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Original Article

Longer anogenital distance in female fetus of diabetic and obese pregnant women

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ABSTRACT

Objective: Previous studies revealed that prenatal exposure to androgen excess such as polycystic ovary syndrome (PCOS) is associated with offspring's anogenital distance (AGD) length, and AGD is a biomarker of intrauterine androgen exposure. This study aims to investigate a possible relationship of fetal AGD with maternal diabetes and obesity, and to evaluate whether AGD predicts the fetal androgen exposure related to diabetes and obesity in female fetus. This study is the first to focus on the relationship between offspring's AGD and maternal diabetes and obesity.

Materials and methods: This is a prospective study investigating 218 pregnant women (125 in control group and 93 in study group). Fetal AGD was measured from the center of anus to the posterior convergence of the fourchette by ultrasound. Multivariate linear regression analysis was applied to assess the association of the fetal AGD length with maternal diabetes and obesity.

Results: The control patients had significantly shorter fetal AGD (mean:10.7 mm, $P < 0.001$) compared to diabetic, obese and diabetic obese patients (mean: 12.6 mm, 12.8 mm and 12.9 mm, respectively). The results of regression analysis showed that both maternal diabetes and obesity were significantly correlated with longer AGD in female fetus. The results confirmed also that offspring's AGD measurement in utero by ultrasound is feasible and reliable.

Conclusion: The study findings suggest that both maternal diabetes and obesity are associated with intrauterine androgenic milieu during pregnancy, and fetal AGD may be used as a biomarker to predict this effect. This may provide important advantages in terms of early detection of reproductive system abnormalities related to prenatal androgen exposure.

Introduction

The fetal reproductive system development is susceptible to the inappropriate levels of androgens in maternal circulation. Androgen or anti-androgen exposure causes structural and physiological consequences on the developing fetus [1,2]. There is an androgen-sensitive period in early fetal life, likely between 8 and 14 weeks, during which genital development is programmed and fetus is particularly vulnerable to endocrine disruption [3]. Any endocrine disruption that occurs during this period may have phenotypic consequences later in life [4].

Maternal hyperandrogenism such as congenital adrenal hyperplasia, placental aromatase deficiency, ovarian luteoma can cause varying degrees of virilization of female genitalia and lead to lifelong consequences on the reproductive system [2]. Previous studies provided evidence that even subtle changes in androgen level may have effects on fetal reproductive system development [5]. There is an association between prenatal androgen exposure and long-term health effects such as high AMH levels and increased antral follicles in adult ovaries, masculine behavior and preferences and PCOS [6,7].

Diabetes and obesity are two well-known interrelated chronic diseases that cause hyperandrogenism in women of reproductive age [8,9]. There is a strong correlation between increased body

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mass index (BMI) and insulin resistance and hyperinsulinemia. Hyperinsulinemia and insulin resistance cause an increase in androgen levels by stimulating ovarian LH-induced androgen synthesis and suppressing hepatic sex hormone binding globulin synthesis. If obesity is accompanied by insulin resistance, the risk of developing hyperandrogenism is even higher [10,11]. Maternal hyperandrogenism associated with diabetes and obesity may be a potential source of androgen excess on the fetal development [2,12].

Since there is no direct method that can safely determine the intrauterine hormonal environment, it is necessary to define and develop indirect biomarkers to evaluate fetal androgen exposure in human pregnancies. In recent years, it has been suggested that anogenital distance (AGD) is a sensitive marker reflecting intrauterine androgen levels [13,14]. AGD refers to the distance from the anus to the genitals. It is androgen dependent and sexually dimorphic parameter, with females having shorter length than males. This male-female difference is observed as early as 11 weeks of gestation and the ratio was fixed as male/female = 2/1 between the 17–20 weeks of gestation and ratio remained constant until at least 2 years of age [3]. There is substantial observational evidence supporting the link between AGD and androgen exposure in fetal life in humans. Previous study revealed that prenatal exposure to antiandrogenic factors such as endocrine disrupting agents in males and to androgen excess such as PCOS in females is associated with offspring's AGD length [5,13,15].

