Prenatal screening in twin pregnancies

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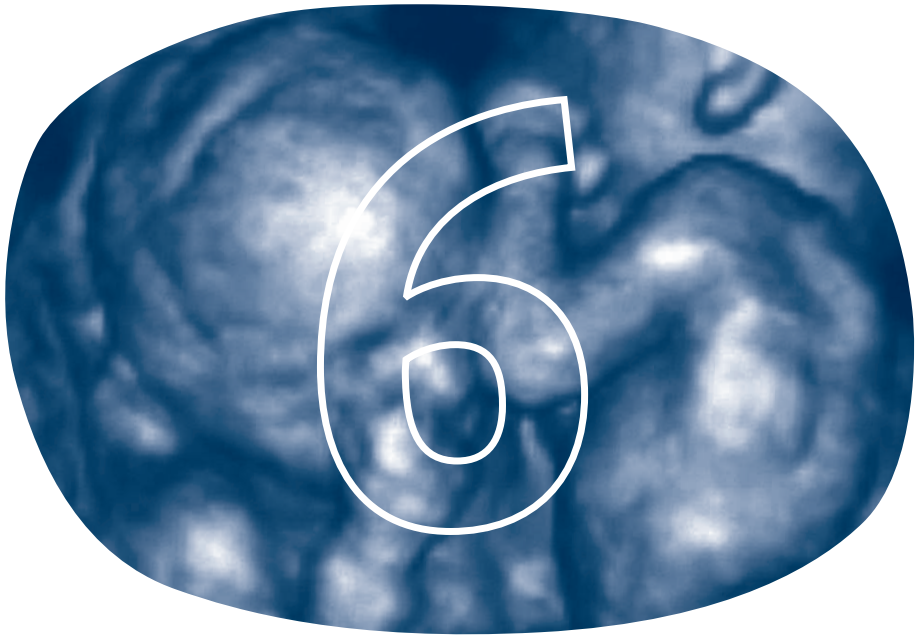

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First trimester maternal serum ADAM12s levels in twin pregnancies

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Abstract

. . . Objective
A Disintegrin And Metalloprotease 12s (ADAM12s) is a potential first trimester serum marker for fetal trisomy and adverse pregnancy outcome in singletons. In this study, ADAM12s levels in first trimester serum of uncomplicated and complicated twins were evaluated.

. . . Methods
ADAM12s was studied in maternal serum of 215 twin pregnancies, collected between 2004 and 2008. ADAM12s was measured 'blind to outcome' using AutoDelfia. As a reference, data from 2423 singletons were used.

. . . Results
The median ADAM12s level was increased in euploid twins (1.61 multiples of the median (MoM); n=209) compared with singletons. The median ADAM12s MoM was significantly lower in monochorionic (1.36 MoM; n=41) compared with dichorionic twins (1.67 MoM; n=168) (Mann-Whitney U, p=0.005). Trisomy 21 was identified in two pregnancies. Median ADAM12s MoM in twins complicated by hypertensive disorders (1.77 MoM; n=35) or small for gestational age fetus (1.54 MoM; n=24) was not significantly different from uncomplicated twins (1.64 MoM; n=134).

. . . Conclusion
Median ADAM12s MoM in euploid twins was increased compared with singletons. Monochorionic had significantly lower median ADAM12s MoM than dichorionic twins. Median ADAM12s MoMs were not significantly different in twins complicated by hypertensive disorders or small for gestational age fetus compared with uncomplicated twins.

