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Liver volume in trisomy 21 and euploid fetuses at 11 to 13 weeks

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Objectives To compare liver volume between trisomy 21 and euploid fetuses at 11 to 13 weeks' gestation.

Methods Fetal liver volume was measured by 3D ultrasound in fetuses at low risk of aneuploidies ($n = 200$) and another group at high risk, including 148 euploid and 37 with trisomy 21. The association of liver volume with fetal nuchal translucency (NT) thickness, tricuspid regurgitation and reversed a-wave in the ductus venosus was investigated.

Results In the low-risk group, fetal liver volume increased exponentially with fetal crown-rump length (CRL) from a median of 0.5 cm^3 at CRL of 45 mm to about 2.5 cm^3 at CRL of 84 mm. In 27 (73.0%) of the trisomy 21 fetuses liver volume was above the 95th percentile of the low-risk group, whereas in the euploid fetuses liver volume was not significantly altered ($P = 0.521$). There were no significant contributions to liver volume from fetal NT ($P = 0.508$), tricuspid regurgitation ($P = 0.958$) or reversed a-wave in the ductus venosus ($P = 0.872$).

Conclusion In trisomy 21 fetuses at 11 to 13 weeks liver volume is increased. Copyright © 2010 John Wiley & Sons, Ltd.

KEY WORDS: first-trimester screening; trisomy 21; liver volume; hepatomegaly; nuchal translucency; myeloproliferative disorder

INTRODUCTION

In the neonatal period, about 10% of babies with trisomy 21 demonstrate transient myeloproliferative disorder which usually disappears within the first 3 months of life but in up to 20% of cases it evolves into acute megakaryoblastic leukemia within the subsequent 4 years (Zipursky, 2003; Massey *et al*., 2006; Roy *et al*., 2009). Prenatal ultrasound studies in the late second and third trimesters of pregnancy reported that some fetuses with trisomy 21 demonstrate hepatomegaly often with hydrops and in these fetuses there is hematological evidence of a myeloproliferative disorder (Zerres *et al*., 1990; Baschat *et al*., 1998; Hartung *et al*., 1998; Hamada *et al*., 2001; Smrcek *et al*., 2001; Robertson *et al*., 2003; Hojo *et al*., 2007). Studies of aborted fetuses at 15 to 18 weeks reported that in all trisomy 21 fetal livers, but not marrows, there was a marked expansion in erythroid–megakaryocyte progenitors suggesting that a 'leukemia-initiating' progenitor population is present within the liver of all trisomic fetuses (Chou *et al*., 2008; Tunstall-Pedoe *et al*., 2008).

Recent studies in the first trimester have reported that trisomy 21 is associated with increased flow in the hepatic artery and this may be the consequence of increased hepatic hematopoietic activity (Bilardo *et al*., 2010; Zvanca *et al*., 2010). Alternatively, the increased hepatic artery flow in trisomy 21 may be a compensatory response to reduced blood supply to the liver from the umbilical vein, due to cardiac dysfunction resulting in abnormal flow in the ductus venosus (Ebbing *et al*., 2008).

The aims of this study are to firstly, construct a normal range of liver volume at 11 to 13 weeks' gestation, secondly, determine possible significant differences in liver volume between trisomy 21 and euploid fetuses and finally, examine the relation of liver volume with fetal nuchal translucency (NT) thickness and Doppler flow pattern in the ductus venosus and across the tricuspid valve.

METHODS

This was a retrospective analysis of 3D volumes of the fetal liver obtained from two groups of singleton pregnancies undergoing screening for aneuploidies at 11 to 13^{+6} weeks by a combination of maternal age, maternal serum free β-hCG and pregnancy-associated plasma protein A (PAPP-A), ultrasonographic measurement of fetal NT thickness and Doppler assessment of the flow pattern in the ductus venosus and across the tricuspid valve (Snijders *et al*., 1998; Kagan *et al*., 2008, 2009; Maiz *et al*., 2009). Approval for the study was obtained from the hospital Ethics Committee.

The entry criteria for the first group of 200 fetuses were NT thickness less than 3 mm, no tricuspid regurgitation or reversed a-wave in the ductus venosus, estimated risk for trisomy 21 less than 1 in 300 and no

