Preliminary Report

Experience on Triple Markers Serum Screening for Down's Syndrome Fetus in Hat Yai, Regional Hospital

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Objectives: *To summarize the experience and evaluate the performance of the Hat Yai maternal serum screening (MSS) program.*
Setting: *The Hat Yai MSS program between 16 February 2003 and 11 March 2004.*
Material and Method: *The uptake of screening was 999 in 1,040 women (96.0%), between 14 to 20 weeks of gestation with the triple markers: Alpha-fetoprotein (AFP), human Chorionic Gonadotropin (hCG), and unconjugated Estriol (uE3) by Immulite chemiluminescent immunoassay system, Diagnostic Product Corporation (DPC). The risk cut-off for Down's syndrome is one in 250 or greater, based on software for prenatal Down's syndrome risk calculation, by Prisca 3.5 DPC.*
Results: *There were 119 in 999 cases (11.9%) of the triple test positive. Amniocentesis had been performed on voluntary basis, and the uptake rate of amniocentesis following a positive Down's syndrome screening was 104 in 119 cases (87.3%). Based on clinical diagnosis of Down's syndrome in the newborns of non-amniocentesis mothers, assuming that normal looking babies were not Down's syndrome, the sensitivity (SENS), specificity (SPEC), positive predictive value (PPV), and negative predictive value (NPV) of all chromosomal abnormalities were 85.7%, 88.6%, 5.0%, and 99.8% respectively. The false positive rate was 113 in 992 cases (11.4%). Whereas, the SENS, SPEC, PPV, and NPV of Down's syndrome were 100%, 88.4%, 3.4%, and 100% respectively. The false positive rate was 115 in 995 cases (11.6%). The mean level, median level, and multiple of median (MoM) of triple markers were demonstrated.*
Conclusion: *The Down's syndrome screening is a systematic application of a test to identify subjects at increased risk of a specific disorder, of course it is not diagnostic, but to benefit making decision regarding further amniocentesis. The sensitivity of Prisca 3.5 software was satisfactory but false positive rate was remarkably high. It needs further standardization with adjusted MoM values.*

Keywords: *Triple test, Second trimester, Down's syndrome, Alpha-fetoprotein, Human chorionic gonadotropin, Unconjugated estriol*

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Prenatal diagnosis of Down's syndrome, trisomy 21, is based on fetal karyotyping, but amniocentesis cannot be performed in all patients because of the risk of fetal loss and the cost. It, therefore, is usually applied only to high-risk (and generally older) patients. Noninvasive assays of maternal serum markers have allowed the extension of screening to mothers of all ages. Alpha-fetoprotein (AFP), human Chorionic Gonadotropin (hCG) or free □-hCG, and unconjugated Estriol (uE3) have been prospectively evaluated during the second trimester in large populations[1-11]. Wald et al[12] proposed an individual risk calculation for Down's syndrome, combining maternal age, maternal serum markers, and gestational age, in which amniocentesis was proposed when the risk was above a cut-off leading to a 60% Down's syndrome detection rate (sensitivity)

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and a 5% amniocentesis rate. Software is necessary for risk calculation and should be routinely validated.

Muller et al has evaluated the influence of software design on risk calculation, and compared six software packages [Prenatal Interpretive Software (Maciel Inc.), Prisca (Diagnostic Product Corporation - DPC), DIANASoft (BioChem Immuno Syst□mes), T21 (Chiron), PrenatScreen (CIS bio international), and MultiCalc (Wallac)] in two populations: 529 control patients (aged 18-37 years) selected randomly from 90,000 screened patients, and all 125 Down's syndrome-affected pregnancies (patient ages, 20-37 years). The present study demonstrates that with the same maternal serum markers, variations are observed between software packages, with a mean detection rate of 54.4-66.4% and a false-positive rate of 2.4-6.8%[13].

