

# Gestational Diabetes Mellitus and Early Hemodynamic Changes in Fetus

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## Abstract

**Background:** Gestational diabetes mellitus (GDM) incidence can increase up to 14%. GDM creates a risk for developing type 2 diabetes mellitus after pregnancy. Umbilical artery (UA) and fetal middle cerebral artery (MCA) changes in GDM have been studied before. The previous studies have contradictory results. In the current study, we aim to detect and define the impairment of color Doppler ultrasound (CDUS) characteristics in UA and MCA for the pregnant with GDM. **Methods:** US examinations were all performed at 18–22 weeks of gestation with a 3.5 MHz convex transducer. We recorded peak systolic velocity (PSV), end diastolic velocity, pulsatility index, resistive index, and systole/diastole ratio values of both UA and MCA at 18–22 weeks of gestation. GDM diagnosis was created according to the American Diabetes Association guidelines. **Results:** Sixty GDM patients and 61 healthy controls were included into the study. Median MCA PSV value was lower in GDM group (28 cm/s vs. 32 cm/s,  $P = 0.37$ ). Among UA CDUS parameters, we cannot find any significant difference. In GDM group, we could not detect any significant correlation between CDUS parameters and HbA1C values. **Conclusion:** GDM changes fetal brain hemodynamics and the change can be detected at 18–22 weeks of gestation. Decreased fetal MCA PSV values can serve as an early warning for GDM.

**Keywords:** Color Doppler ultrasound, gestational diabetes mellitus, middle cerebral artery, second trimester, umbilical artery

## INTRODUCTION

Gestational diabetes mellitus (GDM) is defined as a newly begun impairment in carbohydrate tolerance which is recognized for the first time during pregnancy. Its incidence can increase up to 14%. GDM creates a risk for developing type 2 diabetes mellitus (DM) after pregnancy. Type 2 DM creates cardiovascular risk for females. Seeing that, diagnosing and managing GDM are very crucial.<sup>[1,2]</sup>

Umbilical artery (UA) and fetal middle cerebral artery (MCA) are some of the most evaluated vessels for diagnosing gestational problems. UA color Doppler ultrasound (CDUS) assessment is useful in the diagnosis and management of fetal well-being in the third trimester of pregnancy. Abnormal CDUS result in UA is a marker of uteroplacental insufficiency and consequent intrauterine growth restriction (IUGR), suspected preeclampsia. The examination of UA with CDUS is also recommended in GDM.<sup>[3]</sup> MCA CDUS examination is useful in the

assessment of fetal cardiovascular distress, fetal anemia or fetal hypoxia, and IUGR.<sup>[4]</sup>

UA and MCA changes in GDM have been studied before. Previously performed studies generally examined the relationship between CDUS changes and fetal outcome in GDM patients. They have contradictory results; some indicated that the examination of UA and MCA was not useful in the prediction of abnormal pregnancy outcomes in GDM.<sup>[5]</sup> Whereas, some indicated that UA and MCA vascular impedance changes were related with pregnancy outcome, such as birth weight pregnant with GDM.<sup>[6,7]</sup>

The mentioned studies generally examined the effect of CDUS parameters of UA and MCA above pregnancy outcome. Furthermore, the CDUS examinations in the mentioned studies generally performed at third trimester. Most of the studies were performed almost a decade before. We assume

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that evaluating the change of CDUS characteristics of UA and MCA in pregnant with GDM at routine ultrasound (US) screening, between 18 and 22 weeks of gestation, might help to warn the clinicians for a potential GDM earlier. Furthermore, detecting any possible change in CDUS parameters earlier than 22 weeks of gestation can confirm the negative impact of GDM on the mentioned arteries.

In the current study, we aim to detect and define the impairment of CDUS characteristics in UA and MCA for the pregnant with GDM. Furthermore, evaluating the diagnostic power of mentioned CDUS parameters to predict GDM diagnosis.

## METHODS

The local institutional review board approved the current prospective study (IRB approval number: ABYU/KAEK-19/10-5). Informed consent was acquired from all the participants. The study data were collected between December 2019 and June 2020.

