Thymic–thoracic ratio in fetuses with trisomy 21, 18 or 13

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KEYWORDS: aneuploidy; deletion 22q11; Down syndrome; fetal echocardiography; growth restriction; thymus; trisomy

ABSTRACT

Objectives To assess thymic size expressed as the thymic–thoracic ratio (TT-ratio) in fetuses with trisomy 21, 18 or 13.

Methods The TT-ratio, the quotient of the anteroposterior thymic and the intrathoracic mediastinal diameter, was measured in 65 trisomic fetuses between 15 and 36 weeks’ gestation, including 30 cases with trisomy 21, 19 with trisomy 18 and 16 with trisomy 13. In addition, these 65 fetuses were divided into two groups, according to whether they showed growth that was appropriate-for-gestational age (AGA) (n = 39) or intrauterine growth restriction (IUGR) (n = 26). Measurements were compared with reference ranges from 302 normal fetuses.

Results The TT-ratio was low in 27.7% (n = 18) of the 65 fetuses with aneuploidy. In comparison to normal fetuses (mean TT-ratio, 0.44), those with trisomy 18 or 21 had a significantly smaller TT-ratio (mean, 0.38 [P < 0.001] and 0.40 [P < 0.05], respectively), while those with trisomy 13 did not (mean, 0.43). These values were not as low as those observed previously in fetuses with del.22q11, suggesting a mechanism involving accelerated thymic involution rather than primary thymic hypoplasia. Furthermore, the TT-ratio was significantly lower than normal in both AGA (P < 0.05) and IUGR (P < 0.001) fetuses.

Conclusion Fetuses with trisomy 18 or 21, but not trisomy 13, have a small thymus, suggesting accelerated thymic involution in utero. IUGR may contribute to the reduced thymic size in trisomy 18 fetuses. Trisomy 21 fetuses seem to have additional factors leading to a small thymus which could be a possible confirmation of the reduced immune response observed in fetuses and neonates with Down syndrome. Copyright © 2012 ISUOG. Published by John Wiley & Sons, Ltd.

INTRODUCTION

The thymus is an essential part of the adaptive immune system of the fetus and grows rapidly in utero to reach its greatest relative size at the time of birth. Recently, there has been growing interest in assessing fetal thymic size using high-resolution ultrasound and in examining fetuses with impaired thymic growth. A hypoplastic fetal thymus is typically found in association with deletion 22q11 (del.22q11), and a small thymus has also been reported in fetuses with intrauterine growth restriction (IUGR), prematurity and related chorioamnionitis and pre-eclampsia. Little is known, however, about fetal thymic size in common autosomal trisomies, such as trisomy 21, 18 or 13, apart from the recognition that newborns with trisomy 21 have reduced immunity and a small, functionally abnormal thymus. Thilaganathan et al. showed in 1993 that in trisomy 21 fetuses the immunological deficiency is already present prenatally, expressed by lower T- and B-lymphocytes in the blood of affected fetuses. We recently introduced the thymic–thoracic ratio (TT-ratio) as an easy and reproducible parameter for thymic size assessment in the fetus and found that it was smaller than normal in 19/20 fetuses with del.22q11. The aim of this study was to determine whether thymic size, expressed by the TT-ratio, is also different in fetuses with trisomy 21, 18 or 13.

METHODS

Ultrasound examination

Ultrasound examinations including fetal echocardiography were performed using high-resolution two-dimensional echocardiography 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 written informed consent for fetal examination and agreed to whether they showed growth that was appropriate-for-gestational age (AGA) (n = 39) or intrauterine growth restriction (IUGR) (n = 26). Measurements were compared with reference ranges from 302 normal fetuses.

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to storage of digital images and measurement data for anonymous quality control and later data evaluation. In our setting, standardized planes of the upper mediastinum are documented in the digital image database, according to the recommendations of the German Ultrasound Society. In a previous study we reported in detail the technique of measuring the TT-ratio and assessed the reproducibility of the measurements. Briefly, the thymus is identified in the three vessels and trachea view, between the great vessels posteriorly and the posterior chest wall anteriorly. The intrathoracic mediastinal diameter is measured, along a line traced between the anterior edge of the thoracic vertebral body posteriorly and the internal edge of the sternum anteriorly (Figure 1). Along this line the anteroposterior diameter of the thymus is measured, between the border of the transverse aortic arch posteriorly and the posterior chest wall anteriorly. In fetuses with cardiac defects with possibly abnormal vessels, the thymus can be measured along the midline, from the anterior border of the most posterior vessel to the sternum anteriorly. The TT-ratio is then calculated by dividing the thymic diameter by the intrathoracic mediastinal diameter. In a previous study, data from 302 normal fetuses from uneventful pregnancies were collected to assess the reference range and were used in this paper for comparison with values from the study group.

