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Early vaginal bleeding has no impact on markers used in first trimester aneuploidy screening

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Objective To assess the impact of early vaginal bleeding on the levels of markers used in first trimester screening for aneuploidy.

Methods A retrospective analysis was carried out on the free beta human chorionic gonadotrophin (β-hCG) and pregnancy associated plasma protein-A (PAPP-A) levels and nuchal translucency thickness in 49 653 women with a normal singleton fetus who had first trimester combined screening for Down Syndrome in three centres. Median MoMs and the distribution of log MoMs of the two markers were compared in two groups—7470 women who self-reported vaginal bleeding and 42 183 women who reported no vaginal bleeding at any stage prior to the screening test.

Results The overall median MoM free β-hCG and that in the bleeding and non-bleeding group were 0.9854, 1.0012 and 0.9832, and for PAPP-A were 1.0407, 1.0413 and 1.037. There was no significant difference between the bleeding and non-bleeding group by median test ($p = 0.080$) or by t-test comparing log MoMs ($p = 0.1305$) for free β-hCG and for PAPP-A with median test ($p = 0.5071$) or by t-test comparing log MoMs ($p = 0.1740$). For delta nuchal translucency (NT) there was also no significant difference between the bleeding and non-bleeding group ($p = 0.055$).

Conclusion Vaginal bleeding has little or no impact on first trimester marker levels and no correction is necessary. Copyright © 2010 John Wiley & Sons, Ltd.

KEY WORDS: Downs syndrome; prenatal Screening; PAPP-A; free β-hCG; trisomy; nuchal translucency; vaginal bleeding

INTRODUCTION

Effective screening for Downs syndrome is provided in the first trimester by a combination of fetal nuchal translucency (NT) thickness and maternal serum free beta human chorionic gonadotrophin (β-hCG) and pregnancy associated plasma protein-A (PAPP-A) enabling detection rates of 90% to be achieved at a false positive rate of 5% or less (Spencer *et al*., 1999; Nicolaides *et al*., 2005). In addition, this method of screening identifies a large proportion of the other major aneuploidies such as trisomies 13 and 18 (Tul *et al*., 1999; Spencer *et al*., 2000b), triploidy (Spencer *et al*., 2000d) and various sex aneuploidies (Spencer *et al*., 2000a).

A number of confounding factors are known to influence the maternal serum biochemical markers, including gestational age, maternal weight, ethnicity, smoking status, parity, mode of conception (assisted reproduction), twin pregnancy and chorionicity (Spencer, 2000, 2005; Spencer *et al*., 2000c, 2000f, 2003a, 2004, 2005a, 2008; Liao *et al*. 2001; Kagan *et al*., 2007). Most of these confounders have such a significant influence as to require correction for. Others such as diabetes, fetal sex, previous aneuploid history (Spencer *et al*., 2000e, 2005b, 2009; Cuckle *et al*., 2005; Cowans *et al*., 2009) and previous screening result (Spencer, 2001, 2002) are either too small or too problematical to make correction for.

In the second trimester a relatively small number of studies have identified a potential impact of early vaginal bleeding on the maternal serum biochemical markers used at this time. Alpha-fetoprotein (AFP) levels are significantly increased by around 9%, but although increases have been demonstrated for unconjugated oestriol (5%) and total hCG (12%) the latter two are not statistically significant (Cuckle *et al*., 1994). Elevated levels of AFP in other studies confirm the relationship with vaginal bleeding or threatened abortion (Haddow *et al*., 1986; Bernstein *et al*., 1992; Williams *et al*., 1992; Berry *et al*., 1995).

In the first trimester, de Biasio *et al*. (2003) studied the effect of early vaginal bleeding on a small cohort of 2330 singleton pregnancies undergoing first trimester screening and reported a significant increase by 9% in free β-hCG but no significant difference in PAPP-A or NT. In an identical study of a cohort of 1755 singleton pregnancies, Heinig *et al*. (2007) found no significant difference in delta NT or free β-hCG or PAPP-A MoM between the group with bleeding compared to those without bleeding—although the trend was for a 10% increase in free β-hCG and a 12% increase in PAPP-A.

The aim of this study was to evaluate in a large cohort of women screened in three centres, whether vaginal bleeding at any time prior to screening, as reported by

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the pregnant women, has any impact on the level of the biochemical markers used in first trimester screening.

