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Histomorphometric Analysis of Pulp-Dentin Complex in Relation to Tooth Development and Eruption Patterns

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ABSTRACT

Background: The pulp-dentin complex is a dynamic structure that is important in the development, nutrition, and defense of teeth.
Objective: To examine the histomorphometric characteristics of the pulp-dentin complex, as well as to test the hypothesis of their connection to the tooth development and eruption times.
Methods: The study was an observational cross-sectional study of 80 extracted permanent human teeth that were gathered within one year at Faryal Dental College, Shaikhpura. Teeth that were at different developmental and eruption stages were histologically analyzed after being processed, decalcified, sectioned, and stained using the hematoxylin and eosin stain. The data were processed in SPSS v25; the group comparisons were done with ANOVA, and Pearson correlation was used to evaluate the developmental stage relationships. A p-value that was below 0.05 was notable.
Results: The pulp chamber area was smaller in unerupted (12.5 ± 0.1 mm2) and fully erupted teeth, whereas the dentin thickness, predentin width, and odontoblast densitywere significantly greater in fully erupted teeth. The developmental stage had a negative correlation with the pulp chamber area ($r = -0.82$), whereas dentin thickness, predentin width, and odontoblast density had positive relationships ($p < 0.05$).
Conclusion: Pulp-dentin complex is predicted to experience a systematic structural alteration throughout the development and eruption of the teeth. Such histomorphometric observations can be used in clinical dentistry, age determination, and forensics by offering normative information about pulp and dentin structure..

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Keywords: Pulp-dentin complex; Histomorphometry; Tooth development; Tooth eruption; Pulp chamber.

INTRODUCTION

The pulp-dentin complex is a dynamic and component structure of the tooth, which consists of a special connective tissue (dental pulp) that is surrounded by mineralized dentin.[1, 2] The complex is very important in the development of the teeth, nutrition, sensory, and defense against injury or infections.[3] The pulp-dentin complex plays a role in the formation of dentin in the process of odontogenesis through differentiation of odontoblasts, which form the structural integrity and functional capacity of the tooth.[1, 4] As a measurement of cellular arrangement, dentin thickness, the size of the pulp chamber, and other morphological variables in developing and erupted teeth, histomorphometrical analysis has turned out to be an effective technique that entails evaluation of the quantitative values of the tissue structures.[5]

The formation of teeth and their eruption is a very controlled biological process that is affected by genetics, epigenetics, and the environment.[6] Tooth eruption time and sequence are mandatory indicators of both oral and systemic health, and their abnormalities are usually related to underlying developmental or metabolic pathology.[7] Epidemiological research shows that differences in tooth eruption patterns can impact anywhere between 15%-20% of children worldwide, and this may result

in malocclusion, retarded occlusal development, and impaired oral functioning.[8, 9] Histological analyses have shown that any alteration in the dentin deposition and pulp volume relates to chronological age, type of tooth, and eruption stage, and this has indicated the significant role of the understanding of the microstructure of the pulp-dentin complex in clinical and forensic dentistry.[10]

In spite of its importance, the available amount of quantitative data to correlate the histomorphometric properties of the pulp-dentin complex with particular stages of tooth development and eruption is still low. The available literature is mainly on either gross morphometry or clinical eruption patterns without incorporating accurate histological measurements. A comprehensive histomorphometric analysis may help to reveal normal and pathological changes, inform the course of restorative and endodontic procedures, and provide a source of information regarding age determination in forensic studies.

Considering the pivotal position of the pulp-dentin complex in the development and functioning of the tooth, information on the histomorphometrical properties of the complex as a function of the stage of eruption is important both clinically and in research. This study quantitatively examines the structural characteristics of the pulp-dentin complex at various phases of tooth formation and eruption patterns with the ultimate goal of identifying normative pulp-dentin complex data and determining the relationship between tissue architecture and the stage of development. The aim of the current research was to conduct a histomorphometric study on the pulp-dentin complex of human teeth and to examine the connection between the structure and various eruption sequences and stages of tooth development.

