PAPER REF: 4306

PRENATAL ULTRASOUND SCREENING, TOOTH GERMS HISTOLOGY AND HYPODONTIA

Mariana Seabra [1(*)], António Felino[2], Francisco Valente[3], Rosete Nogueira[4], Paula Vaz[5]

[1] Phd Student of FMDUP, University of Porto, Porto, Portugal
[2] Department of Oral Surgery of FMDUP, University of Porto, Portugal
[3] CHVNG Hospital, Prenatal Diagnosis Unit, Porto, Portugal
[4] CGC Genetics, Porto, Portugal
[5]Department of Medical and Orofacial Genetics of FMDUP, Porto, Portugal
[(*)]*Email:* marianaseabr@gmail.com

ABSTRACT

This research demonstrates the correlation between the ultrasound image and histological image of fetal tooth germs and aims to highlight the importance of the study of hypodontia in abortion products as a sign associated with others, for the early detection of genetic syndromes or congenital abnormalities in prenatal ultrasound.

Keywords: tooth germ, tooth buds, ultrasound, prenatal, diagnosis, histology.

INTRODUCTION

The presence of hypodontia in the prenatal ultrasound might be a sign of congenital malformations, genetic syndromes and chromosomal abnormalities (Ulm, 1995a; Ulm, 1999). There are few studies conducting human dental development and the most of them were done in rodents, so their application in humans is limited (Kapadia, 2007). The published data referring to the presence and distribution of human tooth germ through two-dimensional ultrasonography and its histological correspondence is sparse (Ulm, 1995a; Ulm, 1995b; Ulm, 1998; Ulm, 1999).

The major purposes of the fetal or perinatal autopsy are to determine gestational age, document growth and development, to detect congenital abnormalities, to analyze clinical diagnosis and treatment, to determine the cause of death and possible recurrence risk and identify genetic syndromes (when present) (Désilets, 2011).

The development of the oral cavity begins with the establishment of the frontonasal prominence and the first branchial arch (Chai & Maxson, 2006). As the first branchial arch extends ventral – medially, it gives rise to both mandibular and maxillary prominences. The primitive oral cavity is formed as a consequence of the fusion among intermaxillary segments of the frontonasal prominence and the paired maxillary and mandibular prominences (Chai & Maxson, 2006). The oral epithelium, in contrast to the suboral epithelium, becomes thickened to form the dental lamina, marking the time and location for tooth development to begin. The initiation of each tooth germ is marked by the formation of dental lamina (Chai & Maxson, 2006).

The process of odontogenesis in humans occurs over an extended period of time, from around the 6th week in utero until, at least the late teenage years, in the postnatal period, when the roots of the third molar teeth are formed (Townsend, 2012).
Each tooth undergoes through a series of developmental stages that follows the same pattern - beginning with the formation of the tooth bud, followed by the cap and bell stages of development and subsequently by the laying of the enamel and dentine over the crown of the tooth. Root development follows formation of the dental crown with the teeth emerging into the oral cavity (when around 2/3 of the root has been formed) (Townsend, 2012).
The patterning of dentition depends on the proper development of the oral cavity, where maxillary and mandibular teeth are lodged and involves the determination of location, shape, number, and size of tooth development (Chai & Maxson, 2006).
The mammalian dentition is a serially homologous structure composed of different teeth organized into a single row along the margins of the jaws (Cobourne, 2010). Each tooth belongs to a particular class, defined primarily by coronal morphology and including flat, chisel-shaped incisors, conical canines, bicuspid premolars and multicuspid molars (Cobourne, 2010).
In humans, the primary incisor, canine and molar teeth undergo successional replacement, whereas an accessional permanent molar dentition is added as the posterior jaw dimension increases. Teeth develop in the embryo via a series of interactions between odontogenic epithelium and neural crest-derived ectomesenchyme of the early jaws (Cobourne & Sharpe, 2003; Jernvall & Thesleff, 2000; Tucker & Sharpe, 2004; Tummers & Thesleff, 2009; Cobourne, 2010)
Permanent teeth have primary predecessors and develop as an extension of the dental lamina, associated with the primary tooth germs. In humans formation of these teeth begins between 20th week in utero and the 10 months of postnatal period. The permanent molar dentition has no primary predecessors. These teeth form as an extension of the dental lamina into the posterior region of the jaws (Bailleul-Forestier, 2008).