Sixty GDM patients and 61 healthy controls were included in the study. US examinations were all performed at 18–22 weeks of gestation with a 3.5 MHz convex transducer (Toshiba Medical, Xario). All the pregnant who were referred to the radiology department for detailed morphological abnormality scan were examined to measure CDUS parameters in both UA and MCA during the study period. Then, these pregnant were followed-up until the labor to get information about pregnancy outcome, such as having GDM or any other diagnosis (GDM diagnosis were created according to American Diabetes Association guidelines<sup>[8]</sup> by using the results of oral glucose tolerance test (OGTT) and HbA1C values obtained at 24–28 weeks of gestation). According to OGTT results, we constituted the GDM group. Then, we spontaneously selected 61 healthy pregnant, whose gestational age was similar with

GDM group, to use their data for creating the control group. The exclusion criteria were as follows: (1) having a diagnosis of Type 1 or 2 DM, (2) fetal abnormality detected at US, (3) abnormal double and/or triple test result, (4) coexisting morbidities (pre-eclampsia, thyroidal disorders, coagulation problems, etc.), and (5) multiple gestation pregnancy.

We recorded peak systolic velocity (PSV), end diastolic velocity (EDV), pulsatility index (PI), resistive index (RI), and systole/diastole ratio values of both UA and MCA at 18–22 weeks of gestation.

UA CDUS examinations were performed while the patient was lying in a slightly left lateral position. Measurements were performed in the region of a free-floating loop of cord in one of the UAs [Figures 1 and 2].

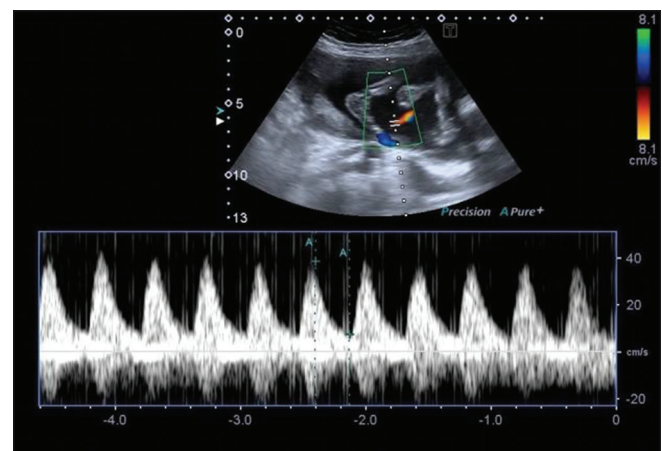
Fetal MCA CDUS examination was performed while the patient was in the supine position. For measurements, the fetal head was imaged in the transverse plane. An axial section of the brain, including the thalami and the sphenoid bone wings, was taken and magnified. The vessels were found with CDUS overlying the anterior wing of the sphenoid bone. The measurements were taken from the portion close to MCA origin in the internal carotid artery [Figures 3 and 4].

UA and MCA measurements were repeated by another author, blindly with the initial one, to test interobserver variability of the measurements. Mean value of the both measurements was used as the final data.

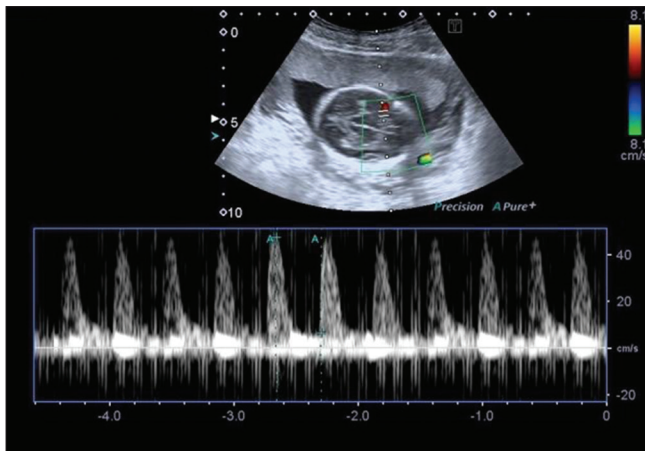
We have also recorded fetal abdominal circumference (AC) values, estimated fetal weight (EFW), and fetal sex at US examination performed at 18–22 weeks of gestation. AC measurements were performed from the standardized view described in the literature.<sup>[9]</sup> EFW was calculated automatically by the US device.