The present study seeks to investigate a possible relationship between maternal diabetes and obesity and fetal AGD length in females. Thus, we aimed to demonstrate whether AGD is a biomarker in the assessment of fetal effect of maternal androgenic milieu related to diabetes and obesity. Since obesity and diabetes often accompany each other, we examined these two causes of hyperandrogenism together. This is the first time that the association of female offspring's AGD with maternal diabetes and obesity has been studied.

Material and method

Study design and participants

This is a prospective study carried out in Ankara City Hospital (Turkey) for a period of one year from September 2019 to September 2020. The participants consisted of Turkish singleton pregnant women with female fetus at between 26 and 35 gestational weeks with normal fetal anatomy according to the performed in second trimester sonogram. The study group was composed of patients with diabetes (pregestational and gestational) and/or obesity. The control group involved pregnant women who met the inclusion criteria, and were not complicated by diabetes (confirmed by negative oral glucose tolerance test result and negative history) or obesity.

In our study, we assessed the confounding factors and excluded those with medical conditions associated with hyperandrogenism, such as PCOS, anovulation, congenital adrenal hyperplasia, history of hypertensive disorders, endocrine disorders, treatment of infertility and high maternal stress during pregnancy. In addition, we assessed the use of medications which might affect the level of hyperandrogenism as oral contraceptive agents, antipsychotic, antiepileptic, steroid hormones, antihypertensive, insulin-sensitizing drugs during pregnancy and habits such as cigarette or alcohol.

Pregestational diabetes refers to type 1 (insulin deficiency caused by the autoimmune process) or type 2 (peripheral insulin resistance and relative insulin deficiency) diabetes diagnosed before pregnancy [16]. Gestational diabetes (GDM) was defined as carbohydrate intolerance that recognized firstly during pregnancy. All pregnant women were tested for GDM by two-step screening protocol between 24 and 28 weeks of gestation as a part of routine prenatal management. Women with a serum glucose level more than 140 mg/dl measured 1 h after the 50-g oral glucose solution administration were evaluated as positive for screening, and 100-g, 3-h OGTT was applied to these women. GDM have been diagnosed when two or more abnormal glucose values were detected according to Carpenter and Coustan criteria: fasting ≥95 mg/dl, 1-h ≥180 mg/dl, 2-h ≥155 mg/dl, and 3-h ≥140 mg/dl [17]. Obesity is defined as body mass index (BMI) is 30 kg/m^2 and above [18].

This study included only pregnant women with female fetuses. Previous studies have shown that AGD is affected by androgen excess in female infants, and by antiandrogenic factors in males [5,13]. Since this study focused on obesity and diabetes, which are hyperandrogenic factors, only the patients with female fetuses were selected as the study group.

All pregnant women who attended to the perinatology clinic during study period were evaluated for eligibility to study. The patients were informed about the study and the written informed consent was obtained. Patients whose fetal AGD could not be measured by ultrasound due to obesity were excluded from the study. A total of 218 patients participated in the study: 36 with diabetes, 32 with obesity, 25 with diabetes and obesity and 125 in control group.

Anogenital distance measurements

Ultrasonographic evaluation was made by Voluson S 10 scanner (Wipro GE Healthcare Private Limited, Karnataka, India) with a 2–8 MHz curvilinear abdominal transducer. All measurement was performed by one expert physician. During the ultrasound examination, the examiner was blinded to the groups. No information was given to the examiner about which group the examined patient belonged to. However, since the obesity is a visible characteristic, complete blindness may have not been provided in this regard.

Previous studies have shown that fetal AGD can be measured reliably from 21 weeks of gestation [19,20]. AGD measurement was performed in the axial plane with the fetal legs open, in the fetal perineum area where the anal sphincter was seen as the target sign, as described by Gilboa, Kivilevitch [20]. AGD was measured from the center of anus to the posterior convergence of the fourchette by using electronic caliper (Fig. 1). In cases where measurement could not be taken due to the fetal position, the mother walked around for a while and the examination was repeated.

