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Dimeric Inhibin A as a Fourth Marker for Down's Syndrome Maternal Serum Screening in Native Japanese Women

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Abstract

Objective: This study was conducted to assess the usefulness of dimeric inhibin A as a fourth marker for Down's syndrome screening in addition to AFP, hCG and uE3 markers for native Japanese women.

Methods: Serum specimens from 367 native Japanese women in the second trimester were assayed for dimeric inhibin A levels. Day specific dimeric inhibin A medians were established for gestational ages 15.0–21.9. Weekly median values for the native Japanese were compared with those of a U.S. population. Selected Japanese specimens from 15 diagnosed Down's syndrome and 3 trisomy 18 cases were also assayed for dimeric inhibin A.

Results: Dimeric inhibin A levels did not vary greatly over the gestational age range as expected. Median value comparison showed that native Japanese dimeric inhibin A medians are higher than the U.S. population medians by an average of 7.95 %. Native Japanese dimeric inhibin A median values in this study are 1.77 times higher in Down's syndrome cases than in unaffected pregnancies. Trisomy 18 dimeric inhibin A levels show no significant difference from the unaffected pregnancies.

Conclusions: This report shows for the first time that dimeric inhibin A can be informative as a fourth marker for Down's syndrome screening in native Japanese women. We expect

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the addition of dimeric inhibin A to a triple marker protocol will increase the accuracy of predicted risk for all pregnancies screened and increase the detection rate of Down's syndrome affected pregnancies.

Key words: dimeric inhibin A, prenatal maternal serum screening, Down's syndrome

Introduction

Prenatal screening in the second trimester of pregnancy to assess the risk of a Down's syndrome (DS) pregnancy using multiple markers has been performed for over 10 years. The commonly used markers include alpha-fetoprotein (AFP), human chorionic gonadotropin (hCG) and often unconjugated estriol (uE3). The DS detection rate progressed from 35% using only AFP, to 54–58% with the addition of hCG and 59–67% when adding uE3.[1]

Recent studies have identified an additional marker. Dimeric inhibin A protein is a corpus luteum secretion which inhibits follicle stimulating hormone (FSH) during the normal menstrual cycle and is a placental hormone of unknown function. Dimeric inhibin A is one of several forms of inhibin found in man. The usefulness of dimeric inhibin A as a potential fourth marker for DS risk assessment was shown by Van Lith *et al.*[2] to be associated with elevated maternal serum levels of total immunoreactive inhibin in pregnant women carrying a DS fetus. The multiple of the normal median (MoM) was 1.9 MoM in their DS affected pregnancies. A report from Cuckle *et al.*[3] found a smaller difference in total inhibin serum levels in their group of women carrying a DS affected fetus.

A series of reports in the literature in 1996 established that dimeric inhibin A is the (most) informative form of inhibin for second trimester DS risk assessment.[4–9] MoM values for DS affected pregnancies from these papers clustered around 2.0. This separation from unaffected pregnancy levels of 1.0 MoM makes dimeric inhibin A a very informative DS marker, equivalent to hCG.

We have previously reported on the difference in maternal serum levels of AFP, hCG and uE3 between a United States population and that of native Japanese. We now report the findings from the evaluation of dimeric inhibin A as a DS risk marker in a screened population of pregnant Japanese women.

Materials and Methods

Second trimester maternal serum samples were collected from pregnant native Japanese women following informed consent. The samples were shipped via express courier to the laboratory in Santa Fe, New Mexico (transit time of 2–3 days).

The dimeric inhibin A assay is based upon published protocols.[10,11] Antibody coated 96 well plates, secondary antibody labeled with alkaline phosphatase and dimeric inhibin A standards were purchased from Serotec (medium sensitivity assay; Kidlington, Oxford, U.K.). The reagents for alkaline phosphatase reaction color development were purchased from Gibco (Gaithersburg, MD.). The assay diluent, assay pretreatment reagents, assay wash buffer and color development stop solution were prepared in the laboratory using vendor reagents (fetal calf serum, Summit Biotechnology, Ft. Collins, CO.; mouse serum, Pel-Freeze, Rogers, AK.; Tween-20, Triton X-100, Citric Acid, Sigma Chemical, St. Louis, MO.; H_2SO_4, H_2O_2, NaCl, VWR, Chicago, IL.). The assay was evaluated and validated following FDA guidelines as a "home brew" test for clinical usage.[12]

All patient and control samples were assayed and the dimeric inhibin A values were determined from a standard curve on each plate. Each sample and standard was assayed in duplicate. Dimeric inhibin A levels from fresh specimens sent from Japan were used to determine gestational age median values for weeks 15.0 through 21.9. Samples with visible hemolysis were not used in this study.

