



REFERENCE CENTILE CHARTS OF PAPP-A AND FREE β -HCG IN INDIAN PREGNANT WOMEN.

Biochemistry

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ABSTRACT

The combined first-trimester screening (CFTS) for fetal aneuploidy involves a combination of maternal age, fetal nuchal translucency (NT), maternal serum pregnancy-associated plasma protein-A (PAPP-A) and free β -human chorionic gonadotropin (β -hCG) from 11+0 to 13+6 weeks of gestation [1]. International prescriptive standards for early fetal linear size and ultrasound dating of pregnancy in the first trimester are available and used throughout the world. Ethnicity has an effect on the biochemical parameters of first trimester screening. As there is a lack of reference chart for the women of Indian ethnicity, we aimed to find out the level of PAPP-A and free β -hCG for fetal crown rump length (CRL) of 40-84 mm in the first trimester of pregnancy and construct the centile charts for both the parameters. A significant but weak correlation between the individual models of PAPP-A and β -hCG and CRL ($P < 0.001$). In conclusion, the normal range of PAPP-A and free β -hCG depending on the gestation (CRL) was derived in this cohort study. This would act as a reference for our population and would be helpful in calculating the risk of an adverse maternal and fetal outcome.

KEYWORDS

First-trimester screening, Reference centile charts, Crown rump length, PAPP-A, Free β -hCG.

INTRODUCTION:

The first-trimester screening (FTS) for fetal aneuploidy involves a combination of maternal age, fetal nuchal translucency (NT), maternal serum pregnancy-associated plasma protein-A (PAPP-A) and free β -human chorionic gonadotropin (β -hCG) from 11+0 to 13+6 weeks of gestation [1]. The lack of correct recall of maternal age which forms the basis of ascertaining a priori risk of screening is a big challenge in developing countries. With multiple birth orders and large family sizes, mothers tend to forget the date and at times even the year of their birth. A proportion of these women also do not remember the exact date of last menstrual period necessitating a dating scan for correct risk assignment [2]. International prescriptive standards for early fetal linear size and ultrasound dating of pregnancy in the first trimester are available and used throughout the world [3]. However, there is lack of such standards for the mothers of Indian ethnicity.

In the present study we aimed to find out the level of PAPP-A and free β -hCG for fetal crown rump length (CRL) of 40-84 mm in the first trimester of pregnancy and construct the centile charts for both the parameters.

METHODS:

We conducted a prospective study, including 1112 consecutive pregnant women who attended the antenatal clinic of the Obstetrics and Gynecology department of Lok Nayak Hospital, New Delhi, India in between 11 to 13+6 weeks of gestation. Prospective mothers were screened for fetal aneuploidy. Ethical clearance was obtained from Institute's Ethical Committee. All the demographic and reproductive information of the participants related to first-trimester screening were recorded in a pre-structured proforma. Singleton pregnancies between the crown-rump length (CRL) of 40-84 mm were included in the study. Informed consent was obtained from all the participants.

Multigravida women, women with insulin dependent diabetes mellitus, cases of fetal death, severe pregnancy complications, multiple congenital abnormalities and those who did not give consent were excluded from the study. The transabdominal ultrasound was done and the CRL was taken in midsagittal section with the neck in neutral position and was measured up to the nearest mm.

Three to four ml of venous blood was collected. It was centrifuged immediately after clot formation and serum was stored at -80°C till analysis was done. Testing was done by time-resolved fluorometry using kits manufactured by M/S Perkin Elmer (Turku, Finland).

Coefficient of variation of less than 5% of the test was taken as acceptable.

Linear regression models were constructed; the mean and standard deviation were derived as a function of CRL. Statistical analysis was done on R Studio Version 1.3.1056.

