Title: Procedure related risk of miscarriage from chorionic villus sampling and amniocentesis

Short title: Procedure-related risk of miscarriage

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Abstract

Objective:

The objective of our study was to estimate the procedure-related risks of miscarriage following CVS and amniocentesis in a large unselected screened and to determine whether these risks are consistent with those reported in systematic reviews and meta-analysis.

Methods:

This was a retrospective cohort study undertaken at a large Fetal Medicine Unit in the United Kingdom during the period of January 2009 to May 2018. We included all singleton pregnancies that booked at our unit before 20 weeks after excluding those with multiple pregnancies, major fetal defects, terminations and lost to follow-up. We estimated the risk of miscarriage in those that had a CVS or amniocentesis as well as those that did not have any invasive procedure, to estimate the procedure-related risk as a risk-difference (95% confidence interval [CI]). Univariate and multivariate regression analysis was used to derive odds ratios (OR) (95%CI) and determine which maternal and pregnancy characteristics provided a significant contribution in prediction of miscarriage and whether CVS or amniocentesis provided a significant independent.

Results:

During the study period, there were 45,120 singleton pregnancies, including 1,546 that had an invasive procedure. We excluded 1,429 pregnancies (3.2%), due to fetal defects, termination of pregnancy or those with missing outcomes. In pregnancies that underwent CVS, the risk of miscarriage was 1.5% (13/861), compared to 1.2% (476/39,152) in pregnancies that did not have a procedure (p=0.437). In pregnancies that underwent an amniocentesis, the risk of miscarriage was 0.8% (3/375), compared to 1.2% (491/42,463) in those that did not (p=0.520). Univariate and multivariate regression analysis demonstrated that there was no significant prediction to the risk of miscarriage from CVS (p=0.399; p=0.592, respectively) or amniocentesis (p=0.543; p=0.550, respectively). The risk of procedure-related loss attributed to CVS was 0.29% (95%CI: -0.53-1.12) and that following amniocentesis was -0.36% (95%CI: -1.26-0.55), which was not significantly different from those that did not have any procedure.

Conclusion:

The procedure-related risks of miscarriage following CVS and amniocentesis are considerably lower than currently quoted. The estimates of risks based on our study are 0.29% for CVS and -0.36 for amniocentesis.

Introduction

Amniocentesis and chorionic villus sampling (CVS) are invasive procedures carried out for prenatal diagnosis. It is essential that women are provided with accurate evidence-based information regarding risks of miscarriage from these invasive procedures. However, there is still considerable variation in recommendations from professional bodies regarding procedure-related risks of miscarriage quoted to women, with some reporting that the additional risk following a CVS is up to 1-2% and that following an amniocentesis is 1%[1-5]. There are recent systematic reviews and meta-analysis, as well as large population and cohort studies, which report that the procedure-related risks of miscarriage following invasive procedures carried out by specialists in Fetal Medicine Centres are much lower than currently reported[6-11]. There is a need to update and standardise information provided to women to allow them to make informed decisions based on accurate figures.

The objective of our study was to estimate the procedure-related risks of miscarriage following CVS and amniocentesis in a large unselected screened population in large specialist Fetal Medicine Unit and to determine whether these risks are consistent with those reported in systematic reviews and meta-analysis.

Materials and Methods

Study population

This was a retrospective cohort study undertaken at the Fetal Medicine Centre at Medway NHS Foundation Trust, in the United Kingdom during the period of 1st January 2009 to 31st May 2018. In our unit, all women booking for their pregnancy care prior to 14 weeks' gestation are offered an appointment at 11-13 weeks' for dating of the pregnancy by measurement of fetal crown-rump length (CRL), assessment of fetal anatomy and combined screening for trisomies 13,18 and 21. The assessment of risk for aneuploidies from combined screening is based on maternal age, measurement of fetal nuchal translucency (NT) thickness and maternal serum free β-human chorionic gonadotrophin (β-hCG) and pregnancy associated plasma protein-A (PAPP-A) [12-14]. Women booking after 14 weeks' are offered scan to date the pregnancy, assess fetal anatomy and assessment of risk of fetal aneuploidies from second trimester serum biochemical

testing[15]. At each of these visits, we record maternal demographic characteristics and medical history on an electronic database (Viewpoint version 5.6; General Electric Company).

