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ORIGINAL ARTICLE

Nomogram of Fetal Thymus Using Thy-Box Technique in South Indian Population

Subapriya Kandasamy[1] · Sherina Paul Raj[1]

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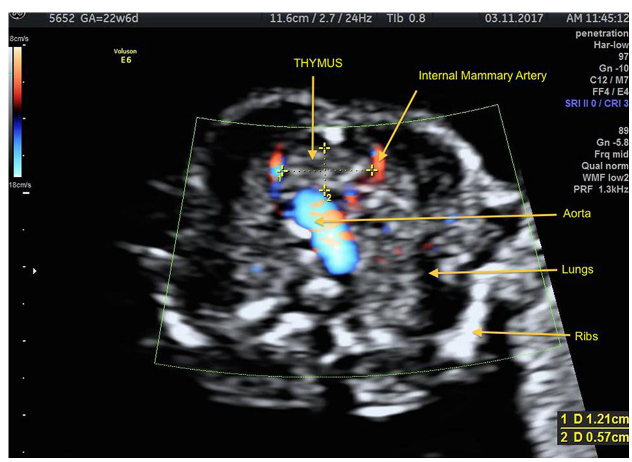
Abstract To create a nomogram of fetal thymus using the thy-box technique from a single center in South Indian population. This is a retrospective study that included 723 singleton pregnancies referred for anomaly scan. The study was conducted at a Fetal Medicine center in South India. The thymus was localized in the axial plane of the upper mediastinum of the fetus. At first, the internal mammary arteries that course laterally to the thymus were located by using color or power Doppler ultrasonography with a low pulse repetition frequency of these vessels. This was used to identify its lateral borders as described by Paladini et al. (Ultrasound Obstet Gynecol 37(4):488–492, 2011). The thy-box, was thereby readily displayed in the fetus. The anteroposterior and transverse diameters of the thymus using thy-box were measured and recorded. The fetal thymus was visualized using the thy-box technique in a total of 723 cases. Thymus transverse diameter as a function of gestational age was expressed by the regression equation: Transverse diameter (in mm) $=0.08115 \times$ Gestational age -0.5366 [R square $=0.98$ and $p<0.0001$]. Similarly the anteroposterior diameter (AP) as a function of gestational age was expressed by the equation: AP (in mm) $=0.0449 \times$ Gestational age -0.2759 [R square$^2=0.9686$ and $p<0.0001$]. The mean, standard deviation and 95% confidence interval for the thymus measurements corresponding to each gestation age were also calculated and tabulated. Linear regression model was used and data was analyzed using IBM SPSS version 22 software (Armonk, NY). The fetal thymus can be quickly measured with reliability and reproducibility in fetuses during the anomaly scan, when the thy-box technique is employed. There is no significant ethnic variation in thymus size. Moreover, an evaluation of the fetal thymus if added as a routine parameter during the performance of the second trimester anomaly scans, would enhance the ability to identify thymic hypoplasia when present.

Graphical Abstract



Keywords Fetal thymus · Thymus measurements · Nomogram · South Indian population · Thy-box · Prenatal ultrasound

✉ Subapriya Kandasamy
ksubapriya@gmail.com

[1] Fetoscan Fetal Medicine Center, Chennai, India

Introduction

The thymus is a homogeneous structure located in the upper mediastinum between the two lungs and in front of the great vessels. The best plane to visualize this structure by ultrasound is the axial plane of the 'three-vessel-trachea view'. The echogenicity of thymus is almost similar to lung in early second trimester [1] i.e. during anomaly scan and later becomes hypo echoic. Maternal habitus is also a determinant factor. This makes the identification of the thymus difficult. Thus, the thy-box technique was developed by Paladini et al. [2], in which the internal mammary arteries located lateral to the thymus were used to demarcate the lateral thymic borders. In the same way, the anteroposterior borders of the thymus were also demarcated between the sternum and the brachiocephalic artery. Applying this technique, the thymus could be identified without much difficulty during the anomaly scan. The size of the thymus varies in conditions like DiGeorge syndrome, Trisomy 18 and 21, congenital heart disease, chondrodysplasia, intrauterine growth restriction, pre-eclampsia, and chorioamnionitis. Intrauterine diagnosis of hypoplastic or absent thymus is important to diagnose and prognosticate in such conditions.

The aim of the present study was to create a nomogram of fetal thymus for Indian population using the thy-box technique.