RESULTS:

Of the 1112 pregnant women, 60 were excluded, resulting in 1052 women who contributed data to the final analysis. The mean maternal age was 27.03 ± 4.47 and ranged from 18-48 yrs. The mean CRL was 59.6 ± 10.6 mm. The mean PAPP-A level was 3748 ± 2700 mU/L and free β -hCG level was 44.9 ± 33.5 ng/ml. The centile charts of PAPP-A and free β -hCG were constructed. Table 1 and 2 show the 5th, 50th and 95th centile value of PAPP-A and β -hCG respectively for different range of CRL.

Table 1: The 5th, 50th and 95th centile, standard deviation and 0.4 multiples of median (MoM) value of pregnancy-associated plasma protein-A (PAPP-A) in mU/L in relation to crown rump length (CRL) in mm.

CRL	n = 1052	Serum PAPP-A in mU/L				
		5 th centile	50 th centile	95 th centile	SD	0.4 MoM
<40	15	698.9	2485.0	7769.3	2430.1	0.5
40-44.9	38	474.7	2212.9	6006.2	2356.3	0.4
45-49.9	133	657.2	2070.7	6245.5	1894.0	0.4
50-54.9	177	774.5	2767.0	7435.5	2185.7	0.4
55-59.9	178	781.3	2572.8	8219.7	2668.8	0.4
60-64.9	178	942.7	3182.1	8845.9	2476.6	0.4
65-69.9	146	972.4	3303.4	9674.4	2644.6	0.4
70-74.9	109	1211.3	4241.1	10048.1	2896.4	0.4
75-79.9	64	1597.7	4625.8	12031.7	3521.0	0.4
80-84	26	2034.2	5238.1	12528.7	3275.5	0.5

Table 2: The 5th, 50th and 95th centile, standard deviation and 2 multiples of median (MoM) value of serum free β -hCG (in ng/ml) in relation to crown rump length (CRL) in mm.

CRL	n = 1052	Serum free β -hCG (in ng/ml)				
		5 th centile	50 th centile	95 th centile	SD	2 MoM
<40	15	19.2	47.6	82.2	25.4	1.8

40-44.9	38	19.9	41.4	96.8	28.3	2.0
45-49.9	133	13.6	38.1	98.7	33.7	1.5
50-54.9	177	15.3	40.3	129.3	41.8	1.9
55-59.9	178	14.2	36.3	95.0	28.1	1.8
60-64.9	178	13.4	32.9	91.4	26.7	1.8
65-69.9	146	12.9	33.3	120.1	39.0	2.0
70-74.9	109	10.4	32.5	99.9	28.8	2.0
75-79.9	64	8.0	29.1	76.2	25.4	1.9
80-84	26	16.8	37.7	96.8	38.0	1.9

The regression analysis showed a significant correlation between PAPP-A and β -hCG and CRL ($P < 0.001$). Table 3 shows the correlation of PAPP-A and Free β -hCG with CRL. There was a positive correlation of CRL with PAPP-A ($y = 55 + 0.0011x$, $R^2 = 0.084$), whereas inverse correlation was seen with free β -human chorionic gonadotropin (β -hCG) ($y = 61 - 0.033x$, $R^2 = 0.01$). Figure 1 and 2 show the regression plot of pregnancy associated plasma protein-A (PAPP-A) and β -human chorionic gonadotropin (β -hCG) in relation to crown rump length (CRL).

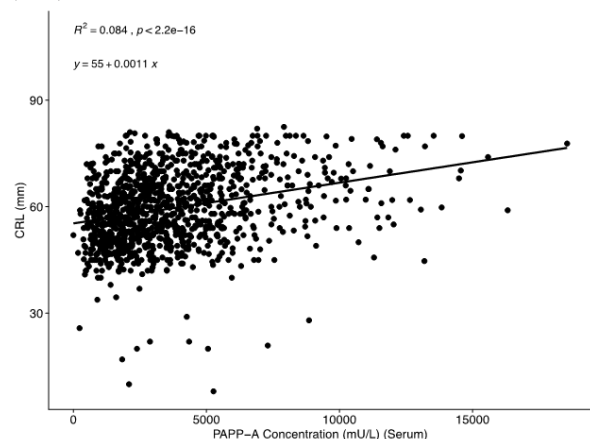


Figure 1: Regression plot of pregnancy-associated plasma protein-A (PAPP-A) with crown rump length (CRL) along the X-axis.

