Ultrasonography of the fetal nose, maxilla, mandible and forehead as markers for aneuploidy

Vos, Fedia

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Prenasal thickness-to-nasal bone length ratio: a strong and simple second- and third-trimester marker for trisomy 21

De Jong-Pleij EA, Vos FI, Ribbert LS, Pistorius LR, Tromp E, Bilardo CM

ABSTRACT

Objectives
To study the ratio of prenasal thickness (PT) to nasal bone length (NBL) in euploid and Down syndrome (DS) fetuses in the second and third trimesters of pregnancy.

Methods
The PT and NBL were measured retrospectively in 106 euploid fetuses (in three-dimensional (3D) volumes) and in 30 DS fetuses (10 on two-dimensional (2D) images and 20 in 3D volumes).

Results
In euploid fetuses the mean PT and NBL increased between 15 and 33 weeks’ gestation from 2.3 to 6.1 mm \( (r = 0.85, p < 0.001) \) and from 3.3 to 9.6 mm \( (r = 0.87, p < 0.001) \), respectively. The PT-NBL ratio was stable throughout gestation, with a mean of 0.61 (95% CI, 0.59 – 0.63; \( r = -0.04, p = 0.7 \)). The 5th and 95th percentiles were 0.48 and 0.80, respectively. In DS fetuses the mean PT and NBL increased between 14 and 34 weeks from 3.0 to 9.2 mm \( (r = 0.86, p < 0.001) \) and from 1.9 to 7.8 mm \( (r = 0.85, p < 0.001) \), respectively. The PT-NBL ratio was significantly higher than in euploid fetuses \( (p < 0.001) \), but also stable throughout gestation, with a mean of 1.50 (95% CI, 1.20 – 1.80; \( r = -0.35, p = 0.07 \)). Twenty-three (77%) of the 30 DS fetuses had a PT above the 95th percentile and 20 (67%) had an NBL below the 5th percentile. All the DS fetuses had a PT-NBL ratio above the 95th percentile. When the 95th percentile of the PT-NBL ratio was used as a cut-off value the detection and false positive rates for DS were 100% (95% CI, 89 – 100%) and 5% (95% CI, 2 – 11%), respectively. The positive likelihood ratio was 21.2.

Conclusions
The PT-NBL ratio is stable in the second and third trimesters of pregnancy in both euploid and DS fetuses, but all DS fetuses in this series had a PT-NBL ratio above the 95th percentile. The ratio is therefore a strong marker for DS.
INTRODUCTION

The word ‘syndrome’ comes from the Greek ‘syn’ (together) and ‘dramein’ (to run) and means ‘run together’. A syndrome is suspected when a combination of anomalies or dysmorphic features occur together in the same patient. The more characteristic features are recognized the higher the chance of a syndromal association. Prenatal identification of a syndrome is important, as it may change the management of pregnancy and perinatal care.

A variety of anomalies and dysmorphic traits are known to be associated with Down syndrome (DS)\(^1,2\). Major structural anomalies like heart defects account for only 27% of affected fetuses\(^3\). In contrast, more subtle deviations of the phenotype are present in the majority of affected individuals\(^3-5\). Currently there is overwhelming evidence that the observations reported by J.L.H. Down in 1866 such as a flat profile, a small nose and redundant skin are useful ultrasound markers\(^2\).

Nasal bone length (NBL) was introduced in 1995 by Guis et al\(^6\) as a possible marker for DS, while prenasal thickness (PT) was proposed in 2005 by Maymon et al\(^7\). Both markers are visualized in the same profile view and even share a landmark, the nasion. Because in DS NBL tends to be smaller while PT tends to be larger than in euploid fetuses, we speculated that their ratio may be a very sensitive and specific indicator for DS.

Recently we showed that three-dimensional (3D) ultrasound enhances the accuracy of facial measurements by enabling definition of the exact midline by multiplanar correction of the volumes\(^8\).

In this study the PT-NBL ratio was evaluated in 3D volumes of second- and third-trimester euploid fetuses and subsequently compared with the PT-NBL ratio of DS fetuses.

