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Maternal Serum Invasive Trophoblast Antigen (Hyperglycosylated hCG) as a Screening Marker for Down Syndrome during the Second Trimester

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Background: **Approximately two million pregnancies in the United States are screened for Down syndrome annually by use of second-trimester maternal serum markers. At present, a combination of four markers can identify 75% of affected pregnancies when 5% of screened women are classified as candidates for amniocentesis. Although not currently included in screening panels, invasive trophoblast antigen (ITA) is a promising screening marker in serum or urine in both the second and first trimesters. This study aims at better defining the screening performance of serum ITA in the second trimester.**

Methods: **In an earlier study, serum samples from an unbiased sampling of 45 Down syndrome (cases) and 238 unaffected (control) pregnancies between 14 and 20 weeks of gestation were collected from various centers in the United States. Samples were aliquoted and stored at −20 °C for 8 years. We measured ITA in these samples and determined the screening performance both univariately and in combination with other screening markers.**

Results: **The median ITA in Down syndrome pregnancies was >3.00 multiples of the median, higher than that found for human chorionic gonadotropin (hCG) or free β-hCG. At a 5% false-positive rate, ITA univariately detected 38% and 40% of Down syndrome pregnancies, respectively, when assigned by date of last menstrual period or ultrasound date. Modeling yielded rates of 45% and 48%. ITA correlated strongly with hCG and free β-hCG. When substituted for either of these in a multiple marker panel, ITA performed comparably.**

Conclusions: **This study indicates that serum ITA is an effective marker for Down syndrome. It is highly correlated with both hCG and free β-hCG and could replace either of them in a multiple marker panel.**

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Multiple marker screening for Down syndrome during the second trimester is now a well-established practice in many countries. In the United States, it is most often performed using a combination of maternal age and three second-trimester maternal serum markers *(1, 2)*: human chorionic gonadotropin (hCG),[3] α-fetoprotein (AFP), and unconjugated estriol (uE_3). Approximately two thirds of affected pregnancies can be detected with this triple test at a false-positive rate of ~5% *(3)*. Unless otherwise noted, all subsequent detection rates quoted in this report will be at a 5% false-positive rate. To improve the detection rate, some laboratories have added measurements of dimeric inhibin-A (DIA) to the triple test *(4, 5)*, yielding up to a 75% detection rate.

Hyperglycosylated hCG is a unique carbohydrate variant of hCG *(6)*; it is produced by poorly differentiated or invasive trophoblast cells and, hence, is called invasive trophoblast antigen (ITA) *(7)*. An initial 1999 study reported an 80% detection rate (at a 5% false-positive rate) for second-trimester urine ITA measurements *(8)*. A second study in 1999 found a lower detection rate of 45% at 14 weeks of gestation and later *(9)*. Urine ITA was also analyzed as part of the Serum, Urine, and Ultrasound Screening Study (SURUSS) in 2003 and was reported to detect 40% of second-trimester Down syndrome pregnan-

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[3] Nonstandard abbreviations: hCG, human chorionic gonadotropin; AFP, α-fetoprotein; uE3, unconjugated estriol; DIA, dimeric inhibin-A; ITA, invasive trophoblast antigen; and MoM, multiple(s) of the median.

cies *(10)*. In a prospective study in 2004 in which fresh urine samples were used *(11)*, a detection rate of 59% was documented. Although urine is routinely collected during pregnancy, most current screening practice relies on serum samples. The present study, therefore, explores the potential usefulness of maternal serum ITA measurements for Down syndrome screening in the second trimester. We used stored serum samples collected during an earlier observational trial involving an unbiased group of 5345 pregnant women who were about to undergo amniocentesis for reasons other than a positive serum screening test. Chromosome results were available on all of these pregnancies.

