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Characteristics of the number of odontoblasts in human dental pulp post-mortem

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ABSTRACT

Estimation of the time since death is important in forensic medicine, and so far not much is known in employing dental pulp for such purposes. The tooth organ is the hardest organ in the human body, with a loose connective tissue of dental pulp situated within a rigid encasement of mineralized surrounding tissues. Human material was obtained from 31 corpses of people who died in car and train accidents and had healthy oral statuses. Samples were divided into two groups at different environmental temperatures. During the autopsy, the jaws were resected to keep teeth *in situ*, and every day one tooth was extracted. After decalcification, serial thin sections stained with hematoxylin and eosin were cut. Odontoblasts in the dental pulp were counted and data analysed. Statistical analysis showed that the number of odontoblasts drops during the time after death, and no odontoblasts remain in the pulp after 5 days.

1. Introduction

The estimation of the time since death has obvious and important implications in the investigation of criminal deaths. The vast amount of labour in this direction has not been rewarded by comparable improvements in accuracy because of permutations of factors that defy exact calculation of the post-mortem interval.

Methods applicable to the early post-mortem period of the first day or two after death include: body temperature; rigor mortis and hypostasis; electrical excitability of muscle; gastric emptying; eye changes—retinal appearance, iris reaction to drugs, vitreous humour chemistry; blood, pericardial and cerebrospinal fluid chemistry; cytological changes in bone marrow [1].

After cessation of vital functions, ischemia causes hypoxia, which interferes with aerobic oxidative respiration. Hypoxia causes cells to pass the nebulous "point of no return" into irreversible injury and cell death. Two phenomena consistently characterize irreversibility: the inability to reverse mitochondrial dysfunction (lack of oxidative phosphorylation and ATP generation) and profound disturbances in membrane function. The injury to lysosomal membranes results in the enzymatic dissolution of the injured cell that is characteristic of necrosis. The events that determine when injury becomes irreversible and progresses to cell death remain poorly understood [2].

The tooth organ is the hardest organ in the human body. It consists of the tooth and its surrounding tissues. Dental pulp is a loose connective tissue situated within a rigid encasement of mineralized dentin, covered with enamel on the crown and cement on the root.

Histologically, beneath the dentin, a layer of odontoblasts circumscribes the outermost part of the pulp. Subjacent to the odontoblast layer, towards the middle of the pulp, the cell-free zone, cell-rich zone and pulp core extend [3].

The odontoblast is considered to be a fixed post-mitotic cell in that once it has fully differentiated, it apparently cannot undergo further cell division. It is responsible for dentinogenesis both during tooth development and in the mature tooth, and is the most characteristic cell of the dentin–pulp complex. It produces a matrix composed of collagen fibrils, non-collagenous proteins and proteoglycans that are capable of undergoing mineralization. The ultrastructural characteristics of the odontoblast exhibit a highly ordered RER, a prominent Golgi complex, secretory granules and numerous mitochondria. In addition, it is rich in RNA, and its nuclei contain one or more prominent nucleoli; these are general characteristics of protein-secreting cells. In contrast to the active odontoblast, the resting or inactive odontoblast has a decreased number of organelles. During dentinogenesis odontoblasts form the dentinal tubules, and their presence within the tubules makes dentin a living responsive tissue [4].