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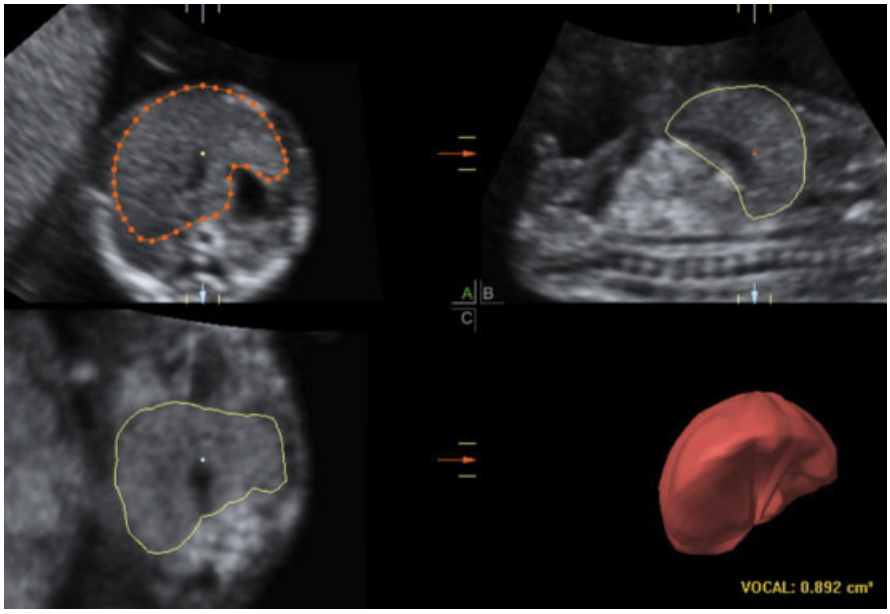

Figure 1—3D measurements of fetal liver volume using the Virtual Organ Computer-aided AnaLysis (VOCAL) technique

obvious fetal defects at the 11 to 13 weeks and 20 to 22 weeks scan (low-risk group). In the second group of fetuses, liver volumes were obtained in the 1 h before chorionic villus sampling (CVS) for karyotyping (pre-CVS group). We subsequently selected all cases of trisomy 21 ($n = 37$) and the next four euploid controls for each case ($n = 148$).

In all cases a mid-sagittal view of the fetal body was obtained by either transabdominal or transvaginal sonography (RAB4–8 and RIC5–9 transducer, Voluson E6, GE Medical Systems, Milwaukee, WI, USA) and a 3D volume of the lower thorax and abdomen acquired and stored for subsequent analysis by sonographers with extensive experience in 3D ultrasound. The VOCAL (Virtual Organ Computer-aided AnaLysis) technique was used to obtain a sequence of 12 longitudinal sections of the fetus around a fixed axis, each after a 15° rotation from the previous one. The contour of the liver was drawn manually in each of the 12 different planes to obtain the 3D volume measurement (Figure 1). All measurements were done offline by operators who were unaware of the fetal karyotype.

Statistical analysis

Continuous and categorical variables were compared using Mann–Whitney U-test with *post hoc* Bonferroni correction and χ^2-test or Fisher's exact test, respectively. The distribution of liver volume was made Gaussian using logarithmic transformation ($\log_{10}$). Normality of distribution was assessed using probability plots and Kolmogorov–Smirnov test. In the screened low-risk group, multiple regression analysis was used to determine the factors among maternal characteristics and fetal crown-rump length (CRL) that provided significant contribution to prediction of $\log_{10}$ liver volume. The only factor providing significant contribution was fetal CRL (see Section on Results) and therefore the measured liver volume in each patient in the pre-CVS and the trisomy 21 group was expressed as a difference from the expected normal mean for the fetal CRL (delta value) in the low-risk group. Similarly, the measured NT in each case was expressed as a difference from the expected normal mean for fetal CRL (delta value) (Wright *et al.*, 2008). The significance of difference in the delta liver volume in the outcome groups was examined using the Mann–Whitney U-test with *post hoc* Bonferroni correction. Multiple regression analysis was then used to determine the significance of contribution to delta liver volume from delta NT, flow through the fetal tricuspid valve (normal or regurgitation), flow through the ductus venosus (normal or reversed a-wave) and fetal karyotype (euploid or trisomy 21).

The statistical software package SPSS 16.0 (SPSS Inc., Chicago, IL, USA) was used for data analysis.

RESULTS

The maternal and fetal characteristics of the study groups are compared in Table 1. In the low-risk group, regression analysis demonstrated that in the prediction of $\log_{10}$ liver volume, there was a significant contribution from fetal CRL but not from maternal age ($P = 0.782$), body mass index ($P = 0.686$), racial origin ($P = 0.548$), mode of conception ($P = 0.075$) or smoking status ($P = 0.228$):

$$\text{Expected } \log_{10} \text{ liver volume} = -1.202 + 0.019 \times \text{fetal CRL in mm; SD} = 0.076,$$
$$R^2 = 0.856,\ P < 0.0001.$$

In both the trisomy 21 and euploid groups, compared to the low-risk group, the delta NT was higher and there

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Table 1—Maternal and pregnancy characteristics of the study populations

