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Assessment of fetal thymus size and BMI in pregnant women with diabetes

Katarzyna Zych-Krekora[1], Mariusz Grzesiak[1], Piotr Kaczmarek[2], Sharon Perlman[3], Ron Bardin[3], Yinon Gilboa[3], Michal Krekora[1]

[1]Department of Perinatology, Obstetrics and Gynaecology, Polish Mother's Memorial Hospital Research Institute in Lodz, Poland
[2]Department of Gynaecology Procreation and Therapy of Fetus, Polish Mother's Memorial Hospital Research Institute in Lodz, Poland
[3]Ultrasound Unit, The Helen Schneider Women's Hospital, Rabin Medical Center, Petach Tikva, Israel

ABSTRACT

Objectives: The purpose of this study is to demonstrate whether diabetes during pregnancy affects the development of the fetal immune system. The background: evaluation of potential complications in diabetic pregnancy. The objective is evaluation of the significance of a new ultrasound method of thymus size in pregnancies complicated by diabetes.

Material and methods: The analysis was performed with the use of IBM SPSS Statistics 25.0 software. The Mann–Whitney U test was used for comparison of two groups, i.e., diabetic pregnancies and non-diabetic pregnancies, whereas Kruskal–Wallis H test was used to compare multiple groups. A linear regression model was used to determine the correlation between the type of diabetes and fetal thymus size as well as between maternal body mass index (BMI) and fetal thymus size. The significance level α was set at 0.05.

Results: A comparison between diabetic and non-diabetic pregnancies was made with the use of Kruskal–Wallis H test. The compared groups included women without gestational diabetes, with pre-gestational diabetes, gestational diabetes managed by diet and gestational diabetes treated with insulin and diet. The analysis revealed significant differences between the compared groups, $H(3) = 23.06$; $p < 0.001$; $\eta^2 = 0.04$. The additional post hoc Dunn's test with Bonferroni correction of the significance level was used to explore specific differences between group means. The results of this detailed analysis indicated that foetuses of diabetic mothers treated with diet had smaller thymus than foetuses of non-diabetic mothers ($p = 0.001$). Linear regression analysis was used to establish whether maternal BMI (defined as the body mass divided by the square of the body height and expressed in units of kg/m^2) affects fetal thymus size. The analysis found no correlation between maternal BMI divided into the following categories: 18.5–24.99 normal weight, 25–29.99 overweight, 30.00–34.99 obese class I, 35.00–39.99 obese class II and ≥ 40.00 very severely obese, and fetal thymus size, $b = -1.82$; $SE = 2.17$; $t = -0.84$; $p = 0.405$; $R^2 < 0.01$.

Conclusions: Thymus size is statistically smaller in foetuses of diabetic mothers when compared to healthy controls. Overweighted and obese pregnancy is not a factor affecting fetal thymus size.

Key words: fetal thymus; pregnancy; diabetes; obesity; ultrasound

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INTRODUCTION

In the era of an increase in incidence of autoimmune diseases and diseases of civilisation the value of ultrasound assessment of the fetal thymus still appears to be underestimated and should be included in the second trimester ultrasound protocol.

MATERIAL AND METHODS

The study group consisted of 63 pregnant women admitted to the Department of Perinatology, Obstetrics and Gynaecology at the Polish Mother's Memorial Research Institute in Lodz — tertiary referral centre.

