PAPER REF: 4305

TOOTH GERMS IN PRENATAL ULTRASOUND – IS ITS IDENTIFICATION POSSIBLE?

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

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

ABSTRACT

The visualization of fetal dental germs in two-dimensional prenatal ultrasonography (2D) may contribute to the complete study of the fetal face. The tooth germs imaging can also be a warning sign for early identification of other signs of pathology and therefore contribute to the timely diagnosis of malformations and orofacial syndromes in pregnancy.

The aim of this study was to demonstrate the efficiency and applicability of the 2D ultrasonography in the identification of the tooth germs and assessment of other parameters as the first line exam for the fetal morphology evaluation and appropriate diagnosis of abnormalities in pregnancy.

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

INTRODUCTION

Craniofacial development has traditionally been a field of research in which many divergent views were expressed and new theories put forward to explain observed normal and abnormal phenomena (Evans & Francis-West, 2005).

The prenatal recognition of facial abnormalities during pregnancy may be determinant in the diagnosis of various genetic and polymalformative syndromes cases and chromosomal abnormalities (Clementi, 2000; Rotten & Levaillant, 2004).

The observation and characterization of the jaws should be an integral part of the usual fetal ultrasound examination in which the fetus is monitored (Andresen, 2012). The mid-sagittal plan allows to study of the facial dismorphology profile, the analysis of the face profile, and the measurement of several biometric parameters such as: facial angles and nasal bone length. The posterior coronal nose-mouth plan is essential to evaluate the interruption of the lip continuity, the nostril deformation and the alveolar crest line-up. Serial axial images allow analysis of several elements of the face such as: the eyes, lips, jaw and tongue (Babcook, 1996; Cash, 2001; Chen, 2001; Ghi, 2002).

The characterization of both jaws and assessment of their size and position can be crucial to corroborate the hypothesis of any genetic syndrome. Similarly, the continuity of the alveolar crests may, especially in the upper jaw, raise the suspicion of cleft palate, particularly when there is a diagnosis of cleft lip. Therefore, the limits of the jaws define the oral cavity and this should be evaluated as soon as possible (Rotten, 2004; Babcook, 1997)

According to some researchers the assessment of tooth germs in prenatal ultrasound may constitute an added value in the early identification of chromosomal syndromes (Ulm, 1995; Ulm, 1998; Ulm, 1999).

Although there is little information regarding the usefulness of visualization of tooth germs, there is still controversy regarding the validity of their identification by 2D and 3D ultrasound to benefit the prenatal diagnosis of craniofacial anomalies (Ulm, 1995; Ulm, 1998; Ulm, 1999).

One of the main purposes of our study was to demonstrate the efficiency and applicability of the 2D ultrasonography in the evaluation of the parameters studied since it is the first line exam for the assessment of fetal morphology. It had less hypothetical side-effects than 3D/4D ultrasonography. Furthermore the 2D ultrasonography had an advantage towards magnetic scan, allowing the investigation in precocious gestational stages, which was crucial for the evaluation of the tooth germs.

MATERIAL AND METHODS

Between May 2011 and August 2012, Caucasian pregnant women, submitted to usual surveillance ultrasound exams of pregnancy in the Prenatal Diagnosis Unit of Centro Hospitalar de Vila Nova de Gaia (CHVNG), were randomly selected to be included in this study. The final sample was composed by 161 women that were carrying unifetal pregnancies from 11^{+0} to 36^{+0} weeks of gestation. So, the inclusion criteria were: Caucasian pregnant women, single fetal pregnancies of both sexes, gestational age (GA) between 11 and 36 weeks gestation.

The two-dimensional (2D) prenatal ultrasounds were performed in GE E8 Voluson® equipment (serial number 0123, 2010, Austria) with C512D, Rab4-8D, 11LD, C1-5 probes and with a normal harmonic frequency. The ultrasounds were performed by trans-abdominal approach, under resting conditions of the pregnant. The images were viewed, captured and archived in Astraia® program (version 1.23.0, Astraia Software Gmbh and parents, Germany) and were processed on the same equipment with dimensions of 640 * 480 VGA pixels.