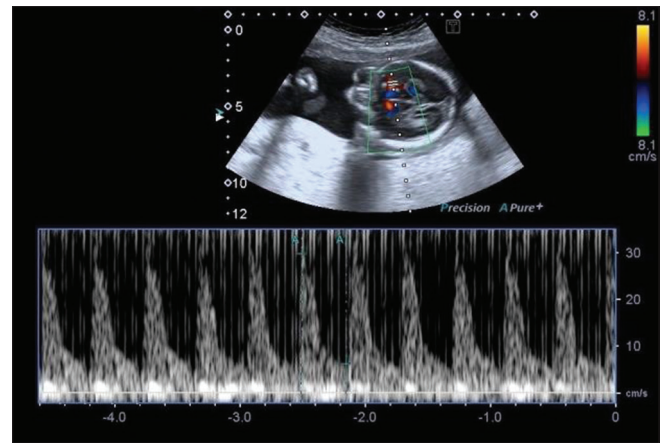
**Figure 1:** (Normal/control patient) umbilical artery color Doppler ultrasound examination of female fetus measured at 22 weeks of gestation, abdominal circumference was 182 mm, estimated fetal weight was 480 g. Twenty-five-year-old pregnant, body mass index 30. Umbilical artery peak systolic velocity: 42.6 cm/s, end diastolic velocity: 9.6 cm/s, resistive index: 0.7, S/D: 4.4, pulsatility index: 1.2



**Figure 2:** (Gestational Diabetes Mellitus patient) Umbilical artery color Doppler ultrasound examination of male fetus measured 21 weeks of gestation, abdominal circumference was 159 mm, estimated fetal weight was 436 g. 29 years old pregnant, body mass index 31. Umbilical artery peak systolic velocity: 38.5 cm/s, end diastolic velocity: 7.2 cm/s, resistive index: 0.8, S/D: 5.3 pulsatility index: 1.3



**Figure 3:** (Normal/control patient) middle cerebral artery color Doppler ultrasound examination of female fetus measured at 22 weeks of gestation, abdominal circumference was 182 mm, estimated fetal weight was 480 g. 25 years old pregnant, body mass index 30. Middle cerebral artery peak systolic velocity: 47.4 cm/s, DV: 6.3 cm/s resistive index: 0.87, S/D: 7.5, pulsatility index: 1.5



**Figure 4:** (Gestational diabetes mellitus patient) middle cerebral artery color Doppler ultrasound examination of male fetus measured 21 weeks of gestation, abdominal circumference was 159 mm, estimated fetal weight was 436 g. Twenty-nine-year-old pregnant, body mass index 31. Middle cerebral artery peak systolic velocity: 29.7 cm/s, DV: 6.1 cm/s resistive index: 0.8, S/D: 4.8, pulsatility index: 1.3

HbA1C and body mass index (BMI) values of patients at 24–28 weeks of gestation were acquired from the medical achieves.

### Statistical analysis

Data were analyzed using the Statistical Package for the Social Sciences (SPSS) software version 25 for Windows (IBM SPSS Inc., Chicago, IL, USA). Normal distribution of the data was evaluated with the Kolmogorov–Smirnov test. The numerical variables with normal distribution were shown as mean  $\pm$  standard deviation. The variables not with normal distribution were shown as median, minimum–maximum values. The categorical variables were shown as number and percentage. For the comparison of numerical variables between two groups, Mann–Whitney U-test were used. We compared the categorical variables between the two groups by using the Pearson Chi-square test. To define the possible correlations between CDUS parameters and the other variables, Spearman correlation was performed. Receiver operating characteristic (ROC) curve analysis was applied for diagnostic performance evaluation of UA PSV value for GDM diagnosis. Youden index was used to define cutoff value of MCA PSV. Categorical correlation analysis (Cohen’s kappa analysis) was performed to test interobserver variability.

A two-tailed value of  $P < 0.05$  was considered statistically significant.

