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Histological changes in dental pulp tissue with age: A comparative study

Abdul Nasser H. Warwar[1], Mohammed I. Abdullah[2], Wesam A. Sami[3], Waleed. K.Mohammed[4*]

[1] *Department of Oral Histology, College of Dentistry, University of Anbar, AL- Ramadi city, Iraq*
[2] *Department of POP, College of Dentistry, University of Anbar, AL- Ramadi city, Iraq*
[3] *Department of POP, College of Dentistry, University of Anbar, AL- Ramadi city, Iraq*
[4] *Department of Basic Science, College of Dentistry, University of Anbar, AL- Ramadi city, Iraq*

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Abstract

The dental pulp undergoes several histological changes with age. These include a reduction in the number of cells and blood vessels, resulting in a decreased capacity for regeneration and repair. Furthermore, there is an increase in collagen fiber density and the formation of secondary dentin, which reduces the volume of the pulp chamber. The study has performed a comparative histological dissection to describe and characterize these age that are related changes in dental pulp tissues. A cross-section of samples was comparatively analyzed to examine the histological changes in dental pulp tissue from two distinct age groups, which are old and young ages, and then extracted teeth were thoroughly cleaned and immediately fixed in 10% formalin for preservation. Each single sample was decalcified by using 10% formic acid and for a duration sufficient to allow sectioning without damaging the tissue, and after decalcification, the teeth were embedded in paraffin wax, and serial sections, with a thickness of 4-6 µm, were prepared using a microtome. The sections have been then stained with Hematoxylin and Eosin to visualize general tissue structure. The histological analysis demonstrated notable differences in dental pulp tissue between the two age groups. Group A (young) samples showed a high cellularity, with numerous fibroblasts and odontoblasts and a clearly defined odontoblastic layer. However, Group B (elderly) samples exhibited a marked reduction in cellularity, with fewer odontoblasts present and evidence of increased fibroblast degeneration. The histological changes observed in our study underscore the impact of aging on dental pulp tissue.

Keywords: Dental pulp tissue, Cellularity reduction, Vascularity reduction, Aging, Pulp stone.

1. Introduction

Dental pulp is a vital soft tissue located at the core of the tooth, encased by dentine and extending from the pulp chamber to root canals. It serves multiple essential functions, including dentine formation through the activity of odontoblast, nutrient supply to the dentine, sensory transmission of pain and other stimuli and immune defense against microbial invasion [1]. The pulp consists of cells (odontoblasts, fibroblasts, immune cells and undifferentiated mesenchymal stem cells), extracellular matrix, blood vessels and nerves[2]. The balance of these components ensures the pulp protective and reparative responses throughout life. With advancing age, notable histological and structural changes occur in the dental pulp. The reduction in cellularity was one of the most primary alterations that can be observed [3], and then the number of odontoblasts and fibroblasts decreases, which is compromises the pulp ability to produce new dentine in response to injuryThe extracellular matrix is highly dynamic, and it also undergoes remodeling with an increase in collagen fibers leading to more fibrotic tissue. Therefore, these changes reduce the overall flexibility and resilience of the pulp [4]. The extracellular matrix is highly dynamic, and it also undergoes remodeling with an increase in collagen fibers leading to more fibrotic tissue. Therefore, these changes reduce the overall flexibility and resilience of the pulp. Another essential mark of aging pulp that can be observed is the formation of dystrophic calcifications and pulp stoneswhich are classified, depended on their structure into true denticles, false denticles, and diffuse calcification, which can range from diffuse calcified deposits to well- defined denticles [5]. In addition, the vascular and nerve supply diminishes, which can lead to decreased sensitivity and reduced ability to mount an effective reparative or inflammatory response [6]. Therefore, these age-related histological transformations have remarkable and significant clinical implications [4]. One of these is that the reduced regenerative capacity of aged pulp may affect treatment outcomes in procedures such as direct pulp capping, root canal treatment and restorative interventions involving deep cavities [7]. Furthermore, the increased fibrotic nature and calcifications can complicate endodontic procedures, making access and instrumentation more challenging [8]. Therefore, Understanding such structural changes in pulp is critical not simply

* Corresponding author.
E-mail address: den.waleed.temem@uoanbar.edu.iq (M. Waleed).
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for making sense of things, but for developing treatment approaches that enhance success while minimizing patient discomfort [9], making this knowledge significant for clinicians and dentistry. This study aims to perform comparative histological analysis of dental pulp tissues among various age groups followed by referencing the age-related changes during young age and old age. Thus, through the identification and characterization of these alterations, the study seeks to evidence these changes in the aging process of dental pulp and their relevance in the clinical context. These findings may influence the development of age-specific treatment modalities leading to superior outcomes in restorative and endodontic procedures.

