Int J Clin Exp Med 2017;10(6):9358-9363
www.ijcem.com /ISSN:1940-5901/IJCEM0051915

Original Article

Absence of nasal bone in fetuses with trisomy 21 at second trimester of pregnancy

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Received March 1, 2017; Accepted June 5, 2017; Epub June 15, 2017; Published June 30, 2017

Abstract: Objectives: To investigate the value of second-trimester ultrasound assessment of fetal nasal bone (NB) in predicting trisomy 21. Methods: A retrospective analysis was performed on the test results of pregnant women who received amniocentesis besides their anomaly scan due to various reasons at the Prenatal Diagnosis Center of Fudan University Obstetrics & Gynecology Hospital from January 2012 to January 2016. The ultrasound screening and chromosome screening results of fetuses with absent NBs were recorded. Results: The rate of NB absence in the fetus group with trisomy 21 was 8.43%, significantly higher than the rate of the euploid fetus group, 0.36% ($P < 0.05$). The rate of trisomy 21 in the fetus group with non-isolated NB absence was 87.5% (14/16), far higher than the rate of the group with isolated NB absence, 4.5% (1/22) ($P < 0.05$). Conclusions: Second-trimester ultrasound screening for absence of the fetal NB, especially for non-isolated NB absence is useful in detecting fetuses with trisomy 21.

Keywords: Nasal bone, trisomy 21, sonography, prenatal screening, second trimester

Introduction

Aneuploidy is the main cause of perinatal or neonatal morbidity and mortality, imposing great costs on pregnant women, their families as well as society. The most common abnormalities are trisomy 18, 13 and 21. The prenatal ultrasound detection rate of trisomy 21 is lower than that of trisomy 18 and 13 [1, 2]. Cicero first proposed in 2001 that there existed a big difference in the rate of nasal bone (NB) absence between normal fetuses and fetuses with trisomy 21. The rate of NB absence in Trisomy 21 fetuses was 73%, while the figure for normal fetuses was merely 0.5% [3]. Previous articles have concluded that including NB into the early pregnancy ultrasound assessment of trisomy 21 contributed to high sensitivity and would significantly reduce the false positive rate [4-6], reducing the cases of invasive tests on villi, amniotic fluid and umbilical cord blood for "high-risk" pregnant women during their prenatal chromosome screening. Considering that in most areas of Mainland China, local hospitals haven't started providing early pregnancy ultrasound screening, and that the best time for diagnosing fetal malformation is the middle period of the second trimester [7], this study examined the value of second-trimester ultrasound screening for NB absence in detecting fetuses with trisomy 21.

Data and methods

Study subjects

A retrospective analysis was performed on the test results of a total of 6665 pregnant women who received amniocentesis after their anomaly scan at the Prenatal Diagnosis Center of Fudan University Obstetrics & Gynecology Hospital from January 2012 to January 2016 due to various reasons including maternal age, previous abnormal pregnancies and deliveries, serologic abnormalities and abnormal first-trimester or second-trimester ultrasound results. The average maternal age was 30.9 ± 4.4 years (ranging from 21 to 46 years old). The average gestational age was 22 weeks 6 days ± 1 week 6 days (ranging from 18 weeks 3 days to 27 weeks 6 days).

Instruments and methods

All examiners were skillful and experienced senior sonographers who had passed the

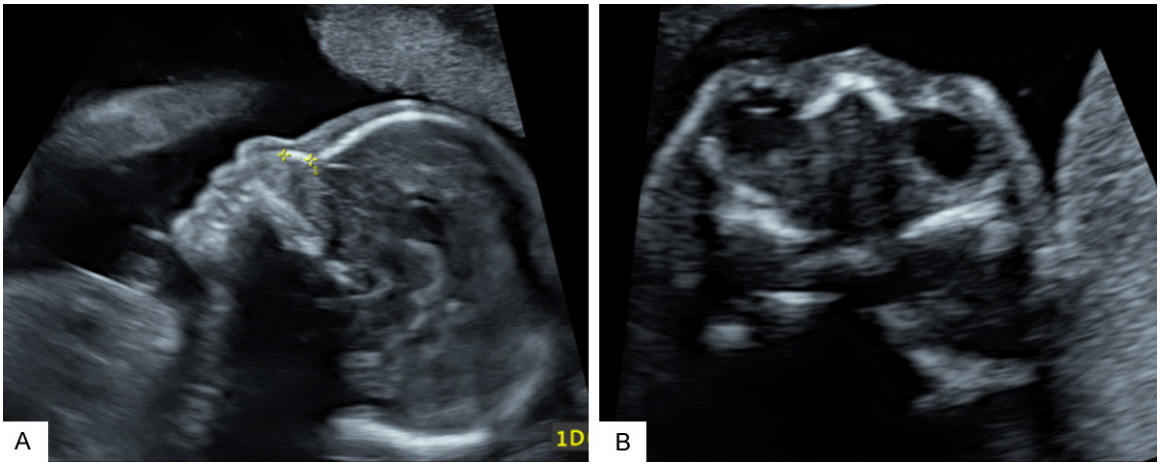

Figure 1. Fetal profiles at 22 weeks of gestation in a normal fetus, showing the nasal bone.