Inclusion criteria included patients with pre-gestational diabetes (PGDM) — 11 pregnancies, gestational diabetes managed by diet (GDM1) — 23 pregnancies, and gestational diabetes treated with insulin and diet (GDM2) — 29 pregnancies. Exclusion criteria included pregnant women with gestational diabetes and history of other comorbidities as well as multiple pregnancies, fetal developmental abnormalities and genetic disorders. Each patient received a de-

Corresponding author:
Katarzyna Zych-Krekora
Polish Mother's Memorial Hospital Research Institute in Lodz, 281/289 Rzgowska St, 93-338 Lodz, Poland
e-mail: zychkrekora@gmail.com

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tailed fetal ultrasound performed by the team of ultrasound experts specialising in obstetric ultrasonography. Fetal thymus measurements were obtained between 14 + 5 and 40 + 0 weeks of gestational age. Transabdominal ultrasound was performed using volumetric probe at the level of the fetal sternum and great vessels [the three-vessel view] of the mediastinum with visible main pulmonary artery (MPA) and its branches, the right and left pulmonary arteries (RPA and LPA), aorta (Ao) and superior vena cava (SVC) (Fig. 1).

After the three-vessel view was clearly displayed we assessed longitudinal dimensions of the thymus (Fig. 2). It is the easiest method, reproducible and available for examination even in advanced pregnancy. The obtained measurements were juxtaposed with nomograms for thymus size in healthy foetuses whose mothers had no history of diabetes (the nomograms from 410 foetuses were designed and published by the team of ultrasound and echocardiography experts from the Department of Prenatal Cardiology at the Polish Mother's Memorial Research Institute in Lodz) [1]. Prior to ultrasound examination the participants were asked to complete a questionnaire regarding their body weight status before 10 weeks of gestational age, which allowed us to assess the baseline body mass index (BMI).

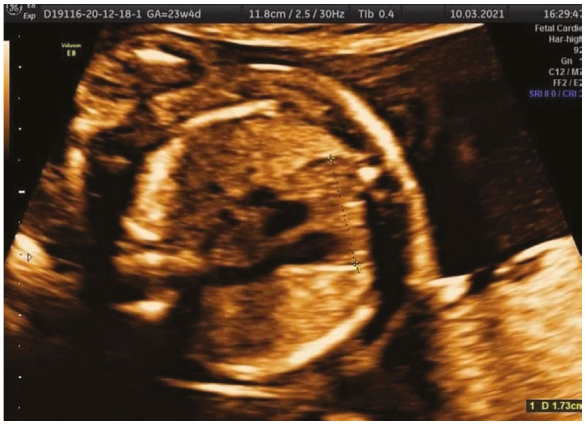

Figure 1. Longitudinal dimensions of the fetal thymus

RESULTS

The analysis was performed with the use of IBM SPSS Statistics 25.0 software. The Mann–Whitney *U* test was used for comparison of two groups, *i.e.*, diabetic pregnancies and non-diabetic pregnancies, whereas Kruskal–Wallis *H* test was used to compare multiple groups. A linear regression model was used to determine the correlation between the type of diabetes and fetal thymus size as well as between maternal BMI and fetal thymus size. The significance level α was set at 0.05.

Comparison of fetal thymus size in diabetic and non-diabetic pregnancies

A comparison between diabetic and non-diabetic pregnancies was made with the use of Kruskal–Wallis *H* test. The compared groups included women without gestational diabetes, with pre-gestational diabetes, gestational diabetes managed by diet and gestational diabetes treated with insulin and diet. The analysis revealed significant differences between the compared groups, H (3) = 23.06; $p < 0.001$; $\eta^2 = 0.04$. The additional post hoc Dunn's test with Bonferroni correction of the significance level was used to explore specific differences between group means. The results of this detailed analysis indicated that foetuses of diabetic mothers treated with diet had smaller thymus than foetuses of non-diabetic mothers ($p = 0.001$). No differences in thymus size were found between foetuses of non-diabetic mothers and mothers with pre-gestational diabetes ($p = 0.235$) and gestational diabetes treated with insulin and diet ($p = 0.065$); between foetuses of mothers with pre-gestational diabetes and gestational diabetes managed with diet ($p = 1.000$) and treated with insulin and diet ($p = 1.000$), as well as between foetuses of mothers with gestational diabetes treated with insulin and diet and treated with diet alone ($p = 1.000$). Descriptive statistics of thymus size in individual groups are presented in Table 1 and Figure 2.