Materials and Methods

Second-trimester maternal serum samples from Down syndrome and unaffected pregnancies were collected as part of a previous Maternal and Child Health-sponsored trial (MCJ-237014) between 1990 and 1992 *(12)*. Briefly, 14 prenatal diagnostic centers in California and elsewhere in the United States recruited women already scheduled to have an amniocentesis between 14 and 21 weeks of gestation, usually because of advanced maternal age. None of the women had undergone any serum screening in their current pregnancy. Demographic and pregnancy-related information was collected, including maternal weight, maternal race and diabetic status, and gestational age estimates based on both biparietal diameter and date of last menstrual period. Maternal serum was collected before amniocentesis and shipped by express mail to the study center in Maine, where it was aliquoted and assayed for AFP, uE3, and hCG. Remaining sera were frozen at −20 °C in a frost-free freezer. Subsequently, a case/control set was constructed, with five samples from pregnancies with normal karyotypes matched for each Down syndrome case as follows: length of freezer storage, maternal age, gestational age, race, and site where sample was obtained. In 1996, an aliquot of this case/control set was retrieved for another Maternal and Child Health-sponsored study comparing hCG and the free β-subunit of hCG (MCJ 230802) *(13)*. In 1997, this same sample set was used to study the usefulness of DIA as an additional Down syndrome marker *(4)*.

For the current study, a never-thawed aliquot from this case/control set was available for each of 45 cases and 238 controls. Three of the case samples and seven of the control samples had been exhausted. Sera from the 283 serum samples were sent on dry ice to Quest Diagnostics, Nichols Institute, for measurement of ITA. The assays were completed without knowledge of whether the sample was from a case or control, and the ITA results were reported to the Foundation for Blood Research for statistical analysis.

Measurements were carried out using an automated immunochemiluminometric assay. The monoclonal antibody (B152) specific for ITA used in this assay has minimal cross-reactivity with hCG and is applicable to many sample types, including serum *(14)*. Singleton samples were assayed. The assay has a calibration range up to 300 μg/L and a lower limit of detection of 0.2 μg/L. CVs were determined with three controls with ITA concentrations of 1.1, 8.5, and 18.2 μg/L. The intra- and interassay CVs were <3.5% and 7.4%, respectively, for all three controls.

All ITA results were converted to multiple(s) of the median (MoM) and corrected for maternal weight *(15)*. Adjustments to the other analytes and summary population variables have been published *(4, 13)*. Correlation coefficients between ITA and serum markers in second-trimester unaffected and Down syndrome pregnancies were derived separately after a logarithmic transformation and exclusion of values outside 3 SD. The Down syndrome screening performance was modeled for selected combinations of serum ITA and other second-trimester serum markers, using the maternal age distribution in the United States for 2000 *(16)*. The modeling methodology is based on overlapping gaussian distributions and has been described previously *(11)*. Continuous variables were compared, after appropriate transformations, by the *t*-test at a two-tailed significance level of 0.05.

Results

The observed ITA measurements in the case and control samples vs gestational age are shown in Fig. 1. Two sets of medians were computed based on gestational age derived from either last menstrual period or biparietal diameter ultrasound measurements: ultrasound median ITA = $10^{3.89519 - 0.0209419 \times \text{days}}$; and last menstrual period median ITA = $10^{3.84743 - 0.0209275 \times \text{days}}$. The weight adjustment equation for ITA is: expected ITA = 68.2934 × 1/weight + 0.5419. The observed ITA performance for detecting Down syndrome was computed after all values were converted to weight-adjusted MoM (Table

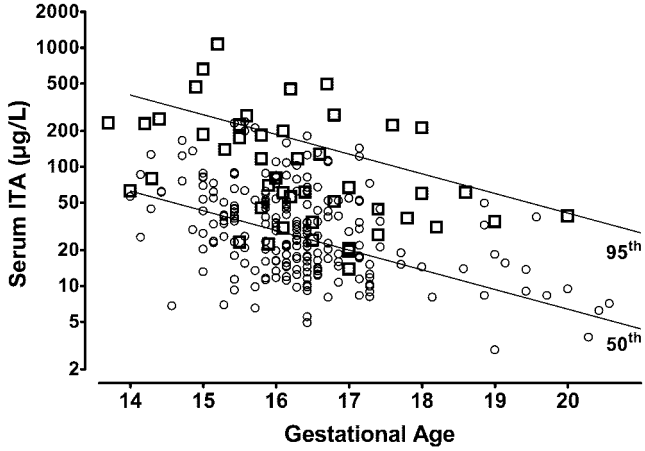

Fig. 1. Second-trimester maternal serum ITA values in unaffected and Down syndrome pregnancies by gestational age as assigned by last menstrual period dating.

