The thymic–thoracic ratio in fetal heart defects: a simple ratio for identification of fetuses at high risk for microdeletion 22q11

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KEYWORDS: congenital heart defects; deletion 22q11; fetal echocardiography; prenatal diagnosis; thymus

INTRODUCTION

The thymus was visualized in the fetus as early as 1989, but interest in it has increased in the last 10 years with the advent of high-resolution probes. The thymus is best visualized during routine sonography in a transverse plane of the upper mediastinum at the level of the three vessels and trachea (3VT) view, in which it appears as a hypoechoic structure between the sternum and the great vessels, with good delineation from the neighboring lung tissue. Reference ranges for normal thymus growth during the second half of gestation have been reported using two-dimensional and three-dimensional ultrasound. Abnormalities of the fetal thymus have been observed mainly in the context of absent or hypoplastic thymus in association with microdeletion 22q11 (DiGeorge syndrome), or small thymus in utero fetal infection in prematurity, and rarely in cases with structural anomalies such as thymic cysts. Evaluation of the thymus during fetal echocardiography, especially in the presence of congenital heart defects (CHD), is better assessed objectively when measurements are performed. The suggested measurements, including diameters, perimeter and volume, always require comparison with reference ranges during the examination and therefore are impractical for use in clinical practice.

The aims of the present study were to propose a new simple ratio, the 'thymic–thoracic ratio' (TT-ratio) and to establish normal reference ranges. In addition, we aimed to evaluate its clinical usefulness by comparing the TT-ratio in fetuses with CHD with and without microdeletion 22q11 (del.22q11), a condition known to be associated with hypoplastic thymus.

ABSTRACT

Objectives To establish reference ranges for the fetal thymic-thoracic ratio (TT-ratio) and to compare results with those from fetuses with congenital heart defects (CHD) with and without microdeletion 22q11 (del.22q11), a condition known to be associated with a hypoplastic thymus.

Methods TT-ratio was defined as the quotient of the anteroposterior thymic to the intrathoracic mediastinal diameter measured in the three vessels and trachea view. This ratio was measured in a prospective cross-sectional study of 302 normal healthy fetuses between 15 and 39 weeks' gestation. The study group comprised two groups: one group (CHDn) consisted of 90 fetuses with CHD and a normal karyotype with no del.22q11 and the other group (CHD22) included 20 fetuses with CHD and a normal karyotype but with proven del.22q11.

Results The TT-ratio of the normal fetuses did not show any statistically significant change during gestation, with a mean value of 0.44. The values of all 90 fetuses of the CHDn group were within the normal range and no different from normal fetuses. However, 19 of the 20 (95%) fetuses in the CHD22 group had a significantly smaller TT-ratio (P < 0.001) compared with both the CHDn group and the normal fetuses, having a mean value of 0.25.

Conclusions The TT-ratio is reliable and easy to obtain during fetal echocardiography. Fetuses with CHD and a low TT-ratio can be considered at high risk of having microdeletion del.22q11. Copyright © 2011 ISUOG.

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PATIENTS AND METHODS

Normal fetuses

In this prospective cross-sectional study, fetal echocardiography was performed between 15 weeks and term on healthy pregnant women referred for routine antenatal ultrasonography, for follow-up in pregnancy for or targeted cardiac examination with a positive family history for heart anomalies. Measurements were collected from singleton pregnancies with structurally normal fetuses of appropriate size for gestational age, showing no cardiac or extracardiac abnormality. Excluded from the study prospectively were pregnant women with chronic diseases affecting fetal growth and excluded retrospectively were pregnancies ending with a premature delivery or a small-for-dates baby. For reliable thymus evaluation, only women with optimal imaging characteristics (i.e. thin abdominal wall; dorsoposterior fetal position and fetal apnea during the exam) were considered for the study. Intraobserver variability was evaluated for TT-ratio measurement in 25 fetuses selected arbitrarily, whose images were retrieved and remeasured on a later occasion.