The *U* Mann Whitney test was used to distinguish the differences between fetal thymus size in pregnant women with diabetes (pre-gestational or gestational) and without diabetes. The analysis indicated significant differences between the groups, $Z = -4.66$; $p < 0.001$; $r = 0.21$. Fetal thymus size in pregnant women with gestational diabetes ($M = 20.71$; $SD = 7.78$; $Me = 21.0$) was significantly smaller than that in non-diabetic women ($M = 27.01$; $SD = 9.29$; $Me = 27.0$) (Fig. 3 and 4).

Correlation between fetal thymus size and type of diabetes

Linear regression analysis was used to determine whether the type of diabetes (pre-gestational vs gestational) affects fetal thymus size. The analysis indicated no relationship between the type of diabetes and fetal thymus size, $b = 0.05$; $SE = 2.60$; $t = 0.02$; $p = 0.985$; $R^2 < 0.01$.

Correlation between maternal BMI and fetal thymus size

Linear regression analysis was used to establish whether maternal BMI (defined as the body mass divided by the square of the body height and expressed in units of kg/m^2) affects fetal thymus size. The analysis found no correlation between maternal BMI divided into the following categories: 18.5–24.99 normal weight, 25–29.99 over-

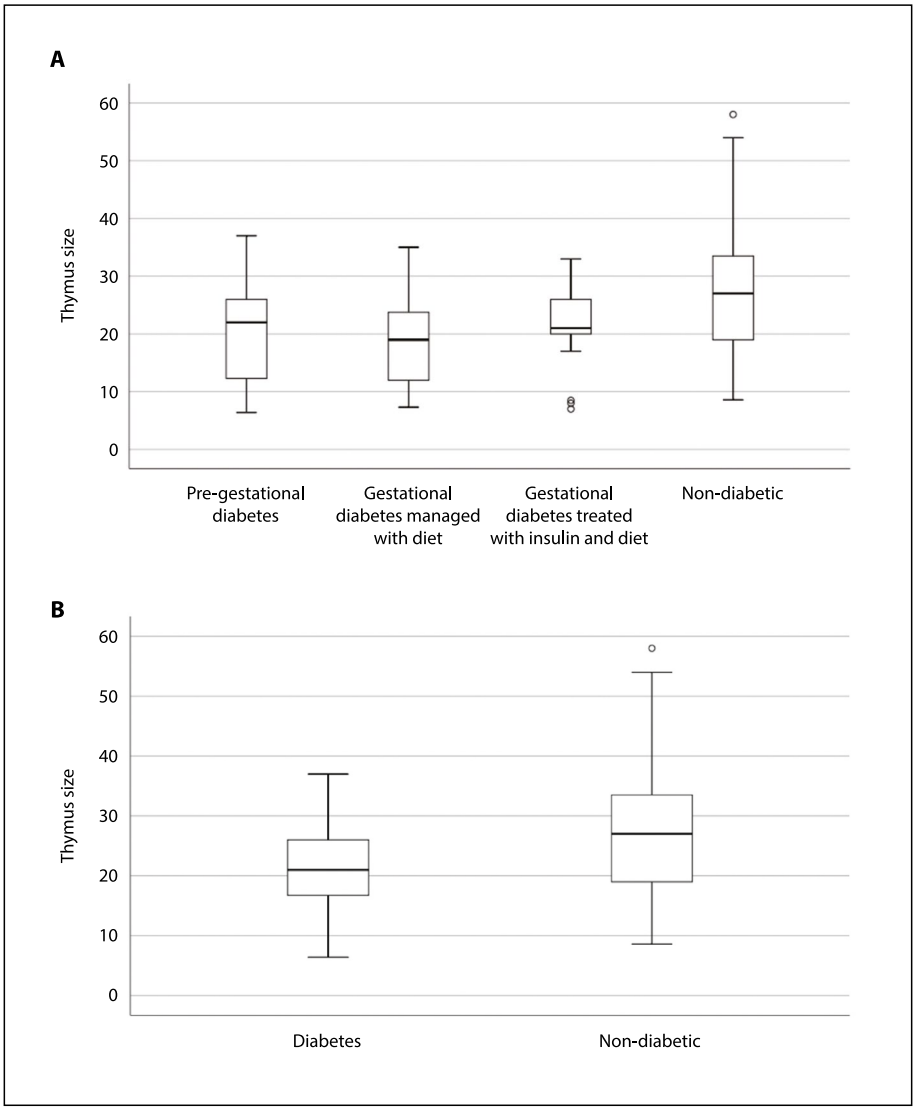

Figure 2 (A, B). Box plot depicting correlation between thymus size and presence/absence of diabetes

weight, 30.00–34.99 obese class I, 35.00–39.99 obese class II and ≥ 40.00 very severely obese, and fetal thymus size, $b = -1.82$; $SE = 2.17$; $t = -0.84$; $p = 0.405$; $R^2 < 0.01$.

A similar analysis was made for maternal BMI divided into two categories: normal body weight vs overweight, $b = -0.14$; SE = 0.85; $t = -0.16$; p = 0.870; $R^2 < 0.01$; and for raw score: $b = -0.03$; SE = 0.17; $t = -0.20$; p = 0.839; $R^2 < 0.01$ with the results indicating no correlation between maternal BMI and fetal thymus size.

