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Immunohistochemical study of Ki67, CD34 and p53 expression in human tooth buds

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Abstract

Aim of the study: Establishment of Ki67, p53 and CD34 expression in human tooth buds of different stages of odontogenetic development. *Materials and Methods*: Tissue samples containing tooth buds were removed from the incisor areas of human fetuses in different stages of development (weeks 9–10, 12–13, 13–16, 21–24), and from the canine and molar areas of 21–24 weeks fetuses. The tissue fragments were fixed using formalin and were processed using common histological techniques with paraffin embedding. Immunostaining for Ki67, p53 and CD34 has been performed using the dextran method and moist heat antigen retrieval (except for CD34). The resulting slides were photographed and quantitatively evaluated. *Results*: Ki67 immunoexpression decreases with advancement of the developmental stage of the tooth bud: in the inner enamel epithelium, between weeks 9 and 16 (IEE), in the preameloblasts (PB) between weeks 13 and 16, in the ameloblasts (AB) between weeks 21 and 24; outer enamel epithelium (OEE); stratum intermedium (SI); in the dental papilla: between weeks 9 and 10 in the dental papilla (DP), between weeks 13 and 16 in the outer layer of the dental papilla (DP1) and in the central layer of the dental papilla (DP2). Likewise, we noted Ki67 expression in the odontoblast layer (O) and pulp (P), between weeks 21 and 24. Concerning CD34 expression, we observed a decrease from weeks 9–10 until weeks 13–16, followed by an increase until weeks 21–24 of intrauterine life. From weeks 9–10, we observed a constant decrease of expression until weeks 13–16, followed by an increase during weeks 21–24. *Conclusions*: All Ki67, p53 and CD34 have been identified in the tooth bud. Ki67 expression gradually decreases with the embryonic development of the tooth, while p53 and CD34 expression decreases from weeks 9–10 to weeks 13–16 of intrauterine life, followed by an increase until weeks 21–24.

Keywords: human tooth bud, Ki67, CD34, p53, tooth development.

Introduction

Tooth eruption is a genetically regulated growth process, being the last stage of odontogenesis. It begins at the time when development of the dental crown is complete, and the development of the root is only complete to a third-to-half of its entire length [1]. Based on the changes occurring in the tooth bud, odontogenesis comprises four stages: dental lamina, the bud stage, the cap stage and the bell stage [2]. Ki67 has been described for the first time in 1991 [3]. The Ki67 antigen appears during phases G1, S, G2 and M of the cell cycle, and it is missing during phase G0. For this reason, this antigen is an important marker of cell proliferation [4, 5]. P53 is a tumor suppressor protein, encoded by the TP53 gene [6–9]; it is very important, regulating the whole cell cycle. Under normal circumstances, this protein is continually produced and degraded [10].

CD34 is a highly glycosylated transmembrane protein encoded by a gene located on the 1q chromosome. It is expressed by hematopoietic stem cells, fetal fibroblasts, vascular endothelial cells [11]. In addition, this protein has been demonstrated in the vascular endothelium of the nasal, oral, pharyngeal and laryngeal mucosa of fetuses [12].

Due to the small number of studies regarding the localization of these markers in the human tooth buds [4, 13, 14], our aim is to study the expression of Ki67, CD34 and p53 in the teeth buds compared to known histological characteristics.

Materials and Methods

For the purposes of the study, three spontaneously aborted human fetuses were used, from the collection of the Department of Anatomy and Embryology, University of Medicine and Pharmacy of Tîrgu Mureş, Romania. The age of the fetuses was estimated based on their length. Tissue samples containing tooth buds were removed from the incisor areas of human fetuses in different stages of development (weeks 9–10, 12–13, 13–16, 21–24) (Figure 1, a–d), and from the canine and molar areas of 21–24 weeks fetuses. The tissue fragments were fixed using 4% buffered formalin, embedded in paraffin and sectioned into 3–4 µm thick sections using a microtome. After a Hematoxylin–Eosin (HE) stained control section, immunostaining has been performed using the two-stage method with dextrane and moist heat antigen retrieval (except for CD34).

