Reference Ranges for Fetal Brain Fissure Development on 3-Dimensional Sonography in the Multiplanar Mode

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Objectives—To determine reference ranges for measurements of fetal cerebral fissures by 3-dimensional (3D) sonography in the multiplanar mode and to evaluate the reliability and concordance of these measurements.

Methods—A cross-sectional study was conducted on 393 women with normal pregnancies at 22 weeks to 33 weeks 6 days. The distances between the internal bone plate of the fetal calvaria and the sylvian, parieto-occipital, hippocampal, and calcarine fissures were assessed. To obtain the distances for the first 3 fissures, a 3D sweep was made in the axial plane, at the level of the lateral ventricles. To obtain the distance for the calcarine fissure, a coronal sweep was used, at the level of the occipital lobes. To evaluate the correlation between the fissures and gestational age, polynomial regression was performed with adjustments using the coefficient of determination ($R^2$). Reliability was determined with intraclass correlation coefficients and concordance with concordance limits.

Results—The mean distances ± SD to the sylvian, parieto-occipital, hippocampal, and calcarine fissures were 10.42 ± 2.28, 22.38 ± 3.23, 24.88 ± 4.67, and 21.19 ± 2.73 mm, respectively. These distances correlated with gestational age such that the best fit with the linear equation produced $R^2$ values of 0.582, 0.627, 0.860, and 0.458 for the sylvian, parieto-occipital, hippocampal, and calcarine fissures. Reliability analyses showed intraobserver and interobserver intraclass correlation coefficients of 0.90 to 0.95 and 0.85 to 0.97. The concordance limits were –1.33 to 1.30 and –2.38 to 2.28 mm for the intraobserver evaluation and –1.60 to 2.57 and –3.51 to 2.73 mm for the interobserver evaluation.

Conclusions—Cerebral fissures can be measured by 3D sonography at 22 to 33 weeks of pregnancy with acceptable reliability and concordance. Reference ranges for this gestational period have thus been described.

Key Words—fetal brain; fissures; reference ranges; reproducibility; 3-dimensional sonography

During embryonic development, it is observed that the cortex increases in size more rapidly than the adjacent white substance and ends up wrapping around and folding over itself, thus forming the cerebral gyri and sulci. During the course of fetal life, the sulci and gyri develop and become increasingly evident, and they come to have relatively constant locations, which enable a precise reference. The lateral or Sylvian sulcus is a deep cleft that starts at the base of the brain, laterally to the anterior perforated substance. It separates the frontal lobe from the parietal lobe and goes toward the superolateral face of the brain. The occipital lobe is separated...
from the parietal lobe by the parieto-occipital sulcus, which makes an acute angle with the calcarine sulcus. The calcarine sulcus starts beneath the splenium of the corpus callosum and follows an arc toward the occipital pole, which marks the center of the visual cortex. The hippocampal sulcus also originates in the region of the splenium of the corpus callosum, continues with the sulcus of the corpus callosum, and heads toward the temporal pole, separating the parahippocampal gyrus from the uncus.3

The process through which neurons move from their originating location (germinal matrix) to their permanent location in the cerebral cortex is known as neuronal migration. Abnormal neuronal migration in the third or fourth month of pregnancy may give rise to lissencephaly, which is characterized by agyria with or without pachygria, minimal hydrocephalus, widening of the cortical mantle, and characteristic dysmorphisms. Lissencephaly is associated with mutations or deletions in the LIS1 (lissencephaly 1) gene, which is located on the short arm of chromosome 17. It may occur alone or in association with the Miller-Dieker or Norman-Roberts genetic syndromes.4

Two-dimensional (2D) sonography makes it possible to show cortical development and diagnose its abnormalities. However, most of the information on fetal cortical development is obtained from descriptive anatomic studies.5 Detection of cerebral fissures and sulci in healthy fetuses by 2D sonography and magnetic resonance imaging presents a delay in comparison with postmortem studies. Absence of a sulcus or presentation of an abnormal appearance at a certain gestational age may raise the suspicion of an abnormality or a delay in cortical development.4