2. Materials and Methods

2.1. Introduction to study design

This study utilized a cross-sectional comparative design to analyze histological alterations in dental pulp tissues from two different aged populations. As noted, the samples of the different age groups were gathered at a single point in time which makes it possible to compare the structural changes associated with aging.

2.2. Ethical considerations and participant consent

The ethical committee of the relevant institution approved the study. Therefore, all participants provided written informed consent for the use of their extracted teeth for research purposes, ensuring compliance with ethical standards for human research.

2.3. Sample selection

This research obtained twenty-six teeth from 16 participants to detect a significant difference with 95% confidence and 80% power. The sample consisted of extracted human teeth divided equally between two age groups:

- **Group A**: Teeth from individuals aged 20-30 years (young adult group).
- **Group B**: Teeth from individuals aged 60 years and above (elderly group). Inclusion criteria included non-carious teeth extracted for orthodontic reasons and teeth with intact pulp chambers and root canals. Exclusion criteria consisted of teeth with advanced caries, visible pulp exposure, prior endodontic treatment, and teeth with external or internal root resorption. All teeth were collected following informed consent from patients and in accordance with ethical guidelines.

2.4. Histological investigation

After extraction, the teeth were cleaned and fixed in 10% formalin for preservation. Each sample was then decalcified with 10% formic acid for a sufficient period to allow sectioning without compromising tissue integrity. The teeth were embedded in paraffin, and serial sections (4-6 µm thick) were prepared. The sections were stained with:

- Hematoxylin and Eosin (H&E) for general tissue structure visualization.
- Van Gieson stain for collagen fiber analysis.

The stained sections were examined under a light microscope to evaluate:

1. Cellularity (number of fibroblasts and odontoblasts).
2. Collagen fiber content.
3. Presence of calcification and pulp stones.
4.

2.5. Data analysis

Data were compared between the two groups using appropriate statistical tests (t-tests).

3. Results

The histological investigation showed distinct differences between the dental pulp tissues of the young (Group A see Fig 1 A and B) and elderly (Group B see Fig 2) samples. Cellularity: In Group A, the dental pulp tissue showed high density of fibroblasts and odontoblasts cells, with a well-defined odontoblastic layer along the periphery. In contrast, Group B exhibited significantly reduced cellularity, with fewer odontoblasts and increased fibroblast degeneration. Collagen content: Increased collagen fibers were evident in the pulp of Group B, contributing to a denser and more fibrotic structure, compared to the looser connective tissue observed in Group A. Calcifications: pulp stones and diffuse calcification were prominent in Group B, while they were rare or absent in Group A. Vascularity: Group A samples showed extensive vascularization, while Group B exhibited reduced vascular networks and narrowed blood vessels as in Table 1. Table 2 presents a comparative analysis of cellularity, collagen fiber density and calcification between Groups A and B. The mean cellularity in group A 45 ± 5.2 cells/ mm^2 was significantly higher than in group B 20 ± 4.3 cells/ mm^2 ($P< 0.01$). Conversely, group B exhibited greater collagen fiber density 60 ± 7.2 compared to group A 30 ± 5.3, with statistically significant ($P< 0.05$). Additionally, calcification was markedly increased in group B 55% relative to group A 5%, showing a significant difference ($P< 0.01$). These findings indicate age-related or condition- specific changes affecting the structural composition of dental pulp tissue.

4. Discussion

The histological analysis of dental pulp tissue in our study distinct differences between younger (group A) and elderly (group B) samples, highlighting age-related changes that significantly affect pulp morphology and function. The results have been demonstrated a significant reductions in cellularity with advancing age. Group A exhibited a high density of fibroblasts and odontoblasts and also a well-defined odontoblastic layer lining the periphery of the pulp tissue. In contrast, Group B was showed markedly fewer odontoblasts and increased degeneration of fibroblasts, resulting in a diminished cellular population. The results of reduction in cellularity can be attributed to the natural aging process, which can leads to a decreased regeneration capacity and reduced metabolic activity in the dental pulp [10]. The loss of odontoblasts which is responsible for dentine formation may compromise the tooth ability to respond to external stimuli, thereby increasing its susceptibility to injury and disease [11The results also showed a significant increase in collagen fiber density that was observed in Group B compared to Group A and the younger pulp tissue displayed sparse, loosely organized collagen fiber, characteristic of a more flexible and resilient connective tissue structure [12]. Conversely, the elderly pulp tissue exhibited dense and fibrotic collagen fibers that are contributing to a stiffer and less adaptable pulp matrix [13]. The aged related fibrosis reflects a progressive shift toward a more fibrotic extracellular environment which can impair nutrient diffusion and cellular communication and overall pulp vitality [14]. The content of increased collagen may also limit the pulp

