Second-Trimester Screening for Trisomy-21 Using Prefrontal Space Ratio

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Introduction

In second-trimester fetuses with trisomy-21 there is increased prenasal skin thickness and midfacial hypoplasia manifested in shortening and/or dorsal displacement of the maxilla [1–5]. Sonek et al. [6] proposed a new method of screening for trisomy-21 which exploits these two features of affected fetuses. In the midline view of the fetal face a mandibulo-maxillary (MM) line is drawn between the leading edge of the mandible and the maxilla and extended in front of the forehead. The prefrontal space ratio (PFSR) is derived by dividing the distance between the skin and the point where the MM line is intercepted (d2) by the distance between the leading edge of the skull and that of the skin (d1). These two measurements are taken in a line which starts just superior to the point where the skin over the forehead turns anteriorly over the fetal nose and runs roughly parallel to the inferior edge of the maxilla.

Key Words
Second-trimester screening · Trisomy-21 · Down syndrome · Prefrontal space ratio

Abstract

Objective: To investigate the potential value of prefrontal space ratio (PFSR) in second-trimester screening for trisomy-21. Methods: A retrospective study utilizing stored mid-sagittal two-dimensional images of fetal profiles in 240 euploid and 45 trisomy-21 pregnancies at 16th–23rd weeks’ gestation. The vertical distance between the leading edge of the skull and that of the skin (D1) and the distance between the skull and the mandibulo-maxillary line (D2) were measured and the D1:D2 ratio (PFSR) was calculated. In euploid pregnancies, regression analysis was used to determine the association between D1, D2 and PFSR with gestational age (GA). D1 and D2 were expressed as delta (Δ) values with gestational age. ΔD1, ΔD2 and PFSR in cases and controls were compared. Results: In trisomy-21, compared to controls, ΔD1 was increased (1.417 vs. 0.000 mm, p < 0.0001), ΔD2 was decreased (–0.842 vs. 0.000 mm, p = 0.003) and PFSR was increased (0.753 vs. 0.463, p < 0.0001). At a false-positive rate of 5%, the detection rates in screening by ΔD1, ΔD2 and PFSR were 80.0% (95% CI 65.4–90.4), 46.7% (95% CI 31.7–62.1) and 100.0% (95% CI 92.1–100.0), respectively. Conclusion: The PFSR is an effective marker in second-trimester screening for trisomy-21.
In both studies the PFSR was highly reproducible, it did not change significantly with gestational age (GA) and it was substantially lower in trisomy-21 than in euploid fetuses, suggesting that this sonographic measurement may provide effective second-trimester screening for trisomy-21. Although the described methodology for measuring d1 and d2 was identical in the two studies there was a substantial difference between them in the distribution of PFSR, with a difference in mean PFSR of 35% in euploid and 44% in aneuploid fetuses. This suggests that in practice the methodology of measuring d1 and d2 in the two studies may not have been the same. One possible cause for variation in measurements is in drawing a line between the skull and the MM line which should be parallel to the inferior edge of the maxilla, because this edge cannot be described by a clearly defined straight line. Another potential problem arises in cases with increased prenasal thickness where d2 is too small for accurate measurement.

In this study we examine the potential performance of PFSR in screening for trisomy-21 but the methodology in deriving the PFSR is modified so that (1) the line between the skull and the MM line is drawn perpendicular to the latter and (2) both D1 and D2 start from the same point in the skull with the first ending at the skin and the second ending on the MM line.

**Methods**

This was a retrospective study utilizing stored 2D images of second-trimester fetal profiles. The ultrasound examinations used in this study were performed at 16°0–23°6 weeks’ gestation at King’s College Hospital and University College London Hospital between April 2007 and November 2012.

Our database was used to identify all cases of trisomy-21 pregnancies and 240 controls (30 for each gestational week between 16 and 23°6 weeks) that were known to have resulted in live birth of phenotypically normal neonates. The ID numbers of the selected cases that were examined at 16–23°6 weeks’ gestation and had stored digital images were given to two doctors with extensive experience in ultrasound scanning who were not aware of the fetal karyotype. The doctors examined the electronic files and selected those cases demonstrating a true midsagittal section of fetal face with clearly identifiable anterior edges of the mandible and maxilla as well as the leading edge of the bony forehead and the skin over the forehead. The digital images were then used to (1) draw the MM line between the leading edge of the mandible and the maxilla and extend this in front of the forehead, (2) draw a line from the inferior most end of the skull in the forehead perpendicular to the MM line, (3) measure the distance between the skull and that of the skin (D1) and the distance between the skull and the MM line (D2), and (4) divide D1 by D2 to derive the PFSR (fig. 1).