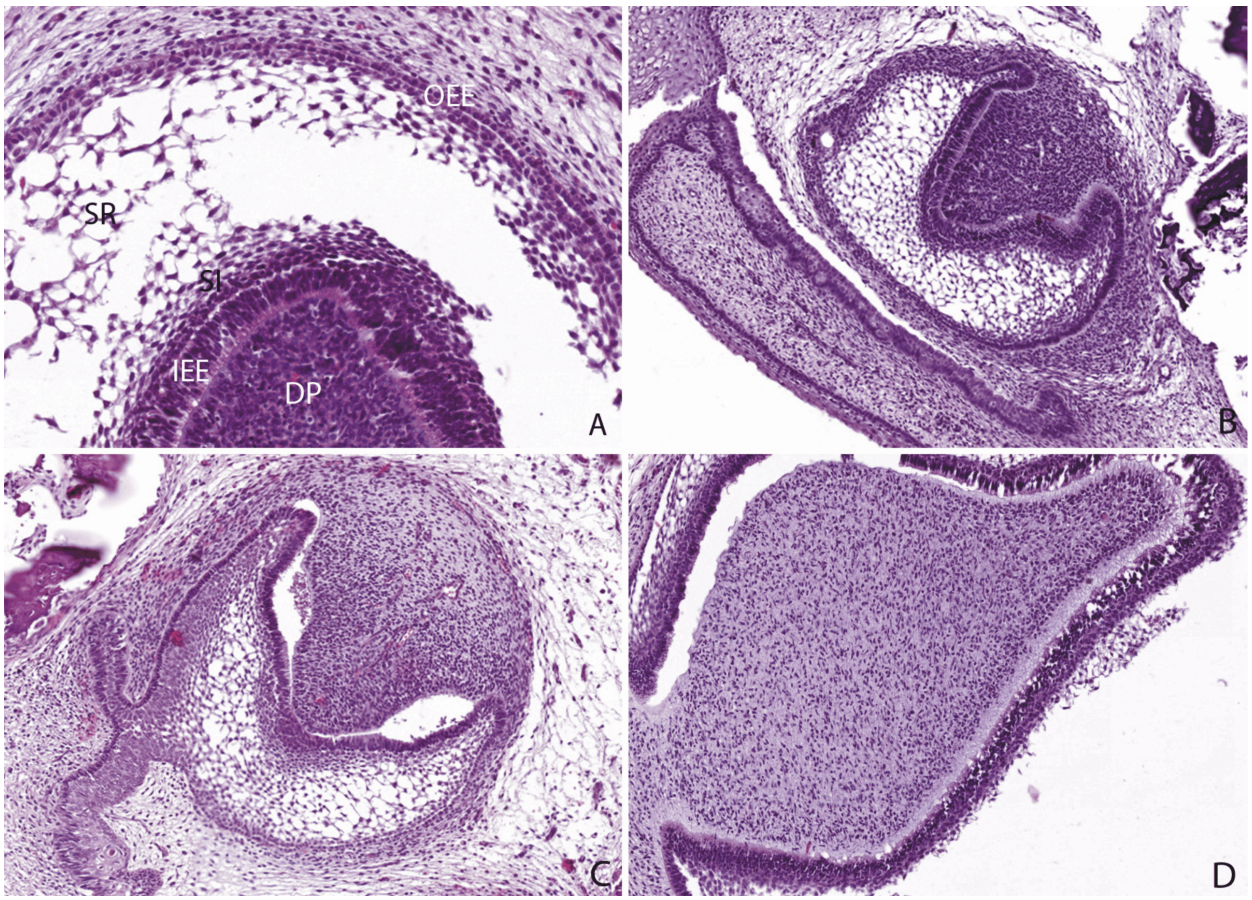

Figure 1 – ***(A) Incisor, W 9–10, HE staining, 200×; (B) Incisor, W 12–13, HE staining, 100×; (C) Incisor, W 13–16, HE staining, 100×; (D) Canine, W 21–24, HE staining, 100×. W – Week; DP – Dental papilla; IEE – Inner enamel epithelium; OEE – Outer enamel epithelium; SI – Stratum intermedium; SR – Stellate reticulum.***

The following primary antibodies were used: Ki67 (Diagnostic Biosystem, Pleasanton, USA, clone SP6, conc. 1/100), p53 (Diagnostic Biosystem, Pleasanton, USA, clone DO-7, conc. 1/100), CD34 (Lab Vision Fremont, CA, USA, clone QBEnd10, conc. 1/200). The following secondary system was used: UltraVision LP Large Volume Detection System HRP Polymer (Ready-To-Use) (Thermo Scientific, Fremont, CA, USA), according to the manufacturer's instructions and development using DAB chromogen.