## RESULTS

Sixty GDM patients and 61 healthy controls were included in the study. Median age of the whole population was 29 years (19–46 years), it was 32 years (20–46 years) in GDM group and 26 years (18–38 years) in the control group. The median age was significantly higher in the GDM group ( $P = 0.00$ ).

Median gestational age was 21 weeks in GDM and control groups.

Median BMI of pregnant in the whole population was 29 (24–35), it was 30 (24–35) in GDM group, and 28 (24–32) in the control group. Median BMI was significantly higher in GDM group ( $P = 0.02$ ).

All the initial fetal sex estimates came true with birth. There were 61 male (50.4%) and 60 (49.6%) female fetus in the whole population. In GDM group, there were 33 male (55%) and 27 (45%) female fetus. In the control group, there were 28 male (45.9%) and 33 (54.1%) female fetus. Sex distribution of fetus was similar between the two groups ( $P = 0.31$ ).

Median AC value of the whole population was 157 mm (122–191 mm). In GDM group, median AC value was 160 mm (122–191 mm), whereas it was 150 mm (129–182 mm) in the control group. Median EFW value was calculated 355 g (126–500 g) in the whole population. Median EFW value was 357.5 g (126–500 g) in GDM group, whereas it was 354 g (244–492 g) in the control group. Median AC and EFW values were similar between two groups ( $P = 0.39$  and  $P = 0.70$ , respectively).

When CDUS parameters of MCA were evaluated, only MCA PSV found to be significantly different between the groups. Median MCA PSV value was lower in GDM group (28 cm/s vs. 32 cm/s,  $P = 0.037$ ). Median MCA CDUS parameter values according to groups are shown in detail in Table 1.

We performed ROC analysis to define the diagnostic effectiveness of MCA PSV values to predict GDM presence risk. A MCA PSV value  $<35.5$  cm/s can predict GDM diagnosis with a sensitivity of 41% and specificity of 78.3%.

Among UA CDUS parameters, we cannot find any significant difference between the groups [Table 2].



According to Spearman correlation results, as the gestational week increases, MCA PSV, UA PSV, and UA EDV values increase, too. On the other hand, as with gestational week increases; UA RI, UA PI, UA S/D ratio values decrease. We found a similar correlation for AC/EFW values. MCA PSV, UA PSV, and UA EDV values were positively correlated with AC and EFW values. Meanwhile, UA RI, UA PI, and UA S/D ratio

values were negatively correlated. We cannot find a significant correlation between parameters and fetal sex, maternal age, and maternal BMI [Tables 3 and 4]. These correlations stayed stable when the analysis performed in both subgroups.

In GDM group, we could not detect any significant correlation between CDUS parameters and HbA1C values [Table 5].

$\kappa$  value for interobserver agreement of the parameters changed between 0.62 and 0.81 ( $P < 0.05$ ). Details of the Cohen's  $\kappa$  values are seen in Table 6.

**Table 1: Median MCA CDUS parameter values in both groups**

	GDM	Control	P
MCA PSV	28 cm/s	32 cm/s	0.037
MCA EDV	6 cm/s	6 cm/s	0.875
MCA RI	0.8	0.8	0.749
MCA PI	1.4	1.4	0.638
MCA S/D ratio	5.2	5.4	0.735

MCA: Median cerebral artery, GDM: Gestational Diabetes Mellitus, PSV: Peak systolic velocity, EDV: End diastolic velocity, RI: Resistive index, PI: Pulsatility index, S/D: Systole/Diastole

**Table 2: Median umbilical artery color Doppler ultrasound parameter values in both groups**

	GDM	Control	P
UA PSV (cm/s)	34	35	0.037
UA EDV (cm/s)	9	9	0.875
UA RI	0.7	0.72	0.749
UA PI	1.1	1.14	0.638
UA S/D ratio	3.8	3.7	0.735

UA: Umbilical artery, GDM: Gestational diabetes mellitus, PSV: Peak systolic velocity, EDV: End diastolic velocity, RI: Resistive index, PI: Pulsatility index, S/D: Systole/diastole

## DISCUSSION

We have evaluated the potential changes in CDUS characteristics of MCA and UA in GDM patients at the second trimester (18–22 weeks), earlier than routine GDM screening tests. According to our results, MCA showed significant changes which can be detected at CDUS examinations performed at 18–22 gestational weeks in GDM patients. MCA PSV value was found to be significantly lower in GDM patients than controls.

