Determination of Medians of Biochemical Serum Markers in First Trimester of Healthy Pregnant Women in Tertiary Care Setup

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ABSTRACT

Objective: To determine medians of pregnancy-associated plasma protein-A (PAPP-A) and free β subunit of Human Chorionic Gonadotropin (hCG-β) in the first trimester of healthy pregnant women visiting tertiary care hospital.

Study Design: Cross-sectional study.

Place and Duration of Study: Department of Chemical Pathology and Endocrinology, Armed Forces Institute of Pathology (AFIP) from May 2018 to May 2019.

Methodology: Healthy pregnant women with a singleton pregnancy reported for an antenatal checkup in Obstetric OPD, Pak Emirates Military Hospital (PEMH) and Combined Military Hospital (CMH) Rawalpindi were included in the study. PAPP-A level was determined by enzyme-linked immunosorbent assay (ELISA) method using PR 4100 Microplate Reader®, and serum hCG-β were analysed on random access IMMULITE 2000®.

Results: A total of 135 pregnant women were enrolled in the current study. The mean age of subjects was 27.66 ± 4.24 years, mean maternal weight was 59.91 ± 8.87 kgs and mean gestational age was 10.37 ± 1.16 weeks. Median value of PAPP-A was 1289.43 mIU/L (range: 510.8-5965.99) and hCGβ was 120985 mIU/ml (range: 23592-290000).

Conclusion: Median values of PAPP-A and HCGβ can be used to calculate (Multiple of Medians) MoM during the first trimester in tertiary care setup.

Keywords: Beta Human Chorionic Gonadotropin, Pregnancy-associated plasma protein A, Threatened abortions.

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INTRODUCTION

Pregnancy-associated plasma protein-A (PAPP-A) in the serum of first trimester women was reported as a vital biochemical marker in screening of fetal Down Syndrome.[1-3] PAPP-A is a metalloprotease that belongs to zinc peptidase.[4,5] It is synthesised by placental syncytiotrophoblasts and acts as an enzyme for binding and cleaving insulin-like growth factor binding proteins 4 and 5 (IGFBP-4 and 5).[6,7] Thus PAPP-A regulates local bioavailability of IGFs. IGF is required for cell differentiation and fetal growth.

Multiple of median (MoM) is used for biochemical serum markers instead of reference values in antenatal screening programs. This is due to the reason that these parameters show immense fluctuation along with gestational age.[8,9] Therefore, it is not possible to find out reference ranges of these parameters during pregnancy. Before starting antenatal screening services for chromosomal anomalies, every region must determine its median values among healthy subjects. There is limited literature in Pakistan about median values of PAPP-A and hCG-β in the first trimester. This study was the first in our setup where the determination of medians of serum biomarkers was done in healthy pregnant women during the first trimester.

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METHODOLOGY

A total of 135 pregnant women were recruited in this cross-sectional study after approval from Institutional Review Board (IRB), AFIP Rawalpindi (reference number FC-CHP 17-6/READ-IRB/18/907). The sample size of 120 was calculated using the area under the curve (AUC)=0.76,10 with margin of error 0.05, study power =80%, with the help of WHO calculator.[11] Patients were recruited in the study by using non-probability, consecutive sampling. Samples were collected from the antenatal unit of PEMH and CMH Rawalpindi Pakistan from May 2018 to May 2019.

Inclusion Criteria: All the primigravid women with age between 18-40 years and gestational age 9 to 12 weeks, were included in this study. All the subjects were normoglycemic (2 hours postprandial glucose

level <11.1 mmol/l), normotensive (BP 120 mm Hg or less) and had haemoglobin level ≥10.5 g/ dL.

Exclusion Criteria: All the multigravida women or women above 40 years of age, with a history of miscarriages, threatened abortions, chronic ailments, those on long term antibiotic therapy and fertility treatments were excluded from the study.

After the verbal explanation, informed consent was taken from each subject or the attendant. Medical history, demographic detail, clinical presentation and weight of study participants were noted. Gestational age was confirmed from the last missed period (LMP) and pelvic ultrasound history.

About 3 ml of venous sample was collected from each subject in a gel vacutainer and allowed to clot. Serum was separated from cells by centrifugation at 3500 rpm. Serum hCG-β was analysed on random access IMMULITE 2000®. IMMULITE 2000® hCG-β assay utilises two monoclonal antibodies, and an enzyme labelled chemiluminescence immunometric assay. Separated serum was then stored at -20°C according to the kits manual for estimation of PAPP-A within three days.