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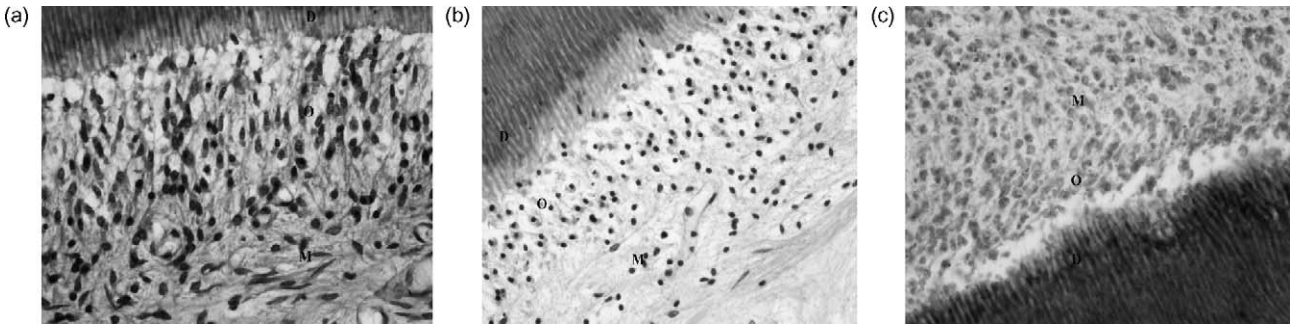

Fig. 1. The layer of odontoblasts in human dental pulp 7 h (a), 55 h (b) and 103 h (c) after death at room temperature. D: dentin, O: layer of odontoblasts, M: middle part of the dental pulp. Hematoxylin and eosin, 40×.

Because of the relatively sparse cellular composition of the pulp, the rate of oxygen consumption is low in comparison with that of most other tissues. During active dentinogenesis, metabolic activity is much higher than after the completion of crown development. The greatest metabolic activity is found in the region of the odontoblast layer, and the lowest is found in the central pulp, where most of the nerves and blood vessels are located. Yu et al. in their study established the O_2 consumption of odontoblasts in the lower incisor pulp of the rat at the rate of 3.2 ± 0.2 ml/min/100 g of pulp tissue [5]. In addition to the usual glycolytic pathway, the pulp has the ability to produce energy through a phosphogluconate (e.g., pentose phosphate) shunt type of carbohydrate metabolism [6], suggesting that the pulp may be able to function under varying degrees of ischemia.

Not much is known about employing dental pulp as a tissue for estimating the time since death. Duffy et al. [7] compared rates of putrefaction of dental pulp in the northwest coastal environment in Canada for extracted human and pig teeth and pig teeth *in situ*. The cells in the pulp were preserved from 96 to 336 h or 4–14 days, according to environmental conditions. Flow cytometric evaluation of dental pulp DNA degradation revealed minimal DNA degradation in dental pulp tissue by 144 h after surgical removal of human third molars; therefore, the pulp tissue was found to be unreliable for determining the early post-mortem interval. Light microscopic examination of the same human dental pulp tissue at 144 h after surgical procedure did not show any morphologic changes of autolysis [8]. The study of Caviedes-Bucheli et al. [9] revealed 41% pulp tissue cell viability 24 h after death.

So far not much has been written about post-mortem changes of human dental pulp in teeth *in situ* in the alveolar bone (jaw). Taking into consideration that odontoblasts are highly differentiated cells, which are usually highly oxygen sensitive, and specific to the dental pulp, and the suggestion of Fisher and Walters [6] that the pulp may be able to function under varying degrees of ischemia, we decided to analyse the basic characteristics of the number of odontoblasts in dental pulp after death.

The article is focused on the dynamics of changes in the number of odontoblasts in human dental pulp *in situ* as being dependent of time. The aim at this point of our research is to check and verify the basic characteristics of this process. The number of cases in our dataset permits these relatively simple analyses, but it is rather low at present to construct a reliable statistical calibration tool.

2. Materials and methods

The study was carried out in accordance with the requirements of the Institute of Forensic Medicine at the University of Ljubljana medical faculty regarding manipulation and research experiments carried out on organs or tissue from dead human bodies, and with the approval of the National Medical Ethics Committee of the Republic of Slovenia, No. 114/12/03.