DISCUSSION

Thymus is the primary organ of the lymphatic system which controls the development of the peripheral lymphoid tissues in the prenatal and pre-adolescence periods. At approx. 8 weeks of gestation T cells migrate to the thymus and interact with thymocytes ensuring their proper development [2]. A defect in hematopoietic stem cell migration may partially or completely compromise thymus function [3]. At the end of the perinatal period and immediately after birth the thymus is large in size. By the age of adolescence, it slowly begins to atrophy, however not entirely [1]. Thymus development is critical for the establishment of a normal immune system in a foetus, neonate and toddler. Ultrasound assessment of an absent or present thymus and its size has, to a certain extent, become an intermediate marker of genetic disorders such as 22q11 deletion syndrome [4, 5] or severe combined immunodeficiency (SCID) [6], which may have practical applications in neonatal assessment and eligibility for immunisation in infants. The underdeveloped thymus has been frequently described as one of the markers of congenital heart defects [7, 8], but also as a predictive factor for IUGR [9], premature birth, chorioamnionitis [10],

Table 1. Descriptive statistics of fetal thymus size in healthy pregnancies and pregnancies complicated by gestational diabetes

Group	N	M	SD	Me	Min	Max
Non-diabetic	410	27.01	9.29	27.00	8.60	58.00
PGDM	11	20.70	9.23	22.0	6.40	37.00
GDM1	23	19.07	8.21	19.00	7.30	35.00
GDM2	29	22.09	6.82	21.00	7.00	33.00

GDM1 — gestational diabetes type 1; GDM2 — gestational diabetes type 2; PGDM — pre-gestational diabetes mellitus; SD — standard deviation

