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Maternal Serum Free Beta-Human Chorionic Gonadotropin Levels between 14 and 21 Weeks of Gestation in Thai Pregnant Women

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ABSTRACT

Objective To determine normative median values for maternal serum free beta-human chorionic gonadotropin (β-hCG) between 14 and 21 weeks of gestation in Thai pregnant women.

Design A prospective descriptive study.

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Methods We measured the concentrations of maternal serum free β-hCG from 836 healthy singleton Thai pregnant women between 14 and 21 weeks of gestation. The maternal age on the day of delivery and maternal weight at the time of sampling were recorded in all cases. Gestational-specific medians for maternal serum free β-hCG were calculated by weighted non-linear regression from the observed medians of each gestational week.

Results The median levels of maternal serum free β-hCG had a downward trend in association with the increase gestational age and these were higher than those of white pregnant women. Of pregnancies with free beta-hCG levels, there were 2.75% and 14.11% less than 0.3 and 0.5 MoM, respectively; 13.40%, 6.34% and 4.90% had serum levels greater than 2.0, 2.5 and 3.0 MoM, respectively.

Conclusion To allow for differences in maternal serum free β-hCG median values at various ages of gestation, establishment of the reference range is essential for further development of maternal serum screening for Down syndrome.

Key words: free beta-human chorionic gonadotropin (β-hCG), multiple of median (MoM), Down syndrome screening

A screening policy for Down syndrome (DS) in Thailand is initially based on the offer of amniocentesis to pregnant women older than 35 years of age at delivery. The recent data document that only 25-30% of all cases of fetal DS could be identified in this old age population. The remaining 70-75% of DS cases occur in pregnant women younger than 35.[1] In order to select young age pregnancies with an increased risk of DS in western countries, maternal serum screening for DS has become an essential prenatal examination.

The second trimester maternal serum screening for DS is a considerable advance over screening using maternal age alone. It has become a regular component of antenatal care in modern obstetric management.[2,3] As the single most discriminating serum marker, human chorionic gonadotropin (hCG) is included in almost all Down syndrome screening strategies.[4,5] Intact hCG consists of 2 noncovalently bound subunits designated as α-subunit and β-subunit. Since the free β-subunit is specific to hCG, it has been proved to achieve a more accurate result in Down syndrome screening.[6-9] Therefore, the measurement of free β-hCG has been demonstrated as having the highest detection capability of all previously reported markers in both retrospective and prospective evaluations.[10]

The concept of converting serum marker concentrations to the unit of multiple of median (MoM) originated from a collaborative study in the United Kingdom.[11] The screening policy of the American Society of Human Genetics stated that the median baseline values in Down syndrome screening should be made by a sufficient number of cases before clinical application for screening purposes.[12] To obtain a more accurate and satisfactory interpretation of the results of maternal serum screening, it is imperative to establish the normative median values from the general population. Therefore, the purpose of this study was to establish normal median values of serum free β-hCG in Thai pregnant women for further clinical application.

Materials and Methods

Serum samples were collected from 836 unselected normal Thai singleton pregnancies which had been included in our maternal serum screening for DS program between 14 and 21 weeks' gestation from March 1998 through January 2000. Informed consent was obtained after proper counseling. Patients were excluded from the study if they had multiple pregnancy or fetuses with congenital anomalies. Gestational age of all fetuses were determined using the ultrasound parameters.

Each blood sample was centrifuged after it was obtained from a single venous blood specimen. The serum was stored at -20°c until it was thawed for measuring free β-hCG levels. The quantitative free β-hCG levels were measured with a solid-phase two-site immunoenzymatic assay (FβHCG ELACT; CIS Ltd., Gif-sur-yvette Cedex, France) in which the monoclonal antibodies used were raised against sterically remote episodes on the β-hCG molecule. The assay involved a 1-hour incubation at room temperature of 50 μL of sample and 200 μL of assay buffer in the coated tube; this was followed by a washing step, a further 1-hour room temperature incubation under continuous shaking (between 300 and 400 rpm). After stopping the enzyme reaction by adding precisely 1 ml of oxalic acid solution to each tube, the optical density of each tube was read by setting the blank in a spectrophotometer at 492 nm. For each groups of tubes, the mean optical density (O.D.) was calculated and the samples values was read directly from the curve after drawing up the standard curve by plotting the standard O.D. versus their concentration.

