False Positive Rate of Serum Markers for Down Syndrome Screening: Does Transportation Have any Effect?

Supatra Sirichotiyakul MD*, Suchaya Luewan MD*,
Rattanaporn Sekararith BSc*, Theera Tongsong MD*

** Department of Obstetrics and Gynecology, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand*

Objective: *To determine the false positive rate of serum markers for Down syndrome screening caused by transportation by analyzing samples sent from different hospitals at various distances.*
Material and Method: *The database of serum markers between March 2008 and August 2010 was retrieved from Maternal-Fetal the Medicine unit, Department of Obstetrics and Gynecology, Faculty of Medicine, Chiang Mai University. The tests were performed using Delfia Xpress system. The cut off value for positive test result was 1 in 250.*
Result: *Two thousand six hundred thirty one samples from hospitals located at various distances ranging from 0 to 449 km were assessed. The overall positive results were 6.1%, ranging from 5.6% among the hospitals near the laboratory to 15.4% among the hospitals far away from the laboratory (p-value < 0.001). The samples from the remote hospitals had significantly higher levels of free beta hCG than those from nearby hospitals (p-value = 0.003).*
Conclusion: *The samples transported from the hospitals far from the laboratory need better storage and shipping procedure to avoid high false positive rate, which leads to unnecessary invasive prenatal diagnosis procedure.*

Keywords: *Serum markers, Down syndrome screening, Stability, Transportation*

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Biochemical markers in serum have been used for Down syndrome screening for decades. Most of first trimester screenings use pregnancy-associated plasma protein A (PAPP-A) and human chorionic gonadotropin (hCG). Second trimester screenings use alpha-fetoprotein (AFP), unconjugated estriol and hCG. The stability of these biochemical markers has been studied. Many reports suggested that hCG level in blood samples may increase to over 100% if stored or shipping under high temperatures such as 30 to 35 degree Celsius[1,2]. In the real practice, not every hospital has a facility to perform these biochemical tests. Therefore, the samples need to be transported and tested in the laboratory that is available in the region. The authors' laboratory has received serum from several hospitals that are located in the northern part of Thailand. The average temperature during the day in Thailand is around 25 to 35 degrees Celsius. Although the protocol for transportation of the samples had been proposed, the methods of transportation in actual practice have still been varied from hospital to hospital. The positive rate of biochemical markers that has been accepted should be around 5% in most studies. The authors hypothesize that the expected positive biochemical markers test in Chiang Mai Hospital laboratory would be the same as other studies. The authors' would like to examine whether the positive rate of serum markers among hospitals at different distances of transportation to Chiang Mai Hospital laboratory were different or not. The aim of the present study was to compare the positive rate of the samples drawn in the authors' setting and the samples that were sent from other hospitals.

Correspondence to:
Luewan S, Department of Obstetrics and Gynecology, Faculty of Medicine, Chiang Mai University, Chiang Mai 50200, Thailand.
Phone: 053-946-429
E-mail: suleuwan@mail.med.cmu.ac.th

Material and Method

A retrospective descriptive study was conducted with approval of the research ethic committee of Faculty of Medicine, Chiang Mai University, Thailand. The data of serum markers between March 2008 and August 2010 were collected from the database of Maternal-Fetal Medicine unit, department of Obstetrics and Gynecology, Faculty of medicine, Chiang Mai University. The measurements

of serum markers were performed using Delfia Xpress system (Perkin Elmer Inc., Waltham, MA, USA). The cut-off value for a positive test result was 1 in 250. All women who had a positive test result were counseled for fetal chromosome study by either chorionic villi sampling or amniocentesis. The samples included in the study were divided into two groups: 1) "Near Group": the samples collected at Maharaj Nakorn Chiang Mai Hospital, where the laboratory was and the nearby hospitals where the laboratory processing time and transportation time not more than 1 hour 2) "Far Group": the samples from the hospitals far away from the laboratory, laboratory processing time and transportation time (from sample collection to the laboratory) of one day or more. All of the specimens were collected following the same protocol. A blood sample was obtained into a glass tube with no additive and centrifuged at 3,000 rpm for 5 to 10 min within 2 to 3 hours after collection. The serum was separated and transferred into new siliconized tubes and then stored in a freezer before transfer by control temperature. Laboratory processing was done every two weeks. The pregnancies with fetal chromosomal abnormalities and structural anomalies were excluded. Data was summarized in term of frequency and median multiple of median (MOM) for all serum markers. Mann-Whitney U test was used to compare the results between Near and Far group and Pearson Chi-square test was used to compare high-risk results. A p-value of less than 0.05 was considered statistical significant.