Statistics

Statistical analyses were carried out with IBM SPSS Statistics for Windows, version 23 (IBM Corp., Armonk, N·Y., USA). The mean and standard deviation were calculated for continuous and normally distributed variables. Categorical variables were presented as frequencies and percentages. To compare the population differences (control group, diabetic group, obese group and diabetic obese group), we used one-way analysis of variance (ANOVA) for continuous variables and Pearson chi square test for categorical variables. A significance level of $p < 0.05$ was applied in all analyses.

Multivariate linear regression analysis was applied to control for the possible confounding effects of covariates. We identified a number of potential covariates based on previous studies examining the relationship between maternal hyperandrogenism and offspring's AGD. As explained above, some potential confounders such as history of PCOS, high maternal stress and maternal cigarette or alcohol use were already excluded from the study. Gestational age and fetal weight were the main covariates included in

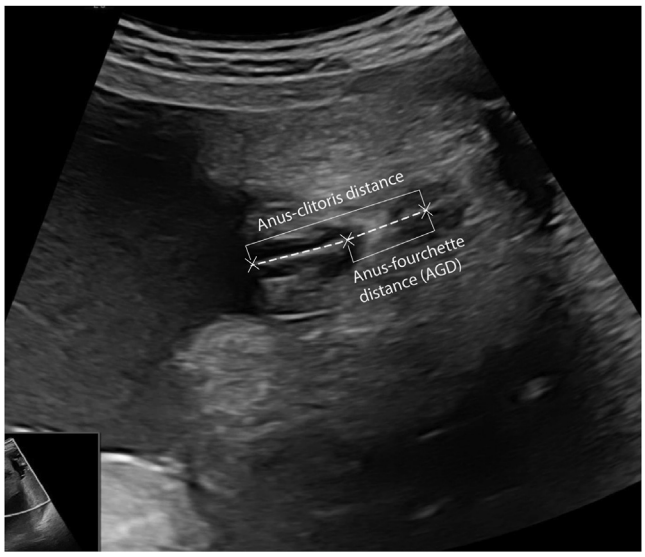

Fig. 1. Sonographic image of the fetal anogenital distance: anus-fourchette distance and anus-clitoris distance.

regression models, as the AGD will increase with the advancement of gestational age and the increase of fetal weight. Maternal age and gravida were also included in the regression models. Because there were significant differences between the control group and the study groups (diabetic, obese and diabetic obese groups) in terms of these two variables (Table 1).

Gestational age and estimated fetal weight are highly correlated variables. Therefore, we did not include these two variables in the same model in order not to reuse a factor that has already been accounted. To evaluate the impact of gestational age and fetal weight on AGD, we created two sets of models in which these are included separately. Similarly, the variables related to obesity and diabetes formed two different sets of models. First, the study group variable (control group; diabetic group; obese group; diabetic obese group) included in a set of regression model. Secondly, the variables of diabetes (diabetic patients; non-diabetic patients) and obesity (obese patients; non-obese patients) were introduced as two separate variables in another set of model. Thus, we set up four different multivariate linear regression models in total. The fetal AGD length was the dependent variable in all models (Table 2).

Table 2
Results of the multivariate linear regression analysis (fetal AGD as the dependent variable).

	β coefficient	95% CI[a]	P-value
Model 1			
Estimated fetal weight	0.612	(0.003, 0.004)	<0.001
Study group[b]	0.392	(0.688, 1.180)	<0.001
Maternal age	−0.020	(-0.057, 0.040)	0.732
Gravida	0.039	(-0.221, 0.450)	0.501
$R^2 = 0{,}497$			
Model 2			
Gestational age	0.618	(0.535, 0.734)	<0.001
Study group[b]	0.403	(0.717, 1.207)	<0.001
Maternal age	0.005	(-0.047, 0.051)	0.934
Gravida	0.035	(-0.231, 0.436)	0.546
$R^2 = 0{,}502$			
Model 3			
Estimated fetal weight	0.615	(0.003, 0.004)	<0.001
Obesity	0.273	(1.005, 2.162)	<0.001
Diabetes	0.251	(0.841, 2.025)	<0.001
Maternal age	−0.033	(-0.063, 0.035)	0.579
Gravida	0.029	(-0.249, 0.421)	0.613
$R^2 = 0{,}505$			
Model 4			
Gestational age	0.623	(0.540, 0.737)	<0.001
Obesity	0.277	(1.034, 2.183)	<0.001
Diabetes	0.266	(0.928, 2.105)	<0.001
Maternal age	−0.009	(-0.052, 0.045)	0.877
Gravida	0.024	(-0.262, 0.403)	0.675
$R^2 = 0{,}512$			

[a] Confidence interval.
[b] The study group variable has four categories: control group, diabetic group, obese group, and diabetic obese group.