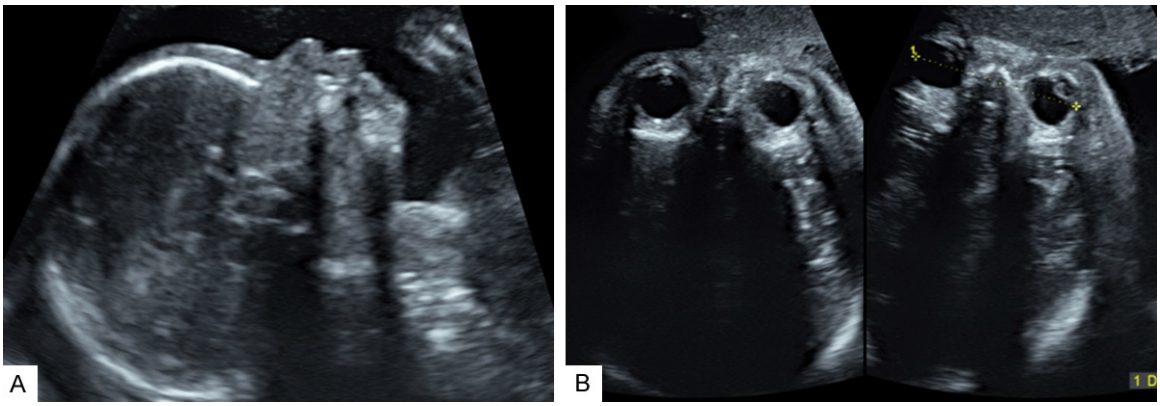

Figure 2. Fetal profiles at 22 + 3 weeks of gestation in a trisomy 21 fetus, showing absence of the nasal bone.

anomaly scan training. The images were obtained using GE Volusou 730 Expert equipped with AB2-7 probe, GE Volusou E6 and GE Volusou E8 with C1-5 probe (GE Medical Systems, Kretz Ultrasound, Zipf, Austria). The examiners had no prior knowledge of the fetal karyotype. The transabdominal ultrasound examination was performed during the second-trimester, measuring fetal biparietal diameter, head circumference, abdominal circumference, femur length, and recording a detailed assessment of brain, spine, face, chest and abdomen, limbs, heart and other related structures. We noted the presence or absence of the NB during the assessment of face.

For examination of the fetal nose, the probe was made to face the fetal head and a midsagitta view of the fetus without the covering of hand or the umbilical cord was obtained. The direction of the incident sound beam was made to be as perpendicular to the long axis of the NB as possible (**Figure 1A**). A slight deflection and lateral movement of the probe would help to show borders of the NB. A sagittal view of the head would show a normal NB and the skin over it as a hyperechoic equal sign. A cross-section plane of the NB was parallel to the horizontal plane through the eyes. The incident sound beam entering perpendicularly from the direction of the forehead would show the arch structure of the two NBs on the inside of the frontal processes of the maxillae in front of the nasal tip (**Figure 1B**). Diagnostic criteria of absent fetal NB: upon examining the sagittal view and the cross-section view of the head, no hyperecho of the NB beneath the skin of the nose was detected in the sagittal view (i.e. no NB hyperecho detected in either of the two orthogonal planes), and no hyperecho of the 2

Table 1. Rate of absence or presence of NB in trisomy 21 and chromosomally normal fetuses in second trimester

	Trisomy 21 (n = 178)		Normal karyotype (n = 6447)		
	NB absent	NB present	NB absent	NB present	*P* value
Number of cases	15 (8.43%)	163 (91.5%)	23 (0.36%)	6424 (99.64%)	< 0.05
Average maternal age (years)	31.5 ± 5.9	32.1 ± 5.1	30.8 ± 4.3	31.1 ± 3.8	> 0.05
Average gestational age (weeks)	23.2 ± 2.3	22.7 ± 2.7	22.4 ± 1.9	23.6 ± 2.4	> 0.05

Table 2. Rate of trisomy 21 in isolated and non-isolated NB absence fetuses

	Trisomy 21	Normal karyotype
Isolated NB absence	1	21
Non-isolated absent NB	14	2

NBs inside the frontal processes of the maxillae in front of the nasal tip was detected in the cross-section view (**Figure 2**).

