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Histological transformations of the dental pulp as possible indicator of post mortem interval: a pilot study

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ABSTRACT

Background: The correct estimation of the post mortem interval (PMI) can be crucial on the success of a forensic investigation. Diverse methods have been used to estimate PMI, considering physical changes that occur after death, such as mortis algor, livor mortis, among others. Degradation after death of dental pulp is a complex process that has not yet been studied thoroughly. It has been described that pulp RNA degradation could be an indicator of PMI, however this study is limited to 6 days. The tooth is the hardest organ of the human body, and within is confined dental pulp. The pulp morphology is defined as a lax conjunctive tissue with great sensory innervation, abundant microcirculation and great presence of groups of cell types.

Aim: The aim of this study is to describe the potential use of pulp post mortem alterations to estimate PMI, using a new methodology that will allow obtainment of pulp tissue to be used for histomorphological analysis. The current study will identify potential histological indicators in dental pulp tissue to estimate PMI in time intervals of 24 h, 1 month, 3 months and 6 months.

Materials and method: This study used 26 teeth from individuals with known PMI of 24 h, 1 month, 3 months or 6 months. All samples were manipulated with the new methodology (Carrasco, P. and Inostroza C. inventors; Universidad de los Andes, assignee. Forensic identification, post mortem interval estimation and cause of death determination by recovery of dental tissue. United State patent US 61/826,558 23.05.2013) to extract pulp tissue without the destruction of the tooth. The dental pulp tissues obtained were fixed in formalin for the subsequent generation of histological sections, stained with Hematoxylin Eosin and Masson's Trichrome. All sections were observed under an optical microscope using magnifications of 10× and 40×.

Results: The microscopic analysis of the samples showed a progressive transformation of the cellular components and fibers of dental pulp along PMI. These results allowed creating a chart of qualitative and quantitative parameters to be used on the estimation on PMI based on microscopic degradation of dental pulp.

Conclusions: The histological transformations of dental pulp as a function of time can be used as PMI indicators.

1. Introduction

The tooth is the hardest organ of the human body. It is formed by pulp tissue, which is within a rigid layer of mineralized dentin, covered by enamel on the crown and cementum on the root [1]. This characteristic of dental pulp gives it a high mechanical resistance against the aggressions of the environment and the surrounding microorganisms. Dental pulp is a non-mineralized oral tissue composed by soft, vascular, lymphatic connective tissue and nerve elements that occupy the central pulp cavity of each tooth. The pulp has a smooth and gelatinous consistency, has great sensory innervation, rich microcirculation and cellular and fibrous variety (loose connective tissue) that makes it a unique tissue on the human body [2]. Some of the properties of the pulp are given by the rigid and mineralized dentin that surrounds and encloses it, hence this tissue may be helpful on the study of time of death [3]. Pulp cavity extends along from the crown to the apex by the root of the tooth, where blood vessels, lymphatic and nerves of pulp come in and out of the tooth, thus creating a communication channel

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between pulp and the tissues around it. Histologically, the types of cells present on the pulp are fibroblasts, odontoblasts and undifferentiated mesenchymal cells, together with other cells from the immune system such as macrophages, lymphocytes, etc. that are needed for the maintenance and defense of the tissue [4].

The fibrous matrix is composed by collagen fibers of type I and II which are present in disaggregated and randomly dispersed form, with greater density around blood vessels and nerves. Collagen type I is believe to be produce by odontoblast and by dentin itself. Collagen type II is most likely produced by fibroblasts of the pulp due to its increase in frequency with the age of the tooth. The oldest pulps contain more of the packaged and diffuse types of collagen. The fundamental substance is the environment that surrounds both cells and fibers of the pulp, rich in proteoglycans, glycoproteins and large amounts of water [4].

The great number of undifferentiated mesenchymal cells (as perivascular cells) in the pulp eases the recruitment of new differentiating cells to replace other when are lost, specifically odontoblasts. Odontoblasts are located on the external region of the pulp, next to the dentin component. These cells are responsible for dentin secretion and formation of dentinal tubes on the crown and root of the tooth [5].

The estimation of PMI has a great importance and implication on the criminal and forensic investigation. After the death of an individual occur different process of degradation and putrefaction of the body, parameters which have been used widely for the determination of PMI, such as *mortis algor*,*livor mortis*, *rigor mortis*, DNA degradation [6], ARN degradation [7], postmortem biomarkers [8–11], bacterial succession and forensic entomology [12,13], among others that can provide an estimate PMI. It has been described that the potential use of molecular markers like ARN degradation as function of time as an indicator for PMI. Recently, Bishop & Cols publication, they studied the characterization of the degradation of RNA as a function of time on porcine dental pulp, however the usefulness of this approximations is limited to 6 days [7,8].