For quantifying the results for Ki67 and p53, we counted 50 cells in each layer, noting that in case of the OEE (outer enamel epithelium), IEE (inner enamel epithelium), PB (preameloblasts), AB (ameloblasts), SI (stratum intermedium), SR (stellate reticulum) we counted cells from the superior third (S), middle third (M) and inferior third (I) of the respective layers.

In case of CD34 expression, we counted all sectioned capillaries in the layers where the marker was expressed.

Digital slides were made with Zeiss MiraxScan digital slide acquisition system (Carl Zeiss Jena GmbH, Jena, Germany) mounted with Marlin F-146C digital camera at a resolution of 1392×1040 pixels with Sony ICX267 sensor (Allied Vision Technologies GmbH, Stadtroda Germany) through Zeiss Plan-Apochromat 20× objective magnification. The acquisition system was controlled by MiraxScan software (3DHistech, Budapest, Hungary) installed on a Fujitsu–Siemens Celsius Workstation computer.

Results

Ki67 expression

Ki67 immunoexpression decreases with advancement of the developmental stage of the tooth bud: in the inner enamel epithelium, between weeks 9 and 16 (IEE); in the preameloblasts (PB), between weeks 13 and 16; in the ameloblasts (AB), between weeks 21 and 24; outer enamel epithelium (OEE); stratum intermedium (SI). In the dental papilla: between weeks 9 and 10, in the dental papilla (DP); between weeks 13 and 16, in the outer layer of the dental papilla (DP1) and in the central layer of the dental papilla (DP2). Likewise, we noted Ki67 expression in the odontoblast layer (O) and pulp (P), between weeks 21 and 24 (Figure 2, a and b; Table 1).

Studying Ki67 expression in the incisors, we noted that it is more intense during weeks 9–10 in the IEE and SI, and between weeks 13–16 in the OEE. After analysis of the results, we observed the decrease of Ki67 expression in all dental areas and all layers according to intrauterine age (Figure 3a). Comparing Ki67 expression in the dental layers according to areas, we saw that the inferior zone of each layer had the most intense proliferation.

After comparing the incisors of different fetuses of same developmental stage, we did not see any significant difference in Ki67 expression. In addition, we observed that the inferior zone and the AB layer had the highest values, and otherwise the values decrease from AB, through SI, OEE, down to RS in all studied areas (Figure 3b).