Fetuses with abnormal karyotypes were all examined for appearance after abortion or delivery; Fetuses with normal karyotypes were all clinically followed until 6 months after birth.

Statistical analysis

Statistical analysis of data was performed using the SPSS 18.0 (SPSS Inc., Chicago, IL, USA) software. Measurement data in normal distribution were denoted as mean ± standard deviation and count data were expressed in median (range). The statistical significance of the difference between groups was compared with chi-square test and ANOVA. $P < 0.05$ was considered statistically significant.

Results

In this study, the average maternal age was 30.9 ± 4.4 years. The average gestational age was 22 weeks 6 days ± 1 week 6 days. Regardless of the presence or absence of the NB, no significant difference of maternal age and gestational age was found between the normal fetus group and the group with trisomy 21. Of the follow-up cases we examined, 38 cases with absent NB had performed chromosome tests. Of the 6447 cases of euploid fetuses, 23 measured NB absence. Of the 178 cases of fetuses with trisomy 21, 15 measured NB absence. Zero of the 29 cases of fetuses with trisomy 18 measured NB absence. Zero of the 11 cases of fetuses with trisomy 13 measured NB absence. The rate of NB absence in the fetus group with trisomy 21 was significantly higher than that of the normal group ($P < 0.05$, **Table 1**), with a likelihood ratio of 23.4.

Of the 38 cases of fetuses with NB absence, 15 were with trisomy 21, 23 had normal chromosomes. The rate of trisomy 21 in this group was 39.5% (15/38). Depending on whether accompanied by other soft markers of chromosomal abnormalities and/or structural abnormalities, the fetus group with absent NB was divided into isolated and non-isolated absence of NB groups. The rate of trisomy 21 in the fetus group with non-isolated absence of the NB was 87.5% (14/16), far higher than the rate of the group with isolated absence of NB, 4.5% (1/22) ($P < 0.05$, **Table 2**).

Ultrasound results of the 23 cases of fetuses with NB absence: 21 had no abnormalities other than the absent NB, 1 with thickened nuchal fold other than the absent NB, and 1 with a widened posterior horn of lateral ventricle other than the absent NB. In five of those cases, the fetus/infant was followed until 6 months after birth and measured a normal NB. Ultrasound findings of the 15 cases of fetuses with absent NB and trisomy 21 included the following types: 6 cases of cardiac abnormalities (4 cases of endocardial cushion absence (**Figure 3A**), 2 cases of ventricular septal defects, 2 cases of tricuspid regurgitation, etc.), 4 cases of thickened nuchal fold (**Figure 3B**), 2 cases of duodenal atresia or stenosis (**Figure 3C**), 2 cases of cavum velum interpositum cyst and minor structural abnormalities including short mandible, single umbilical artery and absence of the middle phalanx of the fifth finger. Detailed ultrasound results are shown in **Table 3**. Fourteen of those fetuses were aborted. One of the fetuses survived after a full-term cesarean section delivery.

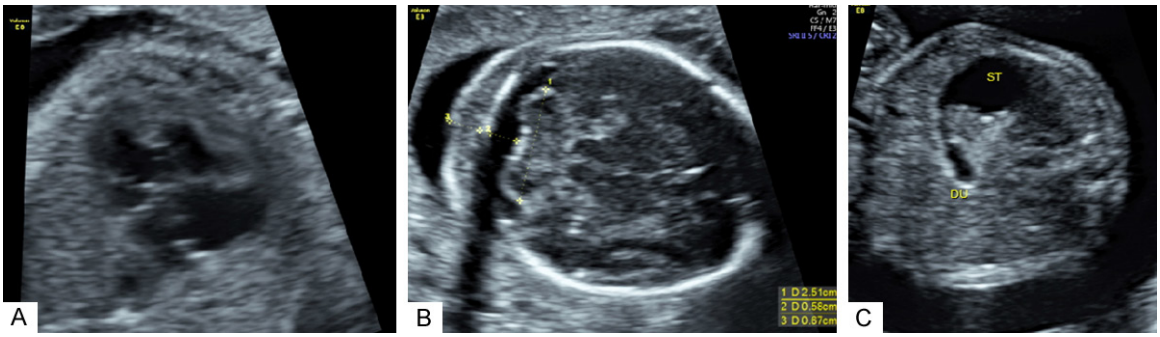

Figure 3. Ultrasound findings in trisomy 21. A. Endocardial cushion defects. B. Thickened nuchal fold. C. Double ball in duodenal atresia or stenosis.