The 2D ultrasound exams were undertaken during usual surveillance of pregnancy and therefore these tests have not been conducted with the specific intention of serving this research. The study was observational, descriptive and transversal. Informed consent was obtained from each woman before the ultrasonography exam.
Two operators were responsible for conducting examinations and registration. The professionals, as experts in fetal medicine, had the same experience degree in obstetric ultrasound and prenatal diagnosis. The calibration of the operators was strict to the interpretation of ultrasound images and the identification of reference aspects linked to this research conduction.
The 2D ultrasonography description of the fetal tooth buds was preceded by fetal biometry and detailed screening for malformations.
The jaws were observed after sagittal, coronal and axial cuts, having been used as a starting point the same plan of the nuchal translucency (NT) and nasal bone. This plan allowed, through oblique deviations, the study of the entire maxilla and mandible. The obtained coronal section should include the mandible, maxilla and nasal triangle.

The differentiation of the alveolar crest of fetal tooth germ was made by the observation and identification of a groove separation between the two structures. The presence of this hypoecogenic groove was considered undoubtful condition to ensure the presence of dental germs.

The data were analysed in the IBM® SPSS® Statistics, version 20.0 (USA).

RESULTS

The sample was composed by 161 pregnant women: 77 women were in the first (1st) trimester and 84 were in the second and third (2nd, 3nd) trimesters of their pregnancy. Four pregnant women were excluded due to the impossibility to observe the dental germs because of the fetal position and poor echogenicity. Therefore the final sample corresponded to 157 pregnant women (77 in the 1st trimester and 80 in the 2nd and 3rd trimesters). The rate of non-observation was 2.5%, mainly in later gestational ages.

The average of the maternal age was 32.1 years (ranged 18–43 years) in the first trimester with a standard deviation of 6.2 years. In the second and third trimesters the average of the maternal age was 31.4 years (ranged 14–47 years) with a standard deviation of 6.7 years. The median age of pregnant women was 32 years and the younger pregnant had 14 years old and the oldest pregnant had 47 years. No statistically significant differences were detected (p value= 0.472) among the average values of the pregnant women ages in the two periods (1st trimester, 2nd and 3rd trimesters).

Whether at the level of the maxilla or mandible, the median gestational age for the detection of 10 tooth germs was of 13 weeks, that means that in 50% of pregnant women the gestational age for detection of 10 tooth germs was less than or equal to 13 weeks of gestation. It was also found that 25% of the pregnant women from our sample exhibited a gestational age less than 12 weeks for the detection of the dental germs.

In the 1st trimester ultrasonography, 10 tooth germs were identified in the maxilla and 10 tooth germs were also recognized in the mandible in all fetuses, with the exception of 1 case that presented 8 maxillary tooth germs. This case was related to a genetic syndrome with a chromosomal abnormality - a trisomy 13 - with bilateral cleft palate.

In the 2nd and 3rd trimesters ultrasonography, for the considered sample, a larger range of tooth germs were identified: 81.2% of cases showed 10 tooth germs in the maxilla and 85.0% of the cases revealed 10 tooth germs in the mandible. In few cases of the 2nd and 3rd trimesters were identified 8 and 9 tooth germs (1.2%; 2.5%). In 15.0% of the 2nd and 3rd trimesters cases, 12 tooth germs were observed in the maxilla and in the mandible, which corresponded to the identification of the first permanent molar (Table 1).

The maxilla was more affected by hypodontia (2.5%) than the mandible. Because of that observation we analyzed the possible association between this variable (hypodontia) with others studied by qualitative 2D ultrasonography (fetal pathology, markers, head, nuchal, face, spine).

Table 1 Distribution of tooth germs by location and gestation period

Evaluation period	Location	Tooth germs	N	%	CI at 95%
1st trimester	Maxilla	8	1	1.3%	0.03% to 7.02%
		10	76	98.7%	92.97% to 99.97%
		Total	77	100.0%	
	Mandible	10	77	100.0%	
		Total	77	100.0%	
2nd and 3rd trimestres	Maxilla	8	1	1.2%	0.03% to 6.77%
		9	2	2.5%	0.30% to 8.74%
		10	65	81.2%	70.96% to 89.11%
		12	12	15.0%	8.00% to 24.74%
		Total	80	100.0%	
	Mandible	10	68	85.0%	
		12	12	15.0%	
		Total	80	100.0%	

To assess the association between hypodontia in the maxilla and fetal pathology in the 2nd and 3rd trimesters of pregnancy it was performed the Fisher exact test and the value found (p value =0.324) revealed the absence of statistically significance (Table 2).