Invasive procedures

Women who were deemed to be at high-risk for fetal aneuploidies or those with major defects were offered the option of an invasive prenatal diagnosis. CVS is offered as the procedure of choice up to 15 weeks' gestation and amniocentesis is offered after this gestational age. All procedures were either carried out either by a specialist in Fetal Medicine or by trainees under direct supervision of the specialist. All procedures were carried out transabdominally under direct ultrasound guidance with a free-hand technique and using standard antiseptic precautions for outpatient procedures with no routine use of antibiotic prophylaxis before or after the procedure. The CVS procedures were carried out with 17G x 17cm linear echo CVS needle (Rocket® LX™ Chorionic Villus Sampling Set, Rocket Medical PLC, Tyne and Wear, NE38 9BZ, United Kingdom). The amniocentesis procedures were carried out with 22G x 15 cm EchoTip® amniocentesis needle, Cook Medical, Bloomington, USA).

Inclusion criteria

The inclusion criteria were all singleton pregnancies which booked at our hospital and Fetal Medicine Centre for their pregnancy care before 20 week's gestation. We excluded multiple pregnancies, pregnancies with major fetal defects, termination of pregnancies and those lost to follow-up. All pregnancies meeting the inclusion criteria were divided into two groups: invasive group, which had either a CVS or an amniocentesis and a group that did not have any invasive procedures. Miscarriage was defined as a pregnancy loss prior to 24 weeks' gestation. We compared the risks of miscarriage in pregnancies undergoing CVS and amniocentesis and compared them to those that did not have any invasive procedure. The procedure-related risk of pregnancy loss following any invasive procedure was calculated as a risk-difference between the two groups.

Statistical analysis

Comparison of the maternal and pregnancy characteristics in the outcome groups was by the χ^2-square test and Fisher's exact test for categorical variables and Mann-Whitney U-test for continuous variables, respectively. Significance was assumed at 5% and *post hoc* Bonferroni correction was used to adjust for multiple comparisons where necessary.

Data for risks of miscarriage were entered into contingency tables and absolute risks were estimated by determining the prevalence of miscarriage in the study groups. Univariate and multivariate logistic regression analysis was used to determine which of the maternal and pregnancy characteristics provided a significant contribution in prediction of miscarriage. To determine whether either CVS or an amniocentesis had any significant independent prediction of miscarriage, we estimated unadjusted and adjusted odds from univariate and multivariate regression analysis to derive odds ratio (OR) (95% confidence intervals [CI]). The estimates of procedure-related risks of miscarriage from CVS or amniocentesis were calculated as a risk-difference (95%CI).

The statistical package SPSS 24.0 (IBM SPSS Statistics for Windows, Version 24.0, Armonk, NY: IBM Corp; 2016) was used for data analyses.

Results

Study population

During the study period of 1st January 2009 to 31st May 2018, 45,120 singleton pregnancies were booked for their pregnancy care at our hospital. In this population, there were 43,574 (96.6%) who did not any invasive procedures and 1,546 (3.4%) who had an invasive procedure prenatal diagnosis, including 1142 (73.9%) who had CVS and 419 (27.1%) who had an amniocentesis. We excluded 1,429 pregnancies (3.2%), due to major fetal defects or those that ended in termination of pregnancies (n=475) and those with missing follow-up data for the pregnancy (n=954). The study population therefore included 43,691 singleton pregnancies with complete outcome data, including 507 (1.2%) that ended in miscarriage prior to 24 weeks' gestation and 43,184 (98.8%) that delivered a phenotypically normal neonate. Out of the study population of 43,691 pregnancies, there were 40,013 (91.6%) that had first-trimester combined screening and 3,678 (8.4%) pregnancies that booked late between 14 and 24 weeks' gestation. In these study groups, we carried out 1,228 invasive procedures, including 861 (70.1%) that had CVS and 375 (30.5%) that had an amniocentesis, including 8 (0.7%) that had both a CVS and amniocentesis.

The maternal and pregnancy characteristics in the study groups are compared in Table 1. In pregnancies that ended in miscarriage compared to those that did not, the median maternal

height was smaller, there were more women with Afro-Caribbean, South Asian, East Asian and Mixed racial origin, more women who conceived following assisted conception and and higher prevelance of pregnancies with chronic hypertension. The maternal characteristics in pregnancies that underwent a CVS or an amniocentesis, compared to those that did not are compared in Supplementary Tables 1 and 2.