Materials and Methods

This is a retrospective study that included 723 singleton pregnancies referred for anomaly scan. The study was conducted at a Fetal Medicine center in South India. The thymus was localized in the axial plane of the upper mediastinum of the fetus. At first, the internal mammary arteries that course laterally to the thymus were located by using color or power Doppler ultrasonography with a low pulse repetition frequency these vessels. This was used to identify its lateral borders as described by Paladini et al. [2]. The thy-box, was thereby readily displayed in the fetus. The anteroposterior and transverse diameters of the thymus using thy-box were measured and recorded. A comparison was also made between thymic measurements obtained in the present study with that of study by Munoz Chapuli et al. [3] using un-paired T test (Tables 3, 4).

The sonographic examination was conducted by a Fetal Medicine Foundation (FMF) accredited Fetal Medicine Consultant, transabdominally by Voluson E6 (GE Healthcare, Kretztechnik, Zipf, Austria). Patients were informed of the study and an informed consent form was signed prior to examination.

Results

A total of 723 singleton pregnancies were studied. The thymus was identified using the thy-box technique in these cases. Doppler was required for clear visualization. The size of the thymus was observed to vary with the gestational age. Thymus transverse diameter as a function of gestational age was expressed by the regression equation: Transverse diameter (in mm) = 0.08115 × Gestational age − 0.5366 [R square = 0.98 and $p < 0.0001$]. Similarly, the anteroposterior diameter (AP) as a function of gestational age was expressed by the equation: AP (in mm) = 0.0449 × Gestational age − 0.2759 [R square^2 = 0.9686 and $p < 0.0001$]. The mean, standard deviation and 95% confidence interval for the thymus measurements corresponding to each gestational age were also calculated and tabulated (Tables 1, 2). Linear regression model was used and data was analyzed using IBM SPSS version 22 software (Armonk, NY).

A comparison made between thymic measurements obtained in the present study with that Munoz Chapuli et al. showed that the difference was found to be statistically insignificant ($p = 0.82$ for the transverse diameter and $p = 0.06$ for the antero-posterior diameter) i.e. no significant difference was found to exist between the two sets of measurements.

Table 1 Anteroposterior diameter/width of thymus according to gestation age (GA)

GA in weeks	No. of cases	Mean	SD	95% CI
16 to 16w6d	4	0.44	0.17	0.17–0.72
17 to 17w6d	3	0.50	0.16	0.10–0.90
18 to 18w6d	3	0.56	0.05	0.42–0.71
19 to 19w6d	19	0.56	0.12	0.50–0.62
20 to 20w6d	164	0.62	0.14	0.60–0.65
21 to 21w6d	274	0.66	0.14	0.64–0.67
22 to 22w6d	169	0.71	0.16	0.69–0.74
23 to 23w6d	56	0.74	0.15	0.70–0.78
24 to 24w6d	22	0.77	0.14	0.71–0.83
25 to 25w6d	9	0.90	0.12	0.80–1.00

Table 2 Transverse diameter/length of thymus according to gestation age (GA)

GA in weeks	No. of cases	Mean	SD	95% CI
16 to 16w6d	4	0.79	0.28	0.33–1.25
17 to 17w6d	3	0.80	0.10	0.55–1.04
18 to 18w6d	3	0.93	0.05	0.78–1.07
19 to 19w6d	19	1.00	0.10	0.95–1.05
20 to 20w6d	164	1.10	0.17	1.07–1.12
21 to 21w6d	274	1.18	0.28	1.15–1.22
22 to 22w6d	169	1.26	0.18	1.23–1.28
23 to 23w6d	56	1.32	0.19	1.27–1.38
24 to 24w6d	22	1.36	0.15	1.29–1.43
25 to 25w6d	9	1.53	0.14	1.43–1.64

Discussion

Thymus develops from third pharyngeal pouch at about sixth embryonic week of gestation. In the developing embryo, neural crest cells migrate into pharyngeal arches. Mesenchymal cells derived from neural crest give rise to smooth muscles of the conotruncal vessels and connective tissue of thymus and also parathyroid gland. So, the genetic and environmental factors affecting the pharyngeal arches will simultaneously affect both thymus and heart [4]. DiGeorge anomaly, the velocardiofacial syndrome (Shprintzen syndrome), the conotruncal anomaly face syndrome, Cayler syndrome, Opitz-GBBB syndrome and CHARGE (Coloboma (eye), Heart anomaly, Atresia (choanal), Retardation (mental and growth), Genital anomaly, Ear anomaly) syndrome are associated with Chr 22q11.2 microdeletion. A heterozygous microdeletion of 30–50 genes in this is responsible for inhibited or arrested migration of neural crest cells into the pharyngeal pouches that leads to thymic hypoplasia/aplasia which is found in 31% of cases with 22q11 microdeletion [5].