Three-dimensional (3D) sonography in the multiplanar mode makes it possible to evaluate the distance from the apex of the fissure to the internal boundary of the calvaria, which enables objective assessment of its development during pregnancy.5,6 The advantages of 3D sonography in the multiplanar mode are that the following become possible: offline evaluation, reference planes in any orientation, a shorter time taken to do analyses, and data volume transmission for analysis by specialists.6 However, only a single study has determined objective reference ranges for the development of the sylvian fissure on 3D sonography in the multiplanar mode,6 and there are no descriptions for the other fissures.

The aim of this study was to determine reference ranges for the distances from the internal faces of the cerebral fissures to the internal bone plate of the fetal cranium by means of 3D sonography in the multiplanar mode between 22 weeks and 33 weeks 6 days of pregnancy.

Materials and Methods

A prospective cross-sectional study was conducted among 393 women with normal pregnancies between 22 weeks and 33 weeks 6 days from September 2010 to January 2012. This study was approved by the Research Ethics Committee of the Federal University of São Paulo under number 1833/10. Patients who voluntarily agreed to participate in the study signed a consent statement. Of the total of 393 pregnant women, 80 formed part of a prior preliminary study conducted by our group that had the aim of evaluating the behavior of objective cerebral fissure measurements over the course of pregnancy.7

The inclusion criteria were as follows: (1) a single pregnancy with a live fetus; and (2) gestational age determined according to the date of the last menstrual period in cases with regular menstrual cycles or dating based on sonography performed before the 13th week of pregnancy in cases with uncertain dates. The following types of cases were excluded: (1) fetuses with structural malformations detected on sonography; (2) pregnant women with chronic diseases that might interfere with fetal growth, such as chronic hypertension, diabetes mellitus, and collagenosis; (3) fetuses with predicted weights below the 10th or above the 90th percentile for gestational age, in accordance with the table proposed by Hadlock et al; (4) oligoamnios (amniotic fluid index <5th percentile) or polyhydramnios (amniotic fluid index >95th percentile), in accordance with the table proposed by Moore and Cayle; (5) 3D volumes of low quality that would prevent clear evaluation of all the cerebral fissures; and (6) changes to the shape of the fetal cranium (dolichocephaly or brachycephy).

The pregnant women who met the inclusion criteria were invented to participate in the study. These patients were from public health care clinics in the municipality of São Paulo. Since this study was cross-sectional, all the pregnant women underwent a single sonographic evaluation, and the postnatal outcomes were not obtained. The examinations were performed in the Fetal Neurology Unit of the Department of Obstetrics at the University of São Paulo and were under the responsibility of a single examiner (C.M.S.A.) with 8 years of experience in obstetric sonography. All examinations were performed abdominally on the same Voluson 730 Expert apparatus (GE Healthcare, Zipf, Austria) equipped with a volumetric convex transducer (RAB 4-8L).

First, a 2D real-time evaluation was made on the fetal morphologic characteristics, fetal biometric measurements, amniotic fluid volume, and predicted fetal weight. A 3D sonographic evaluation was then made, using the fol-
ollowing standard parameters: opening angle from 50° to 70° (varying according to gestational age), normal velocity mode, high quality, harmonic mode, maximum volume depth of 16 cm, and maximum acquisition volume of 3.0 L, with the fetus at rest and asking the pregnant women to remain in apnea for a few seconds. The 3D screen was activated, and a scanning window appeared and was positioned such that it included the entire fetal cranium, ie, the region of interest. To make an objective assessment of the development of the parieto-occipital, sylvian, and hippocampal fissures, an axial plane at the level of the posterior cornua of the lateral ventricles was used as the reference. To evaluate the calcarine fissure, a coronal plane through the occipital lobes was used as the reference. After the 3D sweep had been performed, the image was presented in the multiplanar mode: axial (A), sagittal (B), and coronal (C). Two volumes were obtained for each patient in each plane (axial and coronal), and these were stored in the memory of the apparatus. Subsequently, these volumes were transferred to CDs and were analyzed on a personal computer with 4D View version 10.0 software (GE Healthcare). The volume with the best quality in terms of contrast, brightness, and absence of artifacts was used for the analyses, which were done by a single examiner (C.M.S.A.).

