Thai Journal of Obstetrics and Gynaecology
June 2003, Vol. 15, pp. 69-77

OBSTETRICS

Second Trimester Prenatal Screening for Down Syndrome in Thai Pregnant Women Using Alpha-fetoprotein and Free Beta-hCG

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ABSTRACT

Objective To study the values of second trimester prenatal screening for Down syndrome using alpha-fetoprotein and free beta-hCG in Thai pregnant women.

Design A prospective descriptive study (diagnostic test).

Setting Department of Obstetrics and Gynecology, Charoenkrung Pracharuk Hospital.

Methods Alpha-fetoprotein (AFP) and free beta-hCG (free β-hCG) were measured in serum samples from 1,808 singleton Thai pregnant women at 15-20 weeks of gestation. Gestational ages were determined by ultrasonographic parameters measured at the same visit as the test for all cases. The gestational-age-specific and weight-adjusted medians for serum AFP and free β-hCG were calculated. The risk for fetal Down syndrome (DS) was derived by mathematical modeling of the medians together with maternal age. Amniocentesis were offered to women with a calculated DS risk of 1:270 or greater and to women aged 35 years or over.

Result A total of 141 women (7.8%) were classified as being high risk for DS, including 4.1% (53/1,289) of those younger than 35 years of age and 17.1% (89/519) of those who aged 35 years or over. Five out of six cases of Down syndrome were screen-positive, corresponding to a detection rate of DS of 83% with a 7.5% false positive rate. A case of trisomy13, 1 case of Klinefelter syndrome and 1 case of balanced translocations were found screen-positive for DS. A case of trisomy18 affected by an omphalocele was screened negative for DS with high level of AFP (6.35 MoM) and low level of free β-hCG (0.44 MoM). The additional value of prenatal screening was a detection rate for neural tube defect (NTD) of 80% with a 2.8% false positive rate.

Conclusion Maternal serum screening using double biochemical markers (AFP and free β-hCG) in combination with gestational dating by ultrasonography is effective in the detection of fetal Down syndrome and possibly other abnormalities in Thai pregnant women.

Key words: Down syndrome, second trimester screening, alpha-fetoprotein, free beta-hCG

Down syndrome (DS) is worldwide the most prevalent chromosomal disorder. Since Asians account for a significant proportion of the world population, it is implicated that they have a higher incidence of DS births. For Asian population Taiwan's established study of the first sizeable database (7,232,689 live births) showed that a birth prevalence of DS is 11.8 per 10,000 live births (1:848) and 88.4% of DS were delivered by women younger than 35 years of age.[1] As the study indicated, a maternal serum DS screening program is especially useful for the detection of DS pregnancies since DS babies are born to those younger women. DS in Thailand is the most common and best known chromosomal disorder, however, no sizeable database was established.

Although the incidence of DS increases with advancing maternal age, the use of maternal age alone as a screening tool results in the identification of only about one third of the cases of fetal DS.[2] Maternal serum screening for DS using biochemical markers is now routinely performed in western countries and some countries in Asia.[2,3] The concept of such screening came from the observation of an association between fetal DS and low maternal serum alpha-fetoprotein (AFP) and because of the poor detection rate achieved using alone in either white women or Asians,[4,5] there has been an increasing effort to develop more sensitive markers for DS screening.[6] Although the two-analyte protocol of AFP and total human chorionic gonadotropin (hCG)[7,8] is more accurate than a single analyte, a screening strategy of triple test using AFP, hCG, and unconjugated estriol (UE3) in combination with maternal age currently is the most popular method.[9-13] Because the additional benefit of using UE3 is still under debate[8,14-16] and cost has also become a limitation of the triple test, with significant license fees levied on laboratories running hCG screening tests.[8,17,18] As free β-hCG has been reported as the single most effective marker available in DS screening program[2,19-23] and no licensing fees to be absorbed as additional cost, we intended to use AFP and free β-hCG in combination with maternal age for DS screening in our study.

The objective was to study prospectively the values of AFP and free beta-hCG in DS screening and possibly other abnormalities among Thai population.

Materials and Methods

Data from Charoenkrung Pracharuk Hospital were obtained for the age distribution of women who had live births between the year 1996 and 2001. The relative frequency of maternal age distribution in these years was used as the age distribution of women with unaffected pregnancies and pregnancies affected by DS.