The material was obtained from 32 corpses (cases) of people aged 18–40 years old who died from lethal injuries sustained mostly in car and train accidents, and whose heads were mutilated beyond recognition. Their teeth had to be caries and restoration free; no calculus should be present, and pocket probing depth should not exceed 3 mm. During the autopsy, the jaws were removed and stored in an open plastic bag; thus some airflow was enabled in an attempt to simulate a corpse with a slightly opened mouth. Every day one single-rooted tooth (sample) was extracted from the mandible, starting with a canine as soon as the corpse arrived at the Institute of Forensic Medicine, and followed by premolars every 24 h after the first extraction. Cases were divided into two groups: 15 at room temperature (23 °C) and 17 at a refrigerated temperature of 4 °C.

2.1. Control

For orthodontic reasons, intact mandibular first premolars from a young adult were extracted pain-free under local anaesthesia (Ultracain D-S forte, 2 ml ampoule, Hoechst AG) and examined as a control. The sample was prepared for light microscopy investigation. The time from application of anaesthesia to immersion in fixative was optimized to 5 min.

2.2. Light microscopy

Immediately after extraction, with elevator and forceps, the apical three quarters of the root was chiseled off to facilitate the penetration of the 10% neutral buffered formalin fixative solution in the dental pulp tissue for 3 days at room temperature. After decalcification and preparation of the samples by the classic method, 4-μm-thin paraffin longitudinal serial sections were cut in the vestibulo-lingual plane through the middle of each tooth. The middle of the tooth was considered to be as the dentinal tubules gave their straight and S-shaped course; the best sections were selected and stained with hematoxylin and eosin stain, and then examined at 10× and 40× objective magnifications with a Nikon Eclipse E600 light microscope. Images were digitized and grabbed with a CCD-1300 CB device camera (VDS Vosskühler, GmbH) and analysed with NIS-Elements AR (Nikon) software. Cells in the odontoblast layer were morphologically evaluated, counted and analysed.

According to stereological calculations, in each sample 10 microscopic examination fields in the coronal part of the dental pulp were investigated at 40× objective magnification. After grabbing the photo of the layer of odontoblasts (Figs. 1 and 2), we measured its area in the microscopic field; we then counted odontoblasts with nuclei either intact, with condensed chromatin or pyknotic. We calculated the average absolute (number of odontoblasts per square micrometre and conversion to number of odontoblasts per square millimetre) and relative (the first sample of every case represented 100% of odontoblasts per square micrometre/millimetre) density of odontoblasts for each sample. To diagnose necrosis with classic light microscopy, several hours must elapse, even more than 12 [10].

We carried out statistical analysis of the data using the SPSS statistical package for MS Windows. The two groups of cases (room and refrigerated temperature) were analysed separately.

3. Results and discussion

3.1. Density of odontoblasts

By counting the number of odontoblasts in the samples taken in the first 24 h after death, we estimated the average density of odontoblasts per square millimetre in healthy vital teeth of Caucasian adults aged 18–40 years. Our result of 11,764 odontoblasts per square millimetre in the average of all cases corresponds to the approximately 11,000 odontoblasts per square millimetre after cavity preparation with remaining dentin thickness of 0.501–2.993 mm obtained by Murray et al. [11]. In another study he, with co-workers, reported 350 odontoblasts per 1 mm