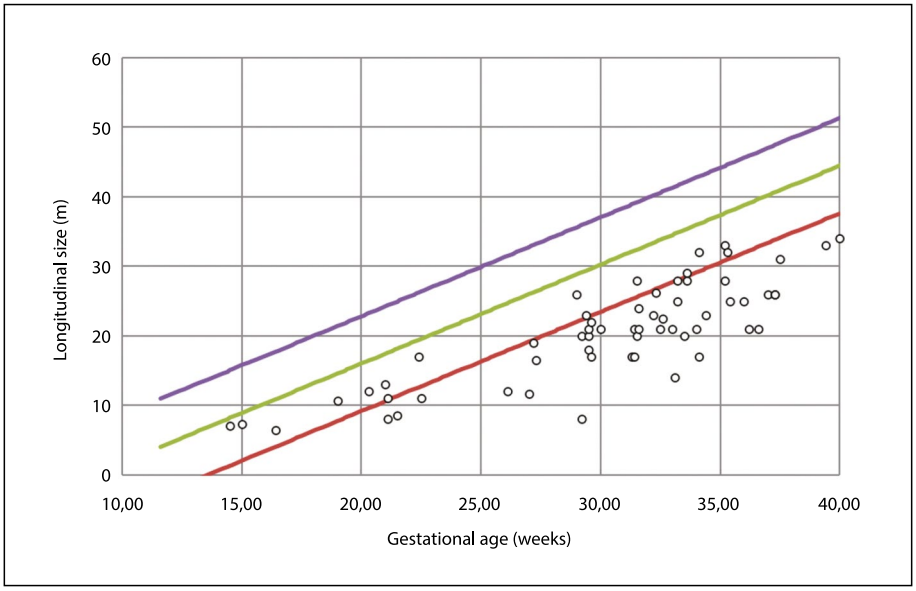

Figure 3. The results for diabetic women with nomogram for non-diabetic women. The results for women with diabetes are depicted below the mean score for women without diabetes

preeclampsia and even neonatal sepsis [11, 12]. Therefore, the size of fetal thymus may be considered as an important indicator of the fetal inflammatory response syndrome [13].

There is a correlation between fetal thymus size and abnormal glycaemia in pregnant women. After a series of steps which involve positive and negative selection, mature T cells with different cell surface markers TCR exit the thymus. Those naïve T cells are populating the periphery where they form a pool of effector cells and/or memory cells after their prior activation by the antigenic stimulus [8]. The main function of Tregs is to modulate other cells of the immune system. Insulin expression in the thymus is involved in regulating the negative selection of autoreactive T cells and in mediating the central immune tolerance towards pancreatic β-cells. The thymus is therefore not a typical endocrine gland, but an organ that connects the immune and neuroendocrine systems. Diemert et al. [14] investigated the association between fetal thymus growth, fetal weight and umbilical cord blood Tregs. The results showed an inverse correlation between fetal thymus size and umbilical cord blood Tregs indicating association between fetal growth, thymus development and immune status at birth. The above observations were confirmed by the ImmunDiabRisk study which aimed to investigate fetal thymus growth and maternal and fetal immune responses in pregnancies with and without type 1 diabetes. The results indicated higher numbers of FOXP3 Tregs, memory Tregs, erythrocytes, and lymphocytes in the cord blood from pregnant women with type 1 diabetes [15]. Those findings correspond with the results of our study which shows statistically significant correlation between maternal diabetes and reduced fetal thymus size. In the current medical literature, there is a limited number of publications on the impact of maternal diabetes on fetal immune system and thymus size. This issue has been addressed only in the recent years. Conclusions of a study similar to ours were published in 2017. The study retrospectively assessed 161 pregnancies with diabetes and 161 pregnancies without diabetes matched by gestational age (control group). Diabetic mothers were allocated to three different groups: 1. gestational diabetes managed with diet, 2. insulin-dependent gestational diabetes and 3. pre-gestational diabetes. In all three groups fetal thy-