Nevertheless, up until now there exists few studies that can establish PMI from the study of the integral degradation of dental pulp.

The studies found on which dental pulp is used to estimate PMI are few and they have limited time intervals where they do not overcome 7 days postmortem. Caballín, Gawande, Mehendiratta and Vavpotic studied histological variations that occur on post mortem pulp, making longitudinal and transversal conventional sections of the demineralized tooth, which allows observing the morphology of dental pulp and dentin. The use of conventional section of the tooth implies the complete destruction of the tooth and limits the analysis to a short PMI [14–17]. The aim of this study is to describe the potential use of dental pulp modifications to estimate PMI, using a new methodology [18] that will allow obtainment of pulp tissue in integral conditions that may be used for histological analysis in time intervals that have never been studied before, from 24 h to 6 months.

2. Material and method

2.1. Study design

In this pilot study collection, manipulation and analyses on human tissues and organs were performed within the framework of the Bioethics committee of the Dental School from Universidad de los Andes. An informed consent was especially designed for the research and was signed by every individual who donated a dental piece for the study and by the principal investigator. Dental samples were donated by patients who had extraction of third molars for orthodontic reasons, at Dental Integral Center Cedin Ltd, Paine, Chile. The ages of the individuals, from both genders, were between 20 and 40 years old.

This experimental design considered the day of extraction as equivalent to the time of death, given that at that moment the neurovascular bundle, which gives vitality to the tooth, is sectioned on the apex initiating a process of anoxia and cellular lysis.

2.2. Postmortem intervals for the study of the samples

The sample size was calculated arbitrary because it is the first study of this type.

This study used 24 samples that were grouped in 4 experimental groups:

1. Experimental group 1: 6 dental pulp 24 h from extraction.
2. Experimental group 2: 6 dental pulp 1 month from extraction.
3. Experimental group 3: 6 dental pulp 3 months from extraction.
4. Experimental group 4: 6 dental pulp 6 months from extraction.

All dental specimens were manipulated and storage under laboratory conditions between 20–25 °C, 1 at. and 40% environmental humidity.

2.3. Obtaining dental pulp

According to the method described previously for the authors [18]. Each dental sample extracted was kept on a 5 ml polypropylene sterile tube (Micro biologic Rubilator, S.L., Barcelona) until completion of 24 h, 1 month, 3 months or 6 months of PMI period, respectively. A digital radiography was taken for each sample in order to accurately plan access to the pulp cavity. The X-ray equipment used was Sirona, Heliodent, and Charlotte, NC 28273, USA. The tooth must be positioned sideways, in other words in its mesial or distal surface for the radiography to show an image on a facial-lingual orientation. The analysis of the distances on the images was made using the software SIDEXIS (Sirona®). Samples were washed on a saline solution buffered at pH 7.4 by phosphate (Phosphate Buffered Saline, PBS 1x, Hyclone) with successive washes on a volume of 10 ml during 1 min and vortexed (Mrc®).

For external rehydration the samples were immersed in buffered external hydration solution (SRE) at pH 7.4 for 16 h at 37 °C. In a vertical flow hood dental samples were placed on petri dish and perforation was performed on the occlusal surface (upper part of the crown) until communication with pulp chamber using turbine with diamond circular burs and micro-motor with carbide circular burs of 1–2 mm of diameter. Also a lateral perforation was made to gain access to the root canal on the apical third of the root using turbine with diamond circular burs, in accordance with the distances measured before. Once perforation was done, communication with pulp chamber and root canal was corroborated using number 20 K type endodontic files. Perforated samples were immersed in sterile internal hydration solution (SRI) for 48 h at 37 °C and 5% CO_2 in an incubator (modelo MCO 17 AC Sanyo®). Once the tissue is hydrated the pulp is removed with Automated Endodontic Files (1.5/25 mm SAF®) and deposited on petri dish to be fixed in formalin buffered at 10% (Sigma). Each tube was labeled with a number for its posterior histological processing.

2.4. Histology

Standard procedures of dehydration and embedding with histologic paraffin were performed. The orientation of the tissue for inclusion was made so that the major axis of the sample was parallel to the cut area of the paraffin block. The cuts were made on a minot-type rotary microtome (Sakura accut Cut) with a thickness