For the sylvian, parieto-occipital, and hippocampal fissures, the axial plane was used as the reference and was shifted craniocaudally and vice versa by means of the Ref slice key. The sylvian fissure was obtained in the standard plane for measuring the biparietal diameter as a linear image in the central portion and an angled image on the periphery posterior to the site of development of the circular sulcus of the insula (Figure 1A). The hippocampal fissure was obtained in a lower plane, close to the level of the cerebral peduncles and was seen as a linear echogenic structure beneath the gyrus of the hippocampus (Figure 1B). The parieto-occipital fissure was obtained in a plane close to the upper margin of the occipital cornua of the lateral ventricles as a lozenge-shaped structure (Figure 1C). The calcarine fissure was obtained in the same coronal acquisition plane at the level of the occipital lobes, with the reference points of the cerebellum and cervical spine. It was seen as a lozenge-shaped structure (Figure 1D).

To identify the fissures, the 3 orthogonal planes were first perfectly aligned so that the interhemispheric line was left in the plane of the horizontal line, ie, at the measurer’s eye level, with the reference point in the median portion. The images were magnified as much as possible within the above-mentioned parameters. Measurements were made

Figure 1. Measurements from the internal boundary of the apex of the fetal cerebral fissures to the internal bone plate of the fetal cranium: sylvian (A), parieto-occipital (B), hippocampal (C), and calcarine (D).
at an angle of 90° in relation to the interhemispheric line. For the sylvian and hippocampal fissures (linear fissures), the distance between the midpoint of the internal face of the fissure and the internal face of the homolateral calvaria was determined. For the parieto-occipital and calcarine fissures (lozenge-shaped fissures), the distance from the external face of the apex to the internal face of the homolateral calvaria was determined.

The data were entered into a study-specific form and were transferred to a spreadsheet within Excel 2007 software (Microsoft Corporation, Redmond, WA). The statistical analysis was done by one of the authors (W.P.M.) using SPSS version 18.0 software (SPSS Inc, Chicago, IL) and GraphPad Prism software (GraphPad Software, Inc, La Jolla, CA). Initially, the mean, standard deviation, maximum, and minimum were determined for each of the variables investigated in the study sample. Graphs were produced as described by Altman and Chitty11 to determine whether the measurements presented a normal distribution according to gestational age. The polynomial regression model with the best fit to the points was determined by comparing the coefficients of determination ($R^2$) and their confidence intervals (CIs), and preference was given to simpler regression models. A first-degree regression model was chosen for all the variables. The residuals between the observed and expected values from the regression were then calculated, and the best polynomial regression model for determining the residual values according to gestational age was chosen. The model chosen for all the residuals was a first-degree polynomial regression, which was multiplied by $\sqrt{(\pi/2)} (\approx 1.253)$ to estimate the standard deviation for each gestational age. Curves corresponding to $±1.654$ SD were then created and inserted in the graphs as references for the 5th and 95th percentiles. The values for the tables for each gestational age were thus determined with these formulas.