Serum ITA results for Down syndrome (□) and unaffected (○) pregnancies are plotted on a vertical logarithmic axis vs gestational age on the horizontal axis. The *thick straight line* indicates the regressed medians (50th centile), and the *thin straight line* indicates the 95th centile.

Table 1. Maternal serum ITA: Observed and modeled Down syndrome detection rates at three false-positive rates, stratified by method of gestational dating.

	Down syndrome detection rate, %			
	LMP[a] dating		BPD dating	
False-positive rate, %	Observed	Modeled	Observed	Modeled
1	28	27	22	31
3	28	38	36	42
5	38	45	40	48

[a] LMP, last menstrual period; BPD, biparietal diameter.

Table 2. Second-trimester maternal serum ITA: Distribution parameters in Down syndrome and unaffected pregnancies, stratified by method of gestational dating.

	Log(mean)	Median (geometric mean)	Log(SD)
LMP[a] dating			
Unaffected	0.0000	1.00 (1.00)	0.3384
Down syndrome	0.4987	3.54 (3.15)	0.4781
BPD dating			
Unaffected	0.0000	1.00 (1.00)	0.3088
Down syndrome	0.4876	2.94 (3.07)	0.4640

[a] LMP, last menstrual period; BPD, biparietal diameter.

1). The observed detection rate of ~40% at a 5% false-positive rate was similar to that found for hCG measurements in serum *(13)*.

The ITA measurements in Down syndrome and unaffected pregnancies were then fitted to straight lines on a probability plot to compute appropriate population parameters. Fig. 2 displays those data when gestational dating is based on the first day of the last menstrual period. In both unaffected and Down syndrome pregnancies, the ITA measurements fit a gaussian distribution reasonably well after a logarithmic transformation. We obtained similar results when we estimated gestational age using biparietal diameter measurements (not shown). The parameters were derived by fitting the data between the 10th and 90th centiles for the two groups and are shown in Table 2. The median ITA measurements in Down syndrome pregnancies differed considerably by method of dating, but the geometric mean ITA values for last menstrual period and ultrasound dating that were used for modeling were nearly identical (3.07 and 3.15 MoM). The geometric mean is more robust than the median for modeling small data sets.

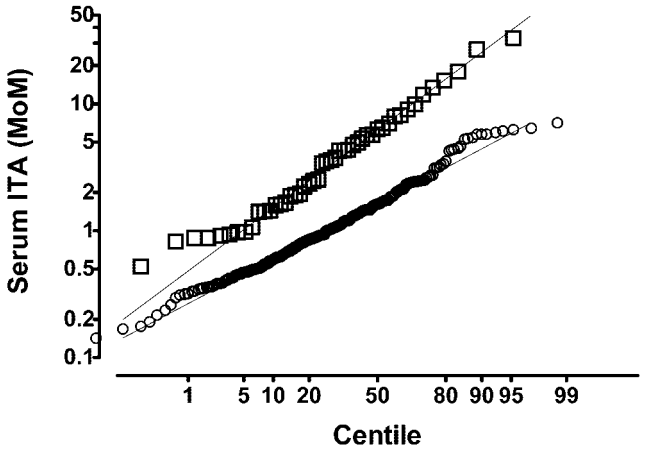

Fig. 2. Probability plot of second-trimester maternal serum ITA measurements in Down syndrome and unaffected pregnancies dated by last menstrual period.

Serum ITA concentrations are shown separately for Down syndrome (□) and unaffected pregnancies (○) on the logarithmic vertical axis vs the expected gaussian centile on the horizontal axis. A straight line indicates that the data fit a logarithmic gaussian distribution.

The population parameters in Table 2 were used to model univariate ITA performance (Table 1). These should be similar to the observed rates. The modeled detection rates were actually somewhat higher in the present comparison. This is explained by the deviation from the expected (Fig. 2, solid line) in the probability plot at higher centiles of unaffected pregnancies. This deviation occurred because the observed data (Fig. 2, ○) were higher than the modeled data (Fig. 2, solid line). Extensive experience with other screening markers indicates that the modeled performance is likely to be the more reliable. Pairwise correlation coefficients between ITA and the other serum measurements are shown in Table 3, and the correlation found between serum ITA and hCG in both unaffected and Down syndrome pregnancies is shown in Fig. 3. The high correlation between ITA and hCG (and ITA and free β-hCG) indicated that including either of these markers with ITA in a Down syndrome risk algorithm would not be useful. Reasonable truncation limits are 0.5–6.0 MoM.

