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Predictive value of cell-free fetal DNA concentration in the maternal plasma for abnormal placental invasion in cases of placenta previa

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SUMMARY

AUTHORS' CONTRIBUTION: (A) Study Design · **(B)** Data Collection · **(C)** Statistical Analysis · **(D)** Data Interpretation · **(E)** Manuscript Preparation · **(F)** Literature Search · **(G)** Funds Collection

Aim of the work: This case control study was done to to evaluate whether measurement of cell-free fetal DNA in the maternal plasma can be predictive of abnormal placental invasion in cases of placenta previa.

Methods: It was conducted on 50 healthy pregnant women. After being provided with an informed consent, they were allocated in two groups. Group one: study group previously diagnosed as placenta previa anterior only or with ultrasound finding suggestive of placental adhesion or invasion (n=25) while group two :matched control with normally situated placenta without ultrasound finding suggestive of placental adhesion (n=25). This study exclusively limited to singleton pregnant women carrying male fetuses, gestational age range from 28-34 weeks, para 1 - para 5, maternal age range from 20-40 years old and body mass index range from 18-25 kg/m². This study excluded women with multifetal pregnancy, hypertension, preterm labor and intrauterine growth restriction and patients were taking a tocolytic agent or those with uterine bleeding at or immediately after blood sampling. The blood samples were processed within 24 h and DNA was extracted from plasma using QIAamp Blood Mini Kit (Qiagen). The TaqMan real time PCR analysis was performed using a PE Applied Biosystems 7500HT Fast Real-Time PCR Sequence, Detector was employed to design SRY primers using the SRY gene sequence.

Results: The main finding of this study was that, there were no statistically significant differences regarding the level of free fetal DNA (F-FDNA) between placenta accreta (PA) group and control group. In this study highly significant statistical differences were found regarding the incidence of preterm delivery with placenta accreta group and highly significant statistical differences regarding the increased maternal age in same group. Significant statistical found positive correlation between level of F-FDNA in maternal plasma and gestional age, F-FDNA increases as gestational age advances. There was no significant statistical correlation between level of F-FDNA in maternal plasma and maternal age while there was significant statistical negative correlation between level of F-FDNA in maternal plasma and body mass index (BMI), F-FDNA decrease in maternal obesity. Comparing between the two groups there were high significant statistical differences regarding the increased number of Cesarean deliveries in placenta accreta group more than control group. Seven women in placenta accreta group were treated with cesarean hysterectomy due to sever intrapartum hemorrage, histopatholgical examination of removed uteri and adherent placenta revealed 1 placenta accreta (14.3%), 3 increta (42.9%) and 3 percreta (42.9%).

Conclusion: No significant association between cell free fetal DNA (cffDNA) and placenta accreta (PA). Obesity during pregnancy is associated with lower ff DNA, gestational age is an additional factor that can affect the ff, levels of fetal DNA increase throughout pregnancy.

Keywords: Maternal serum cell-free fetal DNA; Placental invasion; Placenta previa

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INTRODUCTION

The rate of cesarean delivery has increased substantially over the past few decades. As the cesarean rate increased, so has the incidence of pregnancies complicated by placenta accreta spectrum disorders between the 1960s and 2002, the incidence increased from 1 in 30,000 pregnancies to 1 in 533; this constitutes a nearly 60-fold increase in a matter of 5 decades. As placenta accreta have increased, the indications for peripartum hysterectomy have changed, with accreta accounting for up to 47% of indication. A large multicenter 2006 cohort study has noted that for women presenting with placenta previa and prior cesarean delivery, the risk of accreta placentation is 3%, 11%, 40%, 61%, and 67% for first, second, third, fourth, and fifth or more cesarean deliveries, respectively. These risks are independent of other maternal characteristics, such as parity, body mass index, tobacco use, and coexisting hypertension or diabetes [1].

It has been recently estimated that if the cesarean rates continue to rise as they have in recent years, by 2020 the rate in the US will be 56.2%, leading to an additional 6236 placenta previas, 4504 placenta accretas, and 130 maternal deaths annually [2].