In practice, in a population of 100,000 patients, including 143 cases of Down's syndrome, the most sensitive software will detect 78 cases of Down's syndrome through 2,400 amniocenteses, whereas the least sensitive will detect 95 cases through 6,800 amniocenteses. These differences will have an impact on public health policy[14] and should be minimized. At present, this can be achieved in different ways: use of the same maternal age-related risk, definition for each country of the risk at term or at sampling, use of daily medians, and use of the parameter sets defined by Cuckle[15]. However, if the software obtains the same value, this does not necessarily mean that it is the most accurate one. Hat Yai maternal serum screening (MSS) program aims to use triple markers serum screening: AFP, hCG, and uE3 by Immulite chemiluminescent immunoassay system, DPC, together with a software for prenatal Down's syndrome risk calculation, Prisca 3.5 DPC, determining the feasibility of a screening system.

Material and Method

During the period from 16 February 2003 to 11 March 2004, Hat Yai maternal MSS program voluntarily enrolled 1,040 pregnant women of gestational age between 14 to 20 weeks for triple markers serum screening. The exclusion criteria are diabetes mellitus, dead fetus *in utero*, and multiple pregnancies. Demographic information obtained included patient's date of birth, maternal weight, diabetic status, smoking, and last menstrual period. The gestational age of all subjects was estimated by ultrasound. The protocol and informed consent were approved by the institutional review board of the trial center.

The blood specimens were taken, and serums were collected in the refrigerator for transportation to the central laboratory in Bangkok, once a week. Maternal serum was used to measure level of AFP, hCG, and uE3. The risk cut-off for Down's syndrome is one in 250 or greater, based on software for prenatal Down's syndrome risk calculation, Prisca 3.5 DPC. The performance indicators of the maternal serum screening for Down's syndrome were assessed in terms of accuracy of the test, the blood tested-result delivery time, the result delivered-amniocentesis time, as well as the acceptability of the subjects toward amniocentesis and selective abortion.

A triple test positive result did not provide a diagnosis of trisomy 21 or 18, but indicated that further evaluation, amniocentesis and karyotyping, should be considered. However, because the risk of having a child with trisomy increases with advanced maternal age, pregnancies of mothers aged 35 years or more who were triple test negative, were suggested to voluntarily have amniocentesis performed as a conventional basis. All cases, enrolled in the screening program, were followed until delivery. All newborns were examined whether there were any abnormalities. Descriptive statistics such as median, mean, standard deviation (SD), range, frequency (%), and diagnostic test were used where it was appropriate.

Results

The uptake of screening was 999 in 1,040 women (96.0%). The mean age was 28.5 years with SD = 6.28, and range of age 14-46 years. There were 802 in 999 subjects (80.2%) aged under 35 years old; 173 in 999 subjects (17.3%) aged 35-40 years old; and 24 in 999 subjects (2.4%) aged more than 40 years old.

One hundred and nineteen women (11.9%) were initially classified to be triple test positive. The cut-off for Down's syndrome is one in 250 or greater. Among those who were triple test positive, the age distribution revealed that 42 (35.2%) were less than 35, 57 (47.8%) were 35-40, and 20 (16.8%) were more than 40. Eight hundred and eighty women (88.0%) were triple test negative.

From the 119 positive cases, 104 (87.4%) accepted the offer of amniocentesis, while 15 (12.6%) refused the prenatal diagnosis. In the refusal group, 11 cases (73.3%) were aged 35 years or over. The proportion of age, triple test positive, amniocentesis uptake and amniocentesis refusal are shown in Table 1. In contrast, 19 cases, aged 35 years or over (9.6%) who were triple test negative, had requested or voluntarily had a prenatal diagnosis performed.

The step-by-step of the program comprised

of triple markers serum screening, amniocentesis, karyotyping, and selective abortion. Each step had a period of time consumption. The average blood tested-result delivered time was 12.1 days, range of 2-33 days, and 215 in 999 subjects (21.5%) were longer than 2 weeks. Whereas, the average blood resulted-amniocentesis time was 10.4 days, range of 1-42 days, and 18 in 104 cases (17.3%) were longer than 2 weeks.