Factors predicting risk of miscarriage in study population

The maternal and pregnancy characteristics associated with risk of miscarriage were examined with univariate and multivariate regression analysis (Table 2). After adjustment for confounding factors in multivariate analysis, the maternal characteristics associated with a subsequent risk of miscarriage following a first trimester scan at 11-14 week's gestation were advanced maternal age, weight and height, racial origin, method of conception and parity and chronic hypertension but not cigarette smoking or other medical disorders such as diabetes mellitus, epilepsy, asthma or thyroid disorders. These maternal characteristics providing a significant contribution in the multivariate analysis formed the *a-priori* risk for miscarriage. The components of first trimester combined screening which provided a significant contribution in prediction of miscarriage were $\log_{10}$ *a-priori* risk, an increased fetal NT≥95th percentile, serum PAPP-A MoM≤0.3 and a reversed a-wave in the ductus venosus but not serum free β-HCG MoM (p=0.913).

Procedure-related risk of miscarriage after CVS and amniocentesis

In the study population, the risk of miscarriage following invasive prenatal diagnostic procedures was 1.3% (16/1228) compared to 1.2% (491/42,463) in pregnancies that did not have any invasive procedure (p=0.636). Univariate regression analysis demonstrated that there was no significant prediction to the risk of miscarriage from invasive procedures (p=0.636). Multivariate regression analysis demonstrated that the addition of invasive procedures to the combination of $\log_{10}$ *a-priori* risk from maternal factors and components of first trimester combined screening including fetal NT, serum PAPP-A MoM and flow in ductus venosus, did not provide any significant contribution (p=0.415) to the prediction of miscarriage. The risk of procedure-related loss attributed to any invasive procedure was 0.1% (95%CI: -0.5 to 0.8), which was not significantly different from those that did not have any procedure.

In pregnancies that underwent CVS, the risk of miscarriage was 1.5% (13/861), compared to 1.2% (476/39,152) in pregnancies that had first trimester combined screening and no invasive procedure (p=0.437). Univariate regression analysis demonstrated that there was no significant

prediction to the risk of miscarriage from CVS (p=0.399). Multivariate regression analysis demonstrated that the addition of CVS to the combination of $\log_{10}$ *a-priori* risk from maternal factors and components of first trimester combined screening including fetal NT, serum PAPP-A MoM and flow in ductus venosus, did not provide any significant contribution (p=0.592) to the prediction of miscarriage. The risk of procedure-related loss attributed to CVS was 0.29% (95%CI: -0.53 to 1.12; p=0.483), which was not significantly different from those that did not have any procedure.

In pregnancies that underwent an amniocentesis, the risk of miscarriage was 0.8% (3/375), compared to 1.2% (491/42,463) in pregnancies that did not have any invasive procedure during the pregnancy (p=0.520). Univariate regression analysis demonstrated that there was no significant prediction to the risk of miscarriage from amniocentesis (p=0.543). Multivariate regression analysis demonstrated that the addition of amniocentesis to the combination of $\log_{10}$ *a-priori* risk from maternal factors, did not provide any significant contribution (p=0.550) to the prediction of miscarriage. The risk of procedure-related loss attributed to amniocentesis was -0.36% (95%CI: -1.26 to 0.55; p=0.442), which was not significantly different from those that did not have any procedure.

Discussion

Principal findings of the study

The findings our study demonstrate that first, there is no significant increase in risks of miscarriage following a CVS or amniocentesis compared to pregnancies that had no invasive procedure; second, our findings confirm the association of miscarriage with maternal and pregnancy characteristics and third, the estimate of procedure-related risk of miscarriage from CVS is 0.29% (95%CI -0.53 to 1.12) and from amniocentesis is -0.36% (95%CI: -1.26 to 0.55).

Strengths and limitations

The strengths of the study are first, examination of a large unselected cohort of consecutively screened pregnancies in a large specialist Fetal Medicine Unit; second, procedures were either carried out or directly supervised by specialist Fetal Medicine Consultants; and third, accurate ascertainment of maternal and pregnancy characteristics along with pregnancy outcomes to ensure valid estimation of risks of miscarriage.