The main function of the thymus is the provision of immune support, even before the birth of the fetus [1]. Recent reports have also shown that a relationship between the size of the thymus, and percentage of T-cells in cord blood, are very promising, pointing out to the emergence of newer tools for the assessment of 'immune potential' of the fetus and newborn [6].

It is important to gain insight into the normal measurements of the fetal thymus because of the various congenital anomalies and genetic defects associated with its absence or hypoplasia. Few other anomalies that have been associated with thymus abnormalities include Trisomy 18 and 21 [7], congenital heart defects [8, 9], chondrodysplasia and chorioamnionitis [10]. In addition to this, identification of an absent or hypoplastic thymus gland during the anomaly scan seems to be particularly important as this would hint the sonologist to simultaneously identify and look closely for other existing fetal defects associated with the corresponding congenital anomaly. In this regard, the anomaly scan seems to be the right time to visualize and measure the thymus gland in fetuses. Further, if any discrepancy is noted in the size of the thymus gland in relation to gestational age during the anomaly scan, a repeat examination during the third trimester is advisable. This is because an involution in the size of fetal thymus is associated with maternal complications of pregnancy such as intrauterine infection [11], premature rupture of membranes [12], and even the potential occurrence of clinical pre-eclampsia [13].

We found that the fetal thymus can be successfully visualized during the performance of the anomaly scan from 16 to 25 weeks gestation. Various methods of thymus assessment include the use of volume contrast imaging [14], two-dimensional (2-D) and three-dimensional (3-D) ultrasound [4], magnetic resonance imaging (MRI) [15] and the three-vessel view [16]. The visualization of the thymus using the three-vessel view of the upper mediastinum is not always straightforward and is difficult as the gestation age progresses. In fetuses with cardiac malformation, the outline of the vessels is distorted. Furthermore, the demarcation line present between the borders of the thymus and the more echogenic lungs, is not that clear during the first and early second trimesters. Maternal body habitus is another factor that result in decreased resolution and thereby, difficulty is differentiating the outline of the thymus. With advancing gestation age, there is impairment of the real contours of the gland and thus measurements are not reliable. Moreover, certain maternal factors such as preterm premature rupture of membranes [12] and vitamin D deficiency [17] are associated with decreased size of the thymus gland during the third trimester. Hence, in order to assess the size of the thymus gland with ease, the thy-box technique was proposed by Paladini [2].

Thy-box boundaries are internal mammary arteries on both sides, in the front by sternal plate and on the back by section slightly above three vessels trachea view. Three-vessel trachea view is almost routinely used in cardiac evaluation universally. So, this will not significantly increase the time taken for visualization of thymus. Paladini also suggested that accurate measurements would be obtained after 16 weeks gestation, and prior to third trimester. We have adopted this technique in present study to obtain reliable results.

According to Paladini et al., in case of thymus aplasia, the ductal and aortic arches are displaced anteriorly behind the sternal plate. This makes it difficult to trace the course of the internal mammary arteries. However, in cases of thymus hypoplasia, these vessels converge anteriorly loosing their normal parallel course [2]. The parallel and convergent course of the internal mammary arteries, in normal and hypoplastic fetal thymus respectively, has been illustrated using line diagram in Figs. 1 and 2.

In the present study, we found that the fetal thymus can be successfully visualized using thy- box during this period. The antero-posterior and lateral measurements taken were used to construct a nomogram. A study by Weissman-Brenner et al. [18] has established that it is feasible to visualize and accurately measure the fetal thymus as early as 13 weeks age of gestation. A normal percentile of thymus measurements for this gestation age has also been constructed. Although visualization at this stage has its own benefits, we suggest that identification of the thymus gland be also included as a routine parameter during the performance of the anomaly scan. With this in mind, a

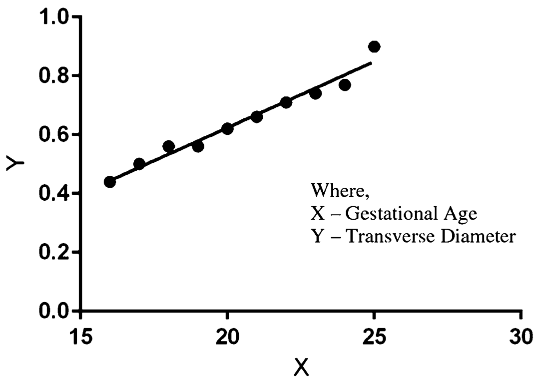


Fig. 1 Graph showing length (transverse diameter) of fetal thymus according to gestation age, where X, gestational age and Y, transverse diameter