The prevalence of GDM has been increasing worldwide. Its increasing prevalence and associated complications are the important problems for health-care providers. Tight glycemic control is the most important feature to reduce obstetric and perinatal complications. Antenatal monitoring is crucial in both the diagnosis and management of GDM.<sup>[10]</sup>

Doppler velocimetry is a widely accepted and used method for the gestational management. CDUS examinations in pregnancy can give information about oxygen metabolism in the maternal-placental-fetal balance. The changes in CDUS characteristics are significantly important in the

**Table 3: Correlations for color Doppler ultrasound parameters**

	MCA PSV	MCA EDV	MCA RI	MCA PI	MCA S/D	UAP SV	UA EDV	UA RI	UA PI	UA S/D
Gestational week										
CC	<b>0.43</b>	0.14	-0.08	0.17	0.07	<b>0.32</b>	<b>0.51</b>	<b>-0.3</b>	<b>-0.41</b>	<b>-0.32</b>
P	<b>0.00</b>	0.10	0.38	0.54	0.42	<b>0.00</b>	<b>0.00</b>	<b>0.00</b>	<b>0.00</b>	<b>0.00</b>
AC										
CC	<b>0.41</b>	0.16	0.11	0.08	0.04	<b>0.33</b>	<b>0.52</b>	<b>-0.3</b>	<b>-0.4</b>	<b>-0.3</b>
P	<b>0.00</b>	0.07	0.19	0.37	0.65	<b>0.00</b>	<b>0.00</b>	<b>0.00</b>	<b>0.00</b>	<b>0.00</b>
EFW										
CC	<b>0.46</b>	0.15	-0.09	0.20	0.07	<b>0.33</b>	<b>0.53</b>	<b>-0.3</b>	<b>-0.4</b>	<b>-0.3</b>
P	<b>0.00</b>	0.09	0.32	0.32	0.38	<b>0.00</b>	<b>0.00</b>	<b>0.00</b>	<b>0.00</b>	<b>0.00</b>
Fetal sex										
CC	-0.31	0.03	-0.17	-0.19	-0.76	-0.13	-0.26	0.13	0.23	0.09
P	0.66	0.68	0.23	0.03	0.41	0.23	0.27	0.14	0.82	0.32
Maternal age										
CC	-0.05	0.15	-0.08	-0.01	-0.17	0.08	0.01	-0.07	-0.04	0.01
P	0.53	0.08	0.38	0.91	0.24	0.37	0.99	0.44	0.61	0.83
Maternal BMI										
CC	0.09	0.17	-0.09	-0.11	-0.09	0.23	0.05	0.05	0.04	0.14
P	0.28	0.06	0.28	0.19	0.28	0.37	0.53	0.56	0.63	0.12

Bold signifies statistically significance. UA: Umbilical artery, MCA: Median cerebral artery, PSV: Peak systolic velocity, EDV: End diastolic velocity, BMI: Body mass index, CC: Correlation coefficient, RI: Resistive index, PI: Pulsatility index, S/D: Systole/diastole, EFW; Estimated fetal weight, AC: Abdominal circumference

**Table 4: Correlations for hemoglobin A1c value in gestational diabetes mellitus group**

	MCA PSV	MCA EDV	MCA RI	MCA PI	MCA S/D	UA PSV	UA EDV	UA RI	UA PI	UA S/D
HbA1C value										
CC	0.099	0.054	0.130	0.027	0.143	0.009	0.008	0.136	0.131	0.162
P	0.453	0.81	0.323	0.835	0.835	0.946	0.950	0.300	0.318	0.216

UA: Umbilical artery, MCA: Median cerebral artery, PSV: Peak systolic velocity, EDV: End diastolic velocity, CC: Correlation coefficient, RI: Resistive index, PI: Pulsatility index, S/D: Systole/diastole, HbA1C: Hemoglobin A1c