Between March 1998 and August 2002, 1,820 Thai pregnant women who attended our hospital for antenatal care consented to biochemical screening. Informed consent was obtained after proper counseling. All had pregnancy at 14 -21 weeks of gestation and had an ultrasound examination before venepuncture. Gestational age was calculated based on sonographic biometric measurements taken on the date of collection of blood sample. Maternal weight was obtained at the time of the biochemical tests.

Each blood sample was centrifuged after it was drawn from a single venous blood specimen. The serum was stored at -20^{o}C until it was thawed for biochemical test. The quantitative AFP levels were measured with a radioimmunoassay (AFP RIA; CIS bio international, France). Measurement of free beta-hCG in maternal serum was carried out using a solid-phase two-site immunoenzymatic assay (FβHCG ELACT; CIS Ltd., Gif-sur-yvette Cedex, France). The performance of this assay has been described previously.[24]

Our previous study[24] revealed that different median values of free β-hCG existed between the data collected from multicenters[25,26] and those of our own hospital. Because MoM (Multiples of Median) conversion is related to DS risk calculation, it is obviously suitable to use the median values provided by own hospital or laboratories. Assay results were converted to MoM for normal pregnancies at the relevant week of gestation. The normative regressed median values for AFP were based on our previous report.[27] Free β-hCG regressed median values were

calculated from our 836 normal singleton Thai pregnancies.[24] The MoM levels were adjusted for maternal weight using the regressed weight correction formula established by our unaffected Thai pregnancies. The weight-adjusted MoM and the risk estimates for DS were calculated with the modified computer software by Spencer et al[23] which uses a mathematical model that combines the two biochemical markers levels expressed as MoM with the primary risk background estimation of DS risk in Asian populations.[1] The first 836 subjects[24], previously using default medians, were recalculated and the new second 972 subjects were calculated for the multivariate risk of DS by our population-specific medians.

Amniocentesis were offered to women with a calculated DS risk of 1:270 or greater and to women aged 35 years or over. After delivery all babies were examined by a neonatologist. All other statistical analyses were performed by the statistical software package.

Results

The total population risk of DS in Charoenkrung Pracharuk Hospital between the year 1996 and 2001 was 1:612 (54 in 33,051 live births). During this period 7.5% (2,471/33,051) of total pregnant women were 35 years or over. Hence 92.5%(30,580/33,051) of the total pregnancies occurred in women younger than 35 years of age and collectively accounted for the high occurrence rate of DS before age 35, which was 77.8% (42/54). Fig.1 shows the frequency distributions of normal and DS live births in their original values and the data after smoothing are plotted.

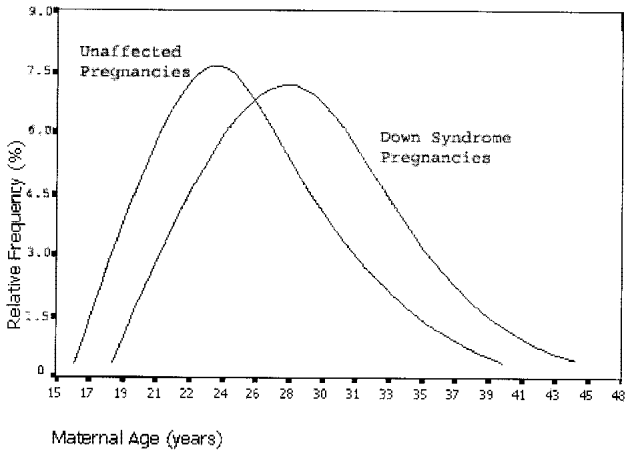

Fig. 1. The smoothed distribution of relative frequency of unaffected and Down syndrome live births at each maternal age.

During March 1998 to August 2002 a total of 1,820 Thai pregnant women were screened; this represents screening uptake of 9.1% (1,820/19,963). Of the 1,820 pregnancies, 1,808 were single pregnancies and 12 were twin pregnancies. The mean (±SD) maternal age (at delivery) of the single pregnancies (n=1,808) in this study was 29.7 ±6.5 years (median 30, range 15.0-48.0) and the mean maternal weight was 55.4±9.3 kg (median 54, range 35-103). The women younger than 35 were 71.3%(1,289) while 28.7% (519) were 35 or over. A total of 141 women were classified as high risk for DS by the test and were offered amniocentesis (a recall rate of 7.8%). The recall rate was 4.1% (53/1,289) among those who were younger than 35 and was 17.1% (89/519) among those who were 35 or over. Sixty-eight women refused

amniocentesis and one of them delivered a DS baby.

