Normative Values of Second-Trimester Maternal Serum Markers Using an Automated Assay Platform for Down Syndrome Screening

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ABSTRACT

Objective: Automated chemiluminescent immunoassay has several advantages over manual ELISA with comparable test performance. Few studies have reported the reference values of the second-trimester serum markers maternal serum alpha-fetoprotein (AFP), human chorionic gonadotropin (hCG), unconjugated estriol (uE3), and inhibin A (Inh A) by automated immunoassay in Asian population. Accordingly, this study aimed to determine the median values of second trimester serum markers as a function of gestational age (GA) in Thai population using an automated immunoassay.

Methods: This prospective cross-sectional study of serum markers in healthy singleton second trimester (14-22 weeks) pregnant women was conducted at Siriraj Hospital from September 2012 to April 2015. Maternal serum AFP, hCG, uE3, and Inh A were analyzed by automated immunoassay. Predicted median values as a function of GA were calculated from best-fit regression equations.

Results: A total of 1,526 women were included. Median values serum markers were constructed from the following optimal models: AFP (ng/mL) = 99.082 - 14.195 GA + 0.662 GA^2, r^2=0.995; hCG (mIU/mL) = 390168.106 - 35 968.397GA + 876.708GA^2, r^2=0.972; uE3 (ng/mL) = -3.388 + 0.274 GA, r^2=0.997; and, Inh-A (pg/mL) = 1206.875 - 114.171 GA + 3.174 GA^2, r^2=0.882. Using the same platform analysis and maternal weight adjustment, the reference values in Thai population were shown to be different from those of other ethnicities.

Conclusion: Median values of second-trimester serum markers for Thai population were determined. Maternal weight and the use of population-specific normal values have to be taken into account for Down syndrome screening in the second trimester.

Keywords: Automated immunoassay; Down syndrome; maternal serum screening; quadruple test; reference values; second trimester (Siriraj Med J 2019;71: 21-24)

INTRODUCTION

Alterations in the serum levels of alpha-fetoprotein (AFP), human chorionic gonadotropin (hCG), unconjugated estriol (uE3), and inhibin A (Inh A) during the mid-trimester (quadruple test), offer a detection rate of 81% for a 5% false positive rate for prenatal detection of Down syndrome.[1,2] The quality of the results from risk calculation is affected by the analytical performance of the assay used for serum marker determination, the accurate dating of pregnancy, population-specific median values of serum analytes, and the reliable relationship between serum markers and gestational age.[3-6] Well-

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established assays often provide automated quantitation of serum AFP, hCG, and uE3, but not Inh A. A new totally automated quantitative assay for these four serum biomarkers has recently been developed. This automated immunoassay is different from the manual enzyme-linked immunosorbent assay (ELISA) in terms of sample treatment, incubations, washes and detection systems, attributed to the advantage of less labor and fast analysis. Up to now, only few studies have been reported regarding the reference values of these four second-trimester serum markers analyzed by an automated immunoassay among Asian population.[7,8] Moreover and importantly, no data derived from an automated immunoassay is available for Thai population. Accordingly, the aim of this study was to determine the median values of second trimester serum markers as a function of gestational age (GA) in Thai population using an automated immunoassay.

MATERIALS AND METHODS

Study population

This prospective validation study was carried out in pregnant women at 14 to 22 weeks' gestation who attended antenatal care at the Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, Faculty of Medicine Siriraj Hospital from September 2012 to April 2015. The study protocol was approved by Siriraj Institutional Review Board (Si 413/2012). In order to generate median values of serum levels for each analyte, at a significance of 5%, standard deviation of 32, margin of error in estimating mean of 5%, and reservation for data loss of 10%, 160 subjects were required for each gestational week (total of 1,600 subjects). Inclusion criteria were women with singleton pregnancy, Thai racial origin, and ≥ 18 years of age. Gestational age was estimated by either a reliable menstrual history and/or by ultrasound examination before 13 +6 weeks of gestation. Exclusion criteria were multiple pregnancies or prior invasive prenatal diagnostic procedures before the time of enrollment. The peripheral blood samples were shipped for analysis to the laboratory no later than 2 hours after blood drawing. Serum sample was transferred at least 500 µL aliquot.

Sample analysis

A new paramagnetic particle chemiluminescent immunoassay on the Access 2 Immunoassay Systems (Beckman Coulter, CA, USA) using Beckman Coulter Access Reagents (cat. No. 33210, 33500, 33570, and A36097 for AFP, hCG, uE3, and Inh-A, respectively) was used. Serum levels of these quadruple markers were then calculated from a stored, multi-point calibration curve. The lowest detection thresholds of AFP, hCG, uE3, and Inh A with 95% confidence were 0.5 ng/mL, 0.5 mIU/mL, 0.017 ng/mL, and < 1 pg/mL, respectively. Results of invasive prenatal genetic testing were obtained from voluntary self-reporting, with an absence of reported abnormalities until the time of birth adjudicated to be euploid.