Results

During such a period, 2,631 samples of normal pregnancies were recruited. Of these, 2,495 samples were sent from our hospitals and nearby ("Near Group") and 136 samples were sent from the hospitals with long distance ("Far Group"). The hospitals with long distance from the laboratory (km; sample size) were as follows: Chiang Rai Hospital (182 km; 41), Tak Hospital (265 km; 21), Phayao Ram Hospital (150 km; 10), Nakorn Sawan Hospital (449 km; 59), Other (5). Means of maternal age of the two groups were 30.89 $\pm$ 4.57 (18-47) and 31.57 $\pm$ 2.81 (24-36) which were not significantly different (Student t-test; p value > 0.05). The overall positive result of serum markers was 6.1%, 161 out of 2,631. The positive result from the "Near Group" was 5.6%, while that from the "Far Group" was 15.4%, as shown in Table 1. The rates had statistically significant difference ($p < 0.001$). Considering each serum marker incorporated into risk calculation for Down syndrome, the levels of serum alpha-fetoprotein, placenta-associated plasma protein A (PAPP-A) and unconjugated estriol (uE3) between both groups were not significantly different, whereas free beta-hCG levels were significantly higher in the "Far Group" (p-value = 0.003), as shown in Table 1. Most of the false positive tests (114/161; 70.8%) were associated with high beta-hCG levels (> 2.0 MoM). Mean maternal age among women with false positive rate was slightly higher than that in the group of low risk but not statistically significant (29.61 $\pm$ 3.95 and 28.79 $\pm$ 6.2 respectively, Student's t-test: p-value 0.093).

Discussion

Down syndrome screening was performed using biochemical markers in maternal serum. First trimester screening at our laboratory included analysis of PAPP-A and free beta-hCG. Second trimester screening was done by using the analysis of AFP, unconjugated estriol, and free beta-hCG. Free beta-hCG constitutes both first and second trimester screening tests. However, the stability of these biochemical markers, especially hCG is one of the concern in validity of Down syndrome screening. Many reports confirmed that a high temperature resulted in increased level of hCG[1-4]. However, whether the temperature during transportation affects the level of hCG and thus affects the risk assessment or not is unclear[1,5-7].

In practice, blood or serum samples are sent to the laboratory from clinics or hospitals located in various distances, usually by post or courier, which may take a few hours or several days to reach the laboratory. The specimens are exposed to variations in ambient temperature during the transportation. Our laboratory is in Thailand where the average temperature is quite high when compared to that in Europe or North America. In fact, the average temperature during summer may reach 30 to 35 degree Celsius. Cowan et al[4] reported that PAPP-A and free beta-hCG levels are stable for 20 days and 12 hours at 30 degree Celsius, respectively. Sancken et al[1] reported that free beta-hCG increased more than 20% when incubated at 20 degrees Celsius for 72 hours and more than 100% increase at 30 degrees Celsius.

Most studies on Down syndrome screening program accept a false positive rate of approximately 5%[8-11]. The present study found the overall positive result was 6.1%, which might be in the acceptable range. However, there were marked differences in the positive rates from hospital to hospital (5.6% vs. 15.4%). High positive results in some hospitals might be due to selective screening in advanced maternal age group.

Table 1. Median multiple of median (MoM) values for all markers and high risk rates between both groups

Serum markers	Groups				p-value
	Near (n = 2,495)		Far (n = 136)		
	n	MoM	n	MoM	
AFP	949	1.005	104	1.065	0.060*
PAPP-A	2,173	1.119	31	1.196	0.966*
uE3	947	1.156	104	1.141	0.700*
Beta-hCG	2,495	1.181	136	1.359	0.003*
High-risk result (> 1:250)	140 (5.6%)		21 (15.4%)		<0.001**

* Mann-Whitney U test, ** Pearson Chi-square test
AFP = alphafetoprotein; PAPP-A = pregnancy-associated plasma protein-A; uE3 = unconjugated estriol; Beta-hCG = beta-human chorionic gonadotropin

