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Increased concentrations of prostate-specific antigen in maternal serum from pregnancies affected by fetal Down syndrome

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Down syndrome is one of the most common causes of mental retardation in the industrialized world. Prenatal serum screening to identify mothers at risk of carrying a fetus affected with Down syndrome is presently part of routine obstetrical care. Prostate-specific antigen (PSA) concentration was measured in stored second-trimester maternal serum samples from 19 pregnancies affected with fetal Down syndrome and in 95 samples from unaffected pregnancies, with each case matched to five controls for gestational age and duration of frozen sample storage. Concentrations of PSA in Down syndrome pregnancy were significantly higher (case median = 2.28 multiples of the median; $P = 0.02$) than in unaffected pregnancy. PSA concentrations were not significantly correlated with the current serum screening analytes, alpha-fetoprotein, unconjugated estriol, or human chorionic gonadotropin in either cases or controls. The increased maternal serum PSA concentrations in Down syndrome pregnancy and their relative independence from other markers suggest the possible utility of PSA as a prenatal screening marker for fetal Down syndrome.

Down syndrome (trisomy 21) is one of the most common causes of mental retardation in the industrialized world, with a birth prevalence of 1 in 700. The prenatal identification of fetuses with Down syndrome by maternal serum screening has become a part of routine obstetrical care. In prenatal screening, maternal serum concentrations of secretory products of the fetoplacental unit in combination with maternal age are used to determine a woman's risk of having a fetus affected with Down syndrome. On average, second-trimester concentrations of alpha-fetoprotein (AFP)[4] and unconjugated estriol (uE3) are low, and concentrations of human chorionic gonadotropin (hCG) and its free β-subunit are high in Down syndrome pregnancy *[1–4]*. Use of various combinations of these markers results in a detection rate of 55–75% at a 5% false-positive rate *[5, 6]*. Efforts are being made to improve prenatal screening; the addition of maternal serum inhibin A, for example, has been found to increase detection by 7–22% *[7, 8]*.

Prostate-specific antigen (PSA), although conventionally considered a specific marker for prostate epithelial cells *[9]*, recently has been measured in female tissue extracts and fluids by an ultrasensitive assay *[10–12]*. Serum PSA concentrations are higher in pregnant women than in healthy nonpregnant women, and amniotic fluid PSA concentrations increase between gestational weeks 11 and 21 *[13, 14]*. These data are consistent with a potential role of PSA in fetal growth. Although a placental origin of PSA during pregnancy has not yet been investigated, possible sources of maternal serum PSA may include diffusion from amniotic fluid, production by the periurethral glands *[15–17]*, and secretion from breast tissue in response to steroid stimulation during pregnancy *[18]*.

Preliminary data suggest that second-trimester amniotic fluid concentrations of PSA are low in cases of fetal Down syndrome *[13]*. Given that maternal serum PSA concentrations are increased in pregnancy and that many

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[4] Nonstandard abbreviations: AFP, alpha-fetoprotein; uE3, unconjugated estriol; hCG, human chorionic gonadotropin; PSA, prostate-specific antigen; and MoM, multiples of the median.

products of the fetoplacental unit have abnormal concentrations in Down syndrome pregnancy, we hypothesized that maternal serum PSA concentrations are also abnormal in Down syndrome pregnancy. This was tested in a sample set of 19 Down syndrome cases through use of a matched case/control statistical design.

Materials and Methods

SAMPLES

Nineteen cases of fetal Down syndrome were identified in women who had maternal serum screening between 15 and 18 completed gestational weeks at Women and Infants Hospital from October 1994 to November 1996. Each case sample was matched to five samples from unaffected singleton pregnancies for length of frozen sample storage (± 1 week) and completed gestational week. Samples had been assayed on receipt for AFP, uE3, and hCG concentrations before freezing at −20 °C, except for one case and nine controls, which had been assayed for AFP only. Fourteen of the cases were identified in our program as screen positive (second-trimester risk, ≥1:270), and five were screen negative by either AFP only or triple marker concentrations. PSA concentrations were measured in residual serum samples with approval by the Women and Infants' Human Studies Review Board. All samples were retrieved from storage and sent to Mount Sinai Hospital, where PSA concentrations were measured without knowledge of whether they were from affected or unaffected pregnancies.

PSA ASSAY

PSA concentrations were measured with an ultrasensitive, time-resolved, immunofluorometric assay, as described previously *[10]*. All calibrators, controls, and patient samples were assayed in triplicate. The intraassay CV was <5% at PSA concentrations from 3 to 7 ng/L. The interassay CVs at 5 and 18 ng/L were 32% and 13%, respectively. The detection limit was 1 ng/L.

The effects of gestational age and duration of frozen sample storage on serum PSA concentrations were assessed by weighted regression analysis. PSA concentrations were expressed as multiples of the control median (MoM) for each case/control set, and the values in Down syndrome and unaffected pregnancies were compared by ANOVA of the ranks of values within each matched set. The extent of correlation between PSA MoM values and AFP, uE3, or hCG MoM values was examined by use of Spearman's rank correlation analysis. $P < 0.05$ was accepted as a significant difference in all tests.