Population day-specific dimeric inhibin A median levels were determined using least-squares polynomial regression. Truncation limits were set at 0.7 and 2.5 MoM. Patient dimeric inhibin A MoM values were adjusted for maternal weight. A patient-specific DS risk is computed beginning with each woman's age-related risk and modifying that risk with the marker results from the serum screening assays, taken from overlapping Gaussian distributions, as described by Haddow *et al.*[13] A patient is considered to be screen-positive by this laboratory if her final mid-trimester DS risk is 1 : 295 or greater.

Serum specimens from several native Japanese pregnancies with a chromosomally confirmed DS

Table 1. Japanese second trimester dimeric inhibin A data and comparison to U.S. data

Week of gestation	Japanese		Non-Japanese	Percent increase of Japanese median
	Specimens N =	Regressed weekly median	Regressed weekly median	
15	88	104	91	14.3
16	80	102	93	9.7
17	72	100	93	7.5
18	48	100	93	7.5
19	36	100	94	6.4
20	27	102	96	6.3
21	16	104	100	4.0
				7.95 avg

Table 2. Dimeric inhibin A median data from Japanese unaffected, Down's syndrome affected and trisomy 18 affected pregnancies

	Controls	Down's syndrome affected	Trisomy 18 affected
Number screened	71	15	3
Dimeric inhibin A median value	103	182	109
Multiple of the control median	1.0	1.77	1.06
		$p < 0.0001$	

fetus or a trisomy 18 fetus were assayed for dimeric inhibin A. These frozen specimens were assayed with matched controls.

Results

A total of 367 specimens were assayed from native Japanese pregnancies between 15.0 and 21.9 weeks of gestation. The mean maternal age at delivery was 32.1 years, with approximately 30% of the specimens from women ≥ 35 years old. Ultrasound dating was provided with 72% of the specimens.

Table 1 presents the median value for each week of gestation screened. The dimeric inhibin A levels do not vary greatly over the gestational age range. Comparison of the weekly and daily median values for the native Japanese to those of a U.S. population showed that Japanese dimeric inhibin A medians are higher by an average of 7.95 % ($p = 0.01$, Table 1). As expected, an inverse relationship between increasing maternal weight and decreasing analyte serum concentration was observed for dimeric inhibin A (data not shown). The median weight of the Japanese patients (53 kg) was used to weight adjust the MoM values for each patient.[5,14,15]

Dimeric inhibin A values for 15 Japanese DS specimens and 71 matched controls were compared (Table 2). These samples were held at < −15°C for 1 to 18 months. Comparison of Japanese DS unaffected vs affected pregnancies showed a dimeric inhibin A MoM value of 1.77 fold higher for the DS group ($p < 0.0001$). Assay of specimens from 3 Japanese trisomy 18 affected pregnancies found no significant difference in the dimeric inhibin A MoM value of the affected pregnancies.

Discussion

This study finds that second trimester levels of dimeric inhibin A in the maternal serum of pregnant Japanese women are significantly higher than those established for a U.S. Caucasian population. These higher values are evident before and after correction for maternal weight. The increase is not due to protocol or method discrepancies because all Japanese and U.S. specimens were analyzed in the same laboratory. Our previously published triple marker data found a similar elevation for AFP, hCG and uE3.[14] Studies of Asian pregnancies tend to confirm that maternal serum marker concentrations are increased in comparison to Caucasian populations.[16,17]

Assay of specimens from 3 Japanese trisomy 18 affected pregnancies show no significant difference in the dimeric inhibin A levels when compared with the unaffected pregnancies, just as previously reported.[6]

As presented in Table 1, levels of dimeric inhibin A are relatively constant over the 15–21 week gestation of our pregnant Japanese population, as in previous reports.[4–9,15] Japanese DS pregnancies result in much higher maternal serum levels of dimeric inhibin A compared to non-DS pregnancies, as reported for other racial

groups.[4–9,15] As the program of 4 marker screening is offered to Japanese women, we fully expect to approximate the prediction of Wald *et al.*[5] of a 78% DS detection rate at a 4.8% referral to amniocentesis rate using a 35 year old mid-trimester risk cutoff (1 : 295).

