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An enlarged fetal thymus may be the initial response to intrauterine inflammation in pregnant women at risk for preterm birth

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SUMMARY

OBJECTIVE: Fetal thymus involvement in prematurity has been studied, and this study aimed to evaluate its relationship with short cervix and amniotic fluid sludge in the second trimester of pregnancy.

METHODS: In this prospective cross-sectional study, 79 pregnant women (19+0 to 24+6 weeks) were included, and cervical length and the presence or absence of amniotic fluid sludge were evaluated. In the three-vessel view of the fetal thorax, the thymus was identified, and its perimeter and transverse diameter were measured and transformed to a zeta score based on gestational age.

RESULTS: Data from 22 women with short cervix (<25 mm) and 57 patients with normal cervix (≥25 mm) were analyzed. The transverse diameter of the fetal thymus was significantly greater in the short cervix group compared to that of the normal cervix group (z-score 2.708 vs. -0.043, p=0.003). There were no significant differences in the perimeter (z-score -0.039 vs. -0.071, p=0.890) or the transverse diameter (z-score 1.297 vs. -0.004, p=0.091) of the fetal thymus associated with the presence (n=21) or absence of sludge (n=58).

CONCLUSION: A short cervix is associated with an increased transverse diameter of the fetal thymus during the second trimester of gestation.

KEYWORDS: Thymus gland. Fetus. Premature birth. Cervix uteri.

INTRODUCTION

Prematurity is a global concern because it leads to high rates of short- and long-term disability and morbidity. In 2014, ~15 million babies were estimated to have been born at <37 weeks of gestation, and this statistics is probably underestimated[1].

Spontaneous preterm birth is considered a syndrome, and the multifactorial aspects involved, such as infection and inflammation, contribute to the difficulty in its prediction and prevention. When clinical chorioamnionitis is present, the diagnosis is clear. However, most cases of subclinical chorioamnionitis are diagnosed only by histological placental examination after premature birth or late miscarriage[2].

The presence of intraamniotic infection or microbial invasion of the amniotic cavity (MIAC) and the presence of pro-inflammatory cytokines in the amniotic fluid perhaps exist in most cases in the long preclinical stage of chorioamnionitis[2]. Some researchers have reported the ultrasonographic image of amniotic fluid sludge (AFS) as a signal of intra-amniotic microbial invasion and an independent risk factor for prematurity[3].

Fetal inflammatory response syndrome (FIRS) represents the involvement of the fetus in this infectious/inflammatory process[4]. Many fetal organs may undergo modifications, particularly organs related to immunity, such as the thymus, which develops early in gestation in humans. The fetal thymus can be visualized by ultrasound since the first trimester, and its size is associated with intrauterine infection in cases of preterm premature rupture of membranes (PROM) in the third trimester. Preterm labor with intact membranes and histological findings of funisitis were associated with a small fetal thymus[5].

Currently, the main strategy to prevent preterm birth is based on ultrasonographic evaluation of cervical length during the second trimester of pregnancy. It is known that the shorter the cervix, the higher the risk of prematurity, and intra-amniotic inflammation/infection has also been identified in these patients[6].

Traditionally, sonographic markers of preterm birth have primarily focused on maternal signs, and fetal involvement in response to the intrauterine infection/inflammation process is less investigated. This study aimed to evaluate fetal thymus size during the second trimester of pregnancy and determine its association with cervical length and the presence of AFS.

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METHODS

A total of 84 pregnant women referred to undergo ultrasound examination were invited to participate in a prospective cross-sectional study involving sonographic measurements of fetal thymus size and preterm birth predictors. Women with low- and high-risk pregnancies were included for ultrasound exams. The study was approved by the Local Ethics Committee, and written informed consent was obtained from each participant.

The inclusion criteria were as follows: gestational age from 19+0 to 24+6 weeks, singleton pregnancy, absence of malformations, absence of signs of PROM, and estimated fetal weight between the 10th and 90th centiles[7]. We analyzed the data of 79 patients and excluded 1 HIV-positive patient, 1 patient undergoing immunosuppressive therapy for kidney transplant, and 3 patients whose neonatal data were unavailable. Other exclusion criteria were suspected or confirmed congenital infections and the presence of malformations at birth.

Gestational age was estimated from the last menstrual period and confirmed by first-trimester ultrasound. The images were obtained using a Voluson 730 Expert (General Electric Medical Systems, Zipf, Austria) and Accuvix V20 (Samsung Corp., Seoul, South Korea). Fetal thymus evaluation was not used for clinical decisions. Patients with short cervixes and AFS received vaginal progesterone and/or cervical pessary and/or antibiotics, in accordance with local protocols.