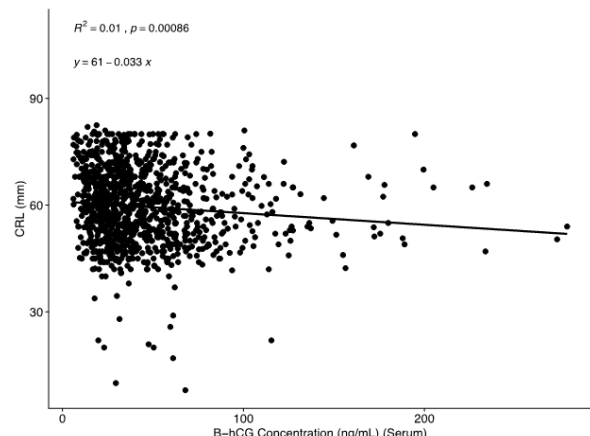


Figure 2: The regression plot of β -human chorionic gonadotropin (β -hCG) with crown rump length (CRL) along the X-axis.

Table 3: Correlation of PAPP-A and Free β -hCG with CRL

Variables	Rho (95% CI)	p-value
PAPP-A	0.29 (0.23, 0.34)	<0.001
Free β -hCG	-0.1 (-0.16, -0.04)	<0.001

DISCUSSION:

In the present study, the reference centile charts of first-trimester screening parameters PAPP-A and β -hCG was constructed in relation to CRL. A proportion of the pregnant women in developing countries

like India do not remember the exact date of last menstrual period. The CRL was preferred over gestational age in weeks, as it was more precise and easier to take in the subgroup of the mothers. Many studies have calculated the parameters with respect to the gestational period [4,5] whereas in the present study the reference centile chart of the parameters was constructed in relation to CRL.

In this study the normal range of values (5th-95th centile) and the median (MoM) of different variables according to CRL was derived, which would act as a reference for Indian women. For free β -hCG, 2 MoM and for PAPP-A 0.4 MoM are taken as cut-off.

The value of PAPP-A increased with the period of gestation. This occurs due to growing placenta. The placental trophoblasts, especially by the extra-villous cytotrophoblasts produce increasing amount of the protein [6]. Free β -hCG levels lowered with increasing gestational period. The β -hCG in maternal serum is also derived from the placenta, however, the concentration of β -hCG starts to decrease after week 12 and stay fairly constant from the 20th week until term [7].

Ethnicity has an effect on the biochemical parameters of FTS [8],[9]. As there is a lack of reference chart for the women of Indian ethnicity, this study may help in risk estimation of aneuploidy in Indian expecting mothers.

In our study, the regression analysis showed a significant correlation between both, β -hCG and PAPP-A with CRL ($P < 0.001$). There was a positive correlation between CRL and pregnancy-associated plasma protein-A (PAPP-A) ($y = 0.001147x + 55.3$, $R^2 = 0.084$), whereas inverse correlation was seen with free β -human chorionic gonadotropin (β -hCG) ($y = -0.0326x + 61.0333$, $R^2 = 0.01$). In the present study, although the correlation of PAPP-A and β -hCG with CRL was significant, the strength of both the models are low, and therefore, the correlation coefficient was low. This was in accordance with the study by M Kumar et al who also observed that the individual models for the study had weak correlation [10].

The strength of the study was that the normal ranges of PAPP-A and free β -hCG were studied in a large number of Indian pregnant women with normal outcome.

CONCLUSION:

The normal range of PAPP-A and free β -hCG depending on the gestation (CRL) was derived, which would act as a reference for our population and would be helpful in calculating the risk of an adverse maternal and fetal outcome.

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Conflicts of interests: The Authors declares that there is no conflict of interest related to this manuscript.

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