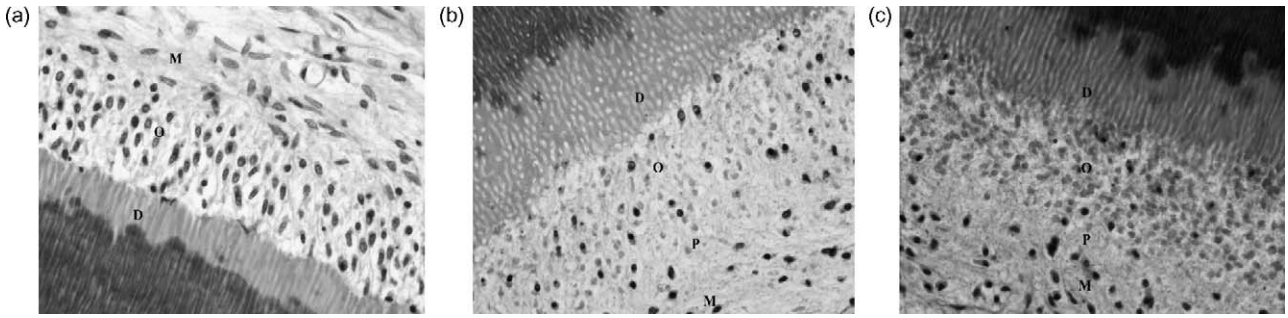

Fig. 2. The layer of odontoblasts in human dental pulp 22 h (a), 70 h (b) and 118 h (c) after death at refrigerated temperature. D: dentin, O: layer of odontoblasts, *P*: cell poor zone, M: middle part of the dental pulp. Hematoxylin and eosin, 40×.

length of pulp dentin border at crown aspect [12]. Studies to establish the density of odontoblasts were also performed in Wistar rats of various ages [13] and Rhesus Macaca monkeys aged between 4 and 5 years [14].

3.2. Linear regression model for absolute values of density of odontoblasts

After estimating the density of odontoblasts in each sample, we calculated the parameters of the linear regression model for estimating the time since death (Table 1).

We used the following form of linear regression model for both groups of cases:

$$OD_s = a + \beta_{OD'} OD'_c + \beta_t t_s + u_s \quad (1)$$

where OD_s is the density of odontoblasts in sample s, OD'_c is the density of odontoblasts in the first sample of each case c, and t_s is the time of the sample taken after death in hours. α, $\beta_{OD'}$ and β_t represent the parameters of the regression model, where α is the intercept, $\beta_{OD'}$ is the regression coefficient of density of odontoblasts in the first sample of each case, and β_t is the regression coefficient which expresses the dependency of odontoblast density on time after death.

The density of odontoblasts in sample (OD_s) is a dependent variable. We used time t_s and the density of odontoblasts in the first sample of each case (OD'_c) as independent variables. The latter represents personal characteristics of each case—each case has its own specifics, including the density (number) of odontoblasts during life. u_s is a disturbance term which represents the difference between the regression model and individual sample (OD_s).

Regression coefficients express the dependency of a dependent variable (density of odontoblasts in our case) and independent variables (density of odontoblasts in the first sample of each case and time of the sample taken after death). Eq. (1) thus states that the density of odontoblasts in each sample s is dependent from the population average number of odontoblasts at the moment of death (intercept α), density of odontoblasts in the first sample of each case c (OD'_c), time of the sample taken after death (t_s) and stochastic disturbances (u_s) which are independent from other variables.

The regression analysis tries to estimate the parameters (regression coefficients) of the regression model. According to the linear regression model (1) we used the following Eq. (2) to estimate the parameters of the model:

$$\bar{OD}_s = a + b_{OD'} OD'_c + b_t t_s \quad (2)$$

where $\bar{OD}_s$ is the estimated density of odontoblasts in sample s. Since this is estimated and not actually measured density, there is no disturbance term u_s in Eq. (2). (We also changed the symbols for regression coefficients α and β to a and b, respectively, to denote the fact that these are estimates and not the true values as they are in Eq. (1).)

The model showed the average drop in the density of odontoblasts by 130 odontoblasts per square millimetre per hour at room temperature, and by 120 odontoblasts per square millimetre per hour in refrigerated conditions. By comparing the $b_{OD'}$ values of both groups we can conclude that personal characteristics were more expressed in the room temperature group.

To find out whether the regression coefficients of both groups for the time after death could be regarded as equal regardless of the environmental temperature, we also tested the hypothesis H_0: $\beta_{tRoom} = \beta_{tRefrig}$. The t-test showed that we could not reject this hypothesis ($t = -1.0937$, $P = 0.276$).