The karyotyping revealed six cases of abnormal chromosome including two cases of 47, XX, +21, two cases of 47, XY, +21, one case of 47, XXX and one case of 46, XX with translocation 46, XX, t(5;15) (q13; q13). Selective abortion was carried out in three of four cases of trisomy 21 fetuses, while the other chromosome abnormalities did not.

The maternal ages of Down's syndrome fetus were 36, 39, 40, and 42 respectively, while a case of triple X and a case of translocation chromosome were both 37 years old. A mother of 36 years old with triple test negative delivered a 47, XY, +13 neonatal dead child. By clinical examination of the newborn, there was no clinical Down's syndrome in the newborns of triple test negative mothers.

The results of triple markers screening and all chromosome abnormalities including Down's syndrome among the fetuses are shown in Table 2. Based on the clinical diagnosis of Down's syndrome in the newborns of non-amniocentesis mothers, assuming that normal looking babies were not Down's syndrome, the sensitivity (SENS), specificity (SPEC), positive predictive value (PPV), and negative predictive value (NPV) of all chromosomal abnormalities were 85.7%, 88.6%, 5.0%, and 99.8% respectively. The false positive rate was 113 in 992 cases (11.4%).

Table 3 shows the results of triple markers and Down's syndrome only. The SENS, SPEC, PPV, and NPV of Down's syndrome only were 100%, 88.4%, 3.4%, and 100% respectively. The false positive rate was 115 in 995 cases (11.6%).

The 6 cases of positive triple markers were excluded, so there were 993 cases to calculate normal mean level, as well as the normal median level of triple

Table 1. Distribution of age, triple test positive, amniocentesis uptake, and amniocentesis refusal

Age	Triple test positive n (%)	Amniocentesis uptake n (%)	Amniocentesis refusal n (%)
Less than 35	42 (35.4)	38 (90.4)	4 (9.5)
35-40	57 (47.8)	50 (87.7)	7 (12.2)
More than 40	20 (16.8)	16 (80)	4 (20)
Total	119 (100.0)	104 (87.3)	15 (12.6)

Table 2. The results of triple markers screening and all chromosome abnormalities including Down's syndrome among the fetuses

Triple markers screening	All chromosomal abnormalities	Normal	Total
Test positive	6 (TP)	113 (FP)	119
Test negative	1 (FN)	879 (TN)	880
Total	7	992	999

TP = True positive, FP = False positive, FN = False negative, TN = True negative

Table 3. The results of triple markers screening and Down's syndrome only among the fetuses

Triple markers screening	Down's syndrome	Normal	Total
Test positive	4 (TP)	115 (FP)	119
Test negative	0 (FN)	880 (TN)	880
Total	4	995	999

TP = True positive, FP = False positive, FN = False negative, TN = True negative

Table 4. Normal mean level of AFP, hCG, and uE3 by gestational age

Gestational age	Number (n = 993)	AFP (ng/mL)	hCG (mIU/mL)	uE3 (ng/mL)
14	16	32.71	57316.75	1.36
15	163	39.9	46018.63	1.87
16	216	43.99	37035.7	2.36
17	189	52.58	32750.3	3.04
18	213	58.74	28632.6	3.92
19	186	71.19	25361.3	4.74
20	10	71.32	30934.2	5.53

Table 5. Normal median value of AFP, hCG, and uE3 by gestational age

Gestational age	Number (n = 993)	AFP (ng/mL)	hCG (mIU/mL)	uE3 (ng/mL)
14	16	30.6	57596.5	1
15	163	36.5	41946	1.6
16	216	41.5	33816	2.2
17	189	48.3	29628	3
18	213	54.7	25017	3.9
19	186	64.8	21433	4.55
20	10	71.25	27496.6	4.9

markers are demonstrated in Table 4 and 5. The normal Multiple of Median (MoM) is normal mean level divided by normal median level, so MoM distributed by gestational age, were calculated and shown in Table 6.

Discussion

Its maximum potential for the reduction of the birth incidence of Down's syndrome is limited by incomplete uptake of screening and compliance with diagnostic testing in the high-risk group[16]. The determining of false negative rate, under diagnosis, and detection rate should be definitely confirmed by karyotyping study of the newborn among triple test negative mothers. This studied software against all chromosome abnormalities/Down's syndrome, the SENSs were 85.5/100%, and the false positive rates were 11.4/11.6%. While other studies, the SENS was around 68% with 5% false positive rate[17,18].