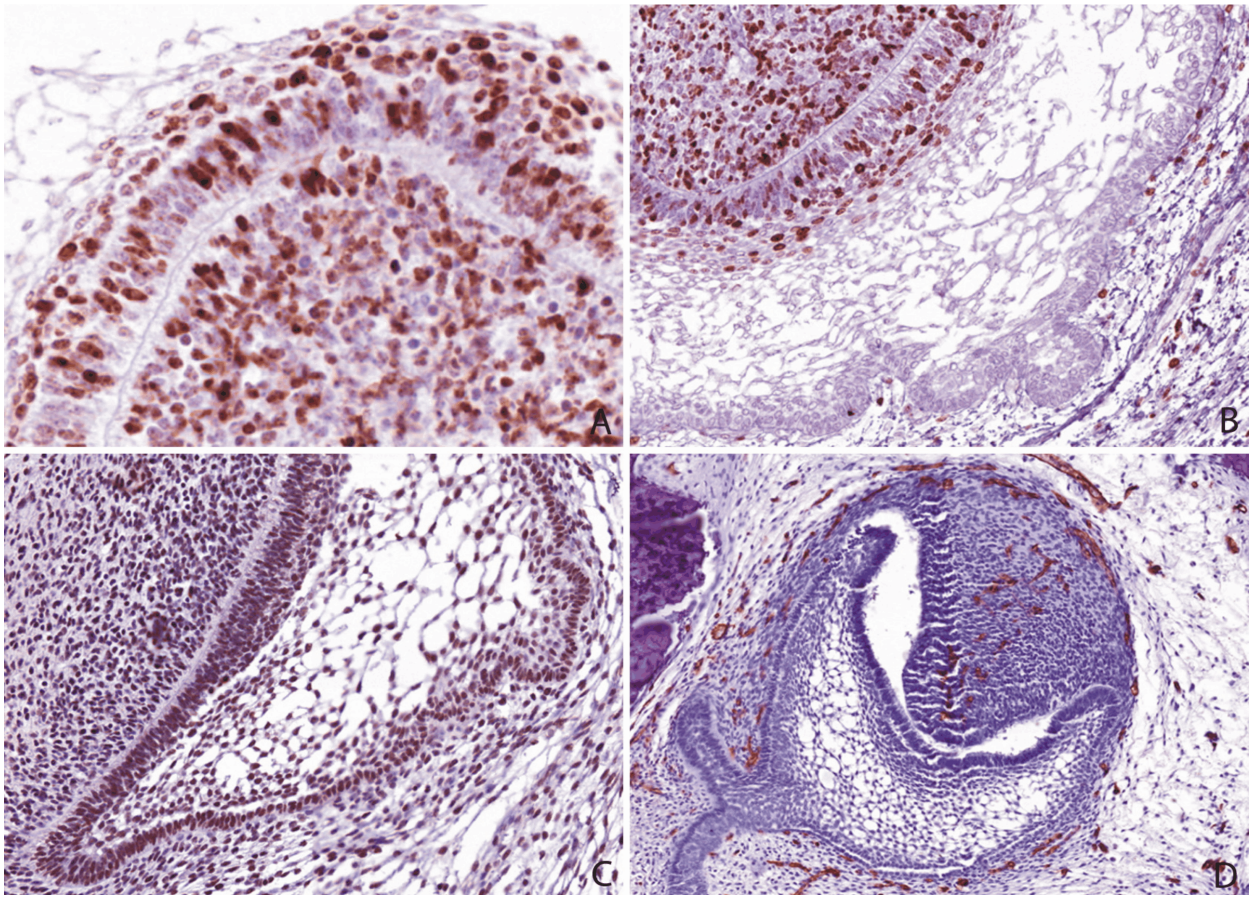

Figure 2 – ***(A) Incisor, W 9–10, Ki67 immunostaining, 200×; (B) Incisor, W 21–24, Ki67 immunostaining, 200×; (C) Incisor, W 21–24, p53 immunostaining, 200×; (D) Incisor, W 13–16, CD34 immunostaining, 100×.***

Table 1 – ***Distribution of Ki67 immunoexpression in different layers of incisors of different developmental stages***

Incisor					
Ki67 [%]					
		W 9–10	*W 12–13*	*W 13–16*	*W 21–24*
IEE/PB/AB	S	58	47	35	34
	M	70	55	41	36
	I	71	58	58	39
	Mean	63	53	45	36
OEE	S	28	32	25	10
	M	30	33	28	11
	I	63	35	65	12
	Mean	42	33	39	11
SI	S	60	30	28	17
	M	61	39	36	20
	I	64	54	36	22
	Mean	62	41	33	20
SR	S	0	0	0	0
	M	0	0	0	0
	I	38	31	0	0
	Mean	13	10	0	0
DP		62			
DP1/O			57	51	28
DP2/P			46	45	29

W – Week; IEE – Inner enamel epithelium; PB – Preameloblasts; AB – Ameloblasts; OEE – Outer enamel epithelium; SI – Stratum intermedium; SR – Stellate reticulum; DP – Dental papilla; DP1 – Outer layer of the dental papilla; DP2 – Central layer of the dental papilla; O – Odontoblasts; P – Dental pulp; S – Superior third; M – Middle third; I – Inferior third.

After comparing teeth in the same developmental stage (weeks 21–24), but from different areas, we noted that the most intense proliferation occurs in the AB layer and the inferior zone of the molar, followed by the canine and incisor (Figure 3c).

