PRENATAL GENETIC SCREENING FOR DOWN SYNDROME AND OPEN NEURAL TUBE DEFECTS USING MATERNAL SERUM MARKERS IN THAI PREGNANT WOMEN

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Abstract. Maternal serum screening has gained widespread acceptance as a major prenatal screening tool for chromosomal abnormalities in the US and Europe since Merkatz et al described an association between low maternal serum alpha fetoprotein (AFP) levels and increased risk for trisomy 21 in 1984. In 1988, Wald et al proposed a screening program based on maternal age in combination with three biochemical markers - AFP, hCG and unconjugated estriol. This study from January 1996 - September 2002 included 1,793 pregnant women (between 14-22 weeks gestation) which were divided into 2 groups - 1,083 women > 35 years (60.40%) and 710 women < 35 years (39.60%). A second trimester risk for trisomy 21 > 1 : 270 was considered a positive screen and genetic counseling to discuss risks and benefits of amniocentesis was offered. This study had 1,376 cases (76.7%) with negative screening (not increased risk for DS and NTD), 21 (1.2%) with negative screening (not increased risk for DS only) ; 292 (16.3%) with increased risk for DS; 5 cases (0.3%) with increased risk for DS and elevated AFP; 19 cases (1.1%) with elevated AFP ; 33 cases (1.8%) with previous DS only; and 9 cases (0.5%) with previous NTD only. Two percent (2.1%) of the results could not be interpreted either because the test was done too early, too late or were grand multiple pregnancies. This study demonstrated that multiple marker screening offers another option for older women who traditionally have all been considered candidates for amniocentesis.

INTRODUCTION

Maternal serum screening began in England in the late 1970s and in the United States in the mid 1980s. Maternal serum alpha fetoprotein (MSAFP) was first used to screen pregnancies at increased risk for the presence of an open neural tube defect (ONTD), spina bifida, or anencephaly. In 1972, elevated amniotic fluid alpha fetoprotein (AFP) levels were shown to be associated with open neural tube defect pregnancies (Brock and Sutcliff, 1972). This led to the first antenatal screening programme for birth defects. MSAFP screening allows detection in about 75 percent of fetuses with open spina bifida and 85 percent of those with ventral wall defects (Brock and Bolton, 1973). Maternal serum AFP screening has become a well accepted part of the prenatal care of a pregnant woman and increased AFP levels indicate the possibilities of having a fetus with neural tube defects, twin pregnancies, gastroschisis, omphalocoeles, fetal renal abnormalities, placental abnormalities and fetal demise (UK collaborative study, 1977).

The association between maternal age and risk of having a Down syndrome (DS) pregnancy was published in 1933 (Penrose, 1933). In 1968, the first antenatal diagnosis of DS was made (Valenti *et al*, 1968). Screening on the basis of advanced maternal age for diagnostic amniocentesis was gradually introduced into medical practice. The usual cut off age was 35 years and an amniocentesis was usually carried out at about 16-18 weeks of pregnancy. This method of screening, based on maternal age only, identified about 20 percent of pregnancies with DS. Therefore, approximately 80 percent of children with DS are born to women who are less than 35 years old and who do not have an identifiable increased risk for delivering a child with DS (Adams *et al*, 1981).

In 1984, it was shown that *low* maternal serum alpha fetoprotein (AFP) was associated with DS (Merkatz *et al*, 1984). However, in women younger than age 35, MSAFP screening identifies only 15-20 percent of fetuses with Down syndrome. Later, elevated maternal serum human chorionic gonadotrophin (hCG), and low unconjugated estriol (μE3) were found to be markers of DS (Canick *et al*, 1988; Wald *et al*, 1988). In 1988, these three biochemical markers were used together with maternal age as a method of screening and have been widely accepted as routine prenatal care in many countries