Table 6. Normal Multiple of Median (MoM) of AFP, hCG, and uE3 by gestational age

Gestational age	AFP	hCG	uE3
14	1.06	0.99	1.36
15	1.09	1.09	1.16
16	1.06	1.09	1.07
17	1.08	1.10	1.01
18	1.07	1.14	1.00
19	1.09	1.18	1.04
20	1.01	1.12	1.12

MoM = normal mean level/normal median level

The software package that yielded the highest detection rate and lowest false positive rate (lowest amniocentesis rate) would be an ideological tool for MSS program. Muller F et al reported that detection and false-positive rate increased substantially with maternal age and differences between software packages[19]. Those were calculated and based on MoM. This was the log-regression of the median value of triple markers, and specific to different ethnic groups[20]. Thai MoM values in this trial with ongoing collection are crucial to utilize and develop the Prisca 3.5. At present, Prisca 4.0 is available, with adjusted MoM values, as well as the factors of chemical assay, gestation, maternal weight, and smoking status.

The advantages of this non invasive method are the aiming to reduce the number of women undergoing invasive prenatal diagnosis, as well as increase the proportion of Down's syndrome detection. Older aged mothers with triple test negative have a decreased risk of having a fetus with Down's syndrome, compared to those calculated by using maternal age alone. The final calculated risk must be carefully considered.

Nevertheless, it is important for each woman to discuss her own particular risks with her physician as part of the process of deciding whether to undergo amniocentesis. Besides, those women should be informed that older aged mothers had not only an increased risk of Down's syndrome, but also other chromosomal-related structural defects.

The amniocentesis for genetic studies is generally done between the 16th and 18th weeks of pregnancy, while the triple screen can be done a bit earlier. The gestational age is very important, because the amount of marker will vary with gestational age. The blood tested-result delivery times should be obtained very soon or within 2 weeks. Nevertheless, a number of blood tested-result delivery times in this trial extended to more than 2 weeks. The reason was the blood specimens for triple tests had been gathered and weekly transported to the central laboratory in Bangkok, about 1,000 kilometers north to the trial center. In addition, a number of the blood resulted-amniocentesis times had also been delayed by more than 2 weeks, with some cases extending up to 6 weeks. The pitfalls included the client-provider communication cap, counseling process of amniocentesis, and second opinion of family members.

However, a number of subjects refused to have prenatal diagnosis. The main reason was maternal anxiety of a miscarriage caused by amniocentesis, especially with women who had experienced infertility, miscarriage, or neonatal death. They may be unwilling to undertake any risk to their present pregnancy. Besides, a number of them refused because of aversion to selective abortion.

In conclusion, the Down's syndrome screening is a systematic application of a test to identify subjects at increased risk of a specific disorder, of course it is not a diagnosis, but to benefit for decision regarding further amniocentesis. Based on the results, the sensitivity of Prisca 3.5 was rather satisfactory but the false positive rate was remarkably high. It needs further standardization with adjusted MoM values.