METHODS

We retrospectively measured PT and NBL in two groups of patients. The first group comprised 219 fetuses with stored volumes collected cross-sectionally from non-smoking, healthy, low-risk Caucasian women with a singleton pregnancy. Only non-anomalous fetuses from uncomplicated pregnancies were included. Volumes were acquired from fetuses facing the transducer, starting as close as possible from the exact midsagittal profile view during periods of quiescence. An attempt was made to collect at least two such volumes per fetus. For each fetus, the volume with the best midsagittal view was selected. At first, all images were corrected by multiplanar mode to the exact midsagittal view and scored from 1 – 5 in terms of quality for contrast and clarity (quality score; 1 being bad and 5 excellent). Only images with an above-average quality (score 4 or 5) were included. Secondly, PT and NBL were scored from 1 – 3 in terms of visualization of landmarks (measurability score; 1 being bad and 3 excellent). Fetuses with score 1 for PT or NBL were excluded. The second group comprised DS fetuses confirmed by karyotyping. In prenatal databases of the Academic Medical Centre, Amsterdam, University Medical Centre, Utrecht and the Saint Antonius Hospital, Nieuwegein, 39 cases of second- and third-trimester DS fetuses were found, 19 on two-dimensional (2D) images and 20 on 3D volumes. Only images with satisfactory quality and with landmark visualization were included. Transabdominal ultrasonography had been carried out by experienced sonographers using a GE Voluson 730 Expert or E8 ultrasound system equipped with a RAB 2-5L.
or RAB 4-8L abdominal transducer (GE Medical Systems, Kretz Ultrasound, Zipf, Austria). Images and volumes were stored and examined either off-line on 4D View software version 7.0 (GE Medical Systems) or on stored images in the GE ultrasound system. The nasal bone was measured from the nasion – defined as the most anterior point of the junction between the frontal and nasal bones – to the distal end of the white ossification line (Figure 1). Care was taken not to include the frontal bone in the measurement as the frontal bone extends posteriorly of the nasal bone. PT was measured as the shortest distance between the nasion (same landmark as used for measuring the NBL) and the frontal skin (Figure 1). Calipers were placed on the outermost borders of the skin or bone, and the mean of three measurements was used for analysis. Multiples of the median (MoM) values were calculated using our own regression equation, but absolute values are reported except where indicated.

Data were analyzed using the statistical software SPSS version 17.0 for Windows (SPSS Inc., Chicago, IL, USA) and Excel for Windows 2000. Correlations were calculated by Pearson’s correlation test after excluding outliers beyond three SD’s from the mean. The statistical significance of the difference of the means of two groups was tested with the unpaired Student’s t-test, and \( p < 0.05 \) was considered statistically significant.

RESULTS

One hundred and eleven of the 219 volumes had an above-average quality score. Five volumes were excluded because of a measurability score of 1 for PT or NBL. Median maternal age and median gestational age at measurement for the groups are given in Table 1. The median birth weight of the
babies was 3450 (range, 1590 – 4885) g, with 91% of the babies having a birth weight between the 5th and 95th percentiles.

The mean PT and NBL increased between 15 and 33 weeks’ gestation from 2.3 to 6.1 mm \( (r = 0.85, \ p < 0.001) \) and from 3.3 to 9.6 mm \( (r = 0.87, \ p < 0.001) \), respectively (Table 1; Figures 2 and 3). There was a highly significant positive correlation between PT and NBL \( (r = 0.83, \ p < 0.001) \) and their MoM values \( (r = 0.50, \ p < 0.001) \) (Table 1). The PT-NBL ratio was stable throughout gestation, with a mean of 0.61 (95% CI, 0.59 – 0.63 (range, 0.36 – 0.85); SD 0.096; \( r = 0.04, \ p = 0.7 \)) (Table 1). The 5th and 95th percentiles were 0.48 and 0.80, respectively (Figure 4).