Ethics

This study was conducted in accordance with the Declaration of Helsinki principles. It was approved by the Ethics Review Committee of Dr. Zekai Tahir Burak Women Health Care, Training, and Research Hospital (approval number: 115/2019).

Results

In total, the data of 125 control and 93 diabetic and/or obese patients were analyzed. The characteristics of study population were presented in Table 1. The control group and the study subgroups (diabetic, obese, and diabetic obese patients) were similar

Table 1
Patient characteristics and fetal AGD measurement.

	Control patients (n = 125)	Diabetic patients (n = 36)	Obese patients (n = 32)	Diabetic obese patients (n = 25)	*P*
Maternal age (years; mean ± SD)	27.2 ± 5.9	31.3 ± 4.8	29.8 ± 5.6	33.7 ± 5.1	<0.001
Pre-pregnancy body mass index (kg/m^2; mean ± SD)	23.6 ± 3.2	25.3 ± 3.1	34.4 ± 4	35 ± 4.7	<0.001
Gestational age (weeks; mean ± SD)	31.2 ± 2.5	30.9 ± 2.2	31.1 ± 2.5	30.2 ± 2.6	0.379
Estimated fetal weight (grams; mean ± SD)	1755 ± 452	1717 ± 457	1752 ± 514	1635 ± 458	0.681
Gravida, *n* (%)					<0.001
1	66 (52.8)	5 (13.9)	10 (31.3]	4 (16)	
2	27 (21.6)	12 (33.3)	8 (25)	7 (28)	
≥ 3	32 (25.6)	19 (52.8)	14 (43.7)	14 (56)	
Diabetes type, *n* (%)					
Type 1 DM	–	3 (8.3)	–	1 (4)	
Type 2 DM	–	8 (22.2)	–	12 (48)	
Gestational DM	–	25 (69.5)	–	12 (48)	
Diabetes treatment, *n* (%)					
Diet	–	15 (41.7)	–	9 (36)	
Insulin	–	21 (58.3)	–	16 (64)	
AGD measure (mm)					<0.001
Mean ± SD	10.7 ± 2.1	12.6 ± 2.6	12.8 ± 2.8	12.9 ± 2.7	
Minimum	7	7.7	7.1	8	
Maximum	16.3	19.5	18.3	17.1	

with regard to gestational age ($P = 0.379$) and estimated fetal weight ($P = 0.681$). But there were significant differences in maternal age ($P < 0.001$) and gravida ($P < 0.001$). The majority of diabetic patients had gestational diabetes (69.5%) and 58.3% of them were receiving insulin therapy.

The patients in control group had significantly shorter fetal AGD (mean:10.7 mm, $P < 0.001$) compared to the study groups (Table 1 and Fig. 2). The mean length of AGD was similar in diabetic, obese and diabetic obese groups: 12.6 mm, 12.8 mm and 12.9 mm, respectively.

The distribution of individual measurements of fetal AGD for the control group and the study groups is plotted in Figs. 3 and 4 according to gestational week and estimated fetal weight. The fitted linear regression lines indicated that AGD had a linear correlation with both estimated fetal weight and gestational age in all groups.

Fetal AGD length was assessed according to diabetes types and no significant difference was found between patients with pre-gestational (mean = 12.9 mm) and gestational (mean = 12.7 mm) diabetes ($P = 0.776$). Since the number of patients with type 1 diabetes was low (n = 4), we could not compare type 1, type 2, and gestational diabetes separately.

Fetal AGD length was also evaluated according to diabetes treatment. Although the fetal AGD was slightly longer in patients who received insulin treatment (mean = 13.1 mm) compared to those who did not receive insulin (mean = 12.2 mm), no significant difference was found ($P = 0.237$).