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References

1. Benn PA, Horne D, Briganti S, Greenstein RM. Prenatal diagnosis of diverse chromosome abnormalities in a population of patients identified by triple-marker testing as screen positive for Down syndrome. Am J Obstet Gynecol 1995; 173: 496-501.
2. Goodburn SF, Yates JR, Raggatt PR, Carr C, Ferguson-Smith ME, Kershaw AJ, et al. Second-trimester maternal serum screening using alpha-fetoprotein, human chorionic gonadotrophin, and unconjugated oestriol: experience of a regional programme. Prenat Diagn 1994; 14: 391-402.
3. Kellner LH, Weiss RR, Weiner Z, Neuer M, Martin GM, Schulman H, et al. The advantages of using triple-marker screening for chromosomal abnormalities. Am J Obstet Gynecol 1995; 172: 831-6.
4. Mooney RA, Peterson CJ, French CA, Saller DN, Jr., Arvan DA. Effectiveness of combining maternal serum alpha-fetoprotein and hCG in a second-trimester screening program for Down syndrome. Obstet Gynecol 1994; 84: 298-303.
5. Herrou M, Leporrier N, Leymarie P. Screening for fetal Down syndrome with maternal serum hCG and oestriol: a prospective study. Prenat Diagn 1992; 12: 887-92.
6. Muller F, Aegerter P, Boue A. Prospective maternal serum human chorionic gonadotropin screening for the risk of fetal chromosome anomalies and of subsequent fetal and neonatal deaths. Prenat Diagn 1993; 13: 29-43.
7. Palomaki GE, Knight GJ, McCarthy JE, Haddow JE, donhowe JM. Maternal serum screening for Down syndrome in the United States: a 1995 survey. Am J Obstet Gynecol 1997; 176: 1046-51.
8. Spencer K, Carpenter P. Prospective study of prenatal screening for Down's syndrome with free beta human chorionic gonadotrophin. BMJ 1993; 307: 764-9.
9. Wald NJ, Kennard A, Densem JW, Cuckle HS, Chard T, Butler L. Antenatal maternal serum screening for Down's syndrome: results of a demonstration project. BMJ 1992; 305: 391-4.
10. Wald NJ, Densem JW, Smith D, Klee GG. Four-marker serum screening for Down's syndrome. Prenat Diagn 1994; 14: 707-16.
11. Wenstrom KD, Williamson RA, Grant SS, Hudson JD, Getchell JP. Evaluation of multiple-marker screening for Down syndrome in a statewide population. Am J Obstet Gynecol 1993; 169: 793-7.
12. Wald NJ, Cuckle HS, Densem JW, Nanchahal K, Royston P, Chard T, et al. Maternal serum screening for Down's syndrome in early pregnancy. BMJ 1988; 297: 883-7.
13. Muller F, Aegerter P, Ngo S, Fort A, Beauchet A,

Giraudet P, et al. Software for prenatal down syndrome risk calculation: a comparative study of six software packages. Clin Chem 1999; 45: 1278-80.
14. Seror V, Muller F, Moatti JP, Le Gales C, Boue A. Economic assessment of maternal serum screening for Down's syndrome using human chorionic gonadotropin. Prenat Diagn 1993; 13: 281-92.
15. Cuckle H. Improved parameters for risk estimation in Down's syndrome screening. Prenat Diagn 1995; 15: 1057-65.
16. Crossley JA, Aitken DA, Berry E, Connor JM. Impact of a regional screening programme using maternal serum alpha fetoprotein (AFP) and human chorionic gonadotrophin (hCG) on the birth incidence of Down's syndrome in the west of Scotland. J Med Screen 1994; 1: 180-3.
17. Wald NJ, Watt HC, Hackshaw AK. Integrated screening for Down's syndrome on the basis of tests performed during the first and second trimesters. N Engl J Med 1999; 341: 461-7.
18. Wald NJ, Cuckle HS, Densem JW, Kennard A, Smith D. Maternal serum screening for Down's syndrome: the effect of routine ultrasound scan determination of gestational age and adjustment for maternal weight. Br J Obstet Gynaecol 1992; 99: 144-9.
19. Muller F, Thalabard JC, Ngo S, Dommergues M. Detection and false-positive rates of maternal serum markers for Down syndrome screening according to maternal age in women over 35 years of age. A study of the agreement of eight dedicated software packages. Prenat Diagn 2002; 22: 350-3.
20. Spencer K. Ethnic differences in outcome of serum screening for Down's syndrome. Median values and maternal weight specific to the ethnic group should be used. BMJ 1996; 312: 1041.