Table 2 Crosstable of maxillary hypodontia *versus* fetal pathology

			Fetal Pathology		Total
			Present	Absent	
Maxilla Hypodontia	**Absent**	N	44	21	65
		% according Maxilla Hypodontia	67.7%	32.3%	100.0%
		% according Pathology	93.6%	100.0%	95.6%
		% of Total	64.7%	30.9%	95.6%
	Present	N	3	0	3
		% according Maxilla Hypodontia	100.0%	0.0%	100.0%
		% according Pathology	6.4%	0.0%	4.4%
		% of Total	4.4%	0.0%	4.4%
Total		N	47	21	68
		% according Maxilla Hypodontia	69.1%	30.9%	100.0%
		% according Pathology	100.0%	100.0%	100.0%
		% of Total	69.1%	30.9%	100.0%

The same test was done to evaluate the association between hypodontia in the maxilla and the markers on the 2nd and 3rd trimesters of pregnancy, which revealed no statistically significant association (p value= 0.229) (Table 3).

The Fisher exact test was also performed in order to assess the association between hypodontia in the maxilla and head anomalies in fetuses of the 2nd and 3rd trimesters of pregnancy (Table 4) The test result did not found statistically significant association (p value = 0.872).

Table 3 Crosstable of maxillary hypodontia *versus* markers

			Markers		Total
			Absent	**Present**	
Maxilla Hypodontia	**Absent**	**N**	39	26	65
		% according Maxilla Hypodontia	60.0%	40.0%	100.0%
		% according Markers	92.9%	100.0%	95.6%
		% of Total	57.4%	38.2%	95.6%
	Present	**N**	3	0	3
		% according Maxilla Hypodontia	100.0%	0.0%	100.0%
		% according Markers	7.1%	0.0%	4.4%
		% of Total	4.4%	0.0%	4.4%
Total		**N**	42	26	68
		% according Maxilla Hypodontia	61.8%	38.2%	100.0%
		% according Markers	100.0%	100.0%	100.0%
		% of Total	61.8%	38.2%	100.0%

Table 4 Crosstable of maxillary hypodontia *versus* head anomalies

			Head		Total
			Normal	**Anomalies**	
Maxilla Hypodontia	**Absent**	**N**	62	3	65
		% according Maxilla Hypodontia	95.4%	4.6%	100.0%
		% according Head	95.4%	100.0%	95.6%
		% of Total	91.2%	4.4%	95.6%
	Present	**N**	3	0	3
		% according Maxilla Hypodontia	100.0%	0.0%	100.0%
		% according Head	4.6%	0.0%	4.4%
		% of Total	4.4%	0.0%	4,4%
Total		**N**	65	3	68
		% according Maxilla Hypodontia	95.6%	4.4%	100.0%
		% according Head	100.0%	100.0%	100.0%
		% of Total	95.6%	4.4%	100.0%

The Fisher exact test also showed no statistically significant association between hypodontia in the maxilla and nuchal anomalies in the fetuses of the 2nd and 3rd trimesters of pregnancy (p value= 0.351) (Table 5).

Table 5 Crosstable of maxillary hypodontia *versus* nuchal anomalies

			Nuchal		Total
			Normal	**Anomalies**	
Maxilla Hypodontia	**Absent**	**N**	57	8	65
		% according Maxilla Hypodontia	87.7%	12.3%	100.0%
		% according Nuchal	96.6%	88.9%	95.6%
		% of Total	83.8%	11.8%	95.6%
	Present	**N**	2	1	3
		% according Maxilla Hypodontia	66.7%	33.3%	100.0%
		% according Nuchal	3.4%	11.1%	4.4%
		% of Total	2.9%	1.5%	4.4%
Total		**N**	59	9	68
		% according Maxilla Hypodontia	86.8%	13.2%	100.0%
		% according Nuchal	100.0%	100.0%	100.0%
		% of Total	86.8%	13.2%	100.0%

As occurred with the other parameters crossed with hypodontia in the maxilla, also the exact test of Fischer found no significant association between hypodontia in the maxilla and anomalies in the face of the fetuses of the 2nd and 3rd trimesters of pregnancy (p value= 0.956) (Table 6).