There were 6 DS pregnancies in this study. Table 1 shows the results of biochemical test of these 6 cases. The screening test was positive for DS in 5 of 6 cases. So the detection rate for DS was 83.3%. The median concentration of free β-hCG in the DS group was 2.74 MoM (indicating a 2.74-fold increase), significantly higher than in unaffected group ($p<0.05$) and that of AFP was 0.79 MoM, significantly lower than in unaffected group ($p<0.05$). The results of free β-hCG levels (MoM) and AFP levels (MoM) in normal and DS pregnancies were plotted against relevant week of gestation. Fig.2 shows the median MoM in free β-hCG levels for 6 DS pregnancies exceeding 2.00 MoM or the 85th percentile of the unaffected pregnancies. Fig.3 shows the median MoM in AFP levels for 6 DS pregnancies being about or below 1.00 MoM or the 50th percentile and for the additional 5 neural tube defect (NTD) pregnancies exceeding 2.00 MoM or the 95th percentile.

Table 1. Biochemical screening results of the six DS pregnancies

Case no.	Maternal age (year)	Gestational age (week)	Free β-hCG MoM	AFP MoM	DS risks	*DS -result screening	Karyotyping
1	38	14.0	2.92	0.70	1/41	+	47,XX, +21
2	28	18.4	2.56	0.95	1/230	+	47,XX, +21
3	36	15.0	2.49	0.35	1/195	+	47,XY, +21
4	37	19.2	3.69	0.63	1/24	+	47,XX, +21
5	42	16.4	3.61	1.01	1/13	+	47,XX, +21
6	38	17.2	2.04	1.02	1/290	-	47,XY, +21

*Positive DS-result screening (+) means a calculated DS risk of 1:270 or over
Negative DS-result screening (-) means a calculated DS risk below 1:270

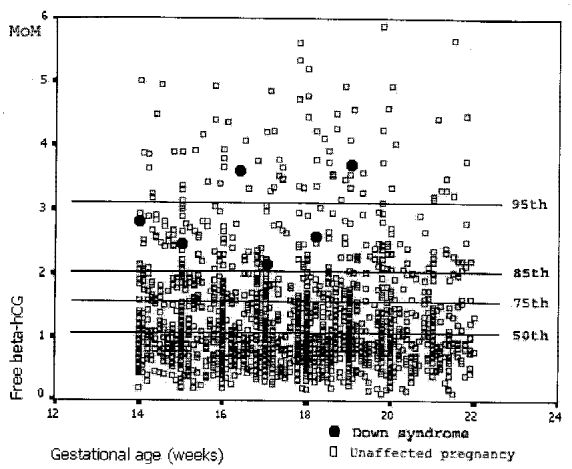

Fig. 2. Free β-hCG levels (MoM) in 6 Down syndrome and unaffected pregnancies.

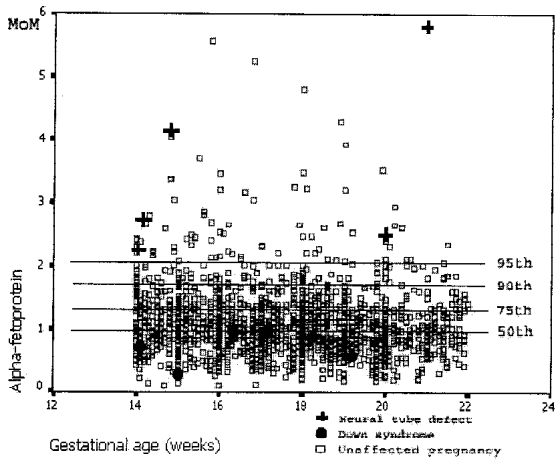

Fig. 3. AFP levels (MoM) in 6 Down syndrome, 5 neural tube defects and unaffected pregnancies.