Characteristics	Low-risk group ($n = 200$)	Pre-chorionic villus sampling groups	
		Euploid ($n = 148$)	Trisomy 21 ($n = 37$)
Maternal age in years, median (IQR)	30.6 (26.6–34.5)	34.0 (30.0–38.0)*	39.1 (34.9–40.8)*
Body mass index in kg/m^2, median (IQR)	24.1 (21.5–28.4)	22.8 (21.1–25.4)*	22.6 (21.3–26.3)
Crown-rump length in mm, median (IQR)	64.4 (57.0–71.9)	66.3 (60.4–74.2)*	67.0 (61.4–75.6)
Racial origin			
Caucasian, *n* (%)	173 (86.5)	125 (84.5)	33 (89.2)
African, *n* (%)	14 (7.0)	10 (6.8)	2 (5.4)
South Asian, *n* (%)	10 (5.0)	3 (2.0)	1 (2.7)
East Asian, *n* (%)	3 (1.5)	6 (1.4)	1 (2.7)
Mixed, *n* (%)	0	4 (2.7)	0
Cigarette smoker, *n* (%)	16 (8.0)	17 (11.5)	0
Conception			
Spontaneous, *n* (%)	198 (99.0)	139 (93.9)	36 (97.3)
Ovulation drugs, *n* (%)	2 (1.0)	9 (6.1)*	1 (2.7)

Comparisons between groups (χ^2-test and Fisher's exact test for categorical variables and Mann–Whitney *U*-test with *post hoc* Bonferroni correction for continuous variables).
IQR, interquartile range.
Significance value * $P < 0.025$.

Table 2—Median and interquartile range (IQR) of delta liver volume, delta nuchal translucency (NT), prevalence of fetuses with tricuspid regurgitation and reversed a-wave in the ductus venosus in the study populations

Characteristics	Low-risk group ($n = 200$)	Pre-chorionic villus sampling groups	
		Euploid ($n = 148$)	Trisomy 21 ($n = 37$)
Delta liver volume, median (IQR)	0.01 (−0.10–0.14)	0.03 (−0.11–0.15)	0.63 (0.37–0.95)*
Fetal delta nuchal translucency, median (IQR)	0.32 (0.10–0.50)	0.52 (0.24–1.08)*	3.09 (1.27–5.58)*
Tricuspid regurgitation, *n* (%)	0	22 (14.9)*	18 (48.6)*
Reversed a-wave in ductus venosus, *n* (%)	0	16 (10.8)*	14 (37.8)*

Comparisons between groups (χ^2-test and Fisher exact test for categorical variables and Mann–Whitney *U*-test with *post hoc* Bonferroni correction for continuous variables).
Significance value * $P < 0.025$.

was an increased prevalence of fetuses with tricuspid regurgitation and reversed a-wave in the ductus venosus. Delta liver volume in trisomy 21 was increased but in euploid fetuses it was not significantly different from the low-risk group ($P = 0.521$) (Table 2, Figure 2).

Multiple regression analysis demonstrated that in predicting delta liver volume, there was significant contribution from fetal karyotype ($P < 0.0001$) but not from delta NT ($P = 0.508$), tricuspid regurgitation ($P = 0.958$) or reversed a-wave in the ductus venosus ($P = 0.872$).

DISCUSSION

This study demonstrates that in normal fetuses at 11 to 13 weeks' gestation the liver volume increases exponentially with CRL and in fetuses with trisomy 21 the liver is bigger than in euploid fetuses.

The fetal liver volume increased from a median of 0.5 cm^3 at CRL of 45 mm to about 2.5 cm^3 at CRL of 84 mm. This is compatible with the results of pathological studies which reported that liver weight increases exponentially with gestation from a mean of 1 g at 12 weeks to 5 g at 16 weeks and about 30 g at 30 weeks (Archie *et al.*, 2006). Previous attempts at assessing fetal liver size by ultrasound relied on the measurement of the length of the liver from the dome of the right diaphragm to the tip of the right lobe (Roberts *et al.*, 1989). The liver length increases linearly with gestation from 13 mm at 13 weeks to 60 mm at 40 weeks (Phatihattakorn *et al.*, 2004). In the 1990s with the advent of 3D ultrasound, attention shifted to the measurement of liver volume (Chang *et al.*, 1997) which was reported to increase exponentially with gestation from a mean of about 10 mL at 20 weeks to 40 mL at 30 weeks and 150 mL at 40 weeks (Chang *et al.*, 2003). There are no previous reports on the use of 3D ultrasound for measurement of liver volume in the first trimester.