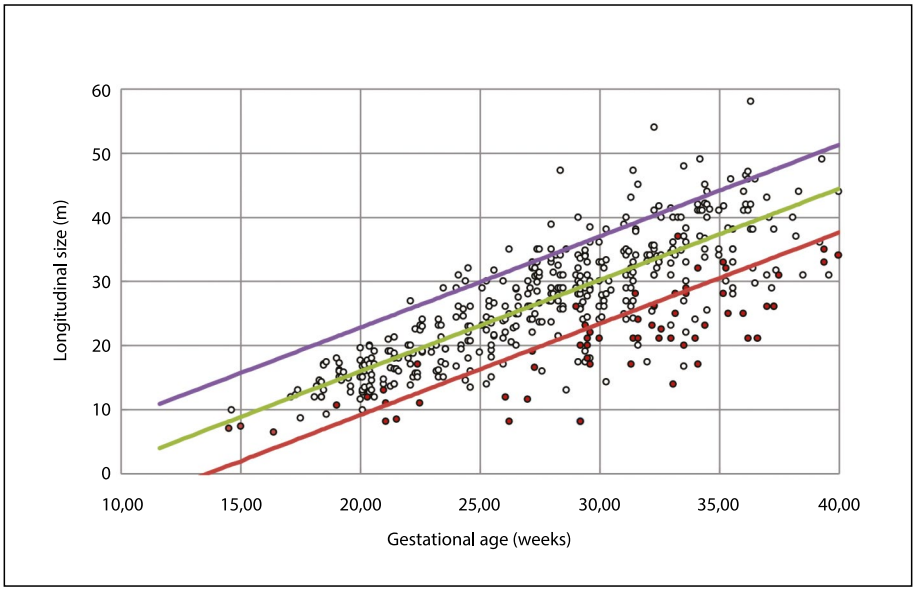

Figure 4. Graph showing the difference in thymus size between foetuses of healthy mothers (white dots) and foetuses of diabetic mothers (red dots)

mus size was smaller when compared to the control group ($p < 0.001$) [16]. Ghalandarpoor-Attar SN et al. [17] measured thymic–thoracic ratio which proved to be significantly lower in foetuses of diabetic mothers as compared to non-diabetic group ($p = 0.001$). The ratio remained significantly lower after subgrouping diabetic mothers to the following groups: pregestational diabetes, insulin-dependent gestational diabetes and gestational diabetes. The above results provide new information on the development of gestational diabetes which affects an estimated 15% of pregnant women worldwide. Aetiology and pathogenesis of the gestational diabetes are still not fully known [18].

Another factor investigated in this study was pre-pregnancy BMI and its correlation with fetal thymus size. The results did not indicate unequivocally the correlation between abnormal BMI ≥ 25 and fetal thymus size in diabetic pregnancies, and between normal BMI and fetal thymus size. However, due to a lack of similar research in medical literature it is difficult to relate to our findings. Further research is needed with larger participant group size of pregnant women with gestational diabetes (GDM). A study of 138 pregnant women without diabetes whose BMI was measured revealed that fetal thymus size in obese women was bigger than that in women with normal body weight [19].

It is also known that for every 1 kg/m^2 increase in BMI, the prevalence of gestational diabetes increases by 0.92% (95% CI 0.73 to 1.10) [20], and the pooled estimate of GDM risk in the underweight, overweight, and obese pregnant women is 0.68, 2.01, and 3.98 using the adjusted OR [21].

Gestational diabetes and abnormal body weight in women who are planning to become pregnant or are pregnant are only two of the discussed in this study factors affecting metabolic programming [22, 23]. Gestational diabetes (GDM) is currently the most common metabolic complication seen in pregnancy, which has a negative effect on structure and maturation of cells, tissues and organs in foetuses and neonates. Changes that occur as a result of metabolic programming in the preconception and prenatal periods can reinforce due to epigenetic regulation of gene expression. Early fetal programming is considered the key element in prevention of the diseases of civilization [24].

CONCLUSIONS

Thymus size is statistically smaller in foetuses of diabetic mothers when compared to healthy controls. Overweighted and obese pregnancy is not a factor affecting fetal thymus size. In the era of an increase in incidence of autoimmune diseases and diseases of civilisation the value of ultrasound assessment of the fetal thymus still appears to be underestimated and should be included in the second trimester ultrasound protocol.

Ethics approval and consent to participate

This study was approved by the ethics committee of Polish Mother's Memorial Hospital Research Institute in Lodz.

Human and animal rights

No animals were used in this research. All human research procedures were followed in accordance with the ethical standards of the committee responsible for human experimentation (institutional and national), and with the Helsinki Declaration of 1975, as revised in 2013.

Consent for publication

Informed consent was obtained from all participants.

Conflicts of interest

All authors declare no conflict of interest.

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