Introduction

A Disintegrin And Metalloprotease 12s (ADAM12s), the short and secreted spliceform of ADAM12 is a placenta-derived glycoprotein produced by throphoblasts, and is involved in growth and differentiation [1]. ADAM12s has been reported to be reduced in trisomy affected singleton pregnancies [2-5]. Moreover, studies reported reduced ADAM12s in singleton pregnancies with adverse pregnancy outcome, preeclampsia (PE) or fetal growth restriction [6-8]. Screening for fetal trisomy in twins is more complicated than in singletons, as both nuchal translucency (NT) measurement, i.e. 'fetus specific', and maternal biochemical markers that are 'pregnancy specific', have to be taken into consideration. The use of NT measurements combined with biochemistry is advocated in Down syndrome screening in twins to reduce the false positive rate [9-12].
Women pregnant with twins are at increased risk of developing gestational hypertension (GH) and PE [13;14]. Moreover, fetal growth restriction is more frequent in twins than in singletons [15;16]. A first trimester maternal serum marker for fetal trisomy screening and for identification of pregnancies at risk of adverse outcome would be highly useful in clinical practice for singletons and especially for twin pregnancies. In this study, we evaluated ADAM12s levels in the serum of twin pregnancies and looked at differences between cases with uncomplicated and complicated outcomes.

Methods

First trimester maternal serum samples of twin pregnancies were identified in the fetal database of the VU university medical center Amsterdam (VUMC) between 2004 and 2008. Blood samples were taken between 63 and 97 days of gestation. Samples were immediately tested for serum pregnancy associated plasma protein-A (PAPP-A) and free β-human Chorionic Gonadotropin (free β-hCG) using commercially available kits on a DelfiaXpress analyzer (PerkinElmer, Turku, Finland). Unused serum had been frozen and stored at -20°C and samples were exposed to a maximum of two freeze/thaw cycles before ADAM12s analysis. Serum ADAM12s was measured 'blinded' for clinical outcome, using a semi-automatically performed time-resolved immunofluorometic assay (AutoDelfia) (PerkinElmer, Turku, Finland). The agreement between these two assay platforms for free β-hCG and PAPP-A has been described earlier [17]. Interassay CV for the ADAM12s assay was below 5% at all levels.
Gestational age at time of serum withdrawal was indicated by the requesting gynecologist based on first day of last menstrual period and early first-trimester dating scan. From all samples information on maternal age, conception, ethnicity and smoking was known. All twins had a first trimester ultrasound performed between 11 and 14 weeks of gestation in which the crown-rump length (CRL) was measured and NT was assessed in each twin if the CRL was between 45 and 79 mm. Chorionicity was classified as monochorionic if there was a single placental mass in the absence of the λ-sign at the inter-twin membrane-placental

junction and dichorionic if there was a single placenta mass or separate placentas with λ-sign present [18;19].
Fetal chromosomal status was determined by chorionic villus sampling or amniocentesis if parents opted for invasive diagnostics after a screen-positive result (cut-off 1:200; based on NT only). Pregnancy course and outcome was evaluated by questionnaires and delivery room records. According to the criteria of the International Society for the Study of Hypertension in Pregnancy, GH was defined as two recordings of diastolic blood pressure above 90 mmHg at least 4 h apart in a previously normotensive woman. The same criteria were used to identify cases of PE, defined as GH combined with proteinuria exceeding 300 mg/24 h or two readings of at least 2+ by dipstick on urine analysis after 20 weeks of gestation [20]. A small for gestational age (SGA) fetus was defined as birth weight below the fifth percentile for gestational age at delivery.

. . . Statistical analysis

Baseline characteristics of twins and reference singleton population are presented as median (ranges), percentages (%) and tested for significance with nonparametric Mann-Whitney U test and chi-square test. To correct for gestational variation of analytes, all parameters are expressed as the corrected multiples of the median (MoM) for unaffected singleton pregnancies. As a reference, data of uncomplicated singleton pregnancies (n=2423) with gestational age between 63 and 97 days at sampling for ADAM12s were selected from the database of a previous study published by Wortelboer et al. (2009) on ADAM12s in trisomy [4]. The following equation, ADAM12s = -308.23 + 8.533 x, with x as gestational age in days was calculated using regression analysis with the concentration of ADAM12s as the dependent variable and gestational age in days as independent variable. Higher order terms for gestational age did not improve the fit of the equation.
Differences in corrected MoMs ADAM12s between singletons and twins, mono- and dichorionic twins and complicated and uncomplicated twins, were assessed for significance with nonparametric Mann-Whitney U tests. Statistical analyses were performed using SPSS version 15.0 and p-values <0.05 were considered significant.

