Demonstration of changes in fetal liver erythropoiesis using echo-planar magnetic resonance imaging

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Duncan, K. R., P. N. Baker, P. A. Gowland, B. Issa, R. Moore, B. Worthington, and I. R. Johnson. Demonstration of changes in fetal liver erythropoiesis using echo-planar magnetic resonance imaging. Am. J. Physiol. Gastrointest. Liver Physiol. 36: G965–G967, 1997.—This study investigated the variation in magnetic resonance characteristics of the fetal liver during a time of changing erythropoietic function. Echo-planar imaging was carried out in 25 normal pregnant women at 20 and 26 wk gestation. The signal intensity from regions of the fetal liver, background image, and maternal back muscle and the highest signal intensity from the maternal spinal cord were measured and compared with the signal intensity of amniotic fluid. Data are expressed as ratios, in arbitrary units (median pixel values; interquartile range shown in parentheses), and analyzed with the use of Wilcoxon’s signed-rank test. At 20 wk, the signal intensity ratio of liver to amniotic fluid was 0.309 (0.231–0.365). At 26 wk, the ratio was 0.544 (0.429–0.616). The change was highly significant (P < 0.0001). No change in the signal intensity ratios of amniotic fluid compared with other measured parameters was noted. These data are consistent with known changes in fetal liver erythropoiesis occurring between 20 and 26 wk gestation and have potential use in early noninvasive physiological assessment of the fetus.

Substantial changes in the dynamics of fetal hematopoiesis, particularly in the production of red blood cells, occur between the first and second trimesters of pregnancy (7). During this period the spleen and bone marrow supersede the liver as the major organs responsible for red blood cell production. Postmortem examination of fetal tissue (10) has shown that this leads to a reduction in the concentration of hemoglobin and its related compounds within the fetal liver. This process, however, has not been demonstrated in vivo. It would be expected that such changes would give rise to a change in the signal from the fetal liver on magnetic resonance images.

The aim of this longitudinal study was to assess such changes in the signal intensity of fetal liver between 20 and 26 wk of pregnancy by measuring the relative change in signal intensity from the fetal liver compared with the amniotic fluid. Echo-planar imaging (EPI) is an ultra high-speed variant of magnetic resonance imaging (MRI) that has previously been used in the assessment of fetal weight (2) and fetal organ volumes (1). Short image acquisition times, typically 64–128 ms, permit a series of contiguous transaxial images of the fetus to be obtained very rapidly, thereby overcoming the problem of fetal motion encountered with conventional MRI.

The images obtained are composed of individual elements referred to as pixels. The intensity of signal obtained at each pixel (pixel value) differs between each individual organ or fluid. The variation in average pixel value between organs and fluid produces changes in contrast, thus allowing anatomic differentiation.

The signal intensity of the individual pixels constituting the image depends on many factors, in particular the proton density (concentration of water molecules), the relaxation times $T_1$ and $T_2$ (reflecting the mobility of proton-containing water molecules, in particular), and $T_2$* (reflecting any small-scale static variations in the local magnetic field). Such variations arise mainly from differences in the magnetic susceptibility between different tissue regions. The local susceptibility (magnetic field induced in the imaging magnet) is altered by many factors, in particular the presence of iron-containing compounds. In the presence of such material, $T_2$* is reduced, leading to a reduction in the signal intensity of EPI images.

With the use of amniotic fluid as a constant reference point, any relative changes in EPI signal intensity measurements from the fetal liver observed between 20 and 26 wk of pregnancy may reflect the changes taking place at the site of hematopoiesis.

Safety guidelines published by the National Radiological Protection Board were adhered to (9), and the project was approved by the local ethics committee.

Methods

Twenty-five normal healthy pregnant volunteers [median age 28; interquartile range (IQR) 26–32 yr], 22 of whom had previously had at least one child, were studied on two successive occasions at 20 and 26 wk of pregnancy (confirmed by an ultrasound scan in early pregnancy) after being fully familiarized with the study protocol and techniques. All of the pregnancies studied were singleton and uncomplicated at the time of investigation or in the remainder of the pregnancy.
Informed written consent was obtained from every patient before scanning. EPI was carried out on each occasion with the use of a purpose-built 0.5-tesla superconducting magnet scanner. A multislice $t_2^*$-weighted modulus-blipped EPI sequence (4) with an acquisition time of 130 ms and an effective echo time of 30 ms was used. The slice thickness was 7 mm, with a field of view of 44.8 × 32.0 cm, for a matrix size of 128 × 128. Nonisotropic resolution was used to reduce the acoustic noise caused by the switched gradient.

Using a specifically designed computer program (ANALYZE) for image analysis, we measured the mean signal intensity (pixel value) from a median of 10 (IQR 8–12) pixels from a region of interest in the middle slice of the liver at the level of the stomach. We also obtained measurements from a similar number of pixels from the background noise and maternal back muscle, as well as the highest pixel value from the maternal spinal cord. The ratio of the amniotic fluid signal intensity to each variable was calculated for each gestational age. It is well established that the use of absolute signal intensity from magnetic resonance images in quantitative studies is difficult, particularly when images are collected over such a long period of time and the stability of the scanner cannot be guaranteed. It is not appropriate therefore to directly compare the signal from the liver at 20 wk to that at 26 wk without standardizing the variables. In this study this was achieved by calculating the ratio of liver signal intensity to the signal intensity from amniotic fluid. The amniotic fluid signal was chosen for three reasons. 1) The amniotic fluid signal is high, reducing random error attributable to noise in the calculation of the final ratio. 2) The amniotic fluid signal can be measured in a region close to the fetal liver, minimizing any effects created due to lack of homogeneity across the image. 3) The signal from the amniotic fluid would not be expected to change with increasing gestation time and does not appear to do so. To confirm this assumption, the amniotic fluid signal was compared with the signal from other parameters that would not be expected to change with increasing gestation, i.e., the maternal muscle and the background noise, which is related to scanner stability. Both of these measurements have a low signal intensity, so the fluid was also compared with the signal from the maternal spinal cord, which has a high intensity and also would not be expected to change.