This study shows that dimeric inhibin A levels are higher in the native Japanese population as are other second trimester DS screening analytes. This investigation of native Japanese pregnant women demonstrates that dimeric inhibin A will be a reliable additional marker that will approximate the data of Wald, *et al.*[5] resulting in a 75–80% DS detection rate.

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References

1. Wald NJ. Antenatal screening for Down's syndrome. Screening brief. *J Med Screen* 1995; 2: 56
2. Van Lith JM, Pratt JJ, Beekhuis JR, Mantingh A. Second-trimester maternal serum immunoreactive inhibin as a marker for fetal Down syndrome. *Prenat Diagn* 1992; 12: 801–806
3. Cuckle HS, Holding S, Jones R. Maternal serum inhibin levels in second-trimester Down's syndrome pregnancies. *Prenat Diagn* 1994; 14: 387–390
4. Wallace EM, Swanston IA, McNeilly AS, Ashby JP, Blundell G, Calder AA, Groome NP. Second trimester screening for Down's syndrome using maternal serum dimeric inhibin A. *Clin Endrocrinol* 1996; 44: 17–21
5. Wald NJ, Densem JW, George L, Muttukrishna S, Knight PG. Prenatal screening for Down's syndrome using inhibin-A as a serum marker. Prenat Diagn 1996; 16: 143–153
6. Aitken DA, Wallace EM, Crossley JA, *et al.* Dimeric inhibin A as a marker for Down's syndrome in early pregnancy. *N Engl J Med* 1996; 334: 1231–1236
7. Lambert-Messerlian GM, Canick JA, Palomaki GE, Schneyer AL. Second trimester levels of maternal serum inhibin A, total inhibin, alpha inhibin precursor, and activin in Down's syndrome pregnancy. *J Med Screen* 1996; 3: 58–62
8. Spencer K, Wallace EM, Ritoe S. Second trimester dimeric inhibin-A in Down's syndrome screening. *Prenat Diagn* 1996; 16: 1101–1110
9. Cuckle HS, Holding S, Jones R, Groome NP, Wallace EM. Combining inhibin A with existing second-trimester markers in maternal serum screening for Down's syndrome. *Prenat Diagn* 1996; 16: 1095–1100
10. Groome NP, O'Brien M. Immunoassays for inhibin and its subunits. Further applications of the synthetic peptide approach. *J Immuno Methods* 1993; 165: 167–176
11. Groome NP, Illingworth PJ, O'Brien M, Cooke I, Ganesan TS, Baird DT, McNeilly A. Detection of dimeric inhibin throughout the human menstrual cycle by two-site enzyme immunoassay. *Clin Endocrinol* 1994; 40: 717–723
12. College of American Pathologists. 1999
13. Haddow JE, Palomaki GE, Knight GJ, Williams J, Pulkkinen A, Canick JA, Saller DN, Jr., Bowers GB. Prenatal screening for Down's syndrome with use of maternal serum markers. *New Engl J Med* 1992; 327:588–593
14. Onda T, Kitagawa M, Takeda O, *et al.* Triple marker screening in native Japanese women. *Prenat Diagn* 1996; 16: 713–717
15. Haddow JE, Palomaki GE, Knight GJ, Foster DL, Neveau LM. Second trimester screening for Down syndrome using maternal serum dimeric inhibin A. *J Med Screen* 1998; 5: 115–119
16. Tompkinson DG, Cunningham GC. Race specific median MSAFP values by gestational age. *Am J Hum Genet* 1992; 51 (Suppl.): A1071
17. O'Brien JE. Drugan A, Chervenak FA, Johnson MP, Isada NB, Hallak M, Evans MI. Maternal serum alpha-fetoprotein screening: The need to use race/ethnic specific medians in Asians. *Fetal Diagn Ther* 1993; 8: 367–370