Transvaginal ultrasound was used to measure the cervical length. Each pregnant woman with an empty bladder was subjected to this examination. A magnified image of the sagittal view of the cervix, including the cervical canal, internal and external os, and without excessive pressure applied over the transducer, was used to obtain the cervical length, excluding the lower uterine segment. Subsequently, three measurements of the cervical length were performed over a period of 3–5 min, and the lowest value was used for further evaluation. Short cervix was defined as a cervical length of <25 mm.

The transvaginal ultrasounds were carefully examined and actively searched for the presence of particulate material near the cervix. If this material was free-floating (confirmed by applying gentle pressure over the anterior uterine wall), it was defined as AFS.

The fetal thymus size was measured in the three-vessel view of the thorax, between the lungs, and in front of the vessels. The perimeter and transverse diameter (TD) of this organ were obtained, as shown in Figure 1. The maximum TD of the thymus was measured perpendicular to the line connecting the sternum and the spine, and the perimeter of the thymus is the line traced around the organ. The measurements were performed three times, and the mean values were taken and then transformed into the Z-scores, which are the standard deviations (SD) from the mean according to gestational age at measurement, based on the normative references[8]. All thymus measurements were performed by the same examiner (TENKH).

Fetal biometry, represented by head circumference (HC), biparietal diameter (BPD), occipito-frontal diameter, abdominal circumference, femur length (FL), and humerus length (HL), was also evaluated and used for fetal weight estimation. An assessment of the P/HC, P/FL, P/HL, TD/HC, TD/FL, and TD/HL ratios was performed.

Statistical analyses were performed using MedCalc® Statistical Software version 19.5.3 (MedCalc Software Ltd., Ostend, Belgium). Comparisons among groups were performed using the chi-square or Fisher's exact test for categorical variables, and the Mann-Whitney U test and Student's t-test for continuous variables. $p<0.05$ was considered statistically significant.

RESULTS

Of the 84 patients invited to participate in the study, 5 were excluded, and data from 79 patients were analyzed. In all, 57 participants presented with normal cervixes (≥25 mm) and 22 with short cervixes (<25 mm). AFS was not observed in 58 women but was present in 21 patients.

Table 1 shows that the between-group characteristics of women with normal and short cervical lengths are similar regarding maternal age, white skin color, parity, BMI, smoking, and high-risk pregnancy. Concerning the AFS, there was a statistically significant difference in maternal age and parity; however, this did not appear to impact the results.

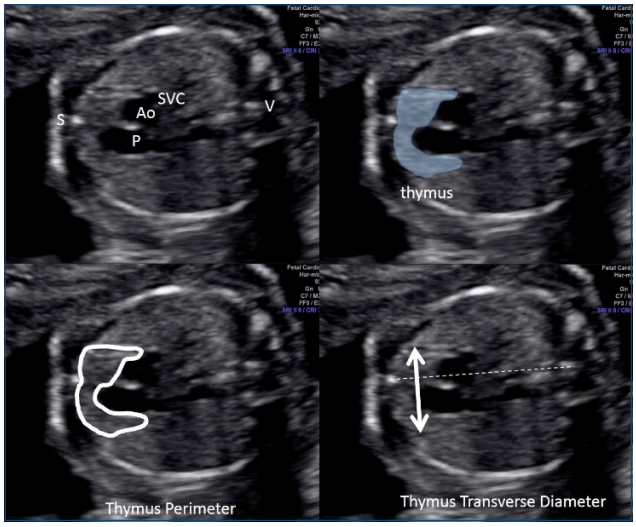

Figure 1. Sonographic fetal thymus measurement. S: sternum; P: pulmonary artery; Ao: aorta artery; SVC: superior vena cava; V: vertebra.

Of the 57 women with cervical length ≥25 mm, 16/57 (28%) presented with AFS, and this ultrasonographic sign was detected in 5/22 (22.7%) women with short cervixes. Of the 79 cases, 10 preterm births occurred (8 spontaneous preterm births, 10.3%). Table 1 also shows that women with short cervixes had births at lower gestational ages, compared to women with normal cervixes.

Table 2 presents the results of thymus measurements (TD and perimeter) and the presence of a short cervix and AFS. In women with a short cervix (<25 mm), the TD of the thymus was enlarged ($p<0.05$), with statistically significant higher TD/HC, TD/FL, and TD/HL ratios. Patients with AFS presented with increased thymus size; however, it was statistically significant only for the TD/HC and TD/FL ratios (Table 2).