(New England Regional Genetics Group Prenatal Collaborative Study on Down Syndrome Screening, 1989; Del Junco *et al*, 1989; Osathanondh *et al*, 1989). Maternal serum screening identifies women with an increased risk of having a pregnancy with DS or an ONTD so that they can be offered a diagnostic test (Heyl *et al*, 1990; Kellner *et al*, 1991; MacDonald *et al*, 1991; Mancini *et al*, 1991). Combining all three biochemical markers with maternal age, Wald *et al* (1997) estimated a detection rate of 67 percent for pregnancies affected with DS at a false positive rate of 7.2 percent. In this age group (<35), multiple marker screening [using either AFP and human chorionic gonadotropin (hCG) or AFP, hCG and unconjugated estriol] can identify about 50 percent fetuses with DS (Mancini *et al*, 1992; Phillips *et al*, 1992; Haddow *et al*, 1992; Cheng *et al*, 1993; Wald *et al*, 1997). There is, therefore, general agreement that multiple marker screening should replace MSAFP screening for women under 35 years (ACOG committee opinion, 1994; Norgaard Pedersen *et al*, 1994; Kellner *et al*, 1995). In addition, all three markers are reduced in patients carrying a fetus with trisomy 18. Although capable of modifying age-related risks for DS and certain other chromosome abnormalities, multiple marker screening fails to detect about 30 percent of DS cases in women ≥35 years old and about 10 percent of those ≥40 years old, as well as 30 percent or more with trisomy 18 and about 50 percent with other chromosome abnormalities (Statement on Multiple Marker Screening in Pregnant Women, 1996)

MATERIALS AND METHODS

From January 1996 through September 2002, 1,793 maternal serum samples were received from Thai obstetricians who offered pregnant women multiple marker screening (alpha fetoprotein, unconjugated estriol and hCG) for Down syndrome and neural tube defects. Samples between 14-22 weeks' gestation were considered in the program. Gestational age at sampling were estimated by ultrasonogram (93.31% of cases) or last menstrual period (6.69% of cases).

Table 1. Median MS-AFP by gestational age.

Gestational age			Median MS-AFP	
Completed weeks	Median days	Number	Units	MoM
14	102	37	32.28	1.069
15	108	179	37.41	1.035
16	114	391	42.16	1.022
17	121	409	49.54	0.995
18	128	299	59.81	1.020
19	135	160	72.58	1.153
20	142	90	83.91	1.032
21	147	59	94.03	1.051
22	157	33	132.60	1.256
14-22		1,657		1.035

AFP and hCG were assayed with microparticle enzyme immunoassay techniques (IMx, Abbott Laboratories). Unconjugated estriol measurements were made with Fluorescence Polarization Immunoassay (FPIA) technology (TDx, Abbott Laboratories). Assay results were converted to multiples of the median (MoM) for normal pregnancies at the relevant week of gestation. The normative regressed median values for AFP were based on our previous report (Wasant *et al*, 1996). The MoM levels were adjusted for maternal weight using the regressed weight-correction formula established by unaffected pregnancies. Gestational age specific medians and a maternal weight correction formula were established for Thai pregnant population. Likelihood ratio for Down syndrome pregnancies in relation to multiples of the median (MoM) levels of these analytes were derived from the overlapping Gaussian frequency distribution curves for Down syndrome and unaffected pregnancies. Modified computer software developed by Wald *et al* (1988, 1997) was used to calculate the multivariate risk of Down syndrome and neural tube defects.

RESULTS AND DISCUSSION

This study has firmly established normal values of AFP, unconjugated estriol and hCG in Thai pregnant women (Tables 1, 2, 3).

The maternal serum report summary (Table 4) demonstrated that 76.7 percent of Thai pregnant women screened negative for DS and NTD and 1.2 percent screened negative for DS only. There were 16.3 percent of pregnant women who screened positive for DS. Of those who screened positive, 16.3 percent had increased risk for DS; 0.3 percent had increased risk for DS and elevated AFP; 1.1 percent with elevated AFP ; 1.8 percent had previous DS only and 0.5 percent had previous NTD only. There were 2.1 percent with uninterpretable results (test done too early, test done too late and grand multiple pregnancy). Increased risk for trisomy 18 was reported in 102 tests which was 5.69 percent. The performance of the screening test is measured by both (i) the detection rate - the proportion of DS birth with positive results and (ii) the false positive rate - the proportion of unaffected birth with positive results. In comparison,

Table 2. Median uE3 by gestational age.