In the previous studies on PFSR [6, 7], their d1 was equivalent to our D1, their d2 was equivalent to our D2-D1 and their PFSR was derived by dividing d2 by d1, which is equivalent to our D2-D1 divided by D1.

To assess the inter-observer reproducibility, two operators (P.C. and M.A.) measured D1 and D2 and both were blinded to the measurements of the other operator and the karyotype results. Operator 1 (P.C.) measured D1, D2 in each case twice to determine the intra-observer repeatability.

**Statistical Analysis**

Intra- and inter-observer reproducibility was examined using 95% limits of agreement [8]. In euploid pregnancies, linear regression analysis was used to determine the association between D1, D2 and PFSR with GA in weeks. D1 and D2 demonstrated a significant association with GA and each measured D1 and D2 value in the cases and controls was expressed as a delta (Δ) value (observed – expected). The mean (standard deviation) of ΔD1, ΔD2 and PFSR in fetuses with trisomy-21 were compared to the distributions in the euploid group using Student’s t-test after confirming that all distributions were gaussian by the Kolmogorov-Smirnov test. Regression analysis was used to determine the significance of association between ΔD1 and ΔD2 with GA in cases of trisomy-21. Detection and false-positive rates were calculated as proportion of cases with risks above certain thresholds. The performance of screening for trisomy-21 by D1, D2 and PFSR was determined by receiver operating characteristic (ROC) curve analysis.

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**Fig. 1.** Measurement of PFSR: (1) the MM line is drawn between the leading edge of the mandible and the maxilla and extended in front of the forehead, (2) the vertical distance between the inferior most end of the skull and the MM line (D1) and the distance between the skull and the MM line (D2) are measured, and (3) D1 is divided by D2 to derive the PFSR. In a euploid fetus at 22 weeks’ gestation, D1 is shorter than D2 (left), whereas in a trisomy-21 fetus at 22 weeks, D1 and D2 are the same (right).
The statistical software package SPSS 20.0 (SPSS, Inc., Chicago, Ill., USA) and MedCalc (MedCalc Software, Mariakerke, Belgium) were used for all data analyses.

Results

In the 240 normal pregnancies the median maternal age was 32 (range 18–48) years, there were 30 cases per gestational week between 16 and 23 +6 weeks, 177 women (73.8%) were Caucasian, 24 (10.0%) were of Afro-Caribbean racial origin, 23 (9.6%) South Asian, 11 (4.5%) East Asian and 5 (2.1%) of mixed racial origin. There were no abnormal ultrasound findings or markers in any of the cases and all pregnancies resulted in healthy live births.

In the 45 cases of trisomy-21 the median maternal age was 35 (range 18–46) years and median GA was 20 (range 16–23) weeks. In 32 cases (71.1%) the women were Caucasian, 8 (17.8%) were of Afro-Caribbean racial origin, 4 (8.9%) South Asian, and 1 (2.2%) East Asian. In 10 cases (22.2%) the diagnosis of trisomy-21 was made in the first-trimester by chorionic villous sampling because the combined test indicated a high risk for this aneuploidy. In 35 cases (77.8%) the diagnosis was made in the second-trimester by amniocentesis because of abnormal ultrasound findings or increased risk indicated by second-trimester serum biochemistry testing. Ultrasound abnormalities or markers were detected in 40 (88.9%) of the cases, including cardiac defects in 18 (40.0%), absent or hypoplastic nasal bone in 23 (51.1%), nuchal fold measurement ≥6 mm in 10 (22.2%), intracardiac echogenic focus in 13 (28.9%), echogenic bowel in 7 (15.6%), ventriculomegaly in 2 (4.4%) and pyelectasia in 5 (11.1%). The parents chose to have pregnancy termination in 35 cases and to continue with the pregnancy in 10, 2 of which resulted in intrauterine death and 8 in live birth of trisomic babies.