### Study group: fetuses with trisomy 21, trisomy 18 and trisomy 13

The study cohort consisted of three groups of fetuses with autosomal trisomies examined between 2005 and 2011, including 30 with trisomy 21, 19 with trisomy 18, and 16 with trisomy 13. In almost all fetuses, a comprehensive ultrasound examination, including fetal echocardiography, was performed before the karyotype was known. The measurements of thymic and mediastinal diameters were stored, but were not taken into account for further patient management. The baseline characteristics of these groups are listed in Table 1. In these fetuses, aneuploidy was suspected on ultrasound at different gestational ages (range, 15–35 weeks). Fetal karyotyping was performed in all cases. In our institution, invasive testing for fetal karyotyping is always offered in cases with findings suspicious of chromosomal anomalies, including congenital heart disease or multiple soft markers, or in cases at increased risk following first-trimester screening. The TT-ratio from each of the three trisomic groups was analyzed separately and compared with the normal reference range. Each fetus was considered only once.

### Table 1 Clinical characteristics of the study group of fetuses with trisomy 21, 18 or 13

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<tr>
<th>Characteristic</th>
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<th>Trisomy 13 (n = 16)</th>
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<tr>
<td>Live birth</td>
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</table>

Data are reported as median (range) or n. Small TT-ratio defined as TT-ratio < 2 SD below the mean. GA, gestational age; IUGR, intrauterine growth restriction; TT-ratio, thymic–thoracic ratio.

Figure 1 Cross-sectional view of the fetal mediastinum at the level of the three vessels and trachea view, showing measurement of the intrathoracic mediastinal diameter and, along the same line, the anteroposterior diameter of the fetal thymus in a normal fetus (a) and a fetus with trisomy 21 (b), which had a smaller thymic–thoracic ratio.
in this study. In view of the association between IUGR and a small thymus, during data analysis for this study we documented whether the fetus was appropriate-for-gestational age (AGA) or small-for-gestational age. IUGR was defined as estimated fetal weight < 5th centile or, before 20 weeks, both abdominal and head circumference < 5th centile, at the time of TT-ratio measurement.

Statistical analysis

The TT-ratio values in cases of trisomy 21, 18 and 13 were plotted and compared with the normal reference range. The Mann–Whitney U-test was used to compare the TT-ratio between the individual groups of fetuses with trisomy 21, 18 or 13 and normal fetuses, as well as to compare it between trisomic fetuses with or without IUGR and normal fetuses. P < 0.05 was considered statistically significant. Analyses were performed using the statistical packages GraphPad Prism 4 and GraphPad InStat for Windows (GraphPad Software, San Diego, CA, USA).

RESULTS

In normal fetuses, as previously reported, the TT-ratio did not vary with gestational age between 15 weeks and term, having a mean ± SD value of 0.4417 ± 0.043 (95% CI, 0.337–0.526).

In the overall study group, the thymus was small (TT-ratio < 2 SD below the mean) in 18 of the 65 (27.7%) fetuses examined (Figure 2). In the individual groups, we found a small TT-ratio in nine of the 30 (30%) fetuses with Down syndrome, in six of the 19 (31.5%) fetuses with trisomy 18 and in three of the 16 (18.7%) fetuses with trisomy 13 (Figure 2) (Table 1). A congenital heart defect was found in 72% (47/65) of cases (Table 1).

Comparison of the TT-ratio in the trisomic fetuses with values measured in normal fetuses showed a significantly smaller TT-ratio in fetuses with trisomy 21 (P < 0.05) and those with trisomy 18 (P < 0.001) but not in those with trisomy 13 (P = 0.165) (Figure 3). The mean (±SD) TT-ratio was 0.404 (±0.069) (95% CI, 0.269–0.539) in trisomy 21 fetuses, 0.377 (±0.079) (95% CI, 0.222–0.532) in trisomy 18 fetuses and 0.426 (±0.056) (95% CI, 0.316–0.535) in trisomy 13 fetuses. In order to assess the possible association between IUGR and thymic size, the 65 fetuses were classified into two groups according to whether they were AGA (n = 39) or IUGR (n = 26) fetuses. Comparison of these groups with normal reference ranges showed a significantly smaller TT-ratio in both subgroups (Figure 4) but the difference in relation to the normal range was more pronounced in the IUGR subgroup (mean ± SD, 0.388 ± 0.081, P < 0.001; 95% CI, 0.229–0.546) in comparison with the AGA group (mean ± SD, 0.411 ± 0.062, P < 0.05; 95% CI, 0.289–0.532).