After we obtained regression coefficient estimates for the regression model (2), it is relatively simple to calculate the average time of persistence of odontoblasts after death. After that time has elapsed, the density of odontoblasts OD is zero. The average time for the observed population can be calculated as directly from Figs. 3 and 4—it is the time where the regression curve crosses the x-axis on both graphs (the logic is the same for the experiment on the room temperature and the one at 4 °C). At that point on the

Table 1
Parameters of the linear regression model for estimating the time since death according to the density of odontoblasts per μm².

Group	Room temperature	Refrigerated temperature
OD' mean	0.01225	0.01139
OD' S.D.	0.00264	0.00267
Adjusted R^2	0.844	0.759
F-test (P value)	130.388 (0.000)	120.559 (0.000)
a (t-test, P value)	0.00990 (8.563, 0.000)	0.01056 (9.121, 0.000)
b_t (t-test, P value)	−0.00013 (−15.742, 0.000)	−0.00012 (−15.486, 0.000)
$b_{OD'}$ (t-test, P value)	0.34372 (4.023, 0.000)	0.19931 (2.127, 0.037)

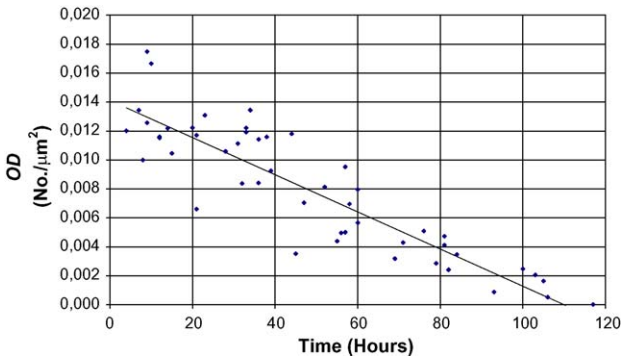

Fig. 3. Density of odontoblasts vs. time of death for cases at room temperature.

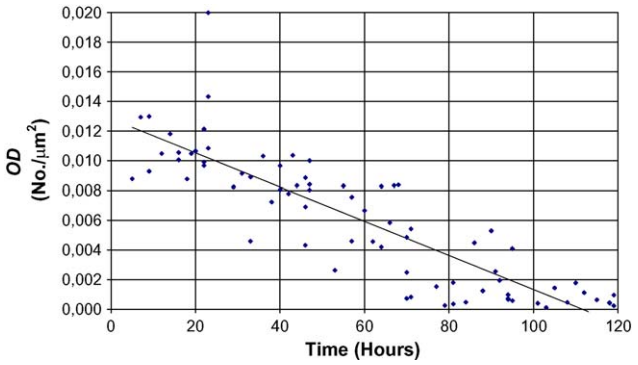

Fig. 4. Density of odontoblasts vs. time of death for cases at 4 °C.

graph, the density of odontoblasts *OD* is zero, so from our regression model (2) which is

$$\bar{OD}_s = a + b_{OD'} OD'_c + b_t t_s$$

we get

$$0 = a + b_{OD'} OD'_c + b_t t_s$$

Since we are calculating the time of persistence of odontoblasts on average (for all cases and samples), instead of using the density of odontoblasts in the first sample of each case c (OD'_c), we should use the average density of odontoblasts in the first sample across all the cases ($\bar{OD}'$):

$$0 = a + b_{OD'} \bar{OD}' + b_t \bar{t}$$

In this manner we will not get the time for each sample t_s, but the average time of persistence of odontoblasts after death $\bar{t}$, so we adopted the above equation accordingly.By rearranging the above equation to

$$-b_t \bar{t} = a + b_{OD'} \bar{OD}'$$

we finally get the formula:

$$\bar{t} = \frac{a + b_{OD'} \bar{OD}'}{-b_t} \quad (3)$$

By using (3). We calculated the time of the persistence of odontoblasts with 95% confidence interval, which was 110.03 ± 14.12 h or 4.58 ± 0.59 days at room temperature, and 111.53 ± 9.35 h or 4.64 ± 0.38 days at 4 °C (Figs. 3 and 4).