Statistical analysis

Demographic characteristics are presented as numbers and percentages for categorical data and as mean ± standard deviation or median and interquartile range (IQR) for continuous data, depending on the distribution. Median values of AFP, hCG, uE3, and Inh A were calculated for each completed gestational week. Regression analysis was used to estimate the relationship of serum markers and gestational age and the optimal model was then selected to predict median values of each marker. Patient results were then stratified into six groups according to maternal weight (< 45, 45-54.9, 55-64.9, 65-74.9, 75-84.9, and ≥ 85 kg). To adjust for maternal weight, predicted multiples of the median (MoMs) values of each marker were calculated from the best fit equation using regression analysis. All data were analysed statistically by using SPSS (IBM SPSS Statistics for Windows version 18, Microsoft Corporation; Chicago, IL, USA) and GraphPad Prism (GraphPad software for Windows version 7.00, San Diego, California, USA).

RESULTS

Of the 1,600 women enrolled in this study, 1,526 (95.38%) women with complete data set were selected for further analysis. Median maternal age, weight, and body mass index (BMI) at the time of study enrollment were 27 years (IQR, 23 - 31), 52 kg (IQR, 47 - 59.25), and 20.82 kg/m^2 (IQR, 18.90 - 23.51), respectively. Only 3% (46/1526) of this study population were obese (BMI ≥ 30 kg/m^2). Most patients (1,417/1,526, 92.9%) were aged less than 35 years and 843 patients (55.24%) were nulliparous. Median gestational age at delivery and birthweight were 39 weeks (IQR, 38 - 39) and 3,110 grams (IQR, 2,890 - 3,330), respectively. All patients included in this study had a naturally conceived pregnancy, no one reported being a current smoker.

The regression equations for serum markers as a function of gestational age (GA) in weeks from 14 to 22 weeks of gestation are described as follows:

AFP (ng/mL) = $99.082 - 14.195\ GA + 0.662\ GA^2$, $r^2 = 0.995$, $P < 0.001$

hCG (mIU/mL) = $390168.106 - 35968.397GA + 876.708GA^2$, $r^2 = 0.972$, $P < 0.001$

uE3 (ng/mL) = -3.388 + 0.274 GA, $r^2 = 0.997$, $P < 0.001$
Inh-A (pg/mL) = 1206.875 – 114.171 GA + 3.174 GA^2, $r^2 = 0.882$, $P = 0.002$.

Median values of all serum markers at each gestational week calculated from the regression equations are shown in Fig 1. Serum levels of AFP and uE3 significantly increased, and serum levels of hCG significantly decreased from 14 to 22 gestational weeks. Serum levels of Inh A continuously dropped until reaching the nadir at 18 weeks of gestation, then continuously elevated.

Significant inverse relationships between multiples of the median (MoMs) serum levels of AFP, hCG, uE3, and Inh A and maternal weight (Wt) (in kilograms) in each category were observed with the best-fit equations described, as follows:

AFP (MoMs) = 1.972 – 0.024 Wt + 0.00011 Wt^2, $r^2 = 0.994$, $P < 0.001$
hCG (MoMs) = 1.484 – 0.008 Wt, $r^2 = 0.921$, $P = 0.002$
uE3 (MoMs) = 1.322 - 0.006 Wt, $r^2 = 0.934$, $P = 0.002$
Inh-A (MoMs) = 1.302 – 0.006 Wt, $r^2 = 0.998$, $P < 0.001$

Predicted MoM values of all serum markers adjusted for maternal weight are shown in Table 1.