ประสบการณ์ในการตรวจคัดกรองตัวชี้วัดสามตัวในซีรั่มเพื่อค้นหาทารกในครรภ์ที่เป็นกลุ่มอาการดาวน์ในโรงพยาบาลศูนย์หาดใหญ่: รายงานเบื้องต้น

สุรชัย ล้ำเลิศกิตติกุล, วีระพล จันทร์ดียิ่ง

วัตถุประสงค์: *เพื่อสรุปและประเมินขีดความสามารถของโรงพยาบาลศูนย์หาดใหญ่เกี่ยวกับโครงการตรวจคัดกรองตัวชี้วัดสามตัวในซีรั่มเพื่อค้นหาทารกในครรภ์ที่เป็นกลุ่มอาการดาวน์*

สถานที่: *โรงพยาบาลศูนย์หาดใหญ่ ระหว่างวันที่ 16 กุมภาพันธ์ พ.ศ. 2546 ถึง 11 มีนาคม พ.ศ. 2547*

วัสดุและวิธีการ: *ผู้ป่วยจำนวน 999 ใน 1,040 ราย (ร้อยละ 96.0) อายุครรภ์ระหว่าง 14 ถึง 20 สัปดาห์ผ่านการตรวจคัดกรองด้วยตัวชี้วัดสามตัวคือ Alpha-fetoprotein (AFP), human Chorionic Gonadotropin (hCG) และ unconjugated Estriol (uE3) ด้วย Immulite chemiluminescent immunoassay system ของ Diagnostic Product Corporation (DPC) จุดตัดความเสี่ยงต่อกลุ่มอาการดาวน์คือ 1 ใน 250 หรือมากกว่า อาศัยการคำนวณด้วยซอฟท์แวร์โปรแกรม Prisca 3.5*

ผลการศึกษา: *ผู้ป่วยจำนวน 119 ใน 999 ราย (ร้อยละ 11.9) ผลการตรวจตัวชี้วัดสามตัวได้ผลบวก การเจาะตรวจน้ำคร่ำเป็นการกระทำด้วยความสมัครใจ อัตราการยอมรับการเจาะตรวจน้ำคร่ำเท่ากับ 104 ใน 119 ราย (ร้อยละ 87.3) อาศัยการตรวจเด็กแรกเกิดจากแม่ที่ไม่ได้รับการเจาะตรวจน้ำคร่ำเป็นกลุ่มอาการดาวน์หรือไม่ด้วยการวินิจฉัยทางคลินิก พบว่าความไวในการวินิจฉัย ความจำเพาะ คุณค่าในการทำนายผลบวก และคุณค่าในการทำนายผลลบต่อความผิดปกติของโครโมโซมทุกชนิดเท่ากับร้อยละ 85.7, 88.6, 5.0 และ 99.8 ตามลำดับ อัตราผลบวกเทียมเท่ากับ 113 ใน 992 ราย (ร้อยละ 11.4) ในขณะที่ความไวในการวินิจฉัย ความจำเพาะ คุณค่าในการทำนายผลบวก และคุณค่าในการทำนายผลลบต่อกลุ่มอาการดาวน์เท่ากับร้อยละ 100, 88.4, 3.4 และ 100% ตามลำดับ อัตราผลบวกเทียมเท่ากับ 115 ใน 995 ราย (ร้อยละ 11.6) ผลการศึกษาแสดงรายละเอียดเกี่ยวกับ ค่าเฉลี่ย ค่ามัธยฐาน และค่า multiple of medians (MoM) ของตัวชี้วัดสามตัว*

สรุป: *การตรวจคัดกรองกลุ่มอาการดาวน์เป็นการทดสอบอย่างเป็นระบบเพื่อระบุผู้ที่มีความเสี่ยงเพิ่มขึ้นต่อความผิดปกตินี้ แน่นอนว่าไม่ใช่การวินิจฉัย แต่เป็นประโยชน์สำหรับการตัดสินใจทำการเจาะตรวจน้ำคร่ำ ความไวของซอฟท์แวร์ โปรแกรม Prisca 3.5 ค่อนข้างเป็นที่พอใจแต่อัตราของบผลบวกเทียมค่อนข้างสูง จำเป็นต้องมีการศึกษาต่อไป เพื่อทำให้เป็นมาตรฐานด้วยค่า MoM ของตัวชี้วัดสามตัวที่ปรับเปลี่ยนให้เหมาะสม*