Fetuses with CHD with and without microdeletion 22q11 (del.22q11)

The study group consisted of fetuses with mild and severe heart defects examined between 2005 and 2010. The distribution of their cardiac anomalies is shown in Table 1. In our institution, when a fetal cardiac anomaly is detected, an invasive procedure for fetal karyotyping is always offered. In addition, since 1996, fluorescent in-situ hybridization (FISH) for del.22q11 has been offered in all cases. In most cases, the TT-ratio was measured at first diagnosis of CHD, before the results of the chromosomal analysis and FISH for del.22q11 were known; in some cases with suspected CHD referred for a second opinion, an invasive procedure for karyotyping with FISH for del.22q11 had already been performed. Fetuses with abnormal chromosomal findings other than del.22q11 were excluded. The remaining group was divided into two: the first group consisted of fetuses with CHD and normal karyotype with no del.22q11 (CHDn); the second group included fetuses with CHD and confirmed del.22q11 (CHD22). We also included in the study group cases with CHD which were first karyotyped on maternal request postnatally.

Ultrasound examination

Fetal echocardiography and measurement of the thymus were performed using high-resolution two-dimensional echocardiography equipment and a convex transabdominal 4–8-MHz transducer (Voluson 730 and Voluson E8 machines, GE Medical Systems, Zipf, Austria). As a standard requirement of our institution, all patients provided signed informed consent for fetal examination and agreed to storage of digital images and measurement data for anonymous quality control and later data evaluation. A fetal echocardiogram was performed by the usual systematic cross-sectional approach. In our setting, standardized planes including the 3VT view are documented in the digital image database, according to the recommendations of the German Ultrasound Society (DEGUM). The thymus can be identified in this plane as a hypoechoic structure with echogenic dots filling the space between the vessels posteriorly and the anterior chest wall (sternum and ribs) anteriorly (Figure 1). The anteroposterior diameter of the thymus was measured along the midline between the transverse aortic arch border posteriorly and the posterior chest wall anteriorly. In addition, the mediastinal sagittal diameter was measured, along the line traced to measure the thymic diameter, as the distance between the anterior edge of the thoracic vertebral body at the level of the transverse arch posteriorly and the internal edge of the sternum anteriorly (Figure 1). The TT-ratio was then calculated as the ratio of the anteroposterior thymic to the intrathoracic mediastinal diameter and related to gestational age. In fetuses with cardiac defects and an abnormal 3VT view, the thymus was measured along the midline starting from the anterior border of the most posterior vessel (Figure 2). All examinations were performed by a single examiner (R.C.) and two consecutive measurements from each fetus were obtained and their average value used in further analysis. Measurements had no impact on medical treatment of the patient.

Statistical analysis

Regression analysis was applied to assess a possible relationship between TT-ratio and gestational age. The two study groups with cardiac defects (CHDn and CHD22) were compared with each other and with the reference group by Kruskal–Wallis one way analysis of variance (ANOVA) for comparing several subgroups. \( P < 0.05 \) was considered statistically significant. A Bland–Altman plot with 95% limits of agreement was applied to evaluate the intraobserver variability for systematic error. Analysis was performed using the statistical packages GraphPad Prism 4 and GraphPad InStat for Windows (GraphPad Software, San Diego, CA, USA).

RESULTS

Normal fetuses and reference range

The reference range for TT ratio was produced using data from 302 fetuses. The ratio did not change significantly throughout gestation (Figure 3) \( (r = 0.108, \ P = 0.059) \) and had a mean \( \pm SD \) value of 0.4417 \( \pm 0.043 \). The Bland–Altman plot with 95% limits of agreement from −0.030 to 0.038 confirmed reliable reproducibility and an absence of systematic error.