DISCUSSION

In pregnant women at risk for preterm birth, if they have short cervixes, the TD of the fetal thymus is increased and is perhaps the initial response to intrauterine inflammation. A short cervix is related to MIAC, and it is believed that patients with this condition are more frequently subjected to inflammatory processes leading to fetal thymus involution, as reported in the literature[5,9]. Some studies have demonstrated that infectious and inflammatory processes involved in prematurity are responsible for FIRS, which is characterized by elevated interleukin 6 (IL-6) levels (>11 pg/mL) in fetal serum[4]. Following the insult, the thymus and adrenals are the first organs to undergo alterations. The endogenous corticosteroids increased by the activation of

Table 1. Population characterization according to the groups.

Characteristics	NC (n=57)	SC (n=22)	p-value	AAFS (n=58)	PAFS (n=21)	p-value
Maternal age, years	28.8 (6.1)	26.2 (8.8)	0.136	27.0 (7.2)	31.1 (5.6)	0.020
White	28 (49.1%)	8 (36.4%)	0.311	23 (39.7%)	13 (61.9%)	0.080
Nulliparous	24 (42.1%)	11 (50.0%)	0.970	31 (53.4%)	4 (19.1%)	0.02
BMI (kg/m^2)	26.3 (4.5)	26.7 (6.0)	0.739	26.3 (4.6)	26.6 (5.9)	0.781
Tobacco use	9 (15.8%)	3 (13.6%)	0.812	9 (15.5%)	3 (14.3%)	0.894
High-risk pregnancy	11 (19.3%)	2 (9.1%)	0.276	10 (17.2%)	3 (14.3%)	0.756
GA, weeks	22.2 (1.3)	22.7 (1.5)	0.204	22.6 (1.3)	21.8 (1.4)	0.035
CL, mm	33.0 (31.4–34.7)	21.1 (17.0–22.4)	<0.001	29.9 (26.8–31.9)	33.0 (27.0–36.5)	0.220
CL<25 mm	–	–	–	16 (27.6%)	6 (28.6%)	0.931
Absence of cervical gland area	0 (0.0%)	1 (4.5%)	0.279	1 (1.7%)	0 (0.0%)	1.0
Funneling presence	0 (0.0%)	8 (36.4%)	<0.001	5 (8.6%)	3 (14.3%)	0.432
Amniotic fluid sludge	1 (1.8%)	6 (27.3%)	0.932	–	–	–

Mean (SD), median (95%CI), n (%). NC: normal cervix; SC: short cervix; AAFS: absence of amniotic fluid sludge; PAFS: presence of amniotic fluid sludge; BMI: body mass index; GA: gestational age; CL: cervical length.

Table 2. Fetal thymus measurements according to groups short cervix versus normal cervix and absence of amniotic fluid sludge versus presence of amniotic fluid sludge.

	NC (n=57)	SC (n=22)	p-value	AAFS (n=58)	PAFS (n=21)	p-value
Perimeter						
P (Z-score)	-0.090 (-0.351 to -0.004)	0.056 (-0.119 to 0.248)	0.199	-0.071 (-0.268 to -0.058)	-0.039 (-0.371 to 0.254)	0.890
P/CC	0.235 (0.226 to 0.244)	0.244 (0.238 to 0.255)	0.238	0.239 (0.227 to 0.245)	0.235 (0.226 to 0.255)	0.965
P/FL	1.251 (1.178 to 1.314)	1.286 (1.180 to 1.334)	0.956	1.267 (1.183 to 1.313)	1.261 (1.136 to 1.386)	0.773
P/HL	1.322 (1.229 to 1.371)	1.371 (1.271 to 1.410)	0.638	1.334 (1.253 to 1.377)	1.325 (1.185 to 1.416)	0.641
Transverse diameter						
TD (Z-score)	-0.043 (-0.194 to 0.194)	2.708 (0.024 to 3.481)	0.003	-0.004 (-0.114 to 0.255)	1.297 (-0.135 to 3.060)	0.091
TD/CC	0.087 (0.084 to 0.092)	0.136 (0.089 to 0.155)	0.005	0.089 (0.084 to 0.094)	0.114 (0.085 to 0.155)	0.042
TD/FL	0.472 (0.444 to 0.501)	0.691 (0.459 to 0.795)	0.021	0.474 (0.446 to 0.507)	0.660 (0.443 to 0.834)	0.042
TD/HL	0.485 (0.469 to 0.539)	0.762 (0.505 to 0.862)	0.024	0.500 (0.475 to 0.543)	0.666 (0.464 to 0.865)	0.126

Median (95%CI). NC: normal cervix; SC: short cervix; AAFS: absence of amniotic fluid sludge; PAFS: presence of amniotic fluid sludge; P: perimeter; CC: cephalic circumference; FL: femur length; HL: humerus length.

the hypothalamus-pituitary-adrenal axis led to thymus involution, possibly due to lymphocyte depletion in the thymic cortex and medulla by apoptosis of the lymphoid tissue[9].