To analyze the intraobserver and interobserver reliability, the intraclass correlation coefficient was determined (mixed bidirectional model, absolute concordance and simple measurements). To analyze the reliability, the mean error and concordance limits of Bland-Altman graphs12 were determined. To calculate the intraobserver reliability, the first examiner (C.M.S.A.) performed a second measurement of 77 volumes selected randomly with a 7-day interval among the first measurements. To calculate the interobserver reliability, a second examiner (L.H.M.) performed a third measurement of the same 77 volumes, without knowledge of the results of the first examiner. Both examiners had the same years of experience in 3D obstetric sonography. In all analyses, the significance level of $P < .05$ was used. To calculate the sample size, the precepts proposed by Royston13 were used. These state that for cross-sectional studies, at least 20 individuals per gestational age are recommended.

**Results**

Initially, 440 pregnant women were selected, but 47 (10.68%) were excluded for the following reasons: macrocrania (12), dolichocephaly (7), microcrania (5), low-quality 3D volumes (14), incorrect positioning of the cephalic pole (4), estimated fetal weight greater than the 90th percentile (4), and ventricular enlargement (1). The mothers’ mean age was 30.07 ± 5.04 years (range, 15.0–44.0 years). The mean gestational age was 28.11 ± 3.68 weeks (range, 22.0–33.8 weeks). The numbers of previous pregnancies and deliveries were 1.61 ± 0.46 (range, 1.0–4.0) and 0.45 ± 0.72 (range, 0.0–3.0), respectively. Most of the women were white (66.41%), followed by mixed race (27.99%) and black (5.90%). The pregnant women’s distribution by gestational age was as follows: 41 (22 weeks–22 weeks 6 days), 42 (23 weeks–23 weeks 6 days), 25 (24 weeks–24 weeks 6 days), 22 (25 weeks–25 weeks 6 days), 18 (26 weeks–26 weeks 6 days), 21 (27 weeks–27 weeks 6 days), 38 (28 weeks–28 weeks 6 days), 37 (29 weeks–29 weeks 6 days), 33 (30 weeks–30 weeks 6 days), 42 (31 weeks–31 weeks 6 days), 39 (32 weeks–32 weeks 6 days), and 35 (33 weeks–33 weeks 6 days).

The mean distances to the sylvian, parieto-occipital, hippocampal, and calcarine fissures were 10.42 ± 2.28 (range, 5.40–16.10), 22.38 ± 3.23 (range, 15.50–30.50), 24.88 ± 4.67 (range, 14.50–33.60), and 21.19 ± 2.73 (range, 14.20–30.80) mm, respectively. All measurements from the fetal cerebral fissures to the internal bone plate of the calvaria were shown to be correlated with gestational age, and the best fit for the polynomial regression was the first-degree equation: sylvian = $-2.851 + 0.472 \times$ gestational age ($R^2 = 0.582$); parieto-occipital = $-2.833 + 0.696 \times$ gestational age ($R^2 = 0.627$); hippocampal = $-8.230 + 1.178 \times$ gestational age ($R^2 = 0.860$); and calcarine = $8.312 + 0.458 \times$ gestational age ($R^2 = 0.458$; Figure 2). Table 1 shows the 5th, 90th, and 95th percentiles for the 4 fissures between the gestational ages of 22 weeks and 33 weeks 6 days.

The objective measurements of the fetal cerebral fissures were shown to have good reliability, with intraobserver intraclass correlation coefficients of 0.95 (95% CI, 0.92–0.97), 0.95 (95% CI, 0.92–0.97), 0.98 (95% CI, 0.97–0.99), and 0.90 (95% CI, 0.85–0.94) and interobserver intraclass correlation coefficients of 0.85 (95% CI, 0.73–0.91), 0.93 (95% CI, 0.87–0.96), 0.97 (95% CI, 0.94–0.99)
Figure 2. Distribution of the measurements of the sylvian (A), parieto-occipital (B), hippocampal (C), and calcarine (D) fissures by gestational age.