The limitations of our study relate to the retrospective study design but we accounted for the potential biases due to this by ensuring that the study population was an unselected screened cohort over a fixed period of time, thus avoiding any selection bias in choosing any cases or controls. Similarly, the possibility of recall bias was unlikely in our study as the risk factors associated with the adverse outcome were recorded systematically in our database before the occurrence of the invasive procedure and pregnancy outcome. Thirdly, all our procedures were carried out transabdominally and therefore the estimates of risks only relate to procedures carried out transabdominally.

Comparison with other studies

The findings of our study are consistent with results of systematic reviews and meta-analysis which report that the procedure-related risk of miscarriage from invasive procedures is much lower than currently quoted[6-11]. A large nationwide population-based study of 147,987 women with a singleton pregnancy including 5,072 who underwent CVS and 1,809 who underwent amniocentesis, reported that the procedure-related risk of miscarriage at 21 days following CVS was -0.21% and that at 28 days following amniocentesis was 0.56%[7]. A recent meta-analysis of large controlled studies, which took into account the results of the large Danish population-based study, reported that there were 623 losses in 64,901 women who underwent amniocentesis and 327 losses in 19,000 women who underwent CVS and the procedure-related risks of miscarriage, after taking into account the miscarriage rate in controls that did not have an invasive procedure, was about 0.35% and 0.30%, respectively.[8] The findings of our study are also consistent with a previous study reporting that the characteristics that are significantly associated with risks of miscarriage, such as increased fetal NT, decreased serum PAPP-A and reversed a-wave in ductus venosus, are the very factors that are associated with increased risk for aneuploidies and therefore an update of a CVS[9]. Therefore, in estimation of procedure-related risk of invasive procedures, it is necessary to adjust for these confounding factors. The findings our study based, on a large unselected cohort of more than 45,000 pregnancies, are consistent with the results from the systematic reviews and meta-analysis, confirming that the procedure-related risks of miscarriage from invasive procedures are considerably lower than what are currently recommended by professional bodies[6,8].

Implications for clinical practice

The main clinical implication from our study is that the procedure-related risks of miscarriage are considerably lower than those currently informed to women and in view of these results as well as those from meta-analysis[6,8], the procedure-related risks associated with CVS and amniocentesis should be revised and made uniform across all recommendations and guidelines. It is important to note that the results from our study are those from a specialist Fetal Medicine Centre, where all the procedures in the study were either undertaken or directly supervised by Fetal Medicine specialists. The results of the meta-analysis also emphasise this point as the studies that were included in the analysis were those that were done by experts in large specialist centres and therefore, the reported procedure-related risks are those from expert operators[6]. There is also evidence from other studies highlighting the fact that the risk of miscarriage from invasive procedures is related to the skill and experience of the operator. Therefore, it may be worthwhile to consider that invasive prenatal diagnostic procedures should be undertaken by skilled operators in specialist centres to ensure low procedure-related complications, as reported in studies, rather than the procedures being carried out by operators who do these procedures infrequently, in which case the procedure-related risks may well be higher.

Conclusion

The procedure-related risks of miscarriage following CVS and amniocentesis in our study are considerably lower than currently quoted and consistent with the estimates of such risks reported from systematic reviews and meta-analysis.

Conflicts of interest

No conflict of interest to report

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Table 1. Maternal and pregnancy characteristics in pregnancies that miscarried compared to those that did not.