Based on these findings, and after reference ranges of fetal thymus size were established[8,10,11], many researchers have applied thymus involution as a marker in histological chorioamnionitis[5,12-15], in cases of preterm birth and PROM, since its sensitivity and specificity are higher than the classic markers, such as erythrocyte sedimentation rate and C-reactive protein levels[14]. However, in the present study, contrary to expectations, in patients with short cervixes, the fetal thymus was larger. In the short cervix group, the TD of the thymus was increased compared to that in women with normal cervical length. Women with AFS also had a tendency to have a larger thymus, and the TD/HC and TD/FL ratios were significantly greater in this group.

It is important to note that the majority of previous studies analyzed the fetal thymus in the third trimester of pregnancy and in cases of preterm PROM or preterm labor[5,12-14,16,17]. However, the present study was performed in the second trimester of pregnancy, before the occurrence of clinical manifestations, except for shrinkage of the cervix.

There are few studies on sonographic measurement of the fetal thymus in the first and second trimesters, and some studies have similar results as ours. Borgelt et al.[18] measured the anteroposterior diameter of the fetal thymus in the first trimester of pregnancy and found a positive relationship between fetal thymus and preterm birth ($p<0.001$). Brandt et al.[19] investigated the fetal thymus in pregnant women during the second trimester to predict prematurity. They did not observe a statistically significant association between small thymus and preterm birth. Nevertheless, the patients presenting with a smaller fetal thymus were more likely to have a greater cervical length. Their study focused on the involuted thymus, and they may not have observed an association with increased fetal thymus size in the cases of short cervix and preterm birth.

The thymus may be affected by various changes in the intrauterine environment. As an important organ in the human immune system, chronic inflammation associated with certain diseases and conditions may influence the size of this organ. One study analyzed HIV-exposed fetuses in the second trimester and observed fetal thymus enlargement[20]. Interestingly, the mean gestational age at examination was 21 weeks, similar to the present study. The authors also observed that HIV-exposed uninfected infants, when older, had reduced thymus sizes and lower CD4+ and CD8+ cell counts.

A large thymus could be caused by true hyperplasia (following recent stress, such as irradiation, corticosteroid therapy, chemotherapy, and infection) and lymphoid hyperplasia (usually related to immunologically mediated diseases, such as myasthenia gravis, Graves' disease, and systemic lupus erythematosus[21]). In true hyperplasia, the thymus becomes atrophic but can grow even larger after such stress – rebound hyperplasia[21]. However, rebound hyperplasia does not explain our findings because the evaluation of the fetuses occurred in the second trimester of pregnancy, during the initial phase of intraamniotic infection/inflammation.

In experimental studies, chorioamnionitis was induced by intraamniotic injection of lipopolysaccharide, and some authors described an increase in the size of the posterior mediastinal lymph nodes in sheep[22]. CD3, CD4, and CD8 T-cell counts increased 2–3 days after exposure. In Rhesus macaques infected with *Ureaplasma parvum* and *Mycoplasma hominis*, the fetal spleen had diffused hyperplasia, with an increase in T-cells after 15 days of exposure, the opposite of the splenic depletion reported in humans[23].

This splenic hyperplasia is a possible hypothesis to explain the thymus enlargement observed in the present study, as both the thymus and spleen are lymphoid organs. In this early period of fetal development, the regulatory mechanisms of the lymphoid response are poorly understood and may differ from the findings during the third trimester of pregnancy. The hypothesis of initial thymus edema is a more reasonable explanation for our findings than rebound hyperplasia[24].

Limitations of our study include the small sample size as well as interventions that could have influenced the outcomes. Furthermore, during the second trimester, the echogenicity of the thymus and lungs is very similar, impairing adequate measures in all cases. As suggested previously[16], we considered the TD of the thymus as the best parameter to evaluate this organ. The strength of this study was the performance of all fetal measurements by a single examiner, removing inter-rater variability.

CONCLUSION

A short cervix is associated with an increased TD diameter of the fetal thymus during the second trimester of gestation and can be the first signal of intra-amniotic inflammation and infection. Not all patients with short cervixes will experience spontaneous preterm birth; however, the association of short cervical length and an enlarged thymus could more accurately predict prematurity. Nevertheless, more studies are needed to elucidate the relationship between these sonographic prematurity markers and fetal thymus size during the second trimester of pregnancy.

AUTHORS' CONTRIBUTIONS

TENKH: Data curation, Visualization, Writing – original draft. **ARH:** Investigation, Visualization. **EAJ:** Visualization, Writing – review & editing. **MSF:** Formal Analysis, Visualization. **SGPS:** Methodology, Visualization. **TMH:** Investigation, Visualization. **RMYN:** Conceptualization, Validation, Visualization. **AFM:** Project administration, Supervision, Visualization.

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