Investigation of craniofacial development uses different animal species as models as with other areas of research in developmental biology (Chai & Maxson, 2006). Studies in mice combine the power of genetics and genome manipulation together with in vitro organ culture techniques, leading to great progress in recent years. Mouse models are extremely valuable in our effort to gain a better understanding of human craniofacial, but humans have a dental formula of 2.1.2/2.1.2 in the temporary dentition (two incisors, one canine, and two molars). Some rodents, such as mice, have a dental formula of 3.0.0.1/ 1.0.0.3 (Chai & Maxson, 2006). So it is difficult to study the dental development in such animals and to extrapolate results for humans, therefore research in human fetuses may arise important information.

The aims of this research were to assess the correspondence of the human tooth germs by two-dimensional ultrasonography to the histology and the study of hypodontia on autopsy products of abortion (spontaneous or resulting for medical termination of pregnancy in a context of syndromic, genetic or malformation fetus) of a population of CHVNG (Centro Hospitalar de Vila Nova de Gaia) Hospital.

MATERIAL AND METHODS

This research project was designed following the legal normative (Helsinki Declaration) and approved by the Ethics Committee of the Faculty of Dental Medicine, University of Porto (FMDUP) and of the CHVNG Hospital.

The fetal tooth germs were evaluated, visualized in both maxilla and mandible, through 2D prenatal ultrasound (GE E8 Voluson® equipment) screening in a Portuguese pregnant population of the CHVNG Hospital. Of all the fetuses, that had autopsy indication of spontaneous fetal death or of medical termination of pregnancy in the CHVNG Hospital, from May 2011 to August 2012, 24 were randomly selected for this study. In these, the routine histopathological study was conducted and was also included the evaluation of their tooth germs. So the final sample was composed by 24 human fetuses with gestational ages between 13 and 30 weeks.

Each fetus was examined according to a predesigned protocol of the pathology laboratory of the CGC Genetics – (Centro de Genética Clínica Porto, Portugal). This included a photograph, a whole body radiograph, and an external and internal examination of every fetus. All post-mortem examinations were carried out with written consent of the father or relatives who brought the fetus.

RESULTS

The sample, consisted of 24 fetal autopsies, from which 14 (58.3%) were male and the remaining 10 (41.7%) were female. The chi-square test revealed no significant differences along the distribution according to gender (x^2= 0.75, df= 1, p value= 0.3865 >0.05). Of the overall of the studied fetus 13 were a result of medical abortion and 11 were spontaneous death. The cases with medical termination of pregnancy were more associated to earlier gestational stages when compared to the cases of fetal death.
To evaluate the association of the clinical information and the gender of the autopsied fetuses was performed the Fisher exact test, that showed a significant statistical association (p value=0.004). That means, spontaneous abortions were associated to male fetuses.

It was possible to determine the exact number, morphology and mineralization of the tooth germs in the 24 histological evaluations of the fetuses. All tooth germs were histologically present at the 13th week of gestation. Of the 24 fetuses autopsied, 41.7% had hypodontia (Table 1).

Table 1 Prevalence of hypodontia

		Frequency	**Percent**	**Valid Percent**	**Cumulative Percent**
Valid	**Absence**	14	58.3	58.3	58.3
	Presence	10	41.7	41.7	100.0
	Total	24	100.0	100.0	

Females were more affected by hypodontia when compared with males, although no statistically significant differences were found (p value= 0.132) (Table 2).
29.1% of fetuses of the sample had hypodontia in the maxilla and 20.9% of fetuses had hypodontia in the mandible. There was no significant association between the presence of hypodontia in the maxilla and hypodontia in the mandible (p value=0.462).

Table 2 Hypodontia versus fetus sex

			Sex		Total
			Female	Male	
Hypodontia	**No**	Count	4	10	14
		% within Hypodontia	28.6%	71.4%	100.0%
		% within Sex	40.0%	71.4%	58.3%
		% of Total	16.7%	41.7%	58.3%
	Yes	Count	6	4	10
		% within Hypodontia	60.0%	40.0%	100,0%
		% within Sex	60.0%	28.6%	41.7%
		% of Total	25.0%	16.7%	41.7%
Total		Count	10	14	24
		% within Hypodontia	41.7%	58.3%	100.0%
		% within Sex	100.0%	100.0%	100.0%
		% of Total	41.7%	58.3%	100.0%