Our study illustrated that the values of the double test using AFP and free β-hCG for second trimester prenatal screening for DS in Thai pregnant women were an accuracy of 92.4%, a sensitivity (detection rate) of 83.3%, a specificity of 92.4%, a false positive rate of 7.5%, a false negative rate of 16.7%, a positive predictive value of 3.8% and a negative predictive value of 99.9%. The additional value of second trimester prenatal screening for NTD was a detection rate of 80% with a 2.8% false positive rate.

Additionally, while following up the outcome of DS, we also identified a number of other abnormalities which had been highlighted by the screening program and had produced report indicating an increased risk of DS and/or NTD (Table 2).

Table 2. Biochemical screening results of other abnormal karyotyping and/or the additional structural abnormalities/fetal outcome

Case No.	Maternal age (year)	Free β-hCG MoM	AFP MoM	DS-result screening	*NTD result screening	Karyotyping	Abnormality/ fetal outcome
1	48	0.44	6.35	-	+	47,XX, +18	Omphalocele
2	44	2.40	2.59	+	+	47,XX, +13	Open NTD
3	38	2.95	1.23	+	-	47,XXY	None
4	26	3.52	0.98	+	-	46,XY,t(9q;13q)	None
5	36	0.49	1.05	-	-	45,X / 46,XY	None
6	35	1.46	2.64	-	+	None	Anencephaly
7	32	1.09	2.78	-	+	None	Anencephaly
8	39	1.18	4.03	-	+	None	Anencephaly
9	30	0.68	5.81	-	+	None	Anencephaly
10	28	0.86	2.20	-	-	None	Anencephaly
11	17	0.89	3.20	-	+	None	Gestroschisis
12	26	3.65	3.46	-	+	None	Hydrops fetalis
13	30	0.54	8.10	-	+	None	Fetal death
14	37	2.54	6.45	-	+	None	Fetal death

*Positive NTD -result screening (+) means a calculated median MoM in AFP level of 2.50MoM or over
Negative NTD -result screening (-) means a calculated median MoM in AFP level below 2.50MoM

Discussion

In our hospital Down syndrome (DS) is the most common chromosomal aberration with a prevalence of 16.3 per 10,000 live births (1:612). Having reviewed the cumulative number and the percentage of DS live births at a specific age (Fig.1), we found that 77.8% (42/54) of our DS live births had been delivered by women younger than 35 years of age. This result correlates well with the other reported data of 69-80%.[28-30] About 92.5% of the total pregnancies were women younger than 35, which corresponded closely to the other reported data (around 92.5-95%)[28,29,31] and collectively accounted for the high occurrence rate of DS before age of 35. If we set the cut-off of maternal age screening for genetic amniocentesis at the age of 35 or over, the detection rate of DS was only 22.2% with a 7.5% false-positive rate. In order to select young age pregnancies with an increased risk of DS in modern countries, maternal serum screening for DS has become an essential prenatal examination.[7-13]

The second trimester maternal serum screening for DS considerably advanced over screening using maternal age alone. It has become more cost effective to use as a regular component of antenatal care in modern obstetric management.[22,23] The general practice in Thailand for screening DS is to offer amniocentesis to women aged 35 or over, which constitute 7.5% of our obstetric population. In the second trimester hCG or free β-hCG is the serum marker of first choice, with AFP as the second marker and UE3 as the third. Statistical models with parameters derived by meta-analysis predict that a three marker combination of AFP, hCG and UE3 will yield a 67% detection rate for a 5% false-positive rate by the model prediction confirmed in 21 large prospective intervention studies.[13] A fourth marker, inhibin A, increases the detection rate by 7% for the same false-positive rate.[32] In the published study by Spencer et al[33], a seven year review of 67,904 pregnancies screened in second trimester using AFP and free β-hCG indicated that the detection rates of DS were 75% for a 5% false positive rate. Our present prospective study of 1,670 Thai pregnant subjects have demonstrated a detection rate for DS of 83% to be achieved with a 7.5% false positive rate.

The use of serum hCG in DS screening is just a current complex matter. Although the use of total hCG is unequivocally under debate for establishment in serum screening for DS,[34-40] a superior detection rate was achieved in several studies by using free β-hCG.[20,41-44] Free β-hCG has been suggested as the major contributor to the increased sensitivity and specificity of the two-marker screening.[33,45] In 6 DS pregnancies we found totally elevated free β-hCG values (exceeding 2.00 MoM or the 85th percentile of the unaffected pregnancies) and the median value of free β-hCG was 2.74 MoM in agreement with the other reports (range 2.22 - 2.79 MoM).[2,23,37,46-49] When we compared our result with the median values of hCG in the other studies (range 1.79 - 2.12 MoM),[10,19,37,40,49-51] free β-hCG values in DS cases gave higher median MoM concentration than hCG. Furthermore, the meta-analysis study[52] in a large amount of DS cases illustrated that the mean free β-hCG level (2.30 MoM) in 477 cases was significantly higher than the mean hCG level (2.02 MoM) in 850 cases.