Table 6 Crosstable of maxillary hypodontia *versus* face anomalies

			Face		Total
			Normal	**Anomalies**	
Maxilla Hypodontia	**Absent**	**N**	64	1	65
		% according Maxilla Hypodontia	98.5%	1.5%	100.0%
		% according Face	95.5%	100.0%	95.6%
		% of Total	94.1%	1.5%	95.6%
	Present	**N**	3	0	3
		% according Maxilla Hypodontia	100.0%	0.0%	100.0%
		% according Face	4.5%	0.0%	4.4%
		% of Total	4.4%	0.0%	4.4%
Total		**N**	67	1	68
		% according Maxilla Hypodontia	98.5%	1.5%	100.0%
		% according Face	100.0%	100.0%	100.0%
		% of Total	98.5%	1.5%	100.0%

The Fisher exact test was also performed to assess the association between hypodontia in the maxilla and spine anomalies in the fetuses of the 2nd and 3rd trimesters of pregnancy. This test revealed no statistically significant association (p value= 0.956) (Table 7).

Table 7 Crosstable of maxillary hypodontia *versus* spine anomalies

			Spine		Total
			Normal	**Anomalies**	
Maxilla Hypodontia	**Absent**	**N**	64	1	65
		% according Maxilla Hypodontia	98.5%	1.5%	100.0%
		% according Spine	95.5%	100.0%	95.6%
		% of Total	94.1%	1.5%	95.6%
	Present	**N**	3	0	3
		% according Maxilla Hypodontia	100.0%	0.0%	100.0%
		% according Spine	4.5%	0.0%	4.4%
		% of Total	4.4%	0.0%	4.4%
Total		**N**	67	1	68
		% according Maxilla Hypodontia	98.5%	1.5%	100.0%
		% according Spine	100.0%	100.0%	100.0%
		% of Total	98.5%	1.5%	100.0%

DISCUSSION

We face a shortage of investigations referring to the parameter evaluation of fetal tooth germs limiting the systematic approach to a small number of authors and their researches.

In Ulm´s researches and his collaborators study the percentage of tooth germs varied according to the ultrasonography used (2D or 3D). The success rate in the evaluation of tooth germs found with 3D ultrasonography, at 19 weeks of gestation, was between 86 and 94%, while using 2D ultrasonography was lower - between 56 to 62% (Ulm, 1998).

Regarding the non-observation rate, our investigation came up with a 2.5%. These results, we managed to achieve with 2D ultrasound, were higher than the ones Ulm presented. We perceived in some cases up to 12 tooth germs the following: these represented the 10 tooth germs of the temporary teeth and apart of those there was the very beginning of the first permanent molar teeth, in the maxilla as well as the mandible.

There is also some discordancy if we confront these results with what Ulm published (Ulm, 1998) when his study refers that the number of tooth germs would keep constant throughout the pregnancy and that they would only be visible from 16 weeks of gestation period, becoming easily identified as the pregnancy went into further stages. In our opinion this discording result might be related to the learning curve of the operators of our research and with the eequipment used during our and Ulm's investigation (Ulm, 1998).

We would like to enhance that during our investigation the number of tooth germs was not constant over the gestational period, which was in agreement to Ten Cate (Ten Cate, 2008), statements that the development of all temporary teeth starts in uterus and that the first permanent molar teeth start developing somewhere around the twentieth week of gestation.

As referred previously, during our study we observed that in the second/third trimesters some fetuses presented 6 tooth germs in each quarter, whilst in the first trimester all fetuses presented 5 tooth germs, except in the case of any anomaly.

We need to point out that the maxilla (2.5%) was more affected by hypodontia than the mandible. Because of that observation we analyzed the possible association between this variable (hypodontia) with others studied by qualitative 2D ultrasonography (fetal pathology, markers, head, neck, face, spine). No relevant association was found in any of the items studied. For these results we do not have terms of comparison because there are no studies similar to ours. It is our conviction that with a broadest sample we may find some kind of association and that this is an emerging area of research.