However, in the present study, the mean maternal age of the two hospital groups were not significantly different. Of note, the samples sent from the hospitals that were located far from the laboratory had a tendency to give higher positive results (Far Group), while the samples from hospitals nearby gave a reasonable positive rate (Near Group). The higher false positive rate is likely caused by significantly high levels of free beta-hCG. This might be associated with dissociation of the hCG during storage and transportation, resulting in an increase in the level of free beta-hCG, as well as a higher positive rate of Down screening. The result of the present study supports the hypothesis that transportation (especially from the remote area) may have a significant adverse effect on the screening program, particularly in the countries that have high average temperature like Thailand.

The authors take this as a very serious issue since the transportation can lead to unnecessary invasive interventions. This is very important particularly in developing tropical countries where there are only a limited number of laboratories are available, long distance transportation is required, and the ambient temperature is high. The authors have incidentally audited, analyzed, and found the effects. The authors have to report this preliminary finding with a small sample size since the authors could no longer go on with this research as an observational study, because it seems to be unethical to do so without more proper intervention. The authors have improved transportation. The authors hope that the lesson from our experience help reduce unnecessary invasive procedures by taking better care of transportation.

Conclusion

The samples for Down syndrome screening may give a higher positive result when sent from a remote area. The present study suggests that samples be transportated from hospitals or clinics that are far from the laboratory in proper storage to avoid a high false positive rate as this leads to unnecessary invasive prenatal diagnosis procedure.

Potential conflicts of interest

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ผลบวกลวงในการตรวจวัดระดับซีรั่มมาร์คเกอร์ในเลือดมารดาเพื่อคัดกรองกลุ่มอาการดาวน์: การขนส่งมีผลหรือไม่

สุพัตรา ศิริโชติยะกุล, สุชยา ลือวรรณ, รัตนาภรณ์ เศขรฤทธ์, ธีระ ทองสง

วัตถุประสงค์: *เพื่อศึกษาผลบวกลวงของการตรวจคัดกรองกลุ่มอาการดาวน์โดยใช้ซีรั่มมาร์คเกอร์ในเลือดมารดาที่เป็นผลจากการขนส่ง โดยการวิเคราะห์ตัวอย่างที่ส่งมาจากโรงพยาบาลต่าง ๆ ที่อยู่ห่างออกไปในระยะทางแตกต่างกัน*

วัสดุและวิธีการ: *เก็บรวบรวมข้อมูลจากฐานข้อมูลการตรวจซีรั่มมาร์คเกอร์คัดกรองกลุ่มอาการดาวน์ ช่วงระหว่างเดือนมีนาคม พ.ศ. 2551 ถึง สิงหาคม พ.ศ. 2553 ของหน่วยเวชศาสตร์มารดาและทารก คณะแพทยศาสตร์ มหาวิทยาลัยเชียงใหม่ ซึ่งตรวจด้วยชุดตรวจของ Delfia Xpress ค่าจุดตัดของผลการตรวจที่เป็นค่าบวก คือ 1 ต่อ 250*

ผลการศึกษา: *จากตัวอย่างเลือดจำนวน 2,631 ราย ที่มาจากหลายแหล่ง คิดเป็นระยะทางตั้งแต่ 0 ถึง 449 กิโลเมตรจากห้องปฏิบัติการพบว่าร้อยละ 6.1 ให้ผลบวกจากการตรวจ โดยที่ร้อยละ 5.6-15.4 เป็นตัวอย่างเลือดที่ส่งมาตรวจจากที่อื่น ๆ จากระยะที่ใกล้จนถึงไกลที่สุด (p-value < 0.001) โดยค่าของ free beta hCG จะสูงขึ้นอย่างมีนัยสำคัญทางสถิติในตัวอย่างเลือดที่ถูกส่งมาจากแหล่งอื่น ๆ (p-value = 0.003)*

สรุป: *ระยะทางในการนำส่งจากแหล่งอื่น มีความสำคัญต่อระดับซีรั่มมาร์คเกอร์ ที่ทำให้เกิดผลบวกลวงได้สูง เป็นผลให้ผู้ป่วยได้รับการตรวจวินิจฉัยก่อนคลอดชนิดรุกล้ำโดยไม่จำเป็น*