METHODOLOGY

The present work was performed through an observational cross-sectional design to measure histomorphometric parameters of the pulp dentin complex between various stages of tooth development and eruption quantitatively. The study was conducted in the Department of Oral Biology at Faryal Dental College, Shaikhpura, Lahore, and all the laboratory processing and microscopic analyses were done.

The sample size was determined with the OpenEpi software because of the 95% confidence interval and the 80% power. It was calculated using the anticipated variations in quantitative pulp and dentin measurements based on past histomorphometric studies on dental research. To illustrate this, histomorphometric pulp tissue parameter comparison studies between various tooth types and conditions have employed between 20 and 60 teeth sample sizes to identify significant differences between tissues in objective morphometry measures.[11] Taking into account the possibility of variation and potential processing loss, the minimum sample size was determined to be 80 extracted permanent teeth in order to provide adequate power to make subgroup analyses.

A non-probability consecutive sampling technique was used to include all the extracted human permanent teeth that were eligible and met the study criteria. The teeth sample was taken in a sequence based on their removal to treat, carry out orthodontic, or prophylactic purposes by the patients who were receiving treatment in the dental clinics at the time they were sampled. All the teeth were screened, and those that had satisfied the inclusion criteria were processed up to the required sample size.

Inclusion criteria included permanent human teeth that were marked to be removed due to a reason not directly related to active deep caries, trauma, or severe periodontal destruction, had intact crowns and roots, which were suitable to undergo histological sectioning. The inclusion of teeth was also to showcase different stages of development and eruptions, such as unerupted, partially erupted, and fully developed teeth. Exclusion criteria were gross carious destruction of teeth, endodontic treatment history, and previous restoration to the pulp chamber, root fracture, or structural deformity that would preclude a proper histomorphometric evaluation. Teeth of patients who had systemic diseases that could lead to dental development, e.g., metabolic bone disease, syndromic growth disorders, were also excluded.

Remnants of the soft tissues on extracted teeth were first removed, and the teeth were transferred into 10% neutral buffered formalin. A standard decalcifying solution was used to decalcify until the softness of the sectioning became optimal. The teeth were then embedded in paraffin wax, and serial sections of about 5 µm thickness were used and achieved using a rotary microtome. Glass slides were used to mount sections and stain sections using hematoxylin and eosin (H&E) to observe the histological structure of the pulp and dentin. The histomorphometric parameters, such as area of the pulp chamber, dentin thickness, integrity of odontoblast layer, and predentin width, were measured using a digital microscope with calibrated imaging software, and measurement was taken by two independent observers who were blinded to the stage of development of the tooth to eliminate observer bias.

All the measurements were then tabulated in SPSS (Statistical Package for the Social Sciences) version 25.0 after data collection. Continuous variables were tested in terms of mean and SD due to the evaluation of the normality with the Shapiro-Wilk test. The comparison of histomorphometric parameters between stages of development and eruption was done with ANOVA of normally distributed data or Kruskal-Wallis test of non-parametric data, with post hoc tests where necessary. Quantitative tissue measurements versus chronological stage of development were tested by the Pearson or Spearman correlation coefficients. A p-value below 0.05 was taken to be statistically significant.

RESULTS

The study contained 80 permanent human teeth, which were at different development stages and at different eruption stages. Among them, 25 teeth (31.2%) were not erupted, 20 teeth (25.0%) were partially erupted, and 35 teeth (43.8%) were fully erupted (Table 1).