Placenta previa increases the risk of antepartum (RR 9.8), intrapartum (RR 2.5), and postpartum hemorrhage (RR 1.9). For this reason, women with placenta previa are more likely to receive blood transfusions (12 versus 0.8 percent without previa) and undergo postpartum hysterectomy, iliac artery ligation, or embolization of pelvic vessels to control bleeding (2.5 versus 0 percent without previa) the risk is particularly high in those with previa-accreta [3].

Multiple studies have shown that maternal morbidity is decreased in patients with a prenatal diagnosis who are transferred to a high volume tertiary care center, delivered in a planned fashion in the late preterm period and cared for by a multidisciplinary care team including obstetricians, gynecological oncologists, obstetrical anesthesiologists, intensivists and interventional radiologists as well as blood bank service [4].

The diagnosis of placenta previa is based on identification of placental tissue reaching or covering the internal cervical os on an imaging study, typically ultrasound. Transabdominal ultrasound examination is performed as the initial examination; if it shows placenta previa or the

findings are uncertain, transvaginal sonography should be performed to better define placental position [5].

The sonographic findings suggestive of placenta accreta in the second and third trimesters include the following:

1. Multiple intraplacental sonolucent spaces contain slow-moving maternal blood (venous lakes or placental lacunae) adjacent to the involved myometrium (This is the most consistent ultrasound finding).
2. Loss or thinning of the normal hypoechoic area behind the placenta.
3. Loss or disruption of the normally continuous white line representing the bladder wall-uterine serosa interface (termed the 'bladder line').
4. Bulging of the placenta into the posterior wall of the bladder [5].

Power and color Doppler are often used in conjunction with gray-scale ultrasound imaging for the diagnosis of abnormal placentation. Doppler studies can highlight areas of increased vascularity and turbulent blood flow through placental lacunae [6]. For many years, investigators have been searching for a biochemical and/or biological marker to improve the accuracy of antenatal diagnosis of placenta accreta. Although elevated levels of maternal serum creatinine kinase, alpha fetoprotein and beta-human chorionic gonadotropin have been reported in patients with abnormal placentation, these markers appear to be too non-specific to be clinically useful [7].

Abnormal levels of cff- DNA may reflect active shedding of trophoblast particles or gradual breakdown of the placental barrier after the 32nd week of pregnancy, independently of pathology [8].

Most of the studies focused on placenta-related pregnancy complications have evaluated cell free plasma DNA (cfp-DNA) concentration relative to plasma volume, as is done for biochemical markers (ie, as copies [or genome equivalents] per milliliter of plasma). Alternatively, cfp-DNA may be normalized to the total amount of cf- DNA in the plasma sample, a ratio referred to as the fetal fraction (ff) [9].

The aim of the work is to evaluate whether measurement of cell-free fetal DNA in the maternal plasma can be predictive of abnormal placental invasion in cases of placenta previa.

PATIENTS AND METHODS

Type of study: Case control study.

Place of the study: Patients were recruited from Ain shams Maternity University Hospital.

Time of the study: Started at beginning of 2016 & ended at the end of 2017.

Study population: 60 women were recruited from Ain shams Maternity University Hospital to carry out the study.

Sample size justification: Based on Sekizawa A et al., [10] the sample size was calculated as 60.

The sample size assumes that the expected effect size is 110 and the standard deviation of outcome variable is 130. To achieve 80% power to detect this difference with a significance level of 5% it is estimated that 22 subjects per group was required. With a withdrawal /non-valuable subject rate of 10% a total of 24 per group subjects were recruited leading to a total required sample size of 48 subjects.

Subjects were divided into 2 groups:

Study group (1): 30 women with one or more previous cesarean section and placenta previa anterior only or with ultrasound finding suggestive of placental adhesion or invasion (accreta, increta or percreta).

Control group (2): 30 matched control with normally situated placenta without ultrasound finding suggestive of placental adhesion or invasion.