Gestational age			Median uE3	
Completed weeks	Median days	Number	Units	MoM
14	101.5	34	4.49	0.864
15	108	167	9.46	1.177
16	114	377	12.89	1.217
17	121	385	15.44	1.274
18	128	272	19.18	1.343
19	135	150	26.58	1.651
20	142	84	32.29	1.213
21	147	57	29.55	1.032
22	157	32	41.47	1.015
14-22		1,558		1.283

Table 3. Median T-hCG by gestational age.

Gestational age			Median T-hCG	
Completed weeks	Median days	Number	Units	MoM
14	102	37	69.50	0.873
15	108	179	50.37	0.904
16	114	391	42.68	0.999
17	121	409	35.34	0.977
18	128	299	29.25	1.001
19	135	160	32.52	1.117
20	142	90	27.66	1.043
21	147	59	30.50	1.170
22	157	33	25.87	0.886
14-22		1,657		1.005

Table 4. Result of maternal serum screening.

Screening result	Reason	Number	Percent
Positive	Increased risk of Down Syndrome	292	16.3%
	Increased risk of Down and raised AFP	5	0.3%
	Raised AFP	19	1.1%
	Previous Down only	33	1.8%
	Previous NTD only	9	0.5%
Uninterpretable	Test done too early	13	0.7%
	Test done too late	23	1.3%
	Grand multiple pregnancy	2	0.10%
Negative (interpretation for Down syndrome and NTD)		1,376	76.7%
Negative (interpretation for Down syndrome only)		21	1.2%
Total		1,793	100.00%

Note : *Increased risk of trisomy 18 was reported in 102 first tests (5.69%)*

the results of this study are consistent with studies in other countries (United States, United Kingdom, Hong Kong, Taiwan and Canada) (Palomaki *et al*, 1997; Wald *et al*, 1997; Lam *et al*, 1998; Sheu *et al*, 1998; SOGC Committee Opinion, 1999).

Majority of Thai pregnant women who participated in this maternal serum screening (MSS) program were referred or recommended by their obstetricians or requested for MSS themselves due to concern regarding the risks of amniocentesis. There were 1,083 women (60.40%) $\geq$ 35 years and 710 women (39.60%) were < 35 years. These data demonstrated fair acceptance from Thai pregnant women and their obstetricians. This study can be considered a pilot program for maternal serum screening in Thailand where the MSS program is quite new and; thus, patient and physician education is equally important and necessary.

CONCLUSION

Maternal serum screening is a *non-invasive screening test* and a more effective and rational approach to aneuploidy screening for DS than screening based on maternal age or MSAFP alone. Multiple serum screening identifies women with an increased risk of having a pregnancy with DS or ONTD so that they can be offerred a diagnostic test. From this study, all women who were screen-positive for DS, NTD (and trisomy 18) received genetic counseling and a referral to an obstetrician for amniotic cytogenetic studies. Only 2 women who were screen-negative had DS babies (out of 1,793 cases screened) and 1 woman who was screen-negative had trisomy 18. Prenatal genetic counseling is of utmost importance. Pregnant women should always be able to choose whether or not to have maternal serum screening after proper prenatal genetic counseling. Proper interpretation of maternal serum screening results requires strict laboratory standards and accurate patient information. Public and physician education and patient counseling will avoid major problems. Our study demonstrated that maternal serum screening offers another option for older women who traditionally have all been considered candidates for amniocentesis. Where feasible, screening for DS, NTD and other chromosomal abnormalities (ie trisomy 18) should be made available to all pregnant women.

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