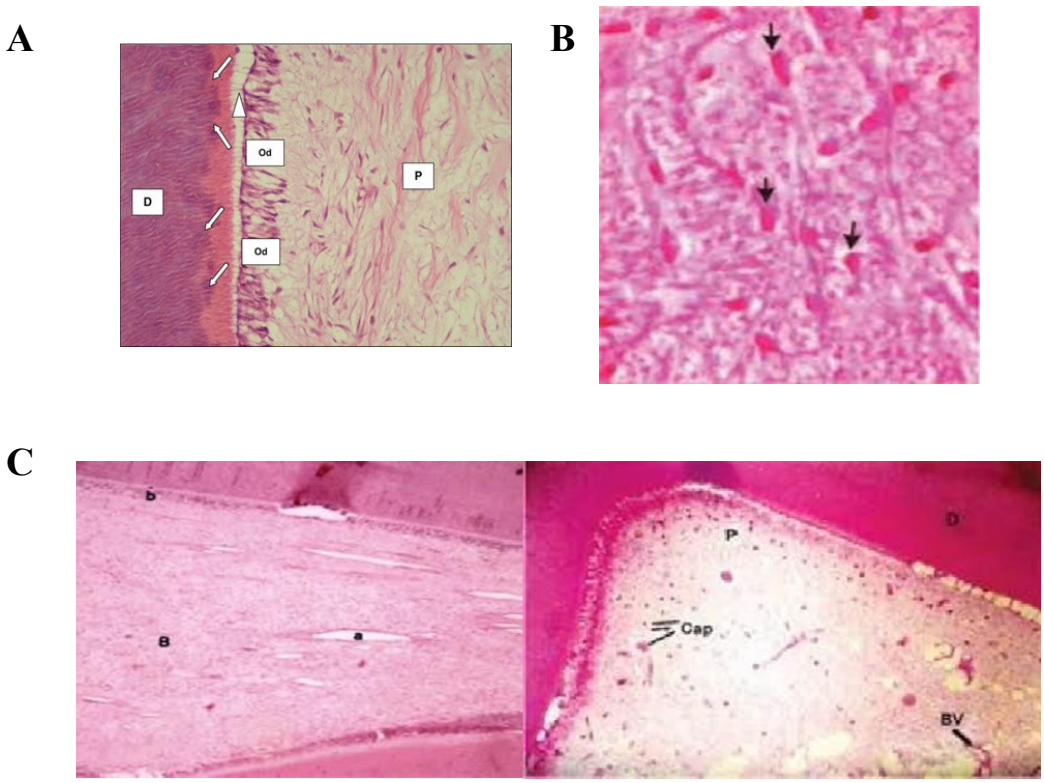

Fig. 1. (A) : Image showing the interface between dentine and pulp tissue. The pointer in D refer to : Dentine that is calcified tissue forms the bulk of the tooth, located adjacent to the pulp ; while Od: Odontoblasts lining the pulpal border of dentine; on the onter hand P: Dental pulp. The arrows indicate the odontoblastic processes extending into the dentinal tubules or may be or the odontoblast layer which are showing the close relationship between the dentine and the odontoblasts. Haematoxylin and eosin stain, original magnification (H&E) ×200. **(B):** The image shows multipolar neurons within the central nervous system. The arrows can indicate that the elongated nuclei of astrocytes, a type of glial cell involved in maintaining the blood-brain barrier, providing nutrients to neurons, and regulating the extracellular ionic balance as well as, the background contains neuropil, composed of a dense network of neuronal and glial processes. Histological section of nervous tissue stained with hematoxylin and eosin (H&E) ×200. **(C)**: This images show Histological of Dental Pulp Tissue in young patient from thw left, image shows low magnification that are showing developing dentin (D) and pulp (P) regions. While the ameloblast layer (a) and odontoblast layer (b) are visible at the interface of the enamel organ and dental papilla. B indicates the dental follicle or surrounding connective tissue. On the other hand, Right image shows that is imag has higher magnification of the dental papilla region. Numerous capillaries (Cap) are seen within the mesenchymal tissue, along with a blood vessel (BV), indicating active vascular support during tooth development. P denotes the dental pulp, and D refers to the surrounding developing dentin matrix. Histological sections of developing tooth tissue stained with hematoxylin and eosin (H&E).