Inclusion criteria:

- Maternal age range from 20-40 years old.
- Parity: Para 1- para 5.
- Body mass index range from 18-25 kg/m^2.
- Singleton pregnancy carrying male fetuses.
- Gestational age range from 28-34 weeks.

Exclusion criteria:

- Multifetal pregnancy.
- Hypertension, preterm labor and intrauterine growth restriction.
- Patients taking a tocolytic agent or those with uterine bleeding at or after blood sampling. Since these complications may increase the level of cell-free fetal DNA.
- Accidental hemorrhage.

Consent: Written informed consent was obtained from all women included in the study.

METHODS

Full history taking especially uterine surgery, cesarean section, uterine curettage, endometrial ablation, myomectomy, hysteroscopic surgery, uterine artery embolization and invasive procedures of the endometrial cavity are considered clinical risk factors for placenta accreta.

Full general examination to assess degree of anemia and blood pressure to exclude hypertension.

Abdominal examination to assess the fundal level to exclude multifetal pregnancy, preterm labor and intrauterine growth restriction.

Routine full laboratory Investigations including:

- Blood grouping.
- Complete blood picture.
- Coagulation profile.
- Kidney and liver function tests.
- Blood glucose level.
- Urine analysis to exclude albuminuria.

The diagnosis of placenta previa was made by ultrasound examination.

Placenta previa is graded into 4 grades [11]:

Grade I: Low lying placenta: placenta lies in lower uterine segment but its lower edge does not about the internal cervical os (i.e., lower edge 2 cm from internal os but does not reach it).

Grade II: Marginal previa: placental tissue reaches the margin of the internal cervical os, but does not cover it.

Grade III: Partial previa: placenta partially covers the internal cervical os.

Grade IV: Complete previa: placenta completely covers the internal cervical os.

Sometimes types I and II are termed a "minor" placenta previa, and types III and IV are termed a "major" placenta previa [11].

Ultrasound criteria suggestive of placenta accrete, were described by Comstock et al., [12]: (1) absence of the clear space, defined as complete focal obliteration of the echolucent area located between the uterus and placenta; (2) placental lacunae, defined as multiple linear, irregular vascular spaces within the placenta; and (3) thinning or focal interruption of the posterior bladder wall–uterine interface.

Color Doppler ultrasound criteria suggestive of placenta accreta included: (1) turbulent or diffuse blood flow through placental lacunae; and (2) vessels crossing the interface disruption site.

The blood samples, 7 ml on average, were processed within 24 h of sampling with Separation of the maternal plasma by centrifugation at 1200 g for 10 min, 1.5 ml plasma aliquots were removed and stored at -80˚C.

DNA was extracted from 400µl plasma using QIAamp Blood Mini Kit (Qiagen) according to the manufacturer's protocol. The DNA preparations were eluted in 50 ml elution buffer (10 mM Tris HCL pH 7.4: 1 mM EDTA), of which 2 µl were used as a template for the PCR reaction.

The TaqMan real time PCR analysis was performed using a PE Applied Biosystems 7500HT Fast Real-Time PCR Sequence Detector (Applied Biosystems, Foster City, California,). For which primers and dual labelled probes which had been designed with the aid of Primer Express software (Perkin Elmer, Branchburg, New Jersey, USA) was used. To determine the amount of male fetal DNA Y chromosome SRY locus (GenBank Accession Nr. L08063) was used. The size of the fragment analyzed was 78 bp.

Ethical & legal issues: Confidentiality of collected data was assured. Specimens were innominate & there was a code number for each specimen. Subjects have the option to withdraw their samples at any time. There is a plan to contact subjects with the findings. There is no future use of the specimens in any other purposes.

Outcome measures: Follow up of 2 groups was done to assess pregnancy course, out come and operative findings for confirmation of placenta accreta. As well as histopathological examination in cases of hysterectomies.