After proper thawing of the sample, PAPP-A was determined by ELISA method by PR 4100 Mic-roplate Reader® Bio-Rad. The accuracy of the result was confirmed using controls run along with samples, and all samples were analysed according to standard operational principles.

Statistical Package for Social Sciences (SPSS) version 24.0 was used for the data analysis. Quantitative variables like age, gestational age, weight, Hb level, BP and 2 hours postprandial plasma glucose level were analysed summarized with mean and standard deviation.

Median PAPP-A and hCG-β were calculated. Scatter plots were generated to measure regression lines and R2. 95% confidence interval was determined for PAPP-A and hCG-β. IQR including 25th, 50th and 75th centiles were calculated for each biomarker at each gestational age.

RESULTS

The sample size of the current study was 135. All the included subjects had successful pregnancies (confirmed via obstetric scan). All the pregnant ladies were normotensive (BP 120 mmHg or less) and normoglycemic (2 hours postprandial glucose level <11.1 mmol/l). The mean age of participants was 27.60 ± 4.22 years, mean gestational age at presentation was 10.37 ± 1.16 weeks, mean weight of participants was 59.91 ± 8.8 kg, and mean haemoglobin level was 11.78 ± 0.62 g /dl (range: 11.0-13.0) (Table-I). The median value of PAPP-A was 1289.43 mIU/l, and hCGβ was 120985 mIU/ml (Table-II).

Table-I: Baseline characteristics of the study subjects (n=135).

Characteristics	Mean ± SD (range)
Age (years)	27.60 ± 4.22 (20-38)
Weight (Kg)	59.91 ± 8.88 (41-79)
Gestational age (Weeks)	10.37 ± 1.16 (9-12)
Hemoglobin (g/dL)	11.78 ± 0.62(11.0-13.0)
Systolic BP (mmHg)	112.96 ± 6.47(100-130)
Diastolic BP (mmHg)	67.78 ± 8.35 (60-80)
2hours PP glucose (mmol/L)	9.13 ± 1.12 (7.0±11.0)

Table-II: Median values of outcome.

Parameters	Median	IQR
PAPP-A (mIU/L)	1289.43	839.76-2312.4100
HCGβ(mIU/mL)	120985	78000-198000

Regression equations were plotted for PAPP-A and hCG-β against the gestational age. The R2 for PAPP-A and the hCG-β equation were 0.694 and 0.791, respectively. The trend of the median value of both PAPP-A and free hCG-β was increasing with gestational age shown in Figure-1 & 2.

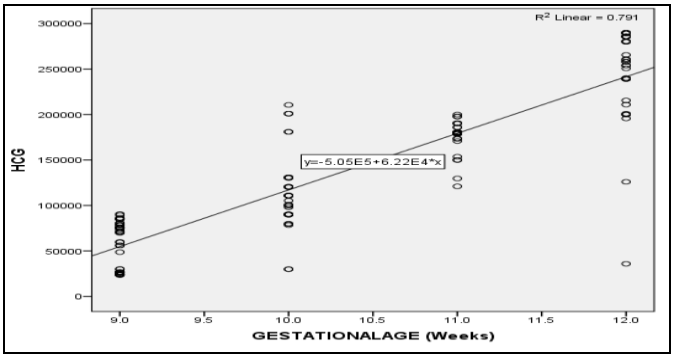

Figure-1: Regression line of hCG- β against Gestational age.

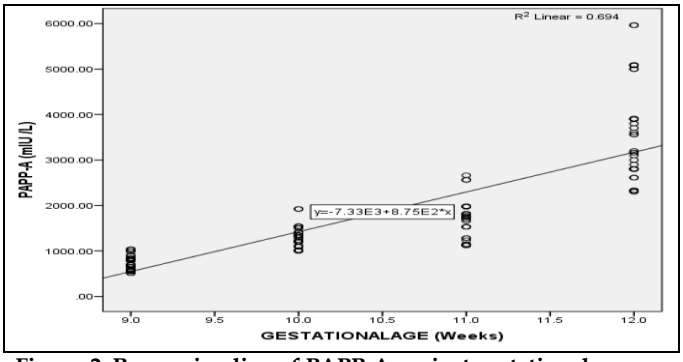

Figure-2: Regression line of PAPP-A against gestational age.