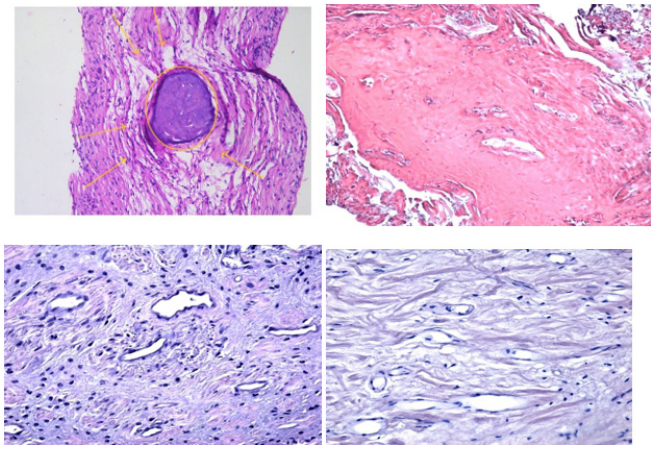

Fig. 2. Histological of Dental Pulp Tissue in adult patient.

ability to mount effective reparative responses following injury [15]. In addition, the prevalence of calcifications was significantly higher in Group B and also where both pulp stones and diffuse calcification were prominent. In contrast, these structures were rare or absent in Group A. The formation of pulp stones and diffuse calcifications that is a common feature of aging pulp and may result from chronic inflammation that is reduced vascularity or dystrophic mineralization processes [16]. Calcifications can be hinder endodontic treatment by obstructing canal systems and also complicating instrumentation [17]. The vascular network that was notably more extensive in Group A was characterized by wide and abundant blood vessels and this can support robust pulp tissue perfusion. In Group B, vascularity was reduced, with narrowed and diminished blood vessels. This reduction in vascular supply with age impairs the delivery of nutrients and oxygen leading to compromised pulp health and decreased capacity for tissue repair [18]. Reduced vascularity also affects the pulp ability to mount inflammatory responses and this can further contributing to diminished pulp vitality [19]. These results align with previous studies indicating that aging significantly affects dental pulp tissue. Another studies by Maeda (2015) and Iezzi et al (2019) also documented decreased cellularity that is increased collagen deposition and higher incidence of pulp calcification in older populations [4, 6]. Biological reasons for observed changes: The age-related decline in pulp cellularity is primarily due to reduced cell proliferation and increased apoptosis of fibroblasts and odontoblasts. Collagen accumulation occurs as part of a reparative or degenerative response, influenced

Table 1. Histological differences in dental pulp tissue according to age.

Feature	Group A(20-30 years)	Group B (60 > years)
Cellularity	High fibroblast and odontoblast count	Reduced fibroblast and odontoblast count
Collagen content	Sparse collagen fiber	Dense fibrotic collagen fiber
Calcifications	Rare or absent	Pulp stone and diffuse calcifications
Vascularity	Extensive network of blood vessels	reduced vascularity with narrowed vessels

Table 2. Comparison of cellularity, Collagen fiber density and calcification between Group A and Group B.

Parameter	Group A Mean ± SD Cells/ mm²	Group B Mean ± SD Cells/ mm²	P- value
Cellularity	45 ± 5.2	20 ± 4.3	0.01*
Collagen fiber density	30 ± 5.3	60 ± 7.2	0.05*
Calcifications	5 %	55 %	0.01*

* Significant at P< 0.05

by decreased matrix turnover and increased fibrotic activity. Calcification that are thought to arise from dystrophic mineralization are driven by chronic inflammation or localized pulp tissue necrosis [20]. The reduction in vascularity results from vascular sclerosis and narrowing of blood vessels that are limiting the pulp ability to maintain homeostasis and repair mechanisms [21, 22]. The statistical analysis confirmed the significance of these age-related differences, with p-values < 0.05 for cellularity, collagen fiber density and calcifications. These findings emphasize the progressive nature of pulp tissue degeneration with age and the corresponding decrease in its functional capabilities.

5. Conclusion

This study provides histological features of dental pulp tissue which are compared with high precision between young and aged individuals, and a clear trend of age changes that is seen as established with the detailed histological study in this work that is decrease in cellularity and vascularity, increased density of collagen fibers and also increased prevalence of pulp calcifications observed in this study reflect the progressive degeneration of pulp tissue with advancing age. All results highlight the need for age specific strategies in dental practice. particularly when deciding the value of preserving pulpal tissues for restorative or endodontic treatment in the elderly. Future research should focus on identifying potential strategies to mitigate these age related changes and preserve pulp health in the aging population.

6. Clinical implication

These histological changes have significant clinical implications. Reduced cellularity and increased fibrosis diminish the pulp regenerative and reparative capacity, making older patients more prone tom pulp necrosis following injury or restorative procedures. Increased calcifications pose challenges in endodontic therapy by obstructing canal access, requiring advanced imaging and instrumentation techniques. Additionally, compromised vascularity can delay healing responses and exacerbate pulp pathologies.

Conflict of Interests

The author has no conflicts with any step of the article preparation.

Consent for publications

The author read and approved the final manuscript for publication.

Ethics approval and consent to participate

No human or animals were used in the present research.

Informed Consent

The authors declare that no patients were used in this study.

Availability of data and material

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Authors' contributions

Abdul Nasser H. Warwar and Mohammed I. Abdullah: Research design and supervision; Wesam A. Sami and Waleed Mohammed: Perform all laboratory procedures

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