Maternal and pregnancy characteristics	No miscarriage	Miscarriage
Sample size	(n=43,184)	(n=507)
Maternal age in years, median (IQR)	28.1 (24.3-32.0)	28.0 (24.1-33.0)
Maternal weight in kg, median (IQR)	68.8 (59.7-81.1)	69.6 (59.1-85.0)
Maternal height in mt, median (IQR)	1.64 (1.60-1.69)	1.63 (1.59-1.67)**
Racial origin		
Caucasian (Reference), n (%)	39,615 (91.7)	427 (84.2)
Afro-Caribbean, n (%)	1,342 (3.1)	26 (5.1)*
South Asian, n (%)	1,860 (4.3)	40 (7.9)**
East Asian, n (%)	147 (0.3)	6 (1.2)**
Mixed, n (%)	220 (0.5)	8 (1.6)*
Conception		
Spontaneous (Reference), n (%)	42,497 (98.4)	489 (96.4)
Assisted conception, n (%)	687 (1.6)	18 (3.6)**
Cigarette smoking, n (%)	6,558 (15.2)	86 (17.0)
History of medical disorders		
Chronic hypertension, n (%)	375 (0.9)	14 (2.8)**
Diabetes mellitus, n (%)	316 (0.7)	6 (1.2)
Connective tissue disorders, n (%)	63 (0.1)	0
Thrombophilia, n (%)	43 (0.1)	0
Asthma, n (%)	2,223 (5.1)	33 (6.5)
Epilepsy, n (%)	254 (0.6)	3 (0.6)
Nulliparous	20,104 (46.6)	209 (41.2)*

IQR = interquartile range; Significance level p ** p<0.0001; *p<0.01.

Table 2. Univariate and multivariate regression analysis in prediction of miscarriage and contribution of chorionic villus sampling and amniocentesis

Variable	Univariate OR (95% CI)	P value	Multivariate OR (95%CI)	P value
Maternal characteristics				
Maternal age ≥40 years	2.48 (1.65-3.74)**	<0.001	1.92 (1.26-2.93)*	0.001
Maternal weight in kg	1.00 (1.00-1.01)	0.225	1.01 (1.00-1.01)*	0.005
Maternal height in cm	0.96 (0.95-0.98)**	<0.001	0.96 (0.95-0.98)**	<0.001
Racial origin				
Caucasian (Reference)	1.00		1.00	
Afro-Caribbean	2.00 (1.35-2.97)*	0.001	1.74 (1.16-2.60)*	0.006
South Asian	2.07 (1.49-2.89)**	<0.001	1.80 (1.28-5.54)*	0.001
East Asian	4.11 (1.80-9.37)*	0.001	3.31 (1.43-7.65)*	0.006
Mixed	3.83 (1.88-7.82)**	<0.001	3.77 (1.84-7.73)**	<0.001
Method of conception				
Spontaneous (Reference)	1.00		1.00	
Assisted conception	2.06 (1.27-3.37)	0.004	1.92 (1.16-3.19)*	0.023
Cigarette smoking	1.20 (0.95-1.52)	0.128	-	-
History of medical disorders				
Chronic hypertension	3.34 (1.95-5.74)**	<0.001	2.70 (1.55-4.71)**	<0.001
Diabetes mellitus	1.40 (0.57-3.39)	0.462	-	-
Asthma	1.31 (0.92-1.87)	0.137	-	-
Epilepsy	1.03 (0.33-3.23)	0.960	-	-
Maternal / pregnancy characteristics				
Maternal characteristics (Log_{10} *a-priori)*	10.61 (6.94-16.23)**	<0.001	10.56 (6.66-16.64)**	<0.001
Fetal NT ≥95th percentile	3.34 (2.18-5.11)**	<0.001	2.91 (1.75-4.86)**	<0.001
Reversed a-wave in ductus venosus	2.58 (1.53-4.35)**	<0.001	2.22 (1.24-4.00)*	0.008
Serum free β-HCG MoM ≤0.3 MoM	1.59 (0.82-3.10)	0.171	-	-
Serum PAPP-A MoM ≤0.3 MoM	2.63 (1.70-4.06)**	<0.001	2.46 (1.59-3.81)**	<0.001
Invasive procedure				
Chorionic villus sampling	1.27 (0.73-2.21)	0.399	-	-
Amniocentesis	0.69 (0.22-2.16)	0.543	-	-

Significance level ** p<0.0001; *p<0.01

Supplementary Table 1. Maternal and pregnancy characteristics in pregnancies undergoing chorionic villus sampling compared to those that did not have an invasive procedure.