Statistical protocol: Statistical analysis is to be performed using Statistical Package for Social Sciences (SPSS® for windows® version 15.0. continuous data are to be presented as range (if parametric). Dichotomous or categorical data are to be presented as number and percentage. Difference between two independent groups is to be estimated using independent student's t-test (for parametric continuous variables) and Mann-Whitney's U-test (for non-parametric continuous variables) and chi-square test (categorical variables). Receiver operator characteristics (ROC) curve is to be constructed to assess the predictability of measured cell-free fetal DNA

Validity of this predictability is to be expressed in terms of sensitivity, specifity, and positive and negative predictive values as well as, likelihood ratios. In addition, Pearson tests were performed to examine the correlation between the cell-free fetal DNA levels in plasma and placental invasion.

Significance level is set at 0.05.

RESULTS

Comparing between the two groups, there were no significant statistical differences regarding Gestational age at first presentation but were found highly significant statistical differences regarding the incidence of preterm delivery with placenta accrete group and highly significant statistical differences regarding the increased maternal age in same group (Tab. 1.).

Highly significant statistical differences were found regarding the increased number of Ceserean deliveries in placenta accreta group. For example (7, 9) patient respectively in placenta accreta group while (3, 0) in control group having previous the four and five CS (Tab. 2.).

Comparing between two groups, there were no significant statistical differences regarding level of F-FDNA (Tab. 3.).

There is no significant statistical correlation between level of F-FDNA in maternal plasma and maternal age while there was significant statistical negative correlation between level of F-FDNA in maternal plasma and BMI, F-FDNA decrease with maternal obesity. There were significant statistical positive correlation between level of F-FDNA in maternal plasma and gestational age-FDNA increases as gestational age advances (Tab. 4.).

Tab. 1. Distribution of the studied groups regarding maternal age and gestational ages.

Parameters	Groups		t Test	P value
	Patients (n=25)	Controls (n=25)		
	Mean ± SD	Mean ± SD		
Maternal age	32.36 ± 4.64	28.76 ± 4.65	2.73	<0.001*
Gestational age at first presentation	31.68 ± 1.57	31.92 ± 1.89	0.48	0.628
Gestational age at delivery	35.60 ± 0.91	38.32 ± 0.69	11.88	<0.001*
*Significant				

Tab. 2. Distribution of the studied groups regarding BMI and number of Ceserean deliveries.

Parameters	Groups				t Test	P-value
	Patients (n=25)		Controls (n=25)			
	Mean ± SD		Mean ± SD			
BMI Kg\m^2	22.95 ± 1.29		22.86 ± 1.54		0.23	0.815
No of CS					χ^2 17.69	0.001*
One	0	0.0	8	32.0		
Two	2	20.0	8	32.0		
Three	7	24.0	6	24.0		
Four	7	24.0	3	12.0		
Five	9	32.0	0	0.0		

Tab. 3. Distribution of the studied groups regarding F-FDNA.

Parameters	Groups		t Test	P value
	Patients (n=25)	Controls (n=25)		
	Mean ± SD	Mean ± SD		
F- FDNA allele	302.20 ± 79.82	266.68 ± 70.04	1.67	0.101
*Significant				

There were (7) patients (28%) who under gone cesarean hysterectomy and (18) patients under gone conservative management (safe cesarean delivery) in placenta accreta group (Tab. 5.).

DISCUSSION

This study is designed to evaluate whether measurement of cell-free fetal DNA in the maternal plasma can be predictive of abnormal placental invasion in cases of placenta previa. The current study included 50 pregnant women with Maternal age range from 20-40 years old, Parity: para 1- para 5, Body mass index range from 18-25 kg/m^2, singleton pregnancy carrying male fetuses and gestational ages range from 28-34 weeks. Not included patients with multifetal pregnancy, Hypertension, preterm labor or intrauterine growth restriction, patients were not taking a tocolytic agent or those with uterine bleeding.

Women were divided into 2 groups:

Study group (1): 25 women with one or more previous cesarean section and placenta previa anterior only or with ultrasound finding suggestive of placental adhesion or invasion (accreta, increta or percreta).

Control group (2): 25 matched control with normally situated placenta without ultrasound finding suggestive of placental adhesion or invasion.