The results of the regression analysis were presented in Table 2. The findings suggest that both estimated fetal weight and gestational age have a strong association with fetal AGD. The impact of these two variables on fetal AGD length were very close to each other. We did not find any relationship between fetal AGD and maternal age. Similarly, there was no association between fetal AGD and gravida.

The results from regression analysis showed that after controlling confounding factors, the study group variable was still significantly associated with fetal AGD ($\beta = 0.392$ [95% CI, 0.688, 1.180], $P < 0.001$ in the Model 1 adjusted with the estimated fetal weight and $\beta = 0.403$ [95% CI, 0.717, 1.207], $P < 0.001$ in the Model 2 adjusted with the gestational age). In the regression models by which we assessed separately the impact of diabetes and obesity, the results revealed that both the diabetes and the obesity were significantly correlated with longer AGD in female fetus. The obesity ($\beta = 0.273$ [95% confidence interval (CI), 1.005, 2.162], $P < 0.001$ in the Model 3 adjusted with the estimated fetal weight and $\beta = 0.277$ [95% CI, 1.034, 2.183], $P < 0.001$ in the Model 4 adjusted with the gestational age) was found to have a slightly stronger association than the diabetes ($\beta = 0{,}251$ [95% CI, 0.841, 2.025], $P < 0.001$ in the Model 3 and $\beta = 0.266$ [95% CI, 0.928, 2.105], $P < 0.001$ in the Model 4).

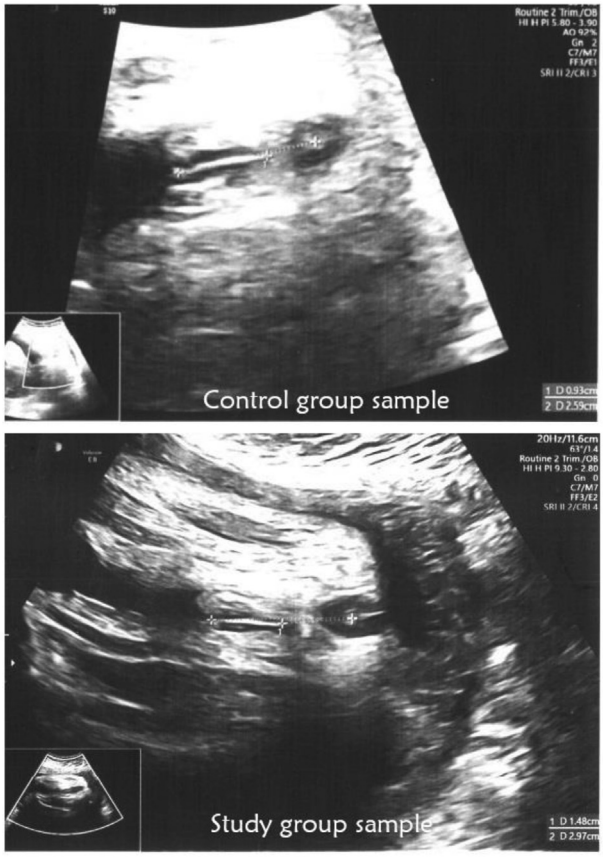

Fig. 2. Ultrasound images of AGD measurement from the study group and the control group. In the study group sample, AGD (anus-fourchette distance) is longer and the ratio of anus-fourchette distance/anus-clitoris distance is bigger than in the control group sample.

Discussion

The results of our study show in the first time in the literature that both maternal diabetes and obesity are associated with increased AGD in female fetus.

In recent years, evidence has accumulated that AGD can be used as a marker of androgen exposure in early fetal life and a predictor of adult reproductive system functions [5]. In their pioneering work, Callegari, Everett [21] showed that the ratio of anus-fourchette distance/anus-clitoris distance was stable (1/3) in normal newborns, but in babies with congenital adrenal hyperplasia, AGD and this ratio increased. They stated that it could be considered as a sign of virilization in fetal life. In recent years, some studies investigated AGD in female infants of mothers with PCOS. They reported that long AGD was observed in these infants and this could be explained by increased maternal androgens accompanying PCOS [22–24]. Other studies found an association between female offspring's AGD length and maternal prenatal stress, maternal smoking habit and pre-pregnancy menstrual irregularities [13,25,26]. A previous study showed the relationship of AGD with maternal age and gravida in male fetuses, but not in girls [27]. Our study did not find any association between maternal age and gravida and AGD in female fetuses, and this is in line with the previous study.