The ratios obtained at 20 wk were compared with those at 26 wk with the use of Wilcoxon's signed-rank test for the analysis of paired nonparametric data. Data are median values (IQR), expressed in arbitrary units.

**RESULTS**

The signal intensity ratio of liver to amniotic fluid changed from 0.309 (0.231–0.365) at 20 wk to 0.544 (0.429–0.617) at 26 wk (Fig. 1). Paired comparison of the data indicated that the changes seen in the ratio of liver signal intensity to amniotic fluid signal intensity (pixel values) from 20 to 26 wk were statistically significant ($P < 0.0001$). The amniotic fluid was compared with the other measurements in a similar fashion. The background noise ratio changed from 0.063 (0.048–0.082) to 0.075 (0.057–0.103), the muscle ratio from 0.106 (0.085–0.147) to 0.126 (0.099–0.141), and the spinal cord ratio from 0.927 (0.854–1) to 0.957 (1.42–0.802) at 20 and 26 wk, respectively. These changes were not significant. The results for the absolute individual pixel values are shown in Table 1. The signal-to-noise ratio in the amniotic fluid (signal of maximum intensity) was 19 (12–26).

**DISCUSSION**

These data were acquired as part of a study investigating the application of MRI in the assessment of normal and compromised fetal development. EPI is a major advance in fetal assessment. It overcomes the problem of fetal motion encountered

Table 1. Individual absolute signal intensity (pixel) values

<table>
<thead>
<tr>
<th></th>
<th>Amniotic Fluid</th>
<th>Fetal Liver</th>
<th>Background Noise</th>
<th>Maternal Muscle</th>
<th>Maternal Spinal Cord</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 wk</td>
<td>124 (89–156)</td>
<td>31 (19–49)</td>
<td>6 (5–12)</td>
<td>13 (11–18)</td>
<td>106 (76–166)</td>
</tr>
<tr>
<td>26 wk</td>
<td>106 (97–195)</td>
<td>61 (48–81)</td>
<td>10 (7–12)</td>
<td>14 (10–20)</td>
<td>142 (135–163)</td>
</tr>
</tbody>
</table>

Data are median pixel values, expressed in arbitrary units (interquartile range given in parentheses).
with conventional MRI while maintaining imaging resolution. Noninvasive in utero fetal assessment is therefore feasible and safe. Follow-up studies of infants scanned in utero have found no ill effects from the imaging technique (3), and no substantial increases in common adverse reproductive outcomes were shown when a large group of female magnetic resonance workers were studied (8).

Because of the nature of the EPI sequence used, high concentrations of iron in hemoglobin and its breakdown products result in strong signal attenuation, i.e., a low signal. The highly significant increase in EPI signal from the fetal liver occurs at the same time as known changes in fetal liver function are occurring. The signal change within the fetal liver could therefore reflect a progressive decrease in the production of hemoglobin within the fetal liver, with a subsequent reduction in overall fetal liver iron concentration. Clearly these changes should be accompanied by opposed changes in the spleen and bone marrow. Unfortunately these tissues could not be detected, due to low contrast and resolution, respectively. The changes seen could also be related to a change in fetal liver architecture or hydration status. We know, however, that liver development is complete well before 20 wk, and little change in architectural structure occurs after this time (10). A change in water content within the liver is also possible, but this could be linked to our original hypothesis. Using amniotic fluid as the comparative constant means that in principle the decrease in the ratio of the signal could be influenced by an increase in the liver signal or a decrease in the amniotic fluid signal. The fact that there was no significant change in the ratio when the amniotic fluid was compared with maternal anatomy and background noise confirms that a real change in fetal liver signal occurred.

It would have been preferable to have performed this study using \( t^2 \) measurements of the liver; however, these data are the result of a retrospective incidental observation that there were changes occurring in the liver so dramatic they were noticed with the naked eye. It would not be ethical to repeat this study now simply to improve this measurement. We recommend that future protocols should measure \( t^2 \) of the fetal liver and spleen to confirm that the changes observed reflect true changes in iron concentration.

Absolute measurements of \( t_1 \) and \( t_2 \) were made on the fetal lungs and placenta. The fetal lung demonstrated an increase (5) and the placenta demonstrated a decrease (6) in relaxation time measurements with increasing gestational age, consistent with the progressive physiological changes taking place therein. The amount of time the volunteers spent inside the imaging magnet was an important ethical consideration, and so relaxation times were assessed in these organs, as these findings would have an immediate clinical application.

These data have shown the potential of EPI as a noninvasive tool for the demonstration of changes in fetal physiology in utero and may have clinical use in the early detection of disordered fetal growth and metabolism.

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