The primary finding of this study regarding the distribution of the studied groups regarding maternal age and gestational ages is that there was statistically significant differences regarding the maternal age between placenta previa accret group (Mean ± SD: 32.36 ± 4.64) and normally situated placenta group (Mean ± SD:28.76 ± 4.65) and (P value ≤ 0.001) this was in agreement with other studies which proved that advanced maternal age increases the inci-dence of placenta previa to 2% after 35 years of age and 5% after age 40 and is associated positively with Placenta accreta [13,14].

Comparing the gestational age at delivery in both groups, we found that there were significant statistical differences regarding the incidence of delivery at early gestational age in PA group (Mean ± SD: 35.60 ± 0.69) more than in normally situated placenta group (Mean ± SD: 38.32 ± 0.69) and (P=value ≤ 0.001). This was in agreement with El Behery et al, [13] and Hull et al. [14] who suggested that repetitive bleeding from placenta previa and previa accreta is a cause of preterm birth due to the need for iatrogenic preterm delivery. Also with agreement with Walfisch et al, [15] as the authors observed that Placental implantation abnormalities are a major cause for indicated preterm delivery, usually in an effort to prevent the anticipated catastrophic complications for both mother and foetus.

Comparing the number of cesarean deliveries in both groups, we found that there were significant statistical correlation between increased incidence of placenta accreta and the increase in cesarean delivery rate. Results were in agreement with Jauniaux et al., [2] who reported that the risks of both placenta previa and placenta accreta in subsequent pregnancies increase with the number of previous cesarean deliveries. A large multicenter cohort study by Bowman et al., [16] has noted that for women presenting with placenta previa and prior cesarean delivery, the risk of accreta placentation is 3%, 11%, 40%, 61%, and 67% for first, second, third, fourth, and fifth or more cesarean deliveries, respectively.

Tab. 4. Correlation between F-FDNA and BMI, Maternal and gestational age.

Parameters	F- FDNA					
	Patients	P value	Controls	P value	Total	P value
	r		r		r	
BMI	-0.632	0.001*	-0.626	0.001*	-0.585	<0.001*
Maternal age	0.356	0.081	0.098	0.640	0.259	0.070
Gestational age	0.792	<0.001*	0.737	<0.001*	0.694	<0.001*
*Significant r: Correlation						

Tab. 5. Distribution of the outcome (cases of hysterectomy) regarding F-FDNA among the studied patients in placenta accreta group.

Parameters	Outcome		Mann-Whitney Test	P value
	Safe cesarean delivery (n=18)	Cesarean hysterectomy (n=7)		
	Mean ± SD	Mean ± SD		
F- FDNA	330.44 ± 79.38	293.14 ± 94.71	0.90	0.389
*Significant				

Results were in agreement with Jauniaux et al., [2] who reported that PA complicates about 5% of pregnancies with placenta previa and expecting that if the CD rate continues to rise as it has in recent years, there will be an additional 6236 placenta previas, 4504 PAs, and 130 maternal deaths annually.

Results regarding the outcome, eighteen placenta accreta (72%) achieved successful conservative surgery. Efforts at conservative surgery with preservation of the uterus and fertility have included a multidisciplinary approach, which had included hemostatic sutures for focal accreta; uterine wedge resection; intrauterine ribbon gauze packing and uterine or internal iliac artery ligation; and control of and support for massive hemorrhage. seven cases (28%) were treated with cesarean hysterectomy without attempting to remove placenta, and pathological diagnosis verified 1 placenta accrete (14.3%) 3 increta (42.9%) and 3 placenta percreta (42.9%).

This was in agreement with Pan et al., [17] who reported that indications for cesarean hysterectomy have changed significantly during his study period from January 2004 to December 2014, that "uterine atony" decreasing from 50.0% (5/10) to 11.1% (1/9) and (P = 0.025), and placenta accreta as the indication for cesarean hysterectomy has increased significantly from 20.0% (2/10) to 77.8% (7/9) and (P = 0.005).