Results

A total of 215 twin pregnancies with known fetal outcome were enrolled. Chorionicity was known in all cases: 41 monochorionic (19.1%) and 174 dichorionic (80.9%). Trisomy 21 was identified in two dichorionic pregnancies. The remaining 213 twin pregnancies had a normal karyotype demonstrated by invasive diagnostics or in cases where a karyotyping was not performed healthy neonates were born without dysmorphic features. Maternal age ranged from 20 to 42 years, median 35.2 years. Conception was spontaneous in 73.2% and in 26.8% conceived by assisted reproductive techniques (ART: IVF and ICSI). Mean CRL was 62.1±7.6mm and median NT was 1.3 mm. Table 1 describes baseline characteristics of the euploid twin pregnancies (n=213) and singletons (n=2423) for gestational age at sampling, ethnicity and smoking. No differences were found in baseline characteristics. Four cases

Table 1 Baseline characteristics of the twin pregnancies and the singletons reference population

	Twins (n 213)	Singletons (n 2423)	p value
Median GA at sampling in days (range)	83 (60-96)	83 (63-97)	0.35
Ethnicity (%)			
>Caucasian	97.2	95.4	0.22
>Non-Caucasian	2.8	4.6	
Smoking (%)			
>not stated	95.8	93.8	0.24
>yes	4.2	6.2	

were excluded from further statistical analysis because the samples were taken before 63 days of gestation. In one of these four cases analysis of free β-hCG and PAPP-A was possible in a second later serum sample.
The median free β-hCG weight-corrected MoM was 2.07 in all euploid twins (n=210), significantly lower in monochorionic (1.67 MoM; n=41) than in dichorionic twins (2.13 MoM; n=169) (Mann- Whitney U, p=0.007). The median PAPP-A weight-corrected MoM was 2.15 in all euploid twins (n=210), significantly lower in monochorionic (1.63 MoM; n=41) than in dichorionic twins (2.42 MoM; n=169) (Mann-Whitney U, p=0.001). ADAM12s concentrations ranged from 64.9 to1377 ng/mL. The median ADAM12s MoM was significantly increased in twins (1.61 MoM; n=209) (Mann-Whitney U, p=0.00).
The median ADAM12s MoM of the twins was in week 9 1.43 MoM (n=29) and in week 10, 11, 12 and 13 it was 1.61 MoM (n=39), 1.56 MoM (n=44), 1.78 MoM (n=68) and 1.60 MoM (n=29) respectively. Figure 1 demonstrates the distribution of ADAM12s MoM with gestation in euploid twin pregnancy cases, subdivided for chorionicity. The median ADAM12s MoM was significantly lower in the monochorionic (1.36 MoM; n=41) compared with dichorionic (1.67 MoM; n=168) (Mann-Whitney U, p=0.005).
Logistic regression analysis demonstrated no significant confounding from maternal age, ethnicity, smoking status on ADAM12s levels in twins. Moreover no significant difference (Mann-Whitney U test, p=0.82) in median ADAM12s MoM was found comparing pregnancies resulting from ART, IVF and ICSI, (1.61 MoM) and spontaneous pregnancies (1.62 MoM).

Table 2 gives an overview of the characteristics of the trisomy 21 affected pregnancies. In case I (discordant) the ADAM12s MoM was 1.83 MoM at 70 days of gestation and in case II (concordant) 0.92 MoM at 70 days of gestation.

Figure 1 Distribution of ADAM12s MoM with gestational age at serum withdrawal in monochorionic (■ square) and dichorionic twin pregnancy cases (● dots)