![Figure 2 and 3](scatterplot of prenasal thickness (PT) and nasal bone length (NBL) against gestational age (GA) for 30 DS fetuses (*) plotted on reference curves (mean, 5th and 95th percentiles) derived from normal fetuses (o) (PT = (0.21 × GA)-0.873; \( r = 0.85, \ p < 0.001 \)), (NBL = –6.927 + (0.830 × GA)-(0.01 × GA²); \( r = 0.87, \ p < 0.001 \)).}
Nine of the 39 DS fetuses were excluded because of unsatisfactory quality or landmark visualization (all 2D images). Of the remaining 30 DS fetuses, 10 were imaged in 2D, 20 on 3D volumes. The PT, NBL and PT-NBL ratio with the corresponding MoM values for each DS fetus are presented in Table 2. The mean PT and NBL increased between 14 and 34 weeks from 3.0 to 9.2 mm \((r = 0.86, p < 0.001)\) and from 1.9 to 7.8 mm \((r = 0.85, p < 0.001)\), respectively. Twenty-three of the 30 (77%) DS fetuses had a PT above the 95th percentile and 20 (67%) had an NBL below the 5th percentile (Figures 2 and 3). In DS fetuses there was a highly significant positive correlation between PT and NBL \((r = 0.81, p < 0.001)\) whereas the positive correlation between the MoM values did not reach significance \((r = 0.35, p = 0.06)\) (Table 1). The PT-MoM values did not differ significantly between fetuses with a normal or small (< 5th percentile) NBL (1.51 and 1.42, respectively; \(p = 0.47\)), whereas the NBL-MoM’s between fetuses with a normal or large (> 95th percentile) PT were significantly different (0.72 and 0.48, respectively; \(p = 0.003\)). In DS fetuses the PT-NBL ratio did not change significantly during gestation, with a mean of 1.50 (95% CI, 1.20 – 1.80 (range, 0.80 – 5.22); \(r = -0.35, p = 0.07\)) (Figure 4). The PT-NBL ratio was significantly higher in DS fetuses \((p < 0.001)\). When the 5th and 95th percentiles were used as cut-off values the detection rate, false positive rate and positive likelihood ratio were 100% (95% CI, 89 – 100), 5% (95% CI, 2 – 11)% and 21.2, respectively. Fifteen DS fetuses had both an abnormal PT and NBL, eight had an abnormal PT but normal NBL, five had a normal PT but an abnormal NBL and two fetuses had both PT and NBL within the normal range. However all the DS fetuses had a PT-NBL ratio above the 95th percentile (Figure 4 and Table 1).
Table 2 | Prenasal thickness (PT), nasal bone length (NBL) and PT-NBL ratio with their multiples of the median (MoM) values of the 30 DS cases. GA, gestational age.

<table>
<thead>
<tr>
<th>GA (weeks)</th>
<th>PT (mm)</th>
<th>PT MoM</th>
<th>NBL (mm)</th>
<th>NBL MoM</th>
<th>PT-NBL ratio MoM</th>
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<tr>
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<td>6.2</td>
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<td>8.2</td>
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<td>1.76</td>
<td>6.3</td>
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</table>
DISCUSSION

In both euploid and DS fetuses the PT-NBL ratio measured on 3D volumes was stable throughout the second and third trimester, and significantly increased in DS fetuses. When the 95th percentile was used as a cut-off value, the detection rate, false positive rate and positive likelihood ratio were 100% (95% CI, 89 – 100), 5% (95% CI, 2 – 11) and 21.2, respectively. The PT-NBL ratio therefore qualifies as a strong second- and third-trimester marker for DS. Another important observation is that in euploid fetuses PT is always about 2/3 (0.6) of NBL, a stable relationship that enables easy recognition of normality.