Results

PSA concentrations in maternal serum increased slightly with duration of frozen sample storage (by ~2% per month; $r = 0.28$, $P = 0.01$) and markedly with gestational age (by ~20% per week; $r = 0.69$, $P = 0.0001$) in the 95 unaffected maternal serum samples. Therefore, data were analyzed as matched case/control sets. Table 1 shows the serum concentrations of PSA for each of the affected pregnancy samples and their five matched unaffected

Table 1. Maternal serum PSA in pregnancies associated with Down syndrome and matched controls.

		PSA concentration (ng/L)						
			Controls					
Case no.	Completed weeks of gestation	Down syndrome pregnancy	1	2	3	4	5	MoM value[a]
1	16	2.43	<1.00	1.26	10.29	10.47	17.13	0.24
2	18	7.14	<1.00	<1.00	17.58	21.06	36.98	0.41
3	17	2.76[b]	<1.00	1.60	6.16	11.20	37.59	0.45
4	16	<1.00[b]	<1.00	<1.00	1.61	7.79	35.69	<0.62
5	16	<1.00	<1.00	<1.00	1.59	2.51	26.25	<0.63
6	15	<1.00	<1.00	<1.00	1.40	4.50	8.54	<0.71
7	16	<1.00	<1.00	<1.00	<1.00	<1.00	1.86	<1.00
8	15	7.67	2.20	3.83	6.85	11.97	20.28	1.12
9	17	33.63[b]	2.34	2.71	16.61	22.21	26.78	2.02
10	16	6.85	1.45	2.87	3.01	4.05	14.33	2.28
11	17	21.57[b]	2.07	6.75	7.97	15.50	39.77	2.71
12	16	28.87	<1.00	4.12	7.75	8.68	16.60	3.73
13	16	33.81[b]	<1.00	1.57	8.40	9.79	17.91	4.03
14	17	25.46	<1.00	4.47	4.51	4.99	5.68	5.65
15	18	28.39	1.90	3.94	4.03	6.36	51.54	7.04
16	16	47.47	1.00	4.41	6.15	7.32	13.98	7.72
17	17	35.42	<1.00	1.53	4.16	6.39	59.68	8.51
18	17	42.24	<1.00	3.31	3.41	17.67	35.69	12.39
19	16	36.89	<1.00	<1.00	1.30	2.09	4.38	28.38
Median								2.28

[a] Down syndrome PSA MoM obtained by dividing PSA value by median of five matched controls.
[b] Cases had screened negative for Down syndrome by AFP, uE3, and hCG serum markers.

controls. Each case value is also given as the MoM of its five matched controls. Twelve of the 19 affected pregnancies (63%) had MoM values >1.0. The median PSA value for the Down syndrome pregnancies was 2.28 MoM, a significant increase above concentrations in unaffected pregnancies ($\chi^2 = 6.87$, $P = 0.02$).

Concentrations of PSA (MoM values) were not significantly correlated with AFP, uE3, or hCG MoM values in either affected or unaffected pregnancy samples (Table 2).

Discussion

In this case/control study, concentrations of second-trimester maternal serum PSA were, on average, more than two times higher in pregnancies affected with fetal Down syndrome than in control pregnancies. This result is similar to the increase of hCG *[3]* and its free β-subunit *[4]*, suggesting that PSA might be useful in screening for Down syndrome pregnancy in the second trimester. In this small sample set, 26% of PSA values in cases were above the 95th centile of the unaffected values.

Maternal serum PSA concentrations were not significantly correlated with the currently used Down syndrome serum markers, AFP, uE3, and hCG. This finding suggests that serum PSA determination might be added to the analysis of other markers to improve sensitivity and specificity of Down syndrome screening. Of the five cases of Down syndrome pregnancy that were screen negative with AFP, uE3, and hCG, three had increased serum PSA concentrations (Table 1). Additional Down syndrome cases and controls must be studied before conclusions can be drawn about the performance of PSA as a serum marker, either univariately or in combination with other markers.

Fourteen of the 19 case samples in this study (74%) were identified on the basis of a positive triple screen. Although it is possible that such a high percentage of screen positive samples could positively bias the PSA concentrations, this is unlikely for two reasons: (*a*) the degree of correlation between PSA and the triple markers, AFP, uE3, and hCG, was very low and was not significant; (*b*) the percentage of Down syndrome case samples from positive screens is similar to the percentage (68%) that is found with triple marker screening at the center that collected the samples *[19]*, indicating that the samples that were used closely reflect the distribution of screen positive and negative results usually seen.

Table 2. Correlation of PSA and AFP, uE3, or hCG MoM values in second-trimester maternal serum samples.

	Down syndrome		Unaffected	
	r	*P*	*r*	*P*
PSA				
versus AFP	−0.02	0.95	−0.02	0.83
versus uE3	0.33	0.89	0.03	0.77
versus hCG	−0.17	0.49	0.12	0.29

The origin of PSA during pregnancy is at present unknown. PSA immunoreactivity in female body fluids was not detected until the recent development of an ultrasensitive assay *[10]*. Substantial concentrations of PSA have now been measured in maternal serum in excess of serum concentrations in nonpregnant women. PSA has also been found in other pregnancy-related fluids, such as amniotic fluid *[13, 14]* and breast milk *[18]*. Studies of breast tumor cells indicate that PSA production can be stimulated by progesterone *[20, 21]*. Therefore, increased serum PSA concentrations in pregnancy might be secondary to increased progesterone concentrations. Possible biological functions of PSA during pregnancy and the reason for its increase in Down syndrome pregnancy have not yet been determined.

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