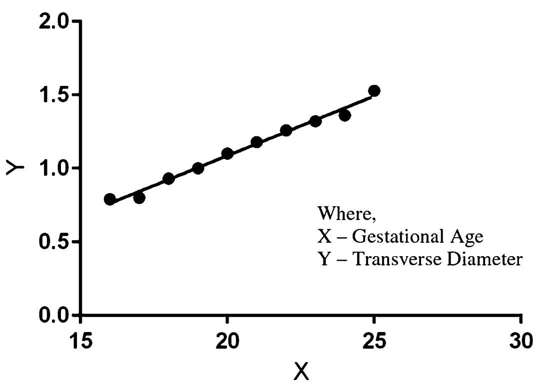


Fig. 2 Graph showing width (antero-posterior diameter) of fetal thymus according to gestation age, where X, gestational age and Y, transverse diameter

nomogram has been constructed (Tables 1, 2) to define the normal thymus measurements in fetuses during the recommended 16–25 weeks gestation. The mean and standard deviations corresponding to each gestational age were also calculated and recorded in the table.

Study published by Cho et al. [1] studied thymic transverse diameter with respect to gestational age, using

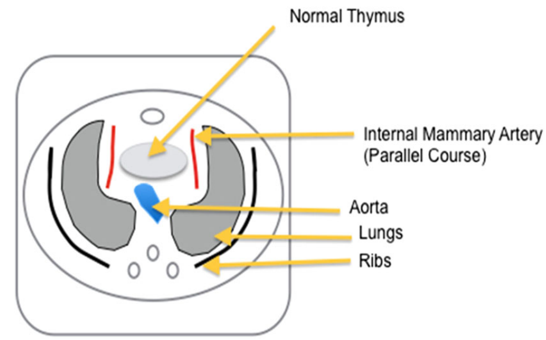


Fig. 3 Line diagram of normal thymus

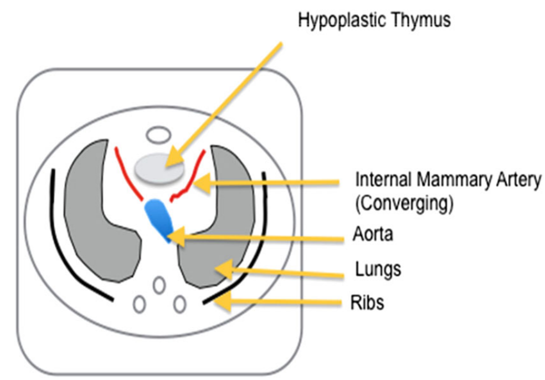


Fig. 4 Line diagram showing Hypoplastic Thymus

the three-vessel view. Moreover, another study by Felker et al. [16] assessed the antero-posterior diameter of the thymus measured in the mid line at the sternum. According to the study, thymic AP diameters ranged from 2 mm at 14 weeks to 20.8 mm at term [16].

A study by Munoz Chapuli et al. [3] used thy-box technique to derive the normative measurements of the thymus in Spanish population. The measurement of the present study have been compared to Chapuli et al.'s study (Tables 3, 4). The means of both the studies were analyzed using un-paired *T*-test. The difference was found to be

Table 3 Comparison of transverse diameters

GA	16	17	18	19	20	21	22	23	24	25
Present study	7.9	8.0	9.3	10.0	11.0	11.8	12.6	13.2	13.6	15.3
Munoz et al.	7.02	7.87	8.71	9.56	10.4	11.24	12.09	12.93	13.78	14.62

$p = 0.82$ (no significant difference was found between the two measurements)

Table 4 Comparison of AP diameters

GA	16	17	18	19	20	21	22	23	24	25
Present study	4.4	5.0	5.6	5.6	6.2	6.6	7.1	7.4	7.7	9.0
Munoz et al.	3.84	4.16	4.48	4.79	5.11	5.43	5.74	6.06	6.38	6.69

$p = 0.06$ (no significant difference was found between the two measurements) statistically insignificant ($p = 0.82$ for the transverse diameter and $p = 0.06$ for the antero-posterior diameter). This shows that there is no significant difference between the thymus measurements of two populations. There is also linear increase in the size of both antero-posterior and transverse diameters of thymus as the gestational age advances as shown in graphs (Figs. 3, 4).

In conclusion, evaluation of the fetal thymus may be added as a routine parameter during the performance of the second trimester anomaly scan using thy-box which is an easy-to-use, quick and reliable technique. Visualization of normal thymus as a routine will enhance our skill to identify thymus when it is abnormal. This in turn would enable simultaneous identification and confirmation of suspected congenital, chromosomal or cardiac anomalies in fetuses. Also, if there were nomograms for each gestational age, it would enable prompt identification of any deviation from the normal. Further studies such as these from other ethnicities and involving larger populations would help to standardize this even more.

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