In fetuses with trisomy 21, the liver was bigger than in euploid fetuses and the increased size was unrelated to fetal NT or blood flow pattern across the tricuspid valve or in the ductus venosus. It is therefore unlikely that the observed hepatomegaly is secondary to heart failure or hypoxia-mediated increased flow in the hepatic artery. A more plausible explanation is that the hepatomegaly is a consequence of the disturbed hematopoiesis with marked expansion of the 'leukemia-initiating' progenitor population within the liver of trisomy 21 fetuses (Chou *et al.*,

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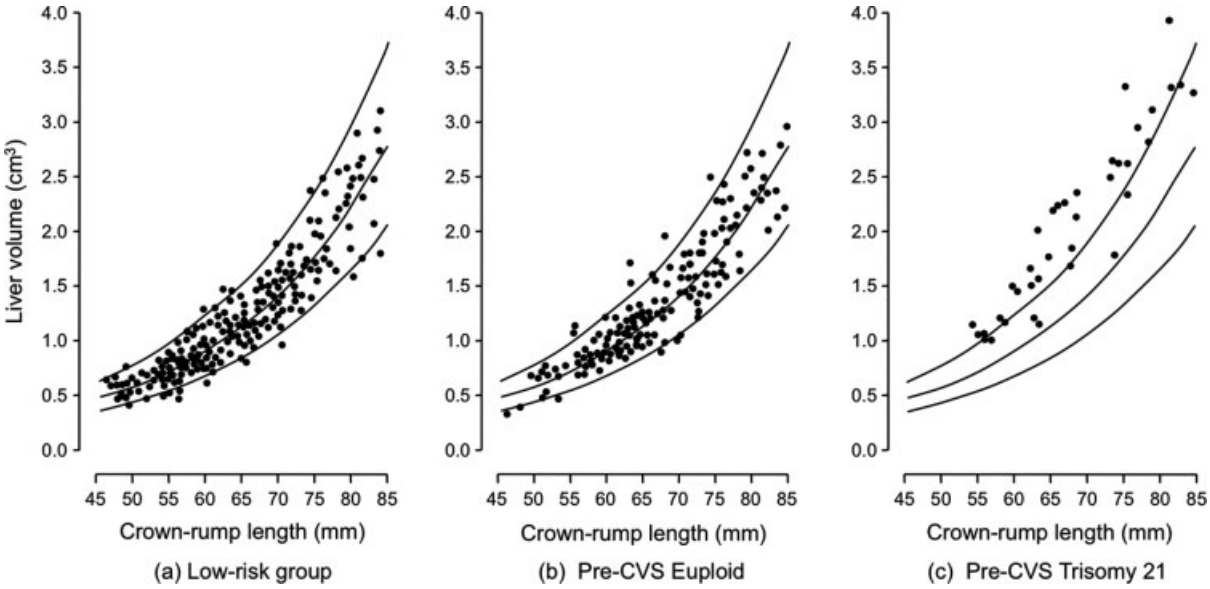

Figure 2—Liver volume with crown-rump length in the low-risk group (a), euploid fetuses (b) and trisomy 21 fetuses (c) plotted on the reference range (median, 5th and 95th percentiles) of the low-risk group

2008; Tunstall-Pedoe *et al*., 2008). Supportive evidence for this concept is the finding that in trisomy 21 pregnancies maternal serum alpha-feto protein (AFP) and unconjugated estriol are reduced. Estriol is a steroid hormone that requires the fetal adrenal, liver and placenta for synthesis and AFP is a glycoprotein synthesized in the fetal liver. Consequently, in trisomy 21 despite the enlarged liver, normal liver function is impaired because of the disturbed hepatic hematopoiesis.

Hematopoiesis in the embryo and fetus is divided into three overlapping periods: mesoblastic, hepatic and myeloid, each corresponding to the major hematopoietic organ of the period, which are the yolk sac, liver and spleen and bone marrow, respectively (Knoll, 1949). The hepatic period extends from the 10th to the 24th gestational week but the liver continues to be involved in hematopoiesis into the first week of postnatal life. It could be postulated that all fetuses with trisomy 21 have a myeloproliferative disorder arising from disturbed hepatic hemopoiesis. In most affected fetuses the hepatomegaly resolves with advancing gestation, since the liver is normally replaced by the bone marrow as the major hematopoietic site. However, in a few cases there is either delay or failure in the switch from hepatic to marrow hematopoiesis and these are the cases that will either present with hepatomegaly, hydrops and death at the end of the second and during the third trimester of pregnancy or a myeloproliferative disorder in the neonatal period. In most affected neonates, the condition resolves over the subsequent few weeks when the liver stops being a hematopoietic organ but in a few cases there is persistence of hepatic hematopoiesis and it is this group that develops acute megakaryoblastic leukemia.

In the first trimester of pregnancy the liver volume of fetuses with trisomy 21 is bigger than in euploid fetuses. Future longitudinal studies of fetal liver volume in fetuses with trisomy 21 diagnosed at 11 to 13 weeks and where the parents choose to continue with the pregnancy can define the potential value of this measurement in predicting the cases that will subsequently develop hydrops in fetal life or transient myeloproliferative disorder in the neonatal period or even acute megakaryoblastic leukemia in childhood.

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