METHODS

Information on vaginal bleeding at any time in pregnancy prior to screening is routinely sought either on the first trimester screening request form or during the patient consultation in the screening centres at King George Hospital, Goodmayes (formerly at Harold Wood Hospital, Romford), at Harris Birthright Research Centre for Fetal Medicine, King's College Hospital, London, and at the Fetal Medicine Centre, London. In all three centres all relevant clinical information is recorded in a fetal database (PIA- Fetal Database, ViewPoint, Wessling, Germany) along with ultrasound information and the biochemical parameters measured as part of a first trimester screening program. At each centre ultrasound is carried out according to the FMF criteria by sonographers who have fulfilled the certificate of competency in the first trimester scan, and crown rump length (CRL) and NT are measured in a standardised way (www.fetalmedicine.com). At King George Hospital and at The Fetal Medicine Centre, the biochemical markers were measured by time-resolved, amplified cryptate emission using the Brahms Kryptor system (Brahms AG, Hennigsdorf, Germany), whilst at King's College the markers were measured using time-resolved fluorescence using the DelfiaXpress system (PerkinElmer Life Sciences, Turku, Finland).

A query of the databases at the three sites was set up to extract relevant data in all live-born singleton pregnancies unaffected by aneuploidy which had been screened during 2008 at the King George centre and during 2006 to end of 2008 for the other two centres and for which information on bleeding had been provided. During the same period data were also extracted for pregnancies identified with Trisomy 21.

The measured concentrations of free β-hCG and PAPP-A were converted to MoMs using the gestation as estimated from CRL and corrected for maternal weight, ethnicity, parity, smoking status, mode of conception (ART) (Spencer, 2000, 2005; Spencer *et al*., 2000c, 2000f, 2003a, 2004, 2005a, 2008; Liao *et al*., 2001; Kagan *et al*., 2007) and type of analyser according to the FMF 2007/8 algorithm (Kagan *et al*., 2008). NT was converted to delta NT according to CRL using a previously derived equation (Spencer *et al*., 2003b).

In order to assess whether marker levels in the early vaginal bleeding and the no bleeding group were different, we performed a median test on the two groups and furthermore logarithmically transformed the individual MoMs and performed a *t*-test on the Gaussian normalised distributions. We considered a p value of 0.05 or less to mean a significant difference between the groups.

RESULTS

Table 1 summarises the study group populations from the three centres. The FMC is a private centre with a higher proportion of Caucasian non-smoking women of an older age group and a higher ART conception, whilst the other two populations are routine NHS screened populations with a wider range of ethnic mix and a higher level of smokers. The proportion of women reporting bleeding varied from 13.3% to 17.8% (15.0% overall), which is similar to other reports in the literature of singleton spontaneously conceived pregnancies (De Sutter *et al*., 2006).

In the Euploid group the overall median MoM free β-hCG was 0.9854 in 49 653 cases. The median MoM in those 7470 women reporting vaginal bleeding was 0.9832 and 1.0013 in those reporting no vaginal bleeding, and this difference was not statistically significant ($p = 0.0800$). When log transformed the mean $\log_{10}$MoM (sd) was 0.00753 (0.2654) in the women reporting vaginal bleeding and 0.00255 (0.2619) in those reporting no bleeding, and this difference also was not significant ($p = 0.1305$). The overall median MoM PAPP-A was 1.0407 in the 49 653 cases. The median MoM in those reporting vaginal bleeding was

Table 1—Summary of the study population

Variable	Fetal Medicine Centre	King's College Hospital	King George Hospital
Number	10 956	18 847	19 849
Period	01/06–12/08	03/06–10/08	01/08–12/08
% Bleeding	13.5	17.8	13.3
Biochemical Analyser	Kryptor	DelfiaXpress	Kryptor
Median maternal age (yrs)	35.9	32.2	30.0
Median maternal weight (kg)	63.0	66.0	65.0
Median GA (days)	87.0	87.0	84.0
% ART	10.5	3.2	2.9
% Nulliparous	42.8	46.8	43.1
% Smoker	2.3	8.3	13.3
Ethnicity			
% Caucasian	92.7	69.8	68.2
% Afro-caribbean	0.7	20.4	11.4
% Asian	4.5	4.8	13.9
% Oriental	0.9	1.8	1.5
% Other	1.2	3.2	5.0

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1.0373 compared to 1.0413 in those reporting no vaginal bleeding, and this difference was not statistically significant ($p = 0.5071$). When log transformed the mean $\log_{10}$MoM (sd) was 0.00146 (0.2531) in the women reporting vaginal bleeding and 0.00563 (0.2427) in those reporting no bleeding, and this difference also was not significant ($p = 0.1740$). The standard deviation of 0.243–0.253 is slightly wider in the vaginal bleeding group, if this unequal variance allowed for the t-test is even less significant ($p = 0.1867$).