The normal regressed median values of free β-hCG at each gestational week were calculated from these uncomplicated pregnancies by weighted non-linear regression of the observed median. Considering the gestational variation of this analyze, the results of all data were expressed in MoM for relevant gestational week. Results are reported as the median, mean, standard deviation(SD) and standard error(SE) of the mean.

Results

The mean and SD of maternal age, gestational weeks and maternal weight in the study population was 29.6±6.6 years (median 29.0, range 15-46), 17.0±1.9 weeks (median 17.0, range 14-21), and 54.6±9.0 kg (median 54.0, range 36-97), respectively. At each week's gestation, we used more than 100 singleton pregnancies to establish normal free β-hCG median values. Serum free β-hCG concentrations were within range of 1-350 ng/mL with a mean and SE value of 19.63±0.67 ng/mL. The number of cases, the median and the mean with SE concentrations of free β-hCG at each week's gestation are depicted in Table1.

Table 1. The Number and Free β-hCG Values of the Median, Mean with Standard Error (SE) and Range between 14 and 21 Weeks' Gestation. (Unit: ng/mL)

GA	No.	Median	Mean	SE	Minimum	Maximum
14	97	26.60	33.65	2.38	8.8	123.6
15	112	22.70	28.34	1.82	3.4	112.5
16	152	16.10	20.62	2.36	3.1	350.0
17	132	13.75	16.65	1.05	2.0	72.3
18	122	12.95	14.67	0.87	1.7	49.0
19	124	10.60	12.94	0.79	1.0	57.0
20	52	10.30	11.48	0.84	1.2	34.5
21	45	8.60	9.55	0.82	1.3	23.9
Total	836	14.70	19.36	0.67	1.0	350.0

There was a trend of decreasing concentration of the median with advancing gestational week. The secretion pattern of free β-hCG in Thai pregnant women was essentially the same as those in previous reports on Taiwanese and white pregnant women[13,14] (Fig.1). The median values of Thai and Taiwanese pregnant women were higher in average than those of white pregnant women. Weighted non-linear regression formulas for free β-hCG levels against gestational week have been obtained for regressed median in MoM levels (Table2).

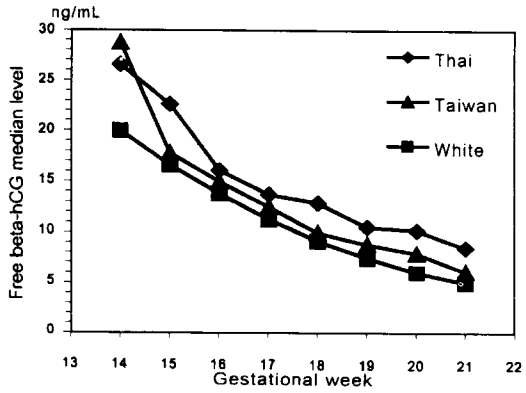

Fig.1. Comparison of the median values of serum free β-hCG in Thai, Taiwanese and white pregnant women between 14 and 21 weeks' gestation.

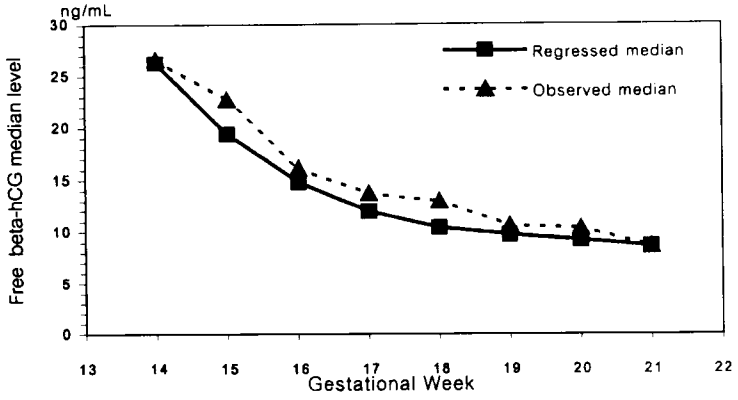

Fig.2. Comparison of observed and regressed median values of serum free β-hCG in Thai pregnant women between 14 and 21 weeks' gestation.