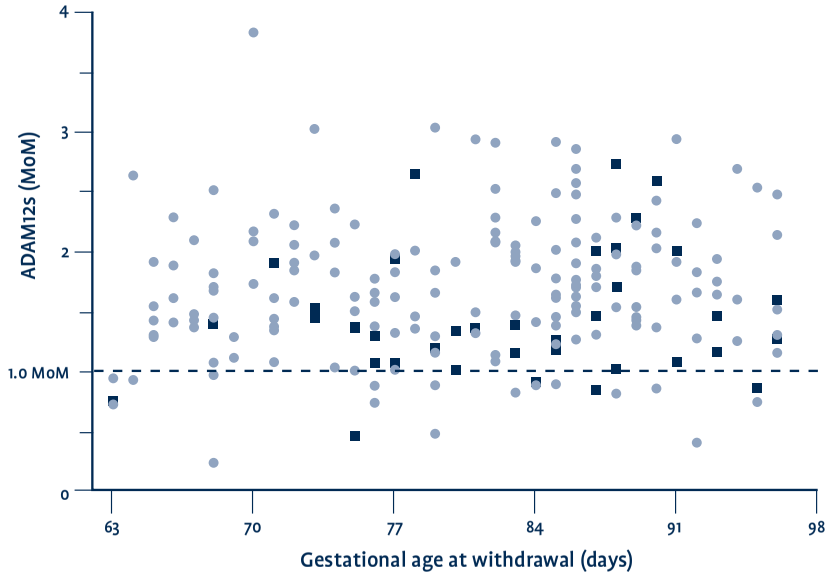

Table 2 Details of trisomy 21 affected pregnancies

	NT1 (mm)	NT2 (mm)	GA (days)	Free β-hCG MoM	PAPP-A MoM	ADAM12s conc (ng/mL)	ADAM12s MoM
I, disc T21	4.8	1.4	70	2.90	2.02	530	1.83
II, con T21	-	-	70	0.79	1.38	265	0.92

disc – discordant; **con** – concordant; **T21** – trisomy 21; **NT** – nuchal translucency; **conc** – concentration; **MoM** – Multiple of the Median; **GA** – gestational age at sampling

In Table 3 the outcome of the euploid twins is presented with median ADAM12s MoM values. The complicated twins (hypertensive disorders/GH/PE/SGA) were compared for median ADAM12s with the uncomplicated group with no significant differences found. In Table 3 16 twins were not listed and those pregnancies were complicated by Twin-to-Twin Transfusion syndrome (n=10), intrauterine fetal death (n=3) or follow-up was incomplete (n=3).

Table 3
Outcome characteristics of twin pregnancies with median ADAM12s MoM

	n	Median ADAM12s MoM	p-value
Uncomplicated	134	1.64	
Hypertensive disorders	35	1.77	0.99
- Gestational Hypertension	15	1.36	0.20
- Preeclampsia	20	1.89	0.26
Small for gestational age fetus	24	1.54	0.52

Discussion

ADAM12s is suggested to be a first trimester marker for fetal trisomy and adverse pregnancy outcome in singletons. This study is the first to describe ADAM12s levels in first trimester maternal serum samples from twin pregnancies. In general, first trimester serum markers used for trisomy screening are approximately doubled in unaffected twins compared with singletons. Weight-corrected MoM values of free β-hCG (2.07 MoM) and PAPP-A (2.15 MoM) reported in this study are corroborated by others [9-12]. We found that the median ADAM12s MoM increased in twins (1.61 MoM) compared with singletons. This increase of 1.61 MoM was previously not reported for other first trimester markers. However, Wald et al. reported on a comparable increase in median MoM for second trimester unconjugated oestriol [21]. In monochorionic twins the median ADAM12s MoM was significantly lower compared with dichorionic. Differences in first trimester biochemical markers between mono- and dichorionic twins have been already described for PAPP-A and free β-hCG [11;22]. In this study the median ADAM12s MoM was not influenced by type of conception (ART vs. spontaneous) in accordance to one previous report concerning conception mode and ADAM12s [23].
ADAM12s can be used as a first trimester marker for trisomy and could improve the test performance of the first trimester combined test. Studies demonstrated reduced ADAM12s levels in first trimester Down syndrome singleton pregnancies [2-5]. The first report by Laigaard et al. (2003) demonstrated extreme reductions in ADAM12s in trisomy 21 affected pregnancies [5]. Other studies did not confirm these reductions to the same extent, but also