Table 1. Distribution of Teeth According to Eruption Status (n = 80)

Eruption Status	Number of Teeth n(%)
Unerupted	25 (31.2%)
Partially Erupted	20 (25.0%)
Fully Erupted	35 (43.8%)

A histomorphometric examination indicated that the pulp chamber area reduced as the tooth developed and decreased to 6.5 ± 1.0 mm² in teeth that had fully erupted. However, on the other hand, the thickness of dentin, predentin, and the density of the odontoblast layer were greater with the maturation of the tooth. The dentin thickness varied between 0.8 ± 0.1 mm in the unerupted teeth and 2.0 ± 0.3 mm in the fully erupted teeth, and predentin width and odontoblast density had the same increasing pattern. The differences between eruption stages were all statistically significant (p 0.05) (Table 2).

Table 2. Histomorphometric Parameters of Pulp–Dentin Complex by Eruption Status

Parameter	Unerupted (n=25)	Partially Erupted (n=20)	Fully Erupted (n=35)	p-value
Pulp Chamber Area (mm²)	12.5 ± 1.8	9.1 ± 1.2	6.5 ± 1.0	<0.001
Dentin Thickness (mm)	0.8 ± 0.1	1.5 ± 0.2	2.0 ± 0.3	<0.001
Predentin Width (µm)	25 ± 5	35 ± 7	40 ± 6	0.002
Odontoblast Layer Density (cells/100 µm)	12 ± 2	18 ± 3	20 ± 2	<0.001

Correlation showed that there is a high degree of negative correlation between pulp chamber area and chronological development stage ($r = -0.82$, $p < 0.001$) whereby the pulp volume declines with the maturity of the teeth. Conversely, the developmental stage had positive relationships with dentin thickness ($r = 0.78$, $p < 0.001$), predentin width ($r = 0.61$, $p = 0.001$), and odontoblast layer density ($r = 0.64$, $p < 0.001$), indicating progressive deposition of dentin and cellular activity in the formation and eruption of teeth (Table 3).

Table 3. Correlation between Histomorphometric Parameters and Chronological Development Stage

Parameter	Correlation Coefficient (r)	p-value
Pulp Chamber Area (mm²)	-0.82	<0.001
Dentin Thickness (mm)	0.78	<0.001
Predentin Width (µm)	0.61	0.001
Odontoblast Layer Density (cells/100 µm)	0.64	<0.001

DISCUSSION

Histomorphometric analysis in the current research revealed that the size of the pulp chamber declined significantly, whereas the thickness of dentin, predentin, and the density of the odontoblast layer grew with the progressions in developmental and eruption stages of the permanent teeth. These patterns are in harmony with the general evidence of these changes of the pulp-dentin complex, closely related to the maturation and structural development of the tooth.

We also found that the degree of progressive pulp reduction with higher tooth maturation corresponded with the volume analysis of the pulp tissue in human and animal models. As an illustration, a micro CT analysis has shown that pulp volume of postmortem human teeth can be predicted to decrease with dentin thickness, increasing with chronological age and maturation of the dentition, which shows that the volume of the pulp is inversely related to the deposition of dentin.[12]

Similarly, equine dental pulp studies indicated that the thickness of the dentin layer is influenced by age, which causes a decrease in the size of the pulp chamber, indicating a pattern of conservation across species.[13]

Our findings are also aligned with age-related histological findings that indicate aging in the dental pulp is characterized by a decrease in cell number, blood vessels, and pulp chamber volume, and a simultaneous increase in secondary dentin. These age-associated changes substantiate that structural maturation is a major factor of pulp and dentin morphology, as is our observation of a growing dentin thickness and constricting pulp space with eruption.[14]

Besides developmental modification, the cellular activity of odontoblasts is another significant factor that dictates the creation of dentin. The animal studies by experiment have demonstrated that the particular progenitor cells in the pulp lead to odontoblast differentiation and dentin regeneration, which means that dental processes in the pulp determine the dentinogenesis during life.[15] Although our research has not addressed molecular markers, the general finding of odontoblast layer density with maturation is consistent with the idea that there is increased odontoblastic activation during appositional dentinogenesis in erupting teeth and maturing teeth.