Maternal and pregnancy characteristics	No invasive procedure	Chorionic villus sampling
Sample size	(n=37,152)	(n=861)
Maternal age in years, median (IQR)	28.1 (24.3-32.0)	33.2 (28.1-37.4)**
Maternal weight in kg, median (IQR)	69.0 (59.9-81.5)	69.0 (59.9-81.5)
Maternal height in mt, median (IQR)	1.64 (1.60-1.69)	1.64 (1.60-1.69)
Racial origin		
Caucasian, n (%)	36,082 (92.2)	755 (87.7)
Afro-Caribbean, n (%)	1,137 (2.9)	46 (5.3)**
South Asian, n (%)	1,635 (4.2)	41 (4.8)
East Asian, n (%)	118 (0.3)	12 (1.4)**
Mixed, n (%)	180 (0.5)	7 (0.8)
Method of conception		
Spontaneous (Reference), n (%)	38,468 (98.3)	848 (98.5)
Assisted conception, n (%)	684 (1.7)	13 (1.5)
Cigarette smoking, n (%)	5,802 (14.8)	110 (12.8)
History of medical disorders		
Chronic hypertension, n (%)	345 (0.9)	18 (2.1)**
Diabetes mellitus, n (%)	285 (0.7)	13 (1.5)*
Connective tissue disorders, n (%)	59 (0.2)	0
Thrombophilia, n (%)	42 (0.1)	1 (0.1)
Asthma, n (%)	2,101 (5.4)	28 (3.3)*
Epilepsy, n (%)	234 (0.6)	4 (0.5)
Nulliparous	17,840 (45.6)	310 (36.0)**
First trimester combined screening		
Fetal crown-rump length in mm, median (IQR)	64.8 (59.3-71.0)	68.3 (61.3-74.3)*
Fetal nuchal translucency ≥ 95th percentile, n (%)	365 (0.9)	201 (25.3)**
Reversed a-wave in ductus venosus, n (%)	417 (1.1)	55 (6.9)**
Serum free β-HCG MoM, median (IQR)	1.03 (0.70-1.53)	1.42 (0.84-2.34)
Serum PAPP-A MoM, median (IQR)	0.99 (0.69-1.41)	0.57 (0.35-0.96)
Pregnancy outcome		
Gestation age at delivery in weeks, median (IQR)	39.6 (38.6-40.5)	39.2 (38.1-40.3)*
Birthweight in grams, median (IQR)	3420 (3075-3760)	3345 (2970-3710)*

IQR = interquartile range; PAPP-A = pregnancy associated plasma protein-A; MoM = Multiple of expected median; Significance level p ** p<0.0001; *p<0.01.

Supplementary Table 2. Maternal and pregnancy characteristics in pregnancies undergoing amniocentesis compared to those that did not have an invasive procedure.

Maternal and pregnancy characteristics	No invasive procedure	Amniocentesis
Sample size	(n=42,463)	(n=375)
Maternal age in years, median (IQR)	28.1 (24.2-32.0)	29.0 (23.6-35.0)
Maternal weight in kg, median (IQR)	68.8 (59.7-81.2)	67.0 (60.4-79.0)
Maternal height in mt, median (IQR)	1.64 (1.60-1.69)	1.64 (1.59-1.67)
Racial origin		
Caucasian (Reference), n (%)	38,958 (91.7)	337 (89.9)
Afro-Caribbean, n (%)	1,307 (3.1)	15 (4.0)
South Asian, n (%)	1,838 (4.3)	21 (5.6)
East Asian, n (%)	140 (0.3)	1 (0.3)
Mixed, n (%)	220 (0.5)	1 (0.3)
Conception		
Spontaneous (Reference), n (%)	41,773 (98.4)	372 (99.2)
Assisted conception, n (%)	690 (1.6)	3 (0.8)
Cigarette smoking, n (%)	6,469 (15.2)	66 (17.6)
History of medical disorders		
Chronic hypertension, n (%)	369 (0.9)	2 (0.5)
Diabetes mellitus, n (%)	303 (0.7)	6 (1.6)
Connective tissue disorders, n (%)	61 (0.1)	2 (0.5)
Thrombophilia, n (%)	42 (0.1)	0
Asthma, n (%)	2,208 (5.2)	20 (5.3)
Epilepsy, n (%)	250 (0.6)	3 (0.8)
Nulliparous	19,844 (46.7)	162 (43.2)
Pregnancy outcome		
Gestation age at delivery in weeks, median (IQR)	39.6 (38.5-40.5)	39.1 (37.5-40.3)
Birthweight in grams, median (IQR)	3415 (3070-3750)	3170 (2682-3630)

IQR = interquartile range; Significance level p ** p<0.0001; *p<0.01.