In 1995 Guis et al published a normal range for NBL between 14 and 35 weeks’ gestation, and absent nasal bone or hypoplasia of the nasal bone became a widely accepted marker for DS. Reference ranges based on a large sample size and on 3D ultrasound have been published. In this study screening with NBL achieved a detection rate of 67% for a 5% false-positive rate. Two prospective midtrimester 2D studies, using the 5th percentile as a cut-off value, reported detection rates of 59% and 41%, respectively. It is noteworthy that in our study no cases with absent nasal bone were found. Also Bunduki et al found no absent nasal bones in 22 cases between 16 and 24 weeks and in 25 cases between 15 and 33 weeks, respectively. Cusick et al found only one case of absent nasal bone out of 11 cases studied between 16 and 21 weeks. In other reports absence of nasal bone during the second trimester ranges from 23 to 56%. The rigorous selection on image quality, the use of 3D ultrasound and especially the more advanced gestational age are the probable explanation for no cases of absent nasal bone – which would result in a grossly abnormal PT-NBL ratio – being found in our study.

Maymon et al introduced the concept of PT measurement and used PT- and NBL-MoM as a way of enhancing NBL screening performance between 14 and 27 weeks’ gestation. In euploid fetuses the PT-NBL ratio was stable at 0.57 and the PT-NBL-MoM in 21 DS fetuses was 1.51. Tables of likelihood ratios based on PT-MoM’s were published in 2009. Recently 3D ultrasound-based reference ranges for PT have been constructed. Combining second-trimester PT measurement with serum and other markers yields a detection rate comparable with that of first-trimester screening.

Our study confirms the diagnostic power of PT measurement. 77% of the 30 DS fetuses had a PT above the 95th percentile, which is similar to the 73% reported in a prospective 3D study by Persico et al. In a meta-analytic study Miguelez et al reported a detection rate of 60% at a 5% false-positive rate.

We found stable PT-NBL ratios in euploid and DS fetuses, but the ratio was significantly higher in the latter. As already mentioned, when 0.8 (the 95th percentile) was used as a cut-off value the sensitivity, specificity and positive likelihood ratio were 100%, 95% and 21.2, respectively. When 1.0 (NBL = PT) was used as the cut-off value the sensitivity and specificity were 90 and 100%, respectively. Maymon et al found a positive likelihood ratio of 13 for a cut-off value of 0.80 for the PT-NBL-MoM. We used absolute values to make recognition of normality simple and the ratio easily applicable in routine settings. Although the results need to be validated by a large prospective study, the PT-NBL ratio appears to be an excellent second- and third-trimester screening test.
In this study 10 DS fetuses were measured with 2D ultrasound although our reference ranges were based on 3D ultrasound. It is known that NBL measurements, obtained by 2D ultrasound, tend to be larger than those obtained by 3D ultrasound\(^9,15\) and that this modality-derived difference happens less for PT\(^15,22\). Therefore the PT-NBL ratios of the DS fetuses would probably have been even higher had 3D ultrasound been used in all cases.

The ratio shows a better screening performance than does NBL or PT alone. However for risk calculations, the sequential use of the two markers (with two likelihood ratios) may yield better results than combining the two measurements into one ratio (with one likelihood ratio)\(^7\). However for sequential use it is important that the markers are independent.

In DS interdependency of the two markers is supported by the theory that accumulation of hyaluronic acid (related to chromosome 21 gene-related overexpression of collagen type VI) in the dermis is responsible for excessive hydration of the extracellular matrix. This causes increased skin thickness and may at the same time influence intramembranous ossification of the nasal bone\(^24-26\). Another theory, suggesting that delayed migration of the neural crest cells alters the membranous ossification of the nasal bones, supports independency of the two markers\(^27\).

Persico et al\(^22\) found no significant difference in delta PT between DS fetuses with and without a nasal bone. Similarly, in this study PT-MoM’s were not different between the DS fetuses with a normal or small NBL. Also, the non-significant correlation between PT-MoM and NBL-MoM of DS fetuses indicates independency of the two markers. However the finding of significantly different NBL-MoM’s in fetuses with a normal or increased PT contradicts this assumption. Therefore more data are needed to clarify the relationship between the two markers.

In conclusion, the PT-NBL ratio is stable in the second and third trimesters in euploid and DS fetuses. In euploid fetuses PT is consistently about 2/3 of the NBL. All DS fetuses in this series had a PT-NBL ratio above the 95\(^{th}\) percentile. The stability and high sensitivity make this ratio a powerful screening tool for DS.
REFERENCES