Table 3 Hypodontia maxilla versus clinical information

			Clinical Information		Total
			MTP	FD	
Hypodontia maxilla	**No**	Count	9	8	17
		% within Hypdontia	52.9%	47.1%	100.0%
		% within Clinical Information	69.2%	72.7%	70.8%
		% of Total	37.5%	33.3%	70.8%
	Yes	Count	4	3	7
		% within Hypodontia	57.1%	42.9%	100.0%
		% within Clinical Information	30.8%	27.3%	29.2%
		% of Total	16.7%	12.5%	29,2%
Total		Count	13	11	24
		% within Hypodontia	54,2%	45.8%	100.0%
		% within Clinical Information	100.0%	100.0%	100.0%
		% of Total	54.2%	45.8%	100.0%

MTP – medical termination of pregnancy; FD – fetal death.

The Fisher test revealed that there was no significant association between the presence of hypodontia in the maxilla and clinical information (p value=1.000) (Table3) and also for the mandible (p value=0.630) (Table 4).

Table 4 Hypodontia mandible versus clinical information

			Clinical Information		Total
			MTP	FD	
Hypodontia mandible	**No**	Count	11	8	19
		% within Hypodontia	57.9%	42.1%	100.0%
		% within Clinical Information	84.6%	72.7%	79.2%
		% of Total	45.8%	33.3%	79.2%
	Yes	Count	2	3	5
		% within Hypodontia	40.0%	60.0%	100.0%
		% within Clinical Information	15.4%	27.3%	20.8%
		% of Total	8.3%	12.5%	20.8%
Total		Count	13	11	24
		% within Hypodontia	54.2%	45.8%	100.0%
		% within Clinical Information	100.0%	100.0%	100.0%
		% of Total	54.2%	45.8%	100.0%

MTP – medical termination of pregnancy; FD – fetal death.

The most frequently missing tooth in the maxilla was the 65 and was the 55 and the 81 in the mandible.

DISCUSSION AND CONCLUSIONS

The number, morphology and mineralization of the tooth germs were identified in all the histological evaluations of our sample, which is consistent with published literature (Ulm, 1995a; Ulm, 1995b).

We also could determine that all the tooth germs were histologically present at the 13th week of gestation. That pleases us comment that this was the proof the prenatal ultrasonography is in agreement with the histology – That is to say that the visualization of the tooth germs in prenatal ultrasonography at the 13th week is a reality that could be in the future one additional marker for surveillance of genetic syndromes.

When epidemiological studies about hypodontia in temporary dentition were checked out, it was understood that most of them occurred in places where there are children such as schools, nurseries. The prevalence of tooth agenesis has been addressed by some studies (Arte, 2004; Mattheeuws, 2004; Polder, 2004). However, the prevalence of hypodontia in temporary dentition never been studied in autopsied fetus.

The term tooth agenesis denotes a condition where deciduous (primary) and/or permanent teeth fail to develop. It is likewise referred to as hypodontia, but the same term is also used to characterize agenesis of up to six teeth (excluding the third molars). In contrast, agenesis of more than six teeth or all teeth of a particular class is referred to as oligodontia, and the term anodontia denotes the extremely rare condition, where all teeth fail to develop (Mitsiadis & Luder, 2011).

