018*
Male (n = 10)	13.4 (12.1–13.9)	12.2 (9.6–12.4)	−1.5 (−4.4–−0.8)	0.048*
14–14.99				
Female (n = 9)	14.4 (14.0–14.9)	12.2 (11.5– 14.1)	−2 (−2.9–−0.9)	0.008*
Male (n = 7)	14.3 (14.1–14.9)	12.5 (10.3–14.3)	−1.9 (−4.7–−0.6)	0.018*

*PPT: Persisted Primary Teeth; Med: Median; Min: Minimum; Max: Maximum; CA: Chronologic age; DA: dental age; Δ: The deviation of age (DA-CA); *$p < 0.05$.*

or a delay in permanent tooth development. To the best of our knowledge, this present study is the first to evaluate the dental age of patients with multiple PPT.

The local or systemic etiological factors of PPT may vary. The most common cause for PPT was reported to be agenesis of a permanent successor, while other factors might include the existence of a pathology (cysts, tumors and odontoma), impaction, transposition, and delayed eruption of successor teeth [4, 15, 21]. In addition, some systemic disturbances, syndromes and metabolic and hormonal disorders have also been reported to be associated with PPT [9, 25]. In this current study, we excluded children with dental anomalies, local pathologies, syndromes or systemic diseases to evaluate the relationship between permanent tooth development and PPT.

Although the persistence of a single tooth may often occur, the occurrence of multiple PPT is rare. Only a limited number of publications on the PPT were found in the literature [4, 15, 16, 26]. In a study conducted on a Turkish subpopulation of 9632 children aged 9–15 years, the prevalence of PPT was found to be 4.5%, in whom 92.6% of the patients had 1–3 PPT, 6.7% had 4–6 PPT, and 0.7% had >6 PPT [15]. Multiple PPT and unerupted permanent teeth are usually associated with syndromes (Cleidocranial dysplasia, Gardner syndrome, Zimmerman Laband syndrome, Noonan's syndrome, Hyper-IgE syndrome) and conditions such as hemifacial atrophy, hypopituitarism, hypothyroidism, cherubism, gingival fibromatosis and cleft palate [27, 28]. Case reports of systemically healthy patients with multiple PPT have also been reported [22, 29]. In this present study, 80 healthy children with two or more PPT were selected and matched with children without PPT.

Dental development is a useful indicator for diagnosing dental anomalies and planning treatments in pediatric dentistry, orthodontics and maxillofacial surgery [30]. It provides additional information in forensic sciences, archeology, anthropology, pediatrics and orthopedics. Of the several methods used to estimate DA, Demirjian's method is the most commonly used radiological method and is based on the anatomical shape of teeth [2, 31]. However, some studies that used the Demirjian method for subjects from different regions and with different ethnicities found some inaccuracies and reported that the Demirjian method tended to overestimate patients' age [32]. Willems modified the Demirjian method's scoring system by creating new tables [11], which was then used in studies comprising various populations and was reported to provide more accurate estimations than the original Demirjian method [13]. Willems method was also found appropriate for estimating the dental age of children from Eastern, Northwestern and Central Anatolia regions of Turkey [13–15]. Therefore, in this study, the Willems method was used for dental age estimation of children from the Aegean region of Turkey.

In this present study, we found that children with PPT exhibited a delay in dental development, and their dental age was lower compared to the chronological age of females by −0.9 years and males by −1.0 years. We also found that as the number of PPT increased, the difference between chronological age and dental age also increased. In particular, the median dental age was 4 years less than the chronological age in males with more than 5 PPT. However, in patients without PPT, the difference between dental age and chronological age was negligible (for females −0.1, for males 0.1 years). Although PPT and delayed tooth eruption are commonly observed in dental practice, there is a lack of corresponding research in the literature. Because dental eruption and tooth development are two distinct processes, it is important to explore both processes to determine the source of the defect [8]. In normal dentition, the roots of primary teeth undergo continuous resorption during the eruption of permanent successors. Several theories have been proposed regarding factors influencing this process, but none clearly explained the cause and effect. The pressure of erupting permanent teeth, occlusal trauma, differentiation of monocytes of the periodontal ligament into odontoclasts, and inflammatory processes have all been considered to play a role in the mechanism [15]. Eruption of succedaneous permanent teeth into the oral cavity is influenced by the presence of primary teeth, whereas the intrabony development of the permanent teeth is influenced by systemic and local changes [8]. Whenever delayed tooth eruption is generalized, the risk of an underlying systemic disease affecting eruption, such as endocrine disorders, organ failures, metabolic disorders, vitamin D deficiency, anemia, drugs and genetic disorders, should be carefully examined [20].

A previous study investigating the distribution of PPT in individuals aged 14–47 reported that PPT was generally found in individuals under the age of 20, and it was determined that the number of PPT decreased proportionally with increasing age [16]. The decrease with age was attributed to the decreasing prognosis of PPT over time and could be extracted for orthodontic and prosthetic treatments. In this present study, the difference between dental age and chronological age was significant, especially in children with multiple PPT and aged 12–14 years. However, the difference might have been affected by the size of the study cohorts. One of the limitations of our study was the heterogeneity in the distribution of multiple PPT among the different age groups.

A disturbed eruption process creates a clinical situation that is challenging to diagnose and treat. If the PPT is free from pathology and has a favorable coronal and root structure, it may survive for many years. In addition, although some clinical problems such as advanced caries, periodontitis, ankylosis, pulp circulation disorder, reduction in pulp size, increase in secondary dentin structure, root resorption and hypercementosis could develop in these teeth [4, 26], these issues could be overcome with surgery, orthodontics, or combined treatments. So, early referral to a multidisciplinary team, including pediatricians and restorative and orthodontic specialists, is advised. Occasionally, a primary tooth can be considered a physical barrier to the eruption of the succedaneous tooth. In most cases, removing the primary tooth allows the spontaneous eruption of the successor tooth [33]. However, the timing of these treatments is also critical, and it is important to determine the developmental stage of the successor permanent tooth. Particularly, according to our present study, it should be kept in mind that tooth development in patients with multiple PPT might be delayed by 0.5–4 years.

There were some limitations in this study. Only children living in a single geographic region were included and matched only by age and sex between the control and study groups. Most children in both the study and control groups were from middle-income families and the same Turkish region. In addition, considering that nutritional, socioeconomic, sociocultural and environmental factors could affect the teeth development of children living in different regions; thus, this study does not adequately represent the general Turkish children population. Additionally, only a single examiner examined the radiographs and used only one method for dental age estimation. Thus, further studies using different methods are required to substantiate the findings of this study.

5. Conclusions

In conclusion, we found that permanent tooth development of children with multiple PPT could be delayed by 0.5–4 years compared to healthy ones. In addition, as the number of PPT increased, the difference between chronological age and dental age also increased, especially in males.

AVAILABILITY OF DATA AND MATERIALS

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

AUTHOR CONTRIBUTIONS

BGT—designed the research study; analyzed the data and wrote the manuscript. AT—performed the research; AT and BGT—final approval of the manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This present study was approved by the Clinical Research Ethics Committee of the Medical School of Afyonkarahisar Health Sciences University (2011-KAEK-2). All patients provided informed consent and permission for data usage from their parents.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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