Table 3. Sonographic findings in trisomy 21 of 15 cases in second trimester

No.	Maternal age (years)	Gestational age (weeks)	Sonographic findings
1	21	26 + 6	NB absence, endocardial cushion defects
2	37	21 + 1	NB absence, endocardial cushion defects
3	28	20 + 6	NB absence, endocardial cushion defects
4	30	22 + 6	NB absence, cavum velum interpositum cyst
5	33	22 + 3	NB absence, thickened nuchal fold, cavum velum interpositum cyst, renal artery variants
6	38	22 + 3	NB absence, thickened nuchal fold absence of the middle phalanx of the fifth finger, cardiac anomaly (left cardiac axis deviation, ventricular septal defects, tricuspid regurgitation)
7	46	22 + 1	NB absence, duodenal atresia or stenosis, thickened nuchal fold, cardiac anomaly (endocardial cushion defects, double outlet right ventricle)
8	27	22 + 2	NB absence, Single umbilical artery, spinal stenosis
9	29	22 + 3	NB absence, facial flat
10	34	20 + 5	NB absence, facial flat, duodenal atresia or stenosis
11	30	18 + 3	NB absence, brachycephaly, alober holoprosencephaly, Short mandible, hypertelorism with right eye dysplasia
12	34	24 + 5	NB absence, brachycephaly, short mandible
13	30	22 + 1	NB absence, right cardiac enlargement, tricuspid regurgitation, ventricular septal defects, high pulmonary velocity
14	32	22 + 1	NB absence, thickened nuchal fold
15	29	23 + 4	NB absence

Discussion

This study has shown that in 8.43% of trisomy 21 fetuses, the nasal bone was not visible in the second-trimester anomaly scan. The rate is lower than what previously published literature has reported [3]. This may be related to the fact that some trisomy 21 fetuses with absent NB were aborted after early pregnancy screening. On the other hand, the NB was not visible in only 0.36% of the euploid fetuses, which is consistent with the results of previously published literature [8, 9], showing that NB absence is a good predicative factor for trisomy 21 and can significantly improve sensitivity and lower false positive rate [6]. Recently, an increasing number of markers have been studied for the assessment of fetal NB [10-15], and prenatal chromosome screening often includes the comprehensive assessment of maternal serum analysis and ultrasound results. Furthermore, with methods like Non-Invasive Chromosomal Evaluation (NICE) using fetus cell-free DNA in maternal blood, the sensitivity of trisomy 21

detection of prenatal screening can be up to 100%, with a false positive rate as low as 0.03% [16]. However, the above mentioned techniques have not reached most areas of our country, and they tend to be very expensive. On the other hand, ultrasound screening for NB absence is simple and easy to conduct. Therefore, the presence or absence of the fetal NB examined in the second-trimester ultrasound screening is still an important marker in predicting trisomy 21. Moreno-Cid et al [17] performed a systematic review and meta-analysis on the performance of NB assessment in predicting trisomy 21 and concluded that NB assessment shows high specificity and low but acceptable sensitivity in identifying fetuses with trisomy 21.

NB absence was found not only in fetuses with chromosomal abnormalities, but also in some normal fetuses. The rates of NB absence in normal fetuses vary according to maternal ethnicity [18]. Although in this study, 8.47% of the trisomy 21 fetuses measured NB absence, far higher than the rate in the normal fetuses, the rate of euploidy in the fetuses with absent NB measured 60.5% (23/38). Among the fetuses with absent NB, we found that most euploid fetuses (21/23) had no other ultrasound abnormalities other than the missing NB, which is what we called isolated NB absence. 95.5% (21/22) of the fetuses with isolated NB absence have normal chromosomes. Non-isolated NB absence is accompanied by other structural abnormalities, such as cardiac abnormalities (especially endocardial cushion absence, ventricular septal defects, etc.), thickened nuchal fold, duodenal stenosis or atresia, cavum velum interpositum cyst, and minor structural abnormalities including short mandible, single umbilical artery and absence of the middle phalanx of the fifth finger, strongly suggesting a high risk of trisomy 21 and requiring an invasive diagnosis. This conclusion is consistent with the findings of previously published literature [19]. A group of "soft markers" represents greatly increased risk than a single "soft marker" [20, 21]. The risk of isolated NB absence compared with non-isolated NB absence is much lower. The result of maternal serum tests should be included for a comprehensive assessment before conducting invasive diagnosis.

There are some limitations in this retrospective study. Firstly, there are biases in statistical data due to high loss to follow-up; Secondly, this study focused only on the presence or absence of the NB with no data on NB dysplasia; Moreover, this study used the classic indicator of NB absence while more new markers which combining NB and other markers such as PNT (the prenasal thickness, PT/NBL) have been used to predict trisomy 21 [20]. Despite the limitations, we conclude that: Second-trimester ultrasound screening for fetal NB absence, especially non-isolated NB absence is very useful in detecting fetuses with trisomy 21.

Disclosure of conflict of interest

None.

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