Study groups

A total of 110 fetuses with cardiac anomalies was recruited for final analysis, which were subdivided into those with...
**Figure 1** Schematic diagram (a) and ultrasound image in a normal fetus (b) showing the upper mediastinum at the three vessels and trachea plane with visualization of the thymus anterior to the great vessels ([a] adapted from Abuhamad and Chaoui5). Red lines and calipers show measurement of the thymic anteroposterior diameter (Caliper 2) and the intrathoracic mediastinal diameter (Caliper 1). A, anterior; Ao, aorta; DA, ductus arteriosus; DAo, descending aorta; L, left; PA, pulmonary artery; R, right; SVC, superior vena cava.

**Figure 2** Two fetuses with cardiac anomalies: (a) with normal chromosomes and a normal sized thymus and (b) with hypoplastic thymus and del.22q11, in which the thymic–thoracic ratio can still be measured, despite the abnormal great vessel anatomy. Sp, spine.

1. normal karyotype \((n = 90)\) and those with del.22q11 \((n = 20)\). The distribution of cardiac defects with respect to subgroup is given in Table 1.
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6. **Fetuses with CHD and normal karyotype (CHDn)**
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8. The values of the TT-ratio in the CHDn fetuses are plotted on the reference range in Figure 4. No case had a severely small thymus. Interestingly, there was no significant difference in TT-ratio between the CHDn group and the normal population.
9.  
10.  
11.  
12.  
13.  
14.  
15. **Fetuses with CHD and del.22q11 (CHD22)**
16. Nineteen of the 20 (95%) fetuses with del.22q11 had a significantly small thymus compared with the normal...
In only two fetuses, one with common arterial trunk and one with pulmonary atresia with ventricular septal defect, was the diagnosis of del.22q11 known at referral. A comparison of the TT-ratio between normal fetuses and the two study subgroups showed a significantly lower ratio in the CHD22 group compared with both the CHDn group and the normal fetuses (P < 0.001) (Figure 5). The TT-ratio in the CHD22 group had a mean ± SD value of $0.25 ± 0.09$.

DISCUSSION

The TT-ratio introduced in this study expresses the extent to which the thymus occupies the upper mediastinum in the anteroposterior direction. We have shown that the TT-ratio is a reliable and easy tool for identifying fetuses at high risk for microdeletion 22q11. In this study 19 out of 20 fetuses with del.22q11 had a decreased TT-ratio as a sign of thymic hypoplasia. A hypoplastic or aplastic thymus is one of the main features found in individuals with microdeletion 22q11, also known as DiGeorge syndrome and described earlier as CATCH-22 (Cardiac anomaly, Abnormal facies, Thymic hypoplasia/aplasia, Cleft palate, Hypothyroidism and microdeletion 22)\textsuperscript{19}. Previous fetal studies have shown that, in expert hands, subjective evaluation of thymus size could be of help in identifying fetuses at risk for microdeletion\textsuperscript{18,19,20} but the diagnostic impact could be improved by using reproducible measurements. In our opinion the proposed ratio, in comparison to perimeter or volume measurements, is easy to measure on a routine scan. Throughout gestation, independent from gestational age, the mean ratio was 0.44 in normal fetuses and in fetuses with CHD and microdeletion 22q11 the ratio was significantly smaller, being lower than 0.3 and with a mean of 0.25, implying ease of use during fetal echocardiography without the need for comparing with charts or Z-scores.

The ultrasonographic assessment of the thymus to detect infants at high-risk for del.22q11 was first reported by two groups in the 1990s\textsuperscript{21,22}. Yeager and Sanders\textsuperscript{21} reported first on thymic measurements in 12 infants with common arterial trunk and 14 with interrupted aortic

Table 1 Study group with congenital heart defects (CHD) classified into subgroups with normal karyotype with or without\textsuperscript{*}deletion 22q11

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*APVS, absent pulmonary valve syndrome; AVSD, atrioventricular septal defect; CoA, coarctation of the aorta; DORV, double outlet right ventricle; HLHS, hypoplastic left heart syndrome; PAVSD, pulmonary valve atresia and ventricular septal defect; TAC, transverse aortic constriction; VSD, ventricular septal defect. 