The modeled Down syndrome screening performance is shown in Table 4 and is based on the maternal age

Table 3. Second-trimester maternal serum ITA measurements vs five other second-trimester serum markers: Correlation coefficients in Down syndrome and unaffected pregnancies, stratified by method of gestational dating.

	Correlation coefficient	
Combination of markers	Unaffected	Down syndrome
LMP[a] dating		
AFP/ITA	−0.013	−0.369
uE3/ITA	−0.301	−0.363
hCG/ITA	0.812	0.814
Free β-hCG/ITA	0.800	0.921
DIA/ITA	0.446	0.309
BPD dating		
AFP/ITA	0.050	−0.214
uE3/ITA	−0.153	−0.117
hCG/ITA	0.795	0.780
Free β-hCG/ITA	0.774	0.878
DIA/ITA	0.480	0.226

[a] LMP, last menstrual period; BPD, biparietal diameter.

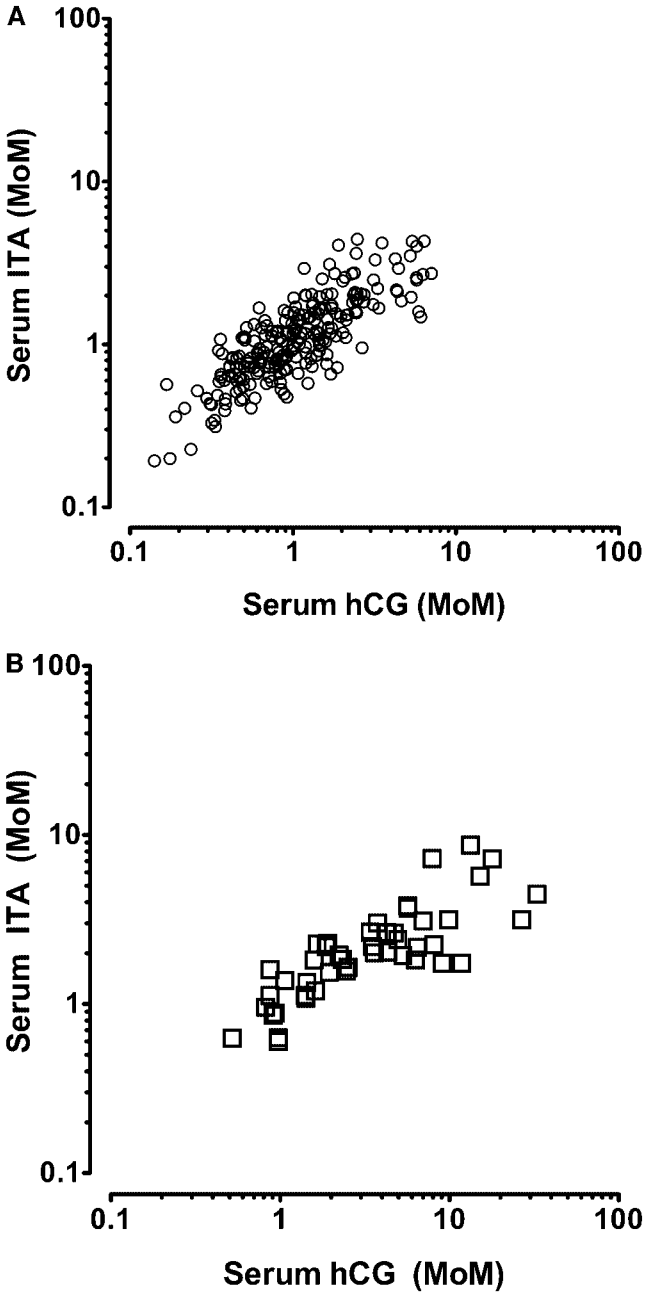

Fig. 3. Correlation of second-trimester maternal serum ITA and hCG measurements in Down syndrome and unaffected pregnancies dated by last menstrual period.