CD34 expression

In our study we noted the presence of the CD34 protein in the tooth bud (Figure 2d) in the endothelium of the vessels found in the dental papilla during weeks 9–10, in the outer layer of the dental papilla during weeks 12–13, 13–16 (DP1), and in the dental pulp (P) during weeks 21–24. Likewise, we demonstrated the presence of CD34 in the outer enamel epithelium (OEE). Regarding CD34 expression, we observed a decrease starting from weeks 9–10 fetal age, until weeks 13–16, followed by an increase until intrauterine weeks 21–24 (Figure 3d). By comparing teeth from the incisor, canine and molar areas, and three different incisors of same developmental stage (weeks 21–24) we did not see any significant results.

P53 expression

Unlike in case of Ki67, we found p53 expression in all layers and all studied stages of the tooth buds (Figure 2c). Starting from intrauterine weeks 9–10, we noted a constant decrease of the expression until weeks 13–16, followed by an increase during weeks 21–24 of intrauterine life (Figure 4b).

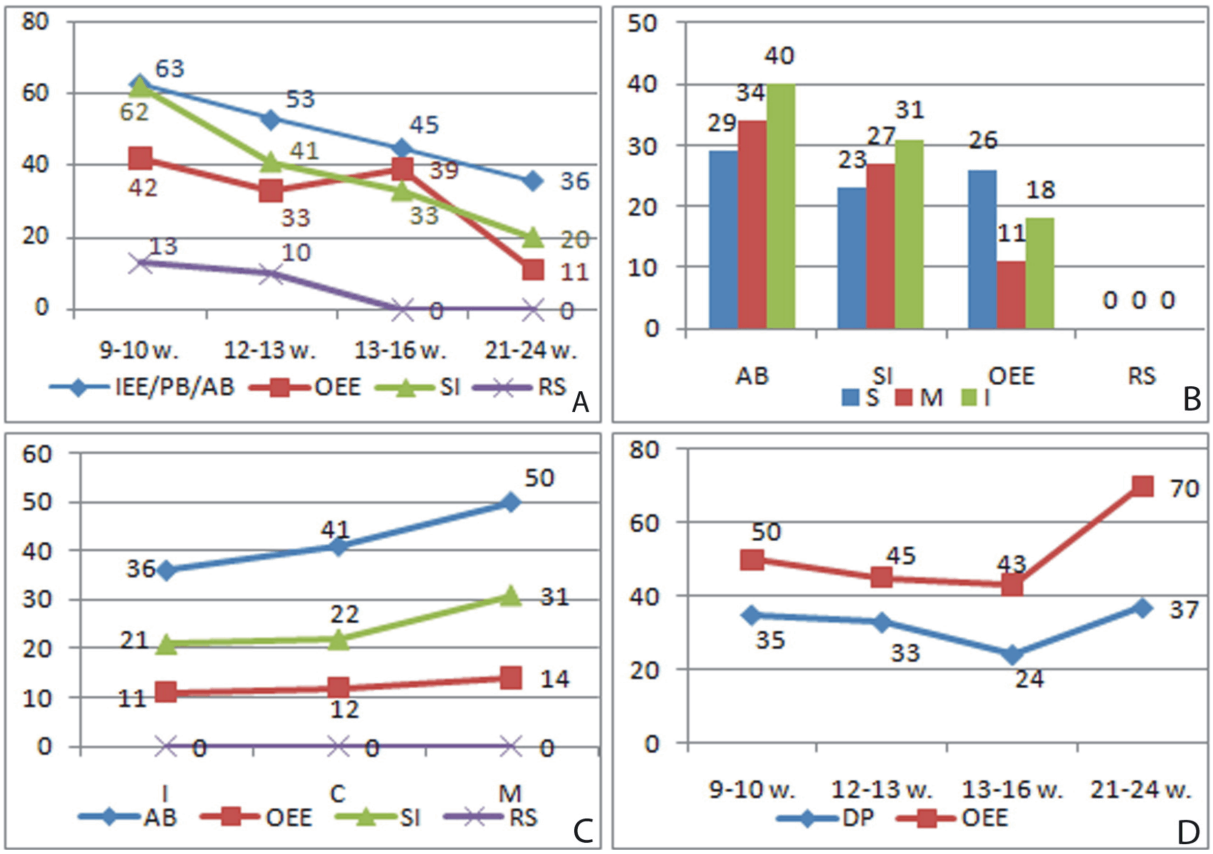

Figure 3 – *(A) Mean Ki67 expression in the incisor layers according to age; (B) Mean Ki67 expression in incisors (weeks 21–24) according to dental zones and layers; (C) Mean Ki67 expression according to dental type and layers during weeks 21–24; (D) CD34 expression according to age.*