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CHDn fetuses and the other two groups. There was no significant difference (NS) between ratios of normal and CHDn fetuses, but there was between CHD and del.22q11. The authors reported on the calculation of a ‘thymic index’, being the ratio of the transverse diameter related to body surface area. A further report performed on 42 infants with tetralogy of Fallot or pulmonary atresia with ventricular septal defect confirmed the efficiency of this thymic index in recognizing the seven infants with DiGeorge syndrome.22

A small thymus is specific to del.22q11. It is not intrinsic to cardiac defects in general (Figure 4). In our study the comparison of thymic size in fetuses with CHD and without del.22q11 showed a low TT-ratio only in the group with del.22q11. Our study does not, therefore, support the recent report in this Journal of a small thymus in fetal cardiac defects9 (Table 2), but unfortunately the study of Li et al.9 lacked karyotyping results and information on del.22q11 in 16 of the 19 reported fetuses with CHD. On the other hand, it must also be borne in mind that some syndromes or conditions other than del.22q11 may also show the combination of a CHD and a hypoplastic thymus, but these were not present in our study. A dilated heart or cardiomegaly in particular could lead to compression of the thymus and give a false-positive result, but this was not observed in our study.

In 1989, Felker et al.1 were the first to report assessment of the fetal thymus. The advent of high-resolution ultrasound a decade ago facilitated reliable visualization of the shape and border of the thymus throughout gestation.2,3,10,11. So far, few papers have been published on fetal thymic size and these are summarized in Table 2. Most of the later studies reporting on the clinical use of fetal thymic measurement focused on its role or importance in the context of prematurity and related chorioamnionitis14,15,23 or growth restriction24. To our knowledge, our study is the largest including fetuses with cardiac defects, and in particular the largest to present thymic measurements in fetuses with del.22q11.

One of the main strengths of our study is that all 110 reported fetuses with CHD underwent karyotyping and a FISH evaluation for del.22q11. We excluded a priori and a posteriori all fetuses with no available karyotype or with aneuploidy. We agree with other authors3,6 that measurements may be challenging due to the irregular shape of the thymus in normal conditions and to the possibly distorted shape in CHD. However, the measurement of the TT-ratio that we propose is standardized to be measured along the midline connecting the spine with the sternum, and the anteroposterior diameter of the thymus in the 3VT plane is merely the distance between the anterior vessel and the thoracic wall (Figure 2). In contrast to studies on thymic perimeter in which the complete shape of the thymus should be identified before achieving a measurement, our ratio permits measurements to be made even under conditions of limited image resolution. Interestingly, in a few cases with del.22q11 with a very small TT-ratio the examiner had the impression that the aorta was abutting the sternal wall, as a sign of thymic aplasia. In such conditions the ‘Thy-box’ recently described by Paladini25 may show in addition the abnormal course of the mammary arteries, on the borders of the thymic region.

One of the main limitations of our study is that the TT-ratio is not truly representative. However, it should be emphasized that this ratio achieved a high suspicion rate of 95% (19/20) of our cases; we think this is sufficiently reliable for this purpose. Another limitation is the possibility of the experienced examiner being biased by knowledge of the cardiac defect while performing the measurements. Interestingly, of the 110 cases with CHD, information on karyotype was available only in 15 at the time of the examination (two with del22q). Yet, two of the 20 fetuses with del.22q11 had just a muscular ventricular septal defect, which is not a conotruncal anomaly and proved to have a small TT-ratio, supporting the reliability of our observation.

In conclusion, our study shows that: (1) in normal fetuses the TT-ratio measured at the level of the 3VT view is constant throughout gestation, with a mean ± SD value of 0.44 ± 0.043 in our population; (2) despite the heterogeneous shape of the thymus in normal fetuses and in fetuses with CHD, the TT-ratio measured in the axial upper thorax plane along the midline is easy to measure and reliable on a routine scan and in targeted fetal echocardiography; (3) fetuses with CHD and del.22q11 in almost all cases show a significantly low TT-ratio of less than 0.3 compared with normal fetuses and with...
Studies have shown a high success rate in the last decade and, except last the two studies, have concentrated mainly on normal fetuses. 22q11, microdeletion 22q11; AP, anteroposterior; CHD, congenital heart defect.