Table 1. Percentile Measurements From the Fetal Cerebral Fissures to the Internal Bone Plate of the Fetal Calvaria

<table>
<thead>
<tr>
<th>GA, wk</th>
<th>Sylvian, mm</th>
<th>Parieto-occipital, mm</th>
<th>Hippocampal, mm</th>
<th>Calcarine, mm</th>
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<tr>
<td></td>
<td>5th</td>
<td>50th</td>
<td>95th</td>
<td>5th</td>
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<td>33</td>
<td>9.90</td>
<td>12.73</td>
<td>15.55</td>
<td>21.86</td>
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GA indicates gestational age.
0.98), and 0.85 (95% CI, 0.77–0.90) for the sylvian, pari-
eto-occipital, hippocampal, and calcarine fissures, respectively. It was also observed that there was good intraobserver concordance between the measurements from the Bland-Altman graphs, with mean differences of −0.01 (limits of agreement, −1.33–1.30), −0.02 (limits of agreement, −1.85–1.80), −0.09 (limits of agreement, −1.60–1.42), and −0.05 (limits of agreement, −2.38–2.28) for the sylvian, parieto-occipital, hippocampal, and calcarine fissures (Figure 3). For the interobserver concordance, the mean differences were 0.48 (limits of agreement, −1.60–2.57), −0.42 (limits of agreement, −2.49–1.65), 0.38 (limits of agreement, −1.52–2.27), and −0.39 (limits of agreement, −3.51–2.73) for the sylvian, parieto-occipital, hippocampal, and calcarine fissures (Figure 4).

Discussion

Studying the development of cerebral fissures is important because delays in their development may have consequences for postnatal neurologic development. In 1977, Dorovini-Zis and Dolman already called attention to fetal cortical brain development in accordance with advancing gestational age. The pioneering study of fetal cerebral development was conducted by Monteagudo and Timor-Tritsch. Those authors assessed the brains of 262 fetuses by transvaginal 2D sonography. The sylvian, calcarine, and parieto-occipital fissures were identified on sonography at 18 weeks. By anatomic study, the sylvian, calcarine, and parieto-occipital fissures were identified at 14, 16, and 16 weeks, respectively. Cohen-Sacher et al assessed the development of the fetal brain from 18 weeks until term.
They observed that the fetal brain at 18 to 20 weeks was completely smooth and homogeneous, with the major fissures present (sylvian, hippocampal, interhemispheric, and parieto-occipital). The period between 22 and 26 weeks is characterized by gradual onset of the calcarine fissure, followed by the central fissure. After 32 weeks, all fissures can be identified on 2D sonography with the exception of the insula fissure. Toi et al.\textsuperscript{16} reported that in fetuses with Miller-Diker syndrome, delayed cortical development can be suspected when delays or abnormal development of the sulci are detected on prenatal sonography before the 24th week of pregnancy, with findings such as absence of the parieto-occipital and calcarine fissures and an abnormal sylvian fissure.

In this study, we measured the distances of the sylvian, parieto-occipital, hippocampal, and calcarine fissures from the internal bone plate as a more objective method, with the aim of improving and simplifying early detection of migration disorders, thereby enabling better prenatal management and better counseling for the parents. We chose to study the interval from the 22nd to the 33rd week of pregnancy. The lower limit was chosen because at this gestational age in healthy fetuses, all the fissures can already be seen on 2D sonography. The parieto-occipital and calcarine fissures are the first ones that may be identified on 2D sonography, at a gestational age of 18.5 weeks, and can always be seen by the ages of 20.5 and 21.9 weeks, respectively. The sylvian fissure presents a characteristic development model: initially, it is seen as a gentle depression; then, after the 17th week, it develops an obtuse angular edge at the location where the circular sulcus of the insula develops, and this edge becomes acute after the 24th week.\textsuperscript{16} The upper

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Figure 4. Mean differences between pairs of measurements made by two examiners (interobserver differences), plotted against the differences in their means, for the sylvian (A), parieto-occipital (B), hippocampal (C), and calcarine (D) fissures.