The pulp dentin complex is also dynamic, as evidenced by the comparative histological studies. Indicatively, subluxation trauma in rat teeth produced an odontoblast layer integrity and accentuation of reactionary dentin, which is paramount in the way mechanical and environmental changes influence pulp and dentin histology. Despite the differences between traumatic changes and normal developmental patterns, such results reveal how sensitive pulp tissue architecture is to both external and internal stimuli, as observed in our changes in structural patterns in the course of eruption.[16]

The restorative and clinical studies also emphasize the significance of dentin thickness in the health of the dentin. The studies comparing pulpal reactions to a range of remaining dentin thickness showed that the histomorphometric alterations in the dentin and pulp tissue in response to the restorative therapies confirmed that the dentin size and pulp shape are interdependent not only in developmental aspects but also in clinical services.[17]

Even though certain studies touch on other areas, e.g., dental pulp response during restorative scenarios, the effects of the restorative material on pulp histology, etc., collectively these studies provide the conclusion that quantitative organization of pulp and dentin is part and parcel of general dental biology and dental pathology. Indeed, histomorphometric analyses of restorative models showed alterations in both inflammatory cell invasion and disorganization of the tissue based on dentin thickness and material biocompatibility, which supports the argument that pulp–dentin structure is a key factor of functional response.[17]

The relevance of our data in the context of developmental and disease conditions is also supported by the fact that the volumetric CBCT-based measurements of the pulpal change can be correlated with periodontal conditions or endodontic conditions, indicating the overall applicability of quantitative analysis of the pulpal in development as well as disease conditions.[18] Although methodologically, imaging and histomorphometry are different, converging information on both techniques suggests that quantitative alteration of pulp and dentin are significant indicator of tooth conditions in the life span.

In general, the current results complement the body of existing literature since the morphometric parameters of the pulp and dentin complex are quantitatively associated with certain eruption and developmental stages in human permanent teeth. Our study reflects normative histomorphometric data of pulp tissue during specific developmental stages, unlike other earlier studies that reported clinical, traumatic, or restorative effects on pulp tissue, which can particularly be of value in estimating age, forensic evidence, and developing normal dental development.

The clinical implications of the findings of this study include a number of factors. Knowledge of the quantitative variations in pulp chamber size, dentin thickness, and odontoblast density through the various phases of tooth development and eruption may assist the practitioner in the field of pediatric dentistry, orthodontics, and endodontics in making an informed decision. Pulp and dentin morphometry are specifically important in age determination in a forensic investigation, minimally invasive restorative dentistry, and the estimation of pulpal reaction to trauma or surgical actions. Additionally, the capacity to understand the normal histomorphometric variations can guide dentists to plan their treatment approach based on the stage in which the tooth has developed, thus limiting the chances of exposing the pulp during cavity preparation or any other dental intervention.

This study has several limitations, although it has strengths. First, the samples were taken from extracted teeth, which might not accurately indicate in vivo conditions of the pulp-dentin relationship, because tooth extraction might change the integrity of the tissue. Second, the methods of the study were histological sectioning and two-dimensional analysis, which might not fully describe the three-dimensional aspect of the pulp-dentin complex. Third, there was no measurement of molecular markers of odontoblastic activity or pulp vitality, which restricted the knowledge of functional correlates of the morphometric change. Lastly, the sample used, though adequate to conduct a preliminary analysis, was still comparatively small and only applied to one population, and this can limit the extrapolation of the findings to greater populations or other age groups.

CONCLUSION

In this study, the pulp-dentin complex was thoroughly studied in terms of histomorphometry during developmental and eruption stages, and it was revealed that there is a sharp decline in the size of the pulp chamber and a sharp increase in the dentin thickness, predentin width, and odontoblast density with the maturation of the tooth. The results of this study indicate

the dynamism of the pulp-dentin complex and its applicability in clinical dentistry, forensic, and developmental biology. This study provides an important contribution to the current understanding of human teeth structural development through the creation of normative histomorphometric data and an initial basis of reference both in clinical practice and future studies on dental development.

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