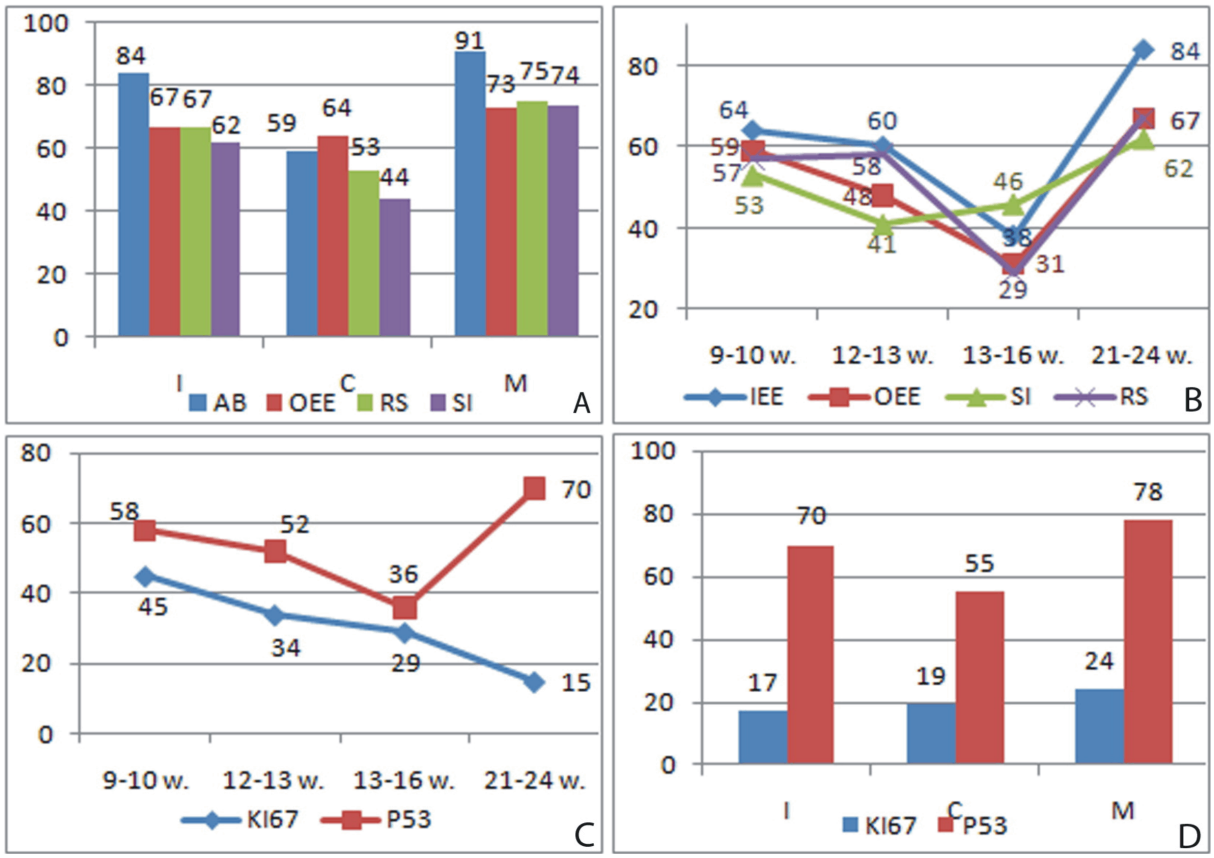

Figure 4 – *(A) Mean p53 expression in the dental layers according to age; (B) Mean p53 expression according to dental type and layers during weeks 21–24; (C) Comparison of Ki67 and p53 immunoexpression according to age; (D) Comparison of Ki67 and p53 expression in case of different teeth of 21–24 intrauterine weeks.*

When studying all enamel layers of teeth of the same developmental stage (weeks 21–24), but from different areas (incisor, canine, molar), we observed the highest expression in the molars (AB: 91%, OEE: 73%, SR: 75%, SI: 74%), followed by the incisors (IEE: 84%, OEE: 67%, SR: 67%, SI: 62%), and lastly the canine (IEE: 59%, OEE: 64%, SR: 53%, SI: 44%) (Figure 4a). Regarding p53 expression in the odontoblast layer, the decreasing order is as follows: canine (33%), molar (23%), incisor (15%). In the pulp, we noticed a decrease of the expression as below: incisor (69%), canine (67%), molar (50%). Comparing three different incisors of 21–24 intrauterine weeks, we did not find any significant results.