Serum hCG measurements in MoM are shown on the logarithmic horizontal axis vs serum ITA concentrations in MoM on the logarithmic vertical axis. (*A*), 238 observations in unaffected pregnancies ($r = 0.812$; $P < 0.001$); (*B*), 45 observations in Down syndrome pregnancies ($r = 0.814$; $P < 0.001$).

distribution for the United States in 2000 *(16)*, along with the ITA parameters from Table 2 and published parameters for AFP, uE3, hCG, and DIA *(4, 13)*. The performance estimates for the triple test (AFP, uE3, and hCG) and the quadruple test (triple test plus DIA) differed slightly from those published previously because the distribution of maternal ages in the United States was different in 2000

Table 4. Second-trimester maternal serum ITA: Modeled Down syndrome screening performance, alone and in combination with other serum markers at fixed false-positive rates, for two methods of gestational dating.

	Down syndrome detection rate, %					
	1% FPR[a]		3% FPR		5% FPR	
Maternal age and	LMP	BPD	LMP	BPD	LMP	BPD
ITA	31	35	46	53	56	58
AFP, ITA	40	45	55	58	62	65
AFP, hCG	44	44	57	59	65	67
AFP, uE3, hCG	45	50	60	65	67	72
AFP, uE3, ITA	44	51	58	65	66	71
AFP, uE3, hCG, ITA	48	55	63	69	70	76
AFP, uE3, hCG, DIA	57	59	71	73	77	79
AFP, uE3, DIA, ITA	57	59	71	73	77	79
AFP, uE3, DIA, hCG, ITA	60	64	74	78	80	83

[a] FPR, false-positive rate; LMP, last menstrual period; BPD, biparietal diameter.

from that available for the earlier calculations. ITA could be substituted for hCG (or free β-hCG) measurements with no loss in performance for any of the test combinations. At a 5% false-positive rate, 77% of Down syndrome pregnancies were detected by either of the "quadruple tests" when the gestational age was estimated by last menstrual period dating; detection improved to 79% when the dating was based on biparietal diameter ultrasound measurements. Although ITA and hCG had a relatively high correlation coefficient in both affected and unaffected pregnancies (~0.8), adding the two markers together in a screening model with other, more independent markers, still led to materially improved performance.

Discussion

Measurements of ITA, a unique carbohydrate variant of hCG, in urine or serum have been reported to be a promising marker for Down syndrome in either the first or second trimester *(8–11, 17, 18)*. The authors of previous studies using second-trimester serum have reported median ITA concentrations of 3.90 MoM and 5.33 MoM, along with detection rates of 60% *(17)* and 81% *(18)*, respectively. However, in the first study, the detection rate was based on only 10 samples from affected pregnancies, and in the second study, assessment of the high detection rate was complicated by the associated unexpectedly high detection for hCG measurements of 75%. Our study explores the potential usefulness of ITA measurements in second-trimester maternal serum, both alone and in combination with other commonly used maternal serum markers. Using standard methods of data analysis that have been shown to be robust in the past, we found that ITA performance was essentially equivalent to hCG. For example, among 533 serum ITA measurements collected as part of another study *(14)*, there was no such

deviation from expected at high ITA concentrations among the unaffected pregnancies, as found in the current study (Fig. 2). The geometric mean ITA MoM for Down syndrome pregnancies was 3.15, higher than that found for either hCG or free β-hCG measurements. Performance, however, was similar regardless of which of these three markers were used because of a higher population variance for ITA measurements. Our median ITA was somewhat lower than that reported in two previous smaller studies *(17, 18)*.

Some laboratories may find that replacing hCG with ITA is more economical or is more easily fit into the laboratory routine. If so, Down syndrome screening performance should not change, providing that the updated population parameters found in our study are used. However, issues such as screening in twin pregnancies or the use of ITA in identifying pregnancies at risk for trisomy 18 should be addressed before ITA is routinely added to the existing triple or quadruple marker panels used to screen for Down syndrome. The highest screening performance for Down syndrome can be obtained by integrating first- and second-trimester serum and ultrasound markers into a single interpretation in the second trimester *(19)*. This integrated test approach can detect 90% of Down syndrome pregnancies at a 3% false-positive rate. The present study indicates that second-trimester measurements of serum ITA might also be substituted for hCG or free β-hCG in such an integrated approach.

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