With the experience acquired it was possible to visualize the tooth germs. In our point of view the study of them could be considered as a detail of the echo-graphic examination from the 1st trimester assessment for changes in the upper face bones.

The visualization of fetal tooth germs using 2D ultrasonography could be an efficient supplementary method of diagnosis for severe syndromes that can be associated to hypodontia or supernumerary teeth. However more studies are needed to reinforce this conviction.

The pre-natal diagnosis of the orofacial cleft was done by 2D ultrasonography and normally 3D, 4D and magnetic ultrasound are used to complement the suspected diagnosis. This diagnosis is done by the end of the 1st trimester or during the 2nd trimester.

The pre-natal characterization of orofacial cleft and the evaluation of the severity of the orofacial clefts could be helped by visualizing the tooth germs through 2D ultrasonography (Rotten, 2004; Sommerlad, 2010).

CONCLUSIONS

The dental germs have a similar echogenicity to that of the bone. Their identification is possible through a groove hypoechoic separation between germs and dental alveolar bone.

The preview of the dental germs may be hampered by the fetal position and maternal echogenicity.

It was possible to visualize, identify and count fetal tooth germs through two-dimensional ultrasound from 13 weeks of gestation.

It is necessary more cases to relate congenital malformations, genetic syndromes and chromosomal abnormalities to integrate this parameter in the routine surveillance of all pregnancies improving the fairness of prenatal diagnosis.

REFERENCES

Andresen C, Matias A, Merz E. Fetal Face: The Whole Picture. Ultraschall Med, 2012, 33, p. 431-440.

Babcook CJ, McGahan JP, Chong BW, Nemzek WR, Salamat MS.Evaluation of fetal midface anatomy related to facial clefts: use of US. Radiology, 1996, 201, p. 113-118.

Babcook CJ, McGahan JP. Axial ultrasonographic imaging of the fetal maxilla for accurate characterization of facial clefts. J Ultrasound Med, 1997, 16, p. 619-625.

Cash C, Set P, Coleman N.The accuracy of antenatal ultrasound in the detection of facial clefts in a low-risk screening population. Ultrasound Obstet Gynecol, 2001, 18, p. 432-436.

Chen ML, Chang CH, Yu CH, Cheng YC, Chang FM. Prenatal diagnosis of cleft palate by three-dimensional ultrasound. Ultrasound Med Biol, 2001, 27, p. 1017-1023.

Clementi M, Tenconi R, Bianchi F, Stoll C. Evaluation of prenatal diagnosis of cleft lip with or without cleft palate and cleft palate by ultrasound: experience from 20 European registries. Prenat Diagn, 2000, 20, p. 870-875.

Evans DJ, Francis-West PH. Craniofacial development: making faces. J Anat, 2005, p. 435-436.

Ghi T, Perolo A, Banzi C, Contratti G, Valeri B, Savelli L, Morselli GP, Bovicelli L, Pilu G.Two-dimensional ultrasound is accurate in the diagnosis of fetal craniofacial malformation. Ultrasound Obstet Gynecol, 2002, 19, p. 543-551.

Rotten D, Levaillant JM. Two- and three-dimensional sonographic assessment of the fetal face. 2. Analysis of cleft lip, alveolus and palate. Ultrasound Obstet Gynecol, 2004, 24, p. 402-411.

Rotten D, Levaillant JM. Two- and three-dimensional sonographic assessment of the fetal face. 1. A systematic analysis of the normal face. Ultrasound Obstet Gynecol, 2004, 23, p. 22-31.

Sommerlad M, Patel N, Vijayalakshmi B, Morris P, Hall P, Ahmad T, Campbell S, Lees C. Detection of lip, alveolar ridge and hard palate abnormalities using two-dimensional ultrasound enhanced with the three-dimensional reverse-face view. Ultrasound Obstet Gynecol, 2010, 36, p. 596-600.

Ten Cate AR. Development of Tooth and its Supporting Tissues. Ten cate`s Oral Histology:development, structure, and function 7th ed. - St. Louis : Mosby, 2008, p. 79-101.

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.

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.