Comparing p53 expression of different zones (S, M, I) of the dental buds we did not find any significant differences.

Comparing Ki67 and p53 expression, we noted that p53 expression increases after week 16, while Ki67 expression gradually decreases during the studied intrauterine period (Figure 4c).

Ki67 expression increases from the incisor, through the canine to the molar, while p53 expression is lower in the canine, and higher in the incisor and molar (Figure 4d).

Discussion

As a result of our study, we observed that the Ki67 antigen displays a gradual decrease in the layers of the tooth bud: in the inner enamel epithelium, between weeks 9 and 16 (IEE); in the preameloblasts (PB) that develop after week 16; in the ameloblasts (AB) that develop after week 21; in the OEE, SI, SR and in the dental papilla as follows: between weeks 9 and 10, in the dental papilla (DP), from which the outer layer of the dental papilla (DP1,) and in the central layer of the dental papilla (DP2) will develop between weeks 12 and 16. Later on, from the outer layer of the dental papilla will give rise to odontoblasts (O), and the central layer will differentiate into the dental pulp (P). Regarding the results presented in the table, we noted a decrease of the expression in all layers from the superior aspect of the bud, towards the middle and inferior layer. This is probably because the superior part is more developed than the middle and inferior parts.

A study on human fetuses has been performed by Guven *et al.* who compared weeks 13, 16, 21 and 30 of intrauterine development, and obtained similar results. They described a decrease of Ki67 expression in the papilla and the outer and inner enamel epithelium, as the developmental stages were more and more advanced. In case of the stellate reticulum and outer enamel epithelium, they did not see any proliferation after week 21 [4].

It is interesting to note that alongside this gradual decrease of Ki67 expression in other layers, in case of the stellate reticulum this marker is completely missing between weeks 13 and 24 of intrauterine life.

In a study concerning tooth development performed on mice, Kwon *et al.* used among others Ki67 as well, and reported the presence of this marker in the epithelium and mesenchyma of molars and incisors of different developmental stages. In the bud stage, they observed a more intense proliferation in the epithelium than in the mesenchyma, just as we did. In the other studied stages (cap, bell), they noted the presence of the marker in the previously mentioned areas, and it was missing from the dental plate [15].

In our study, p53 expression compared to Ki67 had a different tendency. We observed a decrease of the expression starting with week 9 until week 16, followed by an increase until intrauterine week 24. In a similar study, Černochová *et al.* found a relatively constant level of expression between weeks 7 and 9, followed by a decrease until week 13 [13]. Programmed cell death (apoptosis) is an important mechanism of embryonic development. It seems that apoptosis plays an important role in the development of the tooth buds and finalization of the final shape of the dental crown. It is present in all stages of bud development, and an intense p53 activity can be demonstrated in dental buds of mouse embryos [16], as one of the central roles of p53 is to activate cellular apoptosis [17].

We noted a similar tendency of CD34 expression to p53, meaning that CD34 decreased from intrauterine week 9 until week 16, and subsequently increased until week 24. It should be noted that CD43 was confined to the dental pulp and OEE. A similar study of Ide *et al.* performed on tooth buds has demonstrated the presence of CD34 in the dental papilla, near the odontoblasts and ameloblasts [14].

Conclusions

All Ki67, p53 and CD34 have been identified in the human tooth bud. Ki67 expression gradually decreases with the embryonic development of the tooth, while p53 and CD34 expression decreases from weeks 9–10 to weeks 13–16 of intrauterine life, followed by an increase until week 21–24. In case of teeth of the same developmental stage (weeks 21–24), but from different areas of the dental arch, Ki67 expression is most intense in the molars, followed by the canines and incisors. In case of teeth of the same developmental stage (weeks 21–24), but from different areas of the dental arch, p53 expression is most intense in the molars, followed by the incisors and canines.

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