demonstrated reduced ADAM12s levels especially in early first trimester [2-4].
Only one recent report did not find a significant reduction of median ADAM12s in trisomy 21 affected pregnancies (0.96 MoM, n=49) [24]. In the current study two trisomy 21 affected twins were included. In only one case (case II) the ADAM12s MoM was reduced (0.92 MoM) compared with the median MoM in euploid twins (1.61 MoM).
Other than screening for fetal trisomy ADAM12s might also be beneficial when screening for adverse pregnancy outcome. PE is a common complication of pregnancies and is the cause of severe problems for both mother and child. The relationship between low ADAM12s and PE has firstly been reported by Laigaard et al. (2005) in a case controlled study [6].
Spencer et al. also reported on a 0.71 decreased ADAM12s MoM in 64 PE cases, predominantly reduced in those delivered before 35 weeks (0.50 MoM) [7]. Poon et al. in 2008 did not confirm the findings of earlier studies and found no significant reduction in ADAM12s MoM in singletons complicated by hypertensive disorders. The results of the current study correspond with this 2008 report by Poon et al. [25]. With regard to SGA fetus Pihl et al. (2008) found a reduction of 0.74 MoM in median ADAM12s, studying 36 SGA cases and 108 singletons with normal birth weight at delivery [26]. This finding corresponds to the results published by Cowans and Spencer (2007) in 414 growth restricted fetuses and confirmed in 296 cases by Poon et al. (2008) [8;25]. In the current study, we found a reduced, however, not significant median ADAM12s MoM in those pregnancies complicated by SGA fetus (1.54, n=24).
Our study is limited by the small number of cases; it would be most interesting in creating subgroups of adverse outcome and chorionicity. However, no sufficient data were present.

In conclusion, median ADAM12s MoM in euploid twins is significantly increased compared with singletons, however, not doubled like other first trimester markers such as free β-hCG and PAPP-A. Monochorionic have significantly lower median ADAM12s MoM than dichorionic twins. Median ADAM12s MoMs are not significantly different in twins complicated by hypertensive disorders or SGA fetus.

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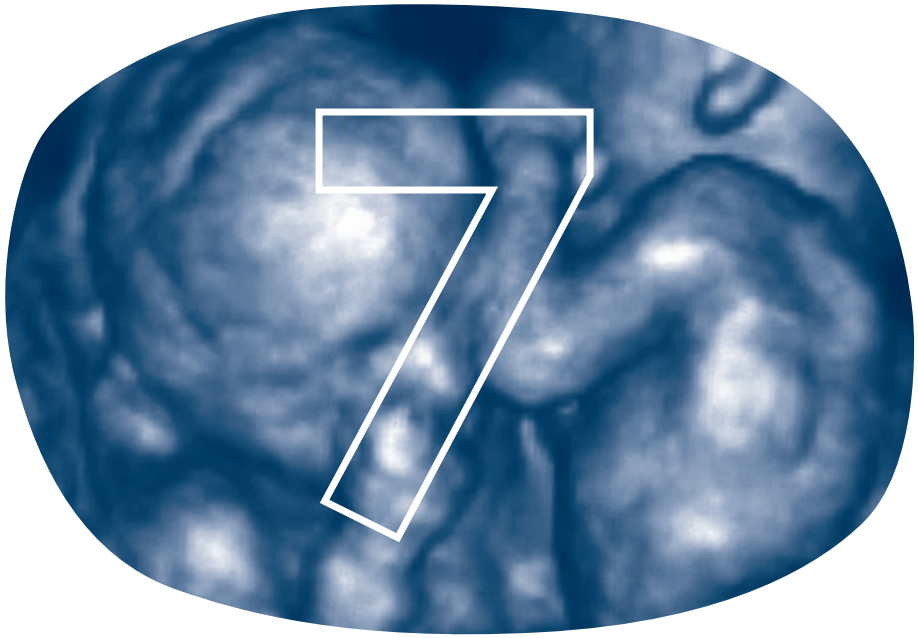

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Discordance in nuchal translucency measurements in monochorionic diamniotic twins as predictor of Twin-to-Twin Transfusion syndrome

Twin Res Hum Genet. 2009 Dec;12(6):605-10