Table 2. The Free β-hCG Values at the Regressed Median, Observed Median, and 0.3, 0.5, 2.0, 2.5 and 3.0 Multiple of the Median (MoM) at Individual Weeks' Gestation. (Unit: ng/mL)

Gestational Weeks	Regressed Median	Observed Median	0.3 MoM	0.5 MoM	2.0 MoM	2.5 MoM	3.0 MoM
14	26.23	26.60	7.98	13.30	53.20	66.50	79.80
15	19.41	22.70	6.81	11.35	45.40	56.75	68.10
16	14.83	16.10	4.83	8.05	32.20	40.25	48.30
17	12.01	13.75	4.13	6.88	27.50	34.38	41.25
18	10.43	12.95	3.89	6.48	25.90	32.38	38.85
19	9.71	10.60	3.18	5.30	21.20	26.50	31.80
20	9.18	10.30	3.09	5.15	20.60	25.75	30.90
21	8.58	8.60	2.58	4.30	17.20	21.50	25.80

Table 3. Distribution(%) of Elevated and Decreased Free β-hCG Multiple of the Median (MoM) in Relation to Gestational Age

Gestational Weeks	≤ 0.3 MoM	≤ 0.5 MoM	≥ 2.0 MoM	≥ 2.5 MoM	≥ 3.0 MoM
14	0	11.32	16.51	8.22	6.22
15	4.51	14.32	15.22	9.82	8.92
16	2.10	12.56	10.53	5.30	5.91
17	2.32	14.41	15.91	8.31	5.36
18	2.54	15.63	10.70	8.22	4.94
19	3.26	14.54	14.53	10.56	4.12
20	1.91	13.56	7.72	1.94	1.93
21	4.40	11.11	8.91	2.28	0
Total	2.75	14.11	13.40	6.34	4.90

Fig.2 shows comparison of observed and regressed median values of serum free β-hCG between 14 and 21 week's gestation. Free β-hCG values in relation to the regressed median, observed median, and 0.3, 0.5, 2.0, 2.5 and 3.0 MoM levels at individual gestational ages are shown in Table 2. There were 2.75% (23/836) of pregnancies with free β-hCG levels less than 0.3 MoM and 14.11% (118/836) with levels less than 0.5 MoM; 13.40% (112/836) among them had serum levels greater than 2.0 MoM, 6.34% (53/836) had serum levels greater than 2.5 MoM and 4.90% (41/836) had serum levels greater than 3.0 MoM (Table3).

Discussion

From early pregnancy, the feto-placental unit synthesizes a variety of pregnancy-specific proteins and hormones which are secreted into the maternal circulation from early in the first trimester. The concentration profile of each of these markers varies and changes as pregnancy advances. Recently, studies of maternal serum from chromosomally abnormal pregnancies in the second trimester have shown that the normal concentration profile of many of these markers is disturbed. The most commonly studied aneuploidy is Down syndrome, which is associated with increased levels of human chorionic gonadotropin (hCG)[15] and free beta subunit of hCG (free β-hCG).[16]