For delta NT the overall median was +0.091 mm with +0.090 mm in the group with no vaginal bleeding compared with +0.100 mm in the group with vaginal bleeding, and this was not significant ($p = 0.055$).

In those pregnancies affected by Trisomy 21 ($n = 103$) there were 12 cases with recorded vaginal bleeding and 81 with no vaginal bleeding. The median PAPP-A MoM was 0.473 compared with 0.481 and was not significantly different ($p = 0.513$). The median free β-hCG MoM was 2.071 compared with 0.2089 and was not significantly different ($p = 0.156$), whilst delta NT was 2.152 and 2.181 and not significantly different ($p = 0.621$).

These results are summarised in Table 2.

DISCUSSION

The results of this study have shown that early vaginal bleeding in women who subsequently deliver a normal baby has no impact on the first trimester markers and consequently there is no need to consider correcting MoMs in such situations. Risks calculated from maternal serum free β-hCG and PAPP-A in conjunction with fetal NT will therefore be accurate in such circumstances. De Biasio *et al.* (2003) in a small study showed a significantly higher median free β-hCG MoM in women with early vaginal bleeding but found no difference in PAPP-A MoM. Heinig *et al.* (2007) in a similar sized study found no significant difference for either free β-hCG or PAPP-A, although the trend was for high values in the early vaginal bleeding group for both markers. When they further performed sub-group analysis after exclusion of those with just spotting, they found a statistically higher (34%) median free β-hCG MoM in 61 cases with heavy and light bleeding but they could not find any significant relationship when comparing free β-hCG MoMs in those with spotting, those with light bleeding and those with heavy bleeding. In this context light bleeding was defined as bleeding of lower intensity than regular menstrual bleeding, and heavy bleeding was defined as an intensity equivalent to or greater than menstrual bleeding. In our study we chose not to characterise the severity of the bleeding because we felt this was a very subjective assessment and we relied solely on the women's self-reporting of this.

Table 2—Summary of marker levels in euploid live-born pregnancies and those with Trisomy 21 in cases of early vaginal bleeding and no bleeding

		Early vaginal bleeding	No bleeding	Probability
Euploid	PAPP-A MoM	1.0413	1.0373	0.507
	Free β-hCG MoM	0.9832	1.0013	0.080
	Delta NT mm	+0.100	+0.090	0.055
T21	PAPP-A MoM	0.473	0.481	0.513
	Free β-hCG MoM	2.071	2.089	0.156
	Delta NT mm	+2.152	+2.181	0.621

In another study (Pedersen *et al.*, 1994) levels of PAPP-A were found to be unaffected in women with early vaginal bleeding or those with a subchorionic haemorrhage. Early vaginal bleeding is a risk factor for miscarriage and we have shown previously that viable pregnancies at 11–13 weeks that are destined to miscarry also have reduced levels of PAPP-A (Ong *et al.*, 2000; Spencer *et al.*, 2006) and to a lesser extent reduced levels of free β-hCG. Indeed low PAPP-A was proposed as a predictor of early pregnancy failure as early as 1983 (Westergaard *et al.*, 1983).

It has also been suggested that vaginal bleeding in pregnancy is also associated with an increased risk of Down Syndrome (Cuckle and Wald, 1987). Whilst the combined relative risk was slightly increased at 1.8, the authors concluded that there may be some reason to regard vaginal bleeding as a risk factor for Down Syndrome—however, in clinical practice this is not normally accounted for. In cases with Trisomy 21 we found a similar incidence of vaginal bleeding to that in euploid pregnancies.

In conclusion, our large population study has found that self-reported early vaginal bleeding in pregnancies that are not destined to miscarry requires no correction to the markers used for first trimester aneuploidy screening.

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