**Table 5: Correlations for HbA1C value in GDM group**

	MCA PSV	MCA EDV	MCA RI	MCA PI	MCA S/D	UA PSV	UA EDV	UA RI	UA PI	UA S/D
HbA1C										
CC	0.099	0.054	0.130	0.027	0.143	0.009	0.008	0.136	0.131	0.162
P	0.453	0.81	0.323	0.835	0.835	0.946	0.950	0.300	0.318	0.216

UA: Umbilical artery, MCA: Median cerebral artery, PSV: Peak systolic velocity, EDV: End diastolic velocity, CC: Correlation Coefficient, RI: Resistive index, PI: Pulsatility index, S/D: Systole/Diastole

**Table 6: Interobserver variability data for the parameters**

	MCA PSV	MCA EDV	MCA RI	MCA PI	MCA S/D	UA PSV	UA EDV	UA RI	UA PI	UA S/D
Kappa	0.78	0.82	0.81	0.81	0.81	0.62	0.67	0.65	0.65	0.65
P	0.001	0.004	0.003	0.003	0.003	0.007	0.004	0.005	0.005	0.005

UA: Umbilical artery, MCA: Median cerebral artery, PSV: Peak systolic velocity, EDV: End diastolic velocity, CC: Correlation Coefficient, RI: Resistive index, PI: Pulsatility index, S/D: Systole/Diastole

diagnosis and management of IUGR, anemia, hypoxemia, and preeclampsia.<sup>[11]</sup>

The change of CDUS characteristics for UA and MCA in GDM patients has been evaluated before. However, the literature mostly contains contradictory results. Pietryga *et al.* reported that CDUS does not seem to be of clinical value for fetal surveillance in GDM patients.<sup>[6]</sup> Leung *et al.* stated that UA-PI, MCA-PI, and MCA-Vmax was not useful in the prediction of abnormal pregnancy outcome in GDM.<sup>[5]</sup> Whereas, Niromanesh *et al.* reported that abnormal UA Doppler assessment is related with poor neonatal outcome.<sup>[7]</sup> In addition Shabani *et al.* emphasized that MCA PI value increased in GDM patients.<sup>[12]</sup> Furthermore, previous studies did not evaluate standardized CDUS parameters, for instance some studies only evaluated PI values, whereas some concentrated on cerebroplacental ratio, etc., In addition, in the literature most of the studies contained CDUS data acquired at the third trimester examinations. We cannot find a satisfactory data about the changes in CDUS parameters detected at 18–22 weeks of gestation. Seeing that we intended to define potential changes of CDUS characteristics of UA and MCA at second trimester, earlier than routine time for GDM screening.

Among CDUS parameters of MCA, we showed that, only PSV values were significantly different in GDM patients. We found that MCA PSV values were lower in pregnant with GDM than controls at the second trimester CDUS examination. We cannot find any similar study performed at second trimester. Shabani *et al.*<sup>[12]</sup> evaluated MCA CDUS characteristics with a similar method at third trimester, they found higher MCA PI values in GDM patients. They also stated that PSV of the

fetal MCA was higher in GDM group. Different from us, they studied with a smaller population and examined the patients at third trimester; yet they mentioned about significantly altered MCA CDUS parameters. Furthermore, Niromanesh *et al.*<sup>[7]</sup> emphasized that UA and MCA CDUS changes were effective in predicting poor neonatal outcome in pregnant with GDM. Their method were different, they have evaluated the effect of CDUS changes over pregnancy outcome. They did not define CDUS parameters independently, instead they defined UA and MCA examinations only as normal/abnormal, and they did not define a precise time for CDUS examination. On the contrary, Leung *et al.*<sup>[5]</sup> could not find a significant change in MCA Vmax values in GDM patients. We think that this contradictory finding might be the result of relatively small GDM patients number (38 GDM cases) of Leung's study.