Some studies, focused on the relationship between AGD length and reproductive functions in adult females, showed that increased AGD was associated with PCOS and high testosterone levels [28–30] and shortened AGD was associated with endometriosis [31].

Our findings provide indirect evidence for the androgen exposure on fetus in diabetic and obese pregnant women and this raised the question of whether diabetes and obesity could adversely affect the development of reproductive system in females. Increased maternal androgen levels in diabetic pregnancies have been shown in many studies [32–34]. The relationship between BMI and maternal androgen level has not been studied much but a positive correlation was shown [35]. In normal pregnancies, the fetus is protected from high maternal androgen levels with placental aromatase activity and high sex hormone binding protein levels. However, it has been shown that hyperinsulinemia and insulin resistance can impair these two protective mechanisms, and this may be a potential cause of the hyperandrogenic intrauterine environment for the fetus [33,36].

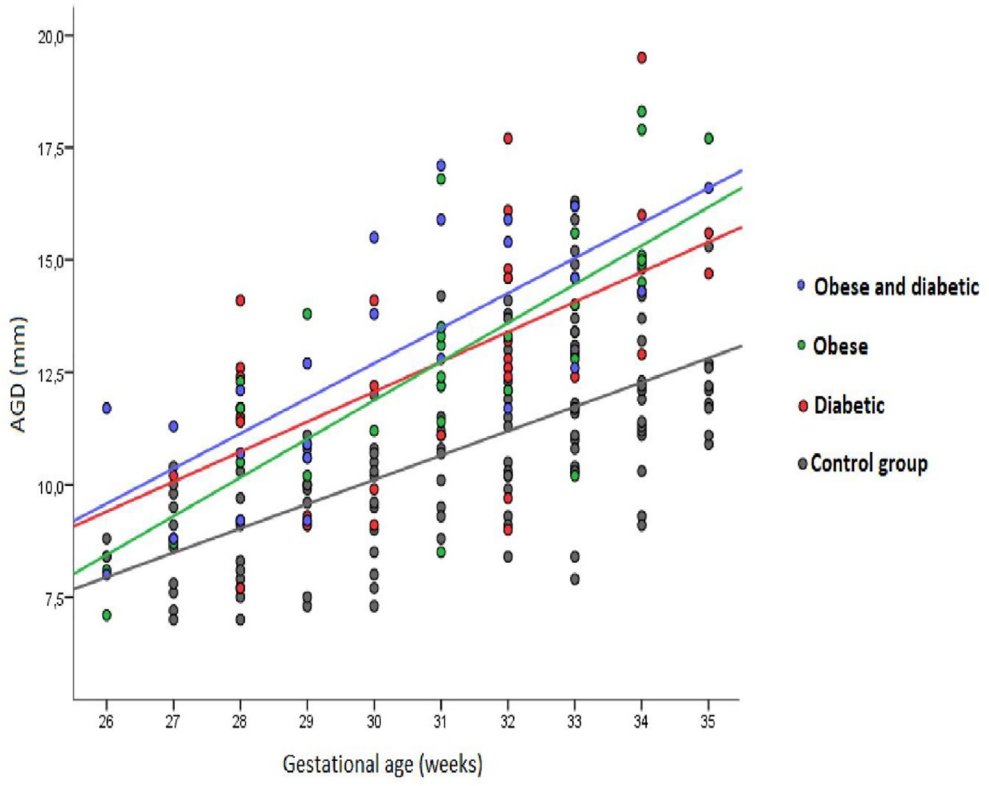

Fig. 3. Scatter plots and fitted linear regression lines of anogenital distance (AGD) measures vs. gestational age (weeks) in groups (control group; diabetic; obese; diabetic obese).