Human chorionic gonadotropin is composed of 2 nonidentical subunits (α and β) that are either free or bound to each other in a noncovalent way. The α-subunit is structurally identical to the α-subunit of other pituitary glycoprotein hormones, i.e., LH, FSH, and TSH. The β-subunit, on the other hand, is hormone-specific and it is this characteristic that confers specific biological activity. The α-subunit gene is expressed in both the pituitary gland and the placenta, whereas the β-subunit genes are expressed only in the placenta. These two subunits are independently produced by the syncytiotrophoblast and secreted into maternal circulation in either a combined or separate form shortly after implantation. Control of secretion of β-subunit is thought to be the rate-limiting step to the production of intact hCG and this is influenced in a positive way by cyclic adenosine monophosphate (AMP), insulin, calcium, interleukin-1, fibroblast growth factor, and placental-derived gonadotropin-releasing hormone and epidermal growth factor. Inhibitory influences include prolactin, progesterone, and inhibin. Morphological changes and abnormal development of trisomic placentae may provide a more general explanation for alterations in the rate of production or secretion of several placental markers such as hCG and free β-hCG in Down syndrome pregnancies.[17]

The free β-hCG concentration declines rapidly from 14 weeks to a plateau after 19 weeks' gestation.[6] Although we had small numbers in each gestational week, our study has confirmed the observation of previous studies[13,14] that median values of free β-hCG levels decline with increasing gestational age. The shape of the free β-hCG decline curve is similar to that of hCG.[15] A possible reason for the fall in hCG at the end of the first 12 weeks' gestation is that the capacity of the syncytiotrophoblast to secrete hCG selectivity diminishes at that time.[18] Free β-hCG is present in serum throughout pregnancy, however, the amounts vary widely and the actual mean and median values are quite different for various gestational weeks. This may be caused by differences in individuals, methodology, and reference standard preparations. In normal second trimester maternal sera, the range of free β-hCG was found to be 1 to 350 ng/mL, which was estimated from 0.5% to 2.0% of the hCG concentration with a range of 1.1 to 348.57 IU/mL.[19] This finding demonstrates the importance in obtaining accurate measurement of free β-hCG using a specific immunoassay in the presence of high concentrations of hCG.

The present study shows that there are racial differences in the median values of free β-hCG. Bogart et al.[20] found that the weighted adjusted hCG MoM levels for Orientals was 16% higher than that for whites. Taiwan's data also found higher median values of either hCG or free β-hCG in Taiwanese pregnant women than those of whites.[13] Our study revealed that different median values of free β-hCG existed

between the data collected from multicenters[13,20] and those of our own hospital. Between MoM conversion is related to Down risk calculation, it is obviously not suitable to use the median valued provided by the reagent manufacturers or other laboratories.

In an Asian population, the median MoM value of free β-hCG in Down syndrome pregnancies was 2.91 MoM[8] and was higher than that of whites (2.64 MoM).[10] Our data found that normal pregnancies of the median free β-hCG levels greater than 2.5 MoM were 6.34%. This result can achieve a predicted false positive rate in calculating Down syndrome risk, however, in general practice, the false positive rate accepted in screening test is 5%.[21]

The use of hCG is unequivocally established in Down syndrome screening.[4,5] However, two independent reports have shown an elevation of free β-hCG levels in Down syndrome pregnancies.[14,16] Since then, the improvement in detection using free β-hCG has been supported by several studies.[1,7,22] In particular, a multicenter study of 480 Down syndrome pregnancies showed that a detection rate of 77.7% can be achieved by combining alpha-fetoprotein (AFP) with free β-hCG and maternal age screening.[10] Recently, a seven year review of 67,904 pregnancies screened in second trimester using these two maternal serum markers indicated that the detection rates of Down syndrome were 75% for a 5% false positive rate.[21] Therefore, free β-hCG has been reported as the most effective marker available in second-trimester Down syndrome screening.[6]

There are several reasons to prefer using free β-hCG in Down syndrome screening. First, a superior detection rate has been achieved using free β-hCG rather than hCG in both retrospective studies[6,14,16,21] and prospective studies.[10,23,24] Second, the procedure for β-hCG assay is simplified and undiluted to minimize human error.[16] Third, the noninvasive technique used with free β-hCG is compatible with the current prenatal screening protocol. And fourth, free β-hCG, unlike intact hCG, can be used for first-trimester Down syndrome screening.[25] In conclusion, free β-hCG is thought to be a potential and powerful serum marker for Down syndrome screening.

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