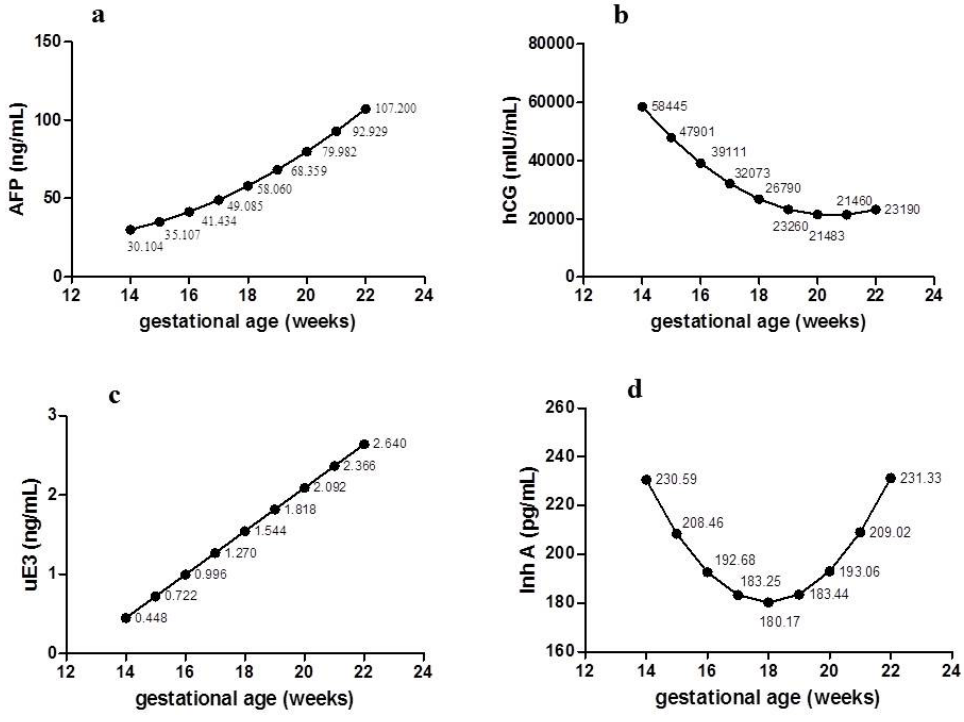

Fig 1. Median values of serum markers in Thai women by gestational age. (a) alpha-fetoprotein (AFP) levels expressed in ng/ml, (b) human chorionic gonadotropin (hCG) levels expressed in mIU/ml, (c) unconjugated estriol (uE3) levels expressed in ng/ml, (d) inhibin A (Inh A) levels expressed in pg/ml.

TABLE 1. Predicted median MoM values of serum markers by maternal weight category.

Maternal weight category		Number of cases	AFP (MoM)	hCG (MoM)	uE3 (MoM)	Inh A (MoM)
I	< 45 kg	199	1.158	1.148	1.070	1.050
II	45 – 54.9 kg	718	1.060	1.092	1.028	1.008
III	55 – 64.9 kg	376	0.950	1.020	0.974	0.954
IV	65 – 74.9 kg	178	0.849	0.940	0.914	0.894
V	75 – 84.9 kg	39	0.769	0.860	0.854	0.834
VI	≥ 85 kg	16	0.710	0.776	0.791	0.771

Abbreviations: AFP: alpha-fetoprotein; hCG: human chorionic gonadotropin; uE3: unconjugated estriol; Inh A: inhibin A; MoM: multiples of the median; kg: kilogram.

DISCUSSION

In this study, median values of serum levels of AFP, hCG, uE3, and Inh A in Thai women carrying singleton pregnancy from 14 to 22 weeks of gestation were generated using a new Access 2 automated chemiluminescence immunoassay system. Previous studies supported the highly correlated results of dimeric Inh A between this new immunoassay platform and manual ELISA, with a comparable performance of prenatal detection of Down syndrome.[9,10] Several advantages of an automated chemiluminescent immunoassay over a manual ELISA with a comparable test performance explain its preference for serum Inh A analysis.[9,10] The effects of gestational age and maternal weight were consistent with previously published data from other platforms.[11,12]

Maternal weight is another influential determinant of these serum marker levels, displaying negative affiliations. The pathophysiology behind these affiliations is at present indistinct. In order to compare the median values of maternal serum markers in our population to those previously reported from other populations using the same automated immunoassay, it is essential to adjust the median values by weight-correction models. Each of four serum markers had a similar pattern of change during the second trimester of pregnancy among various ethnic groups.[7,9,10,13] Nevertheless, unique normal values were reported among different ethnic groups. Higher levels of serum AFP, hCG, and Inh A and a lower level of serum uE3 in our population were observed in comparison of those in the Caucasian groups.[9,10,13] Despite the fact that Thai and Korean people are both racially classified as Asian and have similar serum AFP and hCG levels in the second trimester, there are some more subtle differences in uE3 and Inh A levels between these two ethnic groups.[7] Our population seemed to have a lower level of serum uE3 and a slower progression of serum Inh A after reaching its nadir level at 18 weeks of gestation.[7] These would seem to signify the necessity of population-specific normal values of these serum markers.

The potential impact of this study is reinforced by a number of key strengths. This is the first prospective, well-designed study of normal values of second-trimester serum markers using an automated immunoassay in a large cohort of Thai population with a high rate of available outcomes. Limitations of this study include a highly selected, low-risk study population, no accuracy assessment of these reference values, and no data comparison between the normal values derived from the automated immunoassay and those derived from the manual ELISA method in the studied Thai population. The impact of maternal age, smoking status, and the method of conception on these reference values could not be determined. Further studies on test performance are needed to support the application of these normative values to second-trimester screening for Down syndrome.

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