Tooth agenesis is the most frequent developmental malformation of the orofacial complex. However, prevalence ratios vary considerably between generations and classes of teeth and reveal some characteristic patterns. Thus, with a frequency of less than 1%, agenesis of primary teeth is described as rare (Arte, 2004; Bailleul-Forestier, 2008; Mitsiadis & Luder, 2011).
Among the permanent teeth, absence of at least one third molar (prevalence 20 to 30%) is the most frequent (Arte, 2004; Bailleul-Forestier, 2008; Mitsiadis & Luder, 2011).
In the primary dentition, the maxillary lateral incisors account for over 50% and together with mandibular incisors for 90% of all affected teeth (Järvinen & Lehtinen, 1981; Magnusson, 1984; Whittington & Durward, 1996; Daugaard-Jensen, 1997) by hypodontia.
A correlation of agenesis of primary and secondary teeth it was also referred: agenesis of a primary tooth is nearly always followed by agenesis of the corresponding secondary tooth (Daugaard-Jensen, 1997; Whittington & Durward, 1996). In the primary dentition, the distribution is similar with the majority of cases lacking only one tooth (Daugaard-Jensen, 1997).
Some cases of tooth agenesis occur without association with developmental defects in other organs and are referred to as non-syndromic. However, missing teeth are also observed in association with other malformations, most noticeably with cleft lip with or without cleft palate (Mitsiadis & Luder, 2011).
In our study, the prevalence of hypodontia was of 41.7%, a much higher value than the described in other non-syndromic populations –0.1% (Menczer, 1955), 0.2% (Kapdan, 2012), 0.4% (Grahnen & Granath, 1961), 0.5% (Magnusson, 1981), 0% (Jones 1993), 0.4% (Whittington & Durward, 1996), 0.7% (Plaetschke, 1938), 0.6% (Toth & Csemi, 1967), 0.4% (Carvalho, 1998), 0.6% (Esenlik & Ravn, 2009), 0.6% (Kramer, 2008), 0.3% (Brook, 1974), 2.63% (Altug-Atac & Erdem, 2007), 2.4% (Yonezu, 1997), 4.6% (Clayton, 1956).
This value, that we obtained, may be related to the fact that tooth agenesis can be associated with a lot of documented syndromes. As our sample was composed by fetuses, that had autopsy indication of spontaneous fetal death or of medical termination of pregnancy, it is logical to assume that the sample should have a higher number of pathology associated with hypodontia.
No significant differences were found in the results of hypodontia if considered the gender of the fetal autopsies, but since, in general, the female gender was more affected than the male gender (Polder, 2004). We could hypothetically assume that it would also be the fact that the histological analysis was made in products of abortions and miscarriages.

REFERENCES

Altug-Atac AT & Erdem D. Prevalence and distribution of dental anomalies in orthodontic patients. Am J Orthod Dentofacial Orthop, 2007, 131, p. 510-514.

Arte S, Nieminen P. Hypodontia. orphanet encyclopedia, 2004, p. 1-7.

Bailleul-Forestier I, Berdal A, Vinckier F, de Ravel T, Fryns JP, Verloes A. The genetic basis of inherited anomalies of the teeth. Part 2: syndromes with significant dental involvement. Eur J Med Genet, 2008, 51, p. 383-408.

Bailleul-Forestier I, Molla M, Verloes A, Berdal A. The genetic basis of inherited anomalies of the teeth. Part 1: clinical and molecular aspects of non-syndromic dental disorders. Eur J Med Genet, 2008, 51, p. 273-291.

Brook AH. Dental anomalies of number, form and size: their prevalence in British schoolchildren. J Int Assoc Dent Child, 1974, 5, p. 37-53.

Carvalho JC, Vinker F, Declerck D. Malocclusion, dental injuries and dental anomalies in the primary dentition of Belgian children. Int J Paediatr Dent, 1998, 8, p. 137-141.

Chai Y, Maxson RE Jr. Recent advances in craniofacial morphogenesis. Dev Dyn, 2006, 235, p. 2353-2375.

Clayton JM. Congenital dental anomalies occuring in 3557 children. ASDC J Dent Child, 1956, 23, p. 206-208.

Cobourne MT, Sharpe PT. Making up the numbers: The molecular control of mammalian dental formula. Semin Cell Dev Biol, 2010, 21, p. 314-324.

Cobourne MT, Sharpe PT. Tooth and jaw: molecular mechanisms of patterning in the first branchial arch. Arch Oral Biol, 2003, 48, p. 1–14.

Daugaard-Jensen J, Nodal M, Kjaer I. Pattern of agenesis in the primary dentition: a radiographic study of 193 cases. Int J Paediatr Dent, 1997, 7, p. 3-7.

Désilets V, Oligny LL; Genetics Committee of the Society of Obstetricians and Gynaecology Canada; Family Physicians Advisory Committee; Medico–Legal Committee of the SOGC.Fetal and perinatal autopsy in prenatally diagnosed fetal abnormalities with normal karyotype. J Obstet Gynaecol Can, 2011, 33, p. 1047-1057.

Esenlik E, Sayın MO, Atilla AO, Ozen T, Altun C, Basak F. Supernumerary teeth in a Turkish population. Am J Orthod Dentofacial Orthop, 2009, 136, p. 848-852.

Grahnen H, Granath LE. Numerical variations in primary dentition and their correlation with the permanent dentition. Odontol Revy, 1961, 12, p. 348-357.