3.3. Linear regression model for relative values of odontoblast density

Another possibility to account for personal characteristics for each case is to calculate the relative density of odontoblasts in each sample. Namely, in the previous form of the linear regression model (1) we included in the model absolute values of the odontoblast density in the first sample for each case (OD'_c), to account for the personal characteristics of each case. Another option is to use the relative density of odontoblasts, where the odontoblast density for a case is expressed relative to the first sample for each case.

The linear regression model with absolute values of odontoblast density takes into consideration the personal characteristics of a case in a manner with one additional independent variable. The linear regression model with relative values does not do so, as the values are already corrected in such a way as to exclude the personal characteristics. We changed the linear regression model accordingly:

$$ODR_s = a + \beta_t t_s + u_s \quad (4)$$

Table 2
Parameters of the linear regression model for estimating the time since death according to relative density of odontoblasts per μm^2.

Group	Room temperature	Refrigerated temperature
Adjusted R^2	0.884	0.792
F-test (P value)	365.223 (0.000)	289.583 (0.000)
a (t-test, P value)	1.14462 (37.292, 0.000)	1.14500 (27.758, 0.000)
b_t (t-test, P value)	−0.01025 (−19.111, 0.000)	−0.01015 (−17.017, 0.000)

where ODR_s represents the relative density of odontoblasts. We excluded the density of odontoblasts in the first sample of each case from the model.

Analysis showed (Table 2) that the relative density of odontoblasts after death on average drops by 1.025 percentage points per hour at room temperature and 1.015 percentage points per hour in refrigerated conditions.

As we did for the regression model estimates for absolute density of odontoblasts, we also tested the hypothesis H_0: $\beta_{tRoom} = \beta_{tRefrig}$ for relative density of odontoblasts. The purpose of testing this hypothesis was to find out whether the regression coefficients for the time after death can be regarded as equal, regardless of the temperature of the environment. The t-test showed that we could not reject this hypothesis ($t = -0.1071$, $P = 0.915$).

4. Conclusions

The average density of odontoblasts in healthy vital teeth of Caucasian adults aged 18–40 years corresponds and confirms the data published by Murray et al. [11].

The average drop in the density of odontoblasts is 130 odontoblasts per square millimeter per hour at room temperature, and 120 odontoblasts per square millimeter per hour in refrigerated conditions. Comparing the variability of $\beta_{OD'}$ values of both groups lead us to the conclusion that personal characteristics were more expressed in the room temperature group. The corresponding t-test for the hypothesis H_0: $\beta_{tRoom} = \beta_{tRefrig}$ for absolute density of odontoblasts showed that the regression coefficients for the time after death can be regarded as equal regardless of the temperature of the environment, since we cannot reject the hypothesis.

The estimated time for the persistence of odontoblasts with 95% confidence interval was 110.03 ± 14.12 h or 4.58 ± 0.59 days at room temperature, and 111.53 ± 9.35 h or 4.64 ± 0.38 days at 4 °C. As seen from our data analysis, lower ambient temperature does not slow the disintegration of odontoblasts significantly.

The relative density of odontoblasts after death on average drops by 1.025 percentage points per hour at room temperature and 1.015 percentage points per hour in refrigerated conditions. The corresponding t-test for the hypothesis H_0: $\beta_{tRoom} = \beta_{tRefrig}$ for relative density of odontoblasts showed that the regression coefficients for the time after death can be regarded as equal regardless of the temperature of the environment, since we cannot reject the hypothesis.

We can conclude that, on the basis of the collected data and results of their analysis, the number of odontoblasts and their histological appearance may be one additional parameter in estimating the time since death in the early post-mortem period for up to 5 days. To better estimate the confidence interval, more cases are needed, which may in the future provide us with other exploratory possibilities. Also a research of how odontoblasts die and why/how they do disappear seems to be another issue for the future.

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