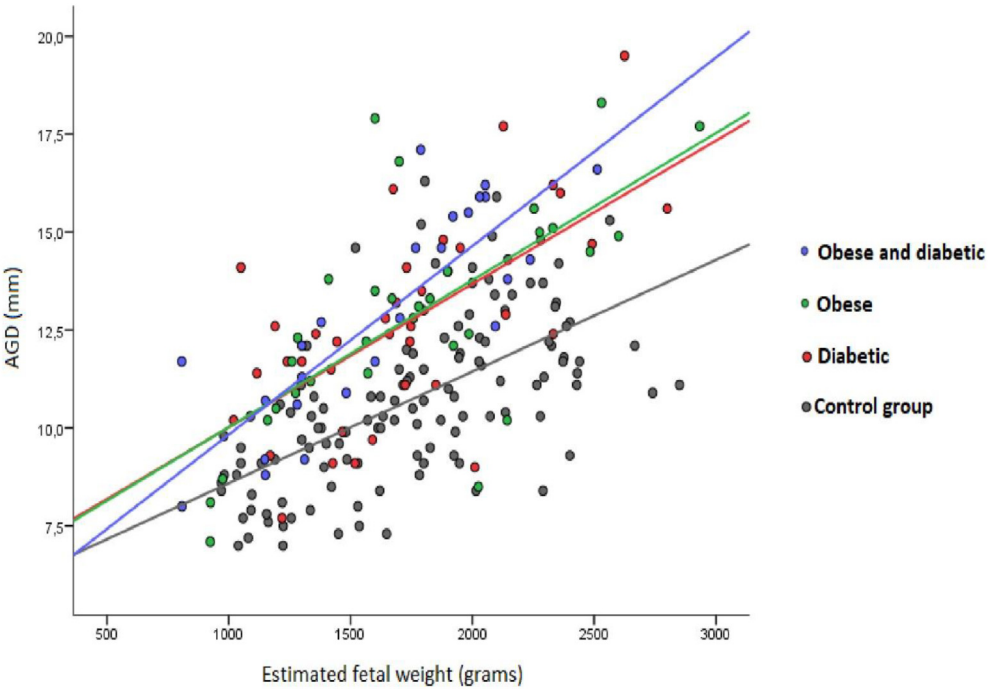

Fig. 4. Scatter plots and fitted linear regression lines of anogenital distance (AGD) measures vs. estimated fetal weight (grams) in groups (control group; diabetic; obese; diabetic obese).

Maternal diabetes and pre-pregnancy obesity are known to have toxic effects on the developing embryo and cause congenital malformations in many organ systems [37,38]. The potential mechanisms underlying to these birth defects is hyperglycemia and hyperinsulinemia. But other metabolic alterations that may accompany to these disorders such as androgen level changes can have an impact on the development of the fetus, particularly on the genital system [37].

The vast majority of studies investigating the effect of maternal factors on AGD were conducted in the postnatal period and prenatal ultrasound studies are rare. These studies proved the feasibility and the reliability of AGD measurement in utero by ultrasound [19,20,24]. Our study supports the previous studies showing that AGD measurement in utero by ultrasound is feasible and reliable, and provides additional insight that AGD can be used as a biomarker of the intrauterine androgen exposure. Currently, the evaluation of fetal hormonal environment during ongoing pregnancy requires invasive procedures. Fetal AGD measurement by ultrasound will make it possible to evaluate the intrauterine androgenic environment starting from the second trimester and in a non-invasive way. This may provide important advantages in terms of early detection of reproductive system abnormalities.

The present study has some limitations due to low number of patients in subgroups according to diabetes type, and obesity classes. Because of the small sample size of the patients with type 1, type 2, and gestational diabetes, we were unable to investigate the effect of different types of diabetes on fetal AGD. Similarly, the effect of obesity severity could not be examined due to the low numbers of patients in subgroups.

In conclusion, our study has revealed that both maternal diabetes and obesity are associated with increased AGD in female fetus, and this provide indirect evidence for the androgen exposure on fetus in diabetic and obese pregnant women. This raised the question of whether diabetes and obesity could adversely affect the development of reproductive system in females.

Conflict of interest

The authors declare that they have no conflict of interest.

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