Intra- and Inter-Observer Reproducibility

Mean (95% confidence interval (CI)) difference between the first and second D1, D2 and PFSR of operator 1 was 0.010 (–0.300 to 0.330), 0.020 (–0.420 to 0.460) and 0.000 (–0.038 to 0.038; fig. 2), respectively. The respective intra-class correlation coefficients were 0.987, 0.992 and 0.959. Mean (95% CI) difference between the D1, D2 and PFSR of operators 1 and 2 was 0.040 (–0.280 to 0.350), 0.010 (–0.500 to 0.530) and 0.003 (–0.038 to 0.045; fig. 2), respectively. The respective intra-class correlation coefficients were 0.986, 0.990 and 0.948.

Distributions of D1 and D2

In euploid pregnancies, linear regression analysis demonstrated that D1 and D2 were significantly associated with GA (fig. 3):

Expected \( D1 = -2.73706 + 0.31497 \cdot GA, \ p < 0.0001, \ R^2 = 0.802; \)

Expected \( D2 = -6.15205 + 0.69398 \cdot GA, \ p < 0.0001, \ R^2 = 0.783.\)

In the trisomy-21 group, compared to the euploid group, the mean Δ value of D1 was significantly increas-
ed and mean ΔD2 was significantly decreased (table 1). There was no significant association between ΔD1 with GA in either cases (r = –0.158, p = 0.300) or controls (r = 0.000, p > 0.999; fig. 4). There was a negative correlation between ΔD2 with GA in trisomy-21 pregnancies (r = –0.314, p = 0.035) but not in euploid pregnancies (r = 0.000, p > 0.999; fig. 4).

**Distribution of PFSR**

In both euploid and trisomy-21 pregnancies, linear regression analysis demonstrated that there was no significant association between PFSR with GA (euploid: r = –0.033, p = 0.612; trisomy-21: r = 0.285, p = 0.058; fig. 3). The mean PFSR was significantly increased in the trisomy-21 group compared to the euploid group (table 1).

**Relation of PFSR in Trisomy-21 with Other Ultrasound Findings**

There was no significant difference in mean PFSR between fetuses with increased and those with normal nuchal fold thickness (0.728, SD 0.126, vs. 0.760, SD 0.139, p = 0.506), between those with cardiac defects including aberrant right subclavian artery and those without such defects (0.749, SD 0.124, vs. 0.755, SD 0.145, p = 0.880), and between those with absent or hypoplastic nasal bone and those with normal nasal bone (0.736, SD 0.124, vs. 0.771, SD 0.148, p = 0.400).

**Performance of Screening for Trisomy-21**

In screening by ΔD1, the area under ROC (AUROC) was 0.903 (95% CI 0.863–0.935), the detection rate was 80.0 (95% CI 65.4–90.4) at a false-positive rate of 5%, with a positive likelihood ratio (LR) of 16.0 (95% CI 9.04–28.31) and a negative LR of 0.21 (95% CI 0.12–0.38). In screening by ΔD2, the AUROC was 0.679 (95% CI 0.621–0.733), the detection rate was 46.7 (95% CI 31.7–62.1) at a false-positive rate of 5%, with a positive LR of 9.3 (95% CI 5.4–16.2) and a negative LR of 0.33 (95% CI 0.22–0.49).

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**Table 1. Mean and SD of D1, D2 and PFSR in trisomy-21 and euploid pregnancies**

<table>
<thead>
<tr>
<th></th>
<th>Euploid (n = 240)</th>
<th>Trisomy-21 (n = 45)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>D1</td>
<td>3.541±0.814</td>
<td>4.900±1.279</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>ΔD1</td>
<td>0.000±0.362</td>
<td>1.417±1.161</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>D2</td>
<td>7.679±1.812</td>
<td>6.710±2.016</td>
<td>0.004</td>
</tr>
<tr>
<td>ΔD2</td>
<td>0.000±0.842</td>
<td>–0.842±1.787</td>
<td>0.003</td>
</tr>
<tr>
<td>PFSR</td>
<td>0.463±0.039</td>
<td>0.753±0.136</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>
Comparison with Previous Publications

The equivalent of the PFSR in previous studies [6, 7], derived from our measurements, was 0.371 (SD 0.249) in trisomy-21 and 1.174 (SD 0.182) in euploid pregnancies (p < 0.0001), the AUROC in screening for trisomy-21 was 0.996 (95% CI 0.981–0.999) and the detection rate was 100% (95% CI 92.1–100.0) at a false-positive rate of 5%, with a positive LR of 20.0 (95% CI 4.95–17.59).