The median value of AFP in our 6 DS pregnancies was 0.79 MoM, which agreed closely with that reported in whites (0.75 MoM)[53] and in Taiwanese (0.77 MoM)[45], however, the serum AFP values were not totally lower than 1.00 MoM or the 50th percentile of the unaffected pregnancies. The performance of the AFP alone in serum screening for DS remains poor, when compared with the other markers.[23] In Asian's study,[2] at a 5% false positive rate, free β-hCG alone would identify 46.8% of DS pregnancies, whilst AFP alone detected 17%. Because of its widespread use in screening with high sensitivity and specificity for neural tube defect (NTD) and ventral wall defect,[54-58] the role of AFP appears secure as a component of maternal serum screening for DS. This supports our data that the detection rate for NTD of 80% with a 2.8% false positive rate and furthermore the serum AFP values of our 5 NTD pregnancies (Anencephaly) exceeding 2.00 MoM or the 95th percentile. We also detected one case of gastroschisis with

the serum AFP value of 3.20 MoM. In the data reported by Saller et al,[58] the median AFP values were significantly higher in pregnancies with gastroschisis and omphalocele compared to unaffected pregnancies (9.42 and 4.18 MoM, respectively).

Screen-positive rates for DS are strongly dependent on the age distribution of the mothers being-screened.[59] In current practice the false positive rate accepted in screening test is about 5%.[33] Our study in screening program showed slightly high false positive rate (7.5%) since the percentage of women aged 35 or over in the screening group was high (28.7%) when compared with those women of the total pregnancies(7.5%). The possible reason is that the calculated risk of DS normally use maternal age-specific rate in the primary risk background estimation of DS risk, therefore the large amount of women aged 35 or over in the screening implicates high false positive rate of the test.

Adding to screening for DS, results of screening for the other abnormal karyotyping and/or the additional abnormalities were identified. Spencer et al[60] reported the median MoM value of AFP and free β-hCG to be significantly lower in pregnancies complicated by trisomy 18 (median values 0.71 and 0.37 respectively). Our study demonstrated one case of trisomy 18 affected by an omphalocele with high maternal serum level of AFP (6.35 MoM) and low level of free β-hCG (0.44 MoM). In other abnormal chromosomes we found 3 cases with positive screen for DS and totally high-elevated free β-hCG values which included trisomy13, Klinefelter syndrome and balanced translocations. At present, no previous studies of free β-hCG had clearly supported these preliminary findings. The risk for outcome of fetal death has been reported to be higher in association with elevated AFP,[61-64] corresponding to our two cases of subsequent fetal death with very high maternal serum level of AFP (8.10 and 6.45 MoM).

At present, there is no national biochemical screening program for DS in Thailand. Free β-hCG is a promising screening test for DS. Free β-hCG could ultimately replace hCG determinations in DS screening. Measurement of free β-hCG may overcome the limitations of hCG, including the comparatively low sensitivity and specificity,[41] the abnormally high licensing fees[8,17,18] and possibly the non-applicability to first-trimester screening.[65-67] AFP and free β-hCG may be a practical test for DS screening. The simple two-marker protocol with sustainable detection rates of 75% in United Kingdom[33] (83% in our study) would seem to be an economical way of moving forward in those health authorities not offering screening policy.

Our preliminary results in this study are encouraging. It appears that maternal serum screening using double biochemical markers (AFP and free β-hCG) in combination with gestational dating by ultrasonography is effective in the detection of fetal DS and possibly other abnormalities in a Thai population. Further study using the present strategy in a larger Thai population is required to substantiate our findings.

Acknowledgements

The authors would like to thank Dr.Siriwat Manotham, Director of Charoenkrung Pracharuk Hospital, for allowing us to complete the study. We also thank CIS bio international for supporting FβHCG EIACT kits and soft wares.

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