Although methods are partly different, when evaluated with above-mentioned recently performed studies, our results indicate that GDM can effect fetal MCA CDUS characteristics even at second trimester, earlier than standard GDM screening time. As stated in the literature, GDM is known to cause fetal polycythemia.<sup>[13]</sup> We think that, the increase in blood viscosity due to polycythemia can change blood flow velocity through the fetal circulation and create a lower MCA PSV value in fetuses of GDM patients.

We also define a cutoff value for MCA PSV to predict future GDM diagnosis. According to our results, a MCA PSV value <35.5 cm/s can predict GDM diagnosis with a relatively good specificity (78.3%). We cannot claim to use MCA PSV value as a new independent diagnostic criterion for GDM, as bigger population sizes and more supporting literature

are needed for such a claim. We believe that the mentioned value for MCA PSV, acquired at second trimester routine US screening, can be used as an early warning factor for a risk of future GDM. Further prospective studies with larger population numbers might confirm the value of MCA PSV or other MCA/UA CDUS parameters in predicting GDM. Then, MCA/UA CDUS parameters can serve as a screening tool for GDM. Examining and following up the patients from the beginning of the pregnancy, until the born (in all trimesters) can strengthen possible future studies and their conclusions.

Some previous studies in the literature found elevated S/D ratio<sup>[14]</sup> and elevated UA RI values<sup>[15]</sup> in GDM patients. However, recent literature found no significant change in UA CDUS parameters in pregnant with GDM.<sup>[12,16]</sup> Furthermore, no relationship was defined between UA CDUS parameters and pregnancy outcome.<sup>[5,6]</sup> Our results were consistent with more recently published articles; we cannot define any significant change in UA CDUS parameters in pregnant with GDM.

The presence of a strong positive correlation between MCA PSV and gestational age was emphasized in the literature.<sup>[17]</sup> UA RI, UA PI, and UA S/D ratio values were known to decrease as the gestational week increases. Meanwhile, it is stated that MCA PI and RI have shown a parabolic curve for with a plateau between 28 and 30 weeks.<sup>[18]</sup> Our correlations were consistent with the literature; we detected a positive correlation between MCA PSV and gestational week. Furthermore, a negative significant correlation was defined between UA RI, UA PI, and UA S/D ratio values and gestational week. We cannot detect any correlation between gestational week and MCA PI and RI values, because of the parabolic trend which has defined previously.

According to our results, MCA PSV, UA PSV, and UA EDV values were positively correlated with AC and EFW values. Meanwhile, UA RI, UA PI, and UA S/D ratio values were negatively correlated. In the literature, there is not so many studies examining the mentioned correlations. Talmor *et al.* reported different results. They stated that, there was no relationship between UA PI and AC in normal fetuses. Whereas in severe fetal growth restriction, Doppler changes are related to absolute fetal AC size.<sup>[19]</sup> Contradictory results might be caused from different CDUS examination times: In Talmor's study, examinations were performed later than us, at 24-week of gestation. We cannot find any similar studies examining the relationship between CDUS parameters other than UA PI and AC/EFW values. Further, prospective studies performing at both second and third trimester can enlighten possible correlations better.

The ability of UA and MCA CDUS parameters in reflecting maternal glycemic control is controversial. In general, studies indicated that there is no correlation between examined UA and MCA CDUS parameters and HbA1C values.<sup>[6]</sup> Our results are similar with the literature; we cannot define any correlation between CDUS parameters and HbA1C values in GDM group.

Interobserver agreement data of the measurements were good or excellent, indicating that the measurements can be repeated safely by others. Reliable reproducibility of the data increases the predictive and diagnostic value of the measurements.

The study has some limitations to mention. Larger sample sizes can reveal more reliable results. We did not evaluate the relationships between CDUS parameters and pregnancy outcome, since the lack of sufficient outcome information for some patients. We did not have third trimester sonographic examination data; hence, we cannot offer a follow-up information about third trimester.

## CONCLUSION

GDM changes fetal brain hemodynamics and the change can be detected at 18–22 weeks of gestation. Fetal MCA PSV values decrease in GDM. Decreased MCA PSV values can predict GDM diagnosis with a specificity of 78.3%; hence, it can serve as an early warning/screening factor for GDM.

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Nil.

## Conflicts of interest

There are no conflicts of interest.

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