This was in agreement with arecent systematic review by Hecht et al. indicated that simple adherence (placenta accreta vera) represents about 60% of PAS, whereas the invasive grades, increta and percreta, represent 16% and 22% of PAS respectively [18].

The main finding of this study was that, there were no statistically significant differences regarding the level of free fetal DNA between patients group (Mean ± SD: 302.20 ± 79.82), control group (Mean ± SD: 266.68 ± 70.04) and non-significant P value = 0.101. Results in agreement with Samuel et al, (2013) who compared the fraction of ff DNA between pathologically confirmed cases of placenta accreta, placenta previa and normal placentation and found in their study the mean fraction of cff DNA for cases of placenta accrete was 19.1%, for previa alone was 27.2% and for prior CD without placenta previa or accrete was 28.9% (p <0.26), which did not differ significantly by groups. The median fraction of cff DNA for the accrete group (17.0%, range 12.6–30.0%) was also lower than the medians fractions for the previa group (30.1%, range 16.1–33.6%) and the prior CD group (22.7%, range 14.1–40.0%) [7].

In contrast Seikazawa et al., [10] observed a higher concentration of cff DNA in cases of placenta previa versus controls. In addition, the two patients with placenta accreta in this study had the highest levels of cff DNA detected. Despite this pilot study suggesting a significant increase in the amount of fetal DNA found in maternal serum in cases of invasive placentation, we could not confirm this correlation.

We were surprised with the lack of an association between cff DNA and abnormal placentation. One possible explanation is that the pathogenesis of invasive placentation is related to the abnormal quality of the maternal myometrium and decidua and not due to any abnormality in the placental body. It is also possible that the abnormality in placental invasion (possibly correlating with increased apoptosis and release of cffDNA) is a process much earlier in gestation than when this group of patients was sampled and perhaps a significant difference between groups would be seen in the first trimester.

Regarding the negative correlation between ff DNA and body mass index of studied groups, Our results were in agreement with Ashoor et al., [19] and Shi et al., [20] who proved that maternal weight and/or maternal body mass index (BMI) has a significant effect on ff DNA.

Obesity during pregnancy is associated with lower ff DNA as has been consistently demonstrated in several independent studies by Haghiac et al., [21] and Canick et al., [22]. Lower ff DNA in gestational obesity may be due to a dilutional effect from increased maternal circulatory volume. In addition, there may be an increase in maternal cell free DNA release from apoptosis/necrosis of stromal vascular cells and adipose tissue in obese pregnant women so decrease the percentage of free fetal DNA.

Results positively correlate level of ff DNA with gestational age are in agreement with Wang and colleagues 2013 who noted that levels of fetal DNA increase throughout pregnancy, with an initial rise of 0.1% per week

from 10 to 20 weeks of gestation, followed by a sharper increase of 1% per week after 21 weeks to term [20-22].

CONCLUSION

Placenta previa and morbidly adherent placenta (MAP) may cause significant maternal morbidity and mortality from postpartum hemorrhage (PPH), which accounts for ~ 29%, of maternal mortality cases. The incidence of placenta accreta is directly linked with the increase in cesarean delivery. Multidisciplinary management of patients with suspected placenta accreta is superior to standard obstetric care. Ultrasound has become the primary screening tool for women at risk of PA Color flow and power Doppler sonography have also been reported to facilitate the diagnosis. MRI is better than ultrasound in defining areas of abnormal placentation and assessing the depth of myometrial invasion, particularly in cases of posterior placentae. Non-significant association between cff DNA and PA. Obesity during pregnancy is associated with lower ff DNA; gestational age is an additional factor that can affect the ff, levels of fetal DNA increase throughout pregnancy.

RECOMMENDATIONS

Recommendations considering a larger cohort study and multicentric studies for estimation of free fetal DNA particularly in the first trimester. The absolute change in quantity in fetal DNA over time throughout the pregnancy may be higher in cases than in controls, so analysis at multiple time points may be useful. Finally, other markers of placental invasion including placental mRNA could be evaluated with a more promising association to abnormal placental invasion.

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