DISCUSSION

In this study we have shown that the thymic size, reflected by the TT-ratio, is small in fetuses with trisomies. A small thymus has been reported previously in fetuses with del.22q11.2[4,9,10], in fetuses with latent infection[8,14] in IUGR fetuses[6] and in fetuses of pre-eclamptic women[3]. This study highlights the finding of reduced thymic size in another fetal condition, namely trisomy. Interestingly, despite many reports of structural abnormalities seen on
Figure 4 Box-and-whisker plot showing distribution of thymic–thoracic ratio (TT-ratio) in normal fetuses (Normal), in fetuses with trisomy 21, 18 or 13 and which were appropriate-for-gestational age (AGA) \((n = 39)\) and in fetuses with trisomy 21, 18 or 13 which had intrauterine growth restriction (IUGR) \((n = 26)\). Median, 25th and 75th centiles and range are shown. The TT-ratio was lower in both AGA and IUGR trisomic fetuses \((P < 0.05\) and \(P < 0.001\), respectively).
fetal blood sampled at midgestation\textsuperscript{13,19}. In trisomy 21 fetuses, other possible mechanisms of immune deficiency may be caused by overproduction of the enzyme copper-zinc superoxide dismutase (SOD)\textsuperscript{25} whose gene is located on chromosome 21q22.1. The resulting decrease in zinc concentration, the reduced bioavailability of thymulin and the suppressed cellular production of prostaglandins E2 and D2 could be the pathogenic mechanism of the immune deficiency\textsuperscript{26}. A recent study found reduced expression of different genes involved in the immune response in thymuses surgically removed from infants with Down syndrome, thus confirming the global thymic hypofunction observed in Down syndrome patients\textsuperscript{22}.

Our study has, however, several limitations, one being the small number of fetuses examined, which did not allow a comprehensive comparison of subgroups within the trisomies. Another limitation is the lack of information regarding the immune status of the fetuses examined, which could have allowed us to compare thymic size and function.

A recent study\textsuperscript{15} of thymic diameter and circumference in 12 Down syndrome fetuses confirms our findings of a small thymus in such fetuses. Those authors reported a small thymic diameter in eight of the 12 cases, suggesting a substantial association between small thymic size and congenital immunodeficiency. However, it is unlikely that all of the fetuses with Down syndrome and small thymic diameter (67% of Down syndrome fetuses in that study and 30% in our study) would have had serious immunodeficiency postnatally. On the other hand, the fetus which was referred at 36 weeks (Figure 2), due to IUGR and polyhydramnios, had a ventricular septal defect and confirmed trisomy 18. After birth, the infant survived 6 months and then died from a liver tumor (hepatoblastoma). It is possible that in this case the small thymus seen in utero was already the expression of a disturbed immune response. Nevertheless, we think that in future, studies addressing the possible relationship between these observed measurements and immunological problems in live-born children may be of interest and might provide additional information for parental counseling in mid-gestation.

In conclusion, our study suggests that the TT-ratio is small not only in fetuses with cardiac defects associated with del.22q11, but also in fetuses with trisomy 21 or trisomy 18, but not in fetuses with trisomy 13. It is likely that the small ratio does not reflect an underlying primarily embryologic underdevelopment like that in del.22q11, but rather that it reflects a thymic involution which is enhanced by growth delay in these fetuses and specifically triggered by the additional chromosome 21 in Down syndrome. It is questionable whether reduced thymic size is an expression of the immune deficiency described in individuals with Down syndrome.

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Queries to Author:

AQ1 Please check that all affiliations are correct and complete

AQ2 ‘these’ changed to ‘trisomy 18’ – ok?

AQ3 Sorry – you seem to have missed a few of the queries, so I left them in.

Here, rather than “The difference in the severity of these findings could be explained primarily by developmental hypoplasia in fetuses with del.22q11 as part of the disease versus an accelerated thymic involution with size reduction in trisomies 21 and 18”, how about…

The difference in the severity of these findings could be explained by there being different underlying mechanisms: primarily developmental hypoplasia in fetuses with del.22q11, as part of the disease, and accelerated thymic involution with thymic size reduction in trisomies 21 and 18.

AQ4 SARAH: I queried ‘an intrinsically deficient immune system since the beginning’, suggesting something like ‘there could/may be an intrinsic deficiency of the immune system from/in/as early as the prenatal period’

Rabih supplies the original quote and we can discuss at proof stage. Thus, our results are in general agreement with the recent proposal by Kusters et al. (6) that “the immune system in DS is intrinsically deficient from the very beginning, and not simply another victim of a generalized process of precocious aging,” as hypothesized by others (7, 8, 54, 55).

AQ5 ‘questionable whether’ changed to ‘possible that’ in response to your reply – OK?

AQ6 SARAH – ‘these observed measurements’ refers to TT-ratio specifically – think that’s clear??

AQ7 Sorry, you missed a few queries here also…

‘It is likely that it is not expressing’ changed to ‘It is likely that the small ratio does not reflect – have i understood correctly?

AQ8 ‘as it is known’ changed to ‘like that’ – have I understood correctly?

AQ9 Is ‘delay’ ok – it shouldn’t be restriction here?

RC: OK

Further query: Do you mean ok change it or it is ok as it is?!

AQ10 Would it be ok to make a similar change here to the one made earlier – changing ‘it is questionable whether’ to ‘It is possible that’?
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