Discussion

The findings of this study demonstrate that in second-trimester fetuses with trisomy-21 (1) prenasal thickness is larger than in normal fetuses, (2) there is shortening and/or dorsal displacement of the maxilla manifested in increased distance between the skull in the forehead and the MM line, (3) the PFSR is increased, and (4) PFSR appears to be unrelated to the presence or absence of other sonographic features associated with this aneuploidy. These results are compatible with those of previous studies [1–7] and suggest that measurement of the PFSR may provide an effective method of screening for trisomy-21 in the second-trimester of pregnancy.

In normal fetuses the mean D1, which is equivalent to the previously reported prenasal thickness, increased linearly with gestation from a mean of 2.4 mm at 16 weeks to 4.7 mm at 23 weeks and the value was above the 95th percentile in 80% of the trisomy-21 fetuses. Two previous studies that specifically examined prenasal thickness reported similar median values in normal fetuses of 2.4 mm at 16 weeks and about 4.5 mm at 24 weeks and in fetuses with trisomy-21 at 16–24 weeks the values were above the 95th percentile in 72% of 18 and 73% of 26 cases, respectively [1, 2].

Indirect evidence of shortening and/or dorsal displacement of the maxilla in trisomy-21 fetuses is provided by the lower D2 than in normal fetuses. In both groups of fetuses the median D2 increases with GA but with advancing gestation trisomy-21 is characterized by progressive relative shortening in D2. One possible explanation for this finding is provided by the suggestion that movement of the tongue plays an important role in normal development of the upper palate and that in trisomy-21 palatal growth is impaired by progressive hypotonia of the tongue [7].

In trisomic fetuses the consequence of increase in D1 and decrease in D2 was a substantial increase in PFSR (D1/D2) which was above the 95th percentile in all affected fetuses. The median PFSR, calculated by the method used in previous publications, was 0.37 in trisomy-21 and 1.17 in euploid pregnancies and the value was below the 5th percentile in all affected fetuses. Consequently, the potentially high performance of PFSR in screening for trisomy-21 is the same irrespective of the method used for calculating the ratio. Nevertheless, the median values for PFSR obtained in the three studies examining this ra-

Fig. 4. The Δ value of D1 and D2 with GA at screening in euploid (○) and trisomy-21 (●) pregnancies, plotted on the 5th, 10th, 50th, 90th and 95th percentiles of the normal range.
tio have been different. In trisomic fetuses the median PFSR in our study was similar to that of Sonek et al. (0.37 vs. 0.36) but higher than that of Yazdi et al. (0.2) [6, 7]. In contrast, our value in the euploid group was similar to that of Yazdi et al. (1.17 vs. 0.97) but lower than that of Sonek et al. (1.48).

A limitation of our study and the previous ones on PFSR resides in their retrospective nature. Prospective studies in normal pregnancies can easily define reference ranges. However, in the era of widespread first-trimester screening for trisomy-21 and selective termination of most affected fetuses, the undertaking of high quality screening studies may ultimately be impossible.

In the last 25 years, several studies have reported that certain features detected during second-trimester ultrasound examination are potential markers for fetal trisomy-21. A recent meta-analysis of such studies reported that (1) if a systematic second-trimester ultrasound examination demonstrates the absence of all major defects and markers there is a 7.7-fold reduction in risk for trisomy-21, (2) the detection of any one of the markers during the scan should stimulate the sonographer to look for all other markers or defects and the post-test odds for trisomy-21 is derived by multiplying the pre-test odds with the positive LR for each detected marker and the negative LR for each marker demonstrated to be absent, (3) in the case of most isolated markers, including intracardiac echogenic focus, echogenic bowel, mild hydronephrosis and short femur or short humerus, there is only a small effect on modifying the pre-test odds, and (4) the strongest marker is absent or hypoplastic nasal bone with a positive LR of 23 and when isolated the LR is 6.6 [9, 10]. In our study the LR associated with PFSR above the 95th percentile was 20 (100% of fetuses with trisomy-21 compared to 5% of euploid fetuses) and in the trisomy-21 group there was no significant difference in mean PFSR between fetuses with absent or hypoplastic nasal bone and those with normal nasal bone. Consequently, the main emphasis in second-trimester assessment of risk for trisomy-21 should be examination of the fetal profile for evaluation of the nasal bone and PFSR.

**Acknowledgement**

The study was supported by a grant from The Fetal Medicine Foundation (UK Charity No. 1037116).

**References**