Thymic–thoracic ratio


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Queries to Author:

AQ1 Sorry – I should have queried this earlier. Should ‘intrathoracic mediastinal diameter’ have ‘anteroposterior’ (or ‘maximum’?) also? This affects wording in a couple of other places.

AQ2 In response to your reply and because in the main methods you state that you excluded Fetuses with abnormal chromosomal findings other than del.22q11. I’ve added here “and a normal karyotype but with” – please check – are you happy with this?

AQ3 I’ve added here “compared with both the CHDn group and the normal fetuses” – OK?

AQ4 Note in your reply you’d typed ‘Please not’ into the text just after the keywords, rather than into a comment box – I’m not sure what it referred to???

AQ5 For the same reasons as in the abstract, I’ve changed ‘and or’ to ‘with or without’ in Table 1 legend and removed ‘normal karyotype’ from the column header, leaving just without/with the deletion – OK?

AQ6 Table 2. Rather than a column heading of ‘Feasibility’ would something like ‘Success rate of visualization’ be better? Note also for Li, singletons, I’ve changed the value from 95.3% to 95.8%. Also regarding Li – sorry, I put ‘including’ in the wrong place. I’ve now consulted the paper itself – you have ‘238 singletons, 64 twins, 19 with CHD’. According to the paper, the 19 with CHD were also singletons. So would you prefer to have 257 singletons, or perhaps add ‘normal’ to the 238 singletons and the 64 twins and say 19 singletons with CHD? Whichever we choose, are similar changes required for any of the other papers? I’ve also added ‘normal’ to your study – i.e. 302 normal singletons – OK? Also, for Cho in the last column – is the wording now ok: “Transverse diameter is easier to measure than perimeter, reference range for transverse diameter”. Note I added ‘to measure’

AQ7 Note rewording to this sentence – is it ok?: “Evaluation of the thymus during fetal echocardiography, especially in the presence of CHD,”

AQ8 ‘(Table 1)’ removed as it didn’t seem to fit here – OK?

AQ9 How about ‘impractical for [use in clinical practice]’ rather than ‘not used’?

AQ10 I’ve gone back closer to your original wording regarding 15 weeks.

AQ11 ‘For reliable thymus evaluation, only women with optimal imaging characteristics (i.e. thin abdominal wall; dorsoposterior fetal position and fetal apnea during the exam) were considered for the study.’ – does this apply also to the study groups?

AQ12 Table 2 changed to Table 1. Note you also refer to the table in the Results – would you prefer it just in the Results?

AQ13 Is my rewording in response to your reply ok here? “In addition, since 1996, fluorescent in-situ hybridization (FISH) for del.22q11 has been offered in all cases.”?

AQ14 How about “We also included in the study group cases with CHD which were first karyotyped on maternal request postnatally”?

AQ15 Sorry – my query about layout was perhaps unclear – I discussed with Sarah and she reckoned give it a go and see what you think. All I’ve done is move this entire paragraph to after introduction of the fetuses. And because you’ve already said above it was a prospective cross-sectional study, I’ve removed ‘In this prospective study’.

AQ16 ‘thymic anteriorly diameter to the mediastinal distance’ has been changed to ‘anteroposterior thymic to the intrathoracic mediastinal diameter’ as you have in the Abstract – is this ok?

AQ17 OK – I’ve thought!! How about something like: “The TT-ratio introduced in this study expresses the extent to which the thymus occupies the upper mediastinum in the anteroposterior direction.”?

AQ18 While removing ‘the above cited measurements’ I also had to change ‘the role’ to ‘its role’ to make sense – have I understood correctly here?

AQ19 I’ve changed ‘a numerical aberration’ to ‘aneuploidy’ – is this what was intended?

AQ20 I’m not sure ‘susicion rate’ is the term to use here?

AQ21 ‘this purpose’ might be a bit vague here??
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