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A

B

C

D


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limit was chosen because of the difficulty in fitting all the fetal cranium into the 3D window after the 34th week and also because the clinical importance of a disorder of neuronal migration after this gestational age becomes of little practical value.

In this study, we used the same method used by Mittal et al6 for evaluating the sylvian fissure, ie, an axial plane of the fetal cranium at the level of biparietal diameter measurement. However, using a similar method, we also evaluated the parieto-occipital and hippocampal fissures. To obtain data for the calcarine fissure, we used the technique described by Ghai et al,17 ie, a coronal plane in the region of the posterior fossa that encompassed the initial portion of the cervical spine. To our knowledge, there are no descriptions in the literature of evaluations on the development of the parieto-occipital, hippocampal, and calcarine fissures by means of this technique. Alonso et al18 proposed a new technique for evaluating fissure development based on the distances from the apices of the sylvian, parieto-occipital, and calcarine fissures to the cerebral midline. Their technique would be advantageous in cases of cranial shape abnormalities such as brachycephaly and dolichocephaly, but those cases were excluded from our study.

In our study, we observed that the measurements from the fissures to the internal bone plate of the fetal cranium increased with advancing gestational age, such that the best fit from polynomial regression was a linear equation. In the study conducted by Mittal et al,6 it was also observed that the sylvian fissure increased with advancing gestational age. However, in comparing the means, the values obtained in our study were larger. One possible explanation may be the sample size of our study (393 pregnant women divided into 12 gestational intervals), whereas in the study by Mittal et al,6 there were only 202 pregnant women but divided into 30 gestational intervals. According to Royston,13 to construct reference intervals requiring 90% variation between the 5th and 95th percentiles of distribution, a sample of 20 per week is recommended. In addition, ethnic differences between populations cannot be disregarded: these may give rise to differences in fetal cranial shape and, consequently, to measurements of distances from the fissures to the internal bone plate. In our study, a large proportion of the pregnant women were of mixed race (27.99%), which may have contributed to these differences. Mittal et al6 did not report the ethnic distribution of their sample group. Alonso et al18 also observed the increasing depth of the sylvian, parieto-occipital, and calcarine fissures between the 19th and 30th weeks, but our results cannot be compared because the measurement techniques were different.

In our study, we observed good intraobserver and interobserver reliability and concordance for the measurements of the 4 fetal cerebral fissures. Mittal et al6 used the same technique we used and also reported good intraobserver and interobserver reproducibility for the measurements of the sylvian fissure. This result validates the new reference curves for use during the prenatal period for fetuses with suspected disorders of neuronal migration so that attempts to reach an earlier diagnosis can be made, as a means of counseling the parents.

In this study, we used 3D sonography to make measurements from the fetal cerebral fissures to the internal bone plate of the cranium. This technique has already been put forward as a method of great importance for assessing the fetal brain, since its multiplanar mode allows reference planes in any orientation to be obtained.19 In our study, gathering a single 3D volume in the axial plane enabled objective measurement of 3 fissures, and this method was confirmed to be feasible for use in clinical practice.

The limitations of this study were as follows: (1) advanced gestational age, because the head is positioned in the pelvis, and the 3D box does not allow inclusion of the entire head in the region of interest; (2) macrocephaly and microcephaly, because these malformations change the relationships between the fissures and internal bone plate of the fetal cranium; and (3) obesity and abdominal scars, because these conditions interfere with the ultrasonic beam.

In summary, we calculated reference values for the distances from the sylvian, parieto-occipital, hippocampal, and calcarine fissures to the internal bone plate of the calvaria as an objective method for assessing the development of these fissures over the course of gestation. Because of the large sample size and because the measurements of the 4 fissures were shown to be reproducible, we believe that these reference values can be used as parameters for normal values in assessing fetuses with suspected disorders of cortical maturation such as lissencephaly as well as other fetal cerebral abnormalities such as Walker-Warburg Syndrome and porencephaly/schizencephaly.

References