Järvinen S, Lehtinen L.Supernumerary and congenitally missing primary teeth in Finnish children. An epidemiologic study. Acta Odontol Scand, 1981, 39, p. 83-86.

Jernvall J, Thesleff I. Reiterative signaling and patterning during mammalian tooth morphogenesis. Mech Dev, 2000, 92, p. 19–29.

Jones ML, Mourino AP, Bowden TA. Evaluation of occlusion,trauma, and dental anomalies in African-American children of metropolitan Headstart programs. J Clin Pediatr Dent, 1993, 18, p. 51-54.

Kapadia H, Mues G, D'Souza R. Genes affecting tooth morphogenesis. Orthod Craniofac Res, 2007, 10, p. 237-244.

Kapdan A, Kustarci A; Buldur B, Arslan D, Kapdan A. Dental anomalies in the primary dentition of Turkish children. Eur J Dent, 2012, 6, p. 178-183.

Kramer PF, Feldens CA, Ferreira SH, Spiguel MH, Feldens EG. Dental anomalies and associated factors in 2- to 5-year old Brazilian children. Int J Paediatr Dent, 2008, 18, p. 434-440.

Magnusson TE. Hypodontia, hyperodontia, and double formation of primary teeth in Iceland. An epidemiological study. Acta Odontol Scand, 1984, 42, p. 137-139.

Mattheeuws N, Dermaut L, Martens G. Has hypodontia increased in Caucasians during the 20th century? A meta-analysis. Eur J Orthod, 2004, 26, p. 99-103.

Menczer L. Anomalies of the primary dentition. J Dent Child, 1955, 22, p. 57-62.

Mitsiadis TA, Luder HU. Genetic basis for tooth malformations: from mice to men and back again. Clin Genet, 2011, 80, p. 319-329.

Plaetschke J. Okklusions anomalien im Milchgebiss. Dtsch. Zahn-, Mund- u. Kieferheilk 1938, 5, p. 435-451 (Abstract).

Polder BJ, Van't Hof MA, Van der Linden FP, Kuijpers-Jagtman AM. A meta-analysis of the prevalence of dental agenesis of permanent teeth. Community Dent Oral Epidemiol, 2004, 32, p. 217-226.

Ravn JJ. Aplasia, supernumerary teeth and fused teeth in the primary dentition. An epidemiologic study. Scand J Dent Res, 1971, 79, p. 1-6.

Toth A, Csemi L. Zwillingszahne im Milchgebiss. Dtsch Zahnarztl Z, 1967, 22, p. 546-554 (Abstract).

Townsend G, Bockmann M, Hughes T, Brook A.Genetic, environmental and epigenetic influences on variation in human tooth number, size and shape. Odontology, 2012, 100, p. 1-9.

Tucker AS, Sharpe P. The cutting edge of mammalian development; how the embryo makes teeth. Nat Rev Genet, 2004, 5, p. 499–508.

Tummers M, Thesleff I. The importance of signal pathway modulation in all aspects of tooth development. J Exp Zool B: Mol Dev Evol, 2009, 312, p. 309–319.

Ulm MR, Kratochwil A, Ulm B, Lee A, Bettelheim D, Bernaschek G. Three- dimensional ultrasonographic imaging of fetal tooth buds for characterization of facial clefts. Early Hum Dev, 1999, 55, p. 67-75.

Ulm MR, Kratochwil A, Ulm B, Solar P, Aro G, Bettelheim D. Three-dimensional ultrasound evaluation of fetal tooth germs. Ultrasound Obstet Gynecol, 1998, 12, p. 240-243.

Ulm MR, Ulm C, Reckendorffer H, Obwegeser R, Plockinger B, Golaszewski T. Ultrasound diagnosis of fetal tooth anlagen and their histologic correlates. Ultraschall Med, 1995, 16, p. 18- 21.a

Ulm, MR, Chalubinski K, Ulm C,Plockinger B,Deutinger J,Bernaschek G. Sonographic depiction of fetal tooth germs. Prenat Diagn, 1995, 15, p. 368-372.b

Whittington BR, Durward CS. Survey of anomalies in primary teeth and their correlation with the permanent dentition. N Z Dent J, 1996, 92, p. 4-8.

Yonezu T, Hayashi Y, Sasaki J, Machida Y. Prevalence of congenital dental anomalies of the deciduous dentition in Japanese children. Bull Tokyo Dent Coll, 1997, 38, p.27-32.