Median values of PAPP-A and hCG-β at 25th, 50th and 75th centiles were compared with gestational age in weeks as shown in Table-III.

Table-III: Comparison of percentiles of PAPP-A and b-hCG with gestational age strata.

Gestational Age (Weeks)	n	Percentiles	PAPP-A	b-Hcg
9th	42	25th	591.08	26700.00
		50th	742.50	70000.00
		75th	837.37	78912.50
10th	33	25th	1155.98	90000.00
		50th	1241.00	110650.00
		75th	1408.18	130400.00
11th	28	25th	1281.61	173339.50
		50th	1721.56	179786.50
		75th	1934.70	185955.00
12th	32	25th	2800.50	221341.25
		50th	3180.05	258000.00
		75th	3908.95	285595.00

DISCUSSION

The current study highlighted the median values of first-trimester serum markers from which multiple of median (MoM) can be calculated in future for the screening programs. MoM can then be used as an early marker of anomalous imbedding or implantation, leading to adverse pregnancy outcomes. It can serve as a non-invasive modality in screening Down syndrome at an early phase of pregnancy.

Serum PAPP-A levels during the first trimester of pregnancy play a cardinal role in fetal growth.[12,13] Under or overproduction of PAPP-A influences fetal growth as it regulates IGF levels.[15] Sluggish growth occurs because of low levels of PAPP-A. Decreased levels of free hCGβ are more indicative of hypertensive disorders related to pregnancy.[16, 17] Therefore, it is essential to determine levels of these biochemical markers to predict pregnancy outcomes and risk of trisomies like Down syndrome.

Free PAPP-A exhibits metalloproteolytic activity and differs from that produced during pregnancy, complex with an endogenous inhibitor called proform of major basic protein (pro-MFP). Insulin-like growth factor (IGF)-binding proteins 4 and 5 are its known substrates in humans, which lead to the release of bound IGF, which has been shown to induce macrophage activation, chemotaxis, LDL uptake by macrophages and release of proinflammatory cytokines.[18-20]

Our study was planned to determine the median of biochemical markers PAPP-A and hCG-β in healthy pregnant women. We did not include cases with miscarriages or ectopic pregnancies. Ugurlu *et al,* 2009 included normal intrauterine pregnant cases and those with miscarriages and ectopic pregnancies. They found that females with spontaneous abortion and ectopic pregnancy had low median PAPP-A 0.05 (0.02–6.0) as compared to those with normal intrauterine pregnancy (Median PAPP-A 0.08 (0.03-0.9)).21

In our study, the median value of PAPP-A was 1289.43 mIU/L (510.8-5965.99), and that of hCG-β was 120985 mIU/mL (23592-290000). Regression equations were plotted for PAPP-A and hCG-β against the gestational age. The R2 for the PAPP-A and the hCG-β equation were 0.694 and 0.791, respectively.

In a study conducted by Yigiter *et al,*[22] data of 1275 pregnant women was collected for hCG-β and maternal serum PAPP-A during the first trimester. Median values of PAPP-A and hCG-β were low, compared to those in our study. However, median PAPP-A and hCG-β levels increased with gestational, which has been noted in our study as well.

Borowski *et al*,[23] included women with 11 to 14 weeks of gestation. Total 800 pregnant women were included in their study. Nomograms for hCG-β and PAPP-A levels were determined in the subjects. CRL was used as a determinant of gestational age. They noticed a positive correlation between PAPP-A and CRL, whereas a weak negative correlation between free hCGβ and CRL was age demonstrated.[23] However, we demonstrated increasing values of both hCG-β and PAPP-A with increasing gestational age. This difference in hCG-β can be due to the confinement of our study group to 12th week gestation.

Shiefa *et al*,[24] demonstrated that as the pregnancy advances, levels of PAPP-A also increase. Its levels increase exponentially and have a doubling time of 3–4 days in the first trimester. After that, the levels continue to rise throughout the pregnancy. The exponential rise in PAPP-A levels in the first trimester causes the interpretation of PAPP-A value to be significantly related and dependent on gestational age. In our study, PAPP-A showed an increasing trend with increasing gestational age.

We have determined the median of PAPP-A and hCG-β levels during the 9th to 12th weeks of gestation. These values can be used to calculate MoM later. There is no recent data in the region about median values of PAPP-A.

Ghasemi-Tehrani *et al*,[25] concluded low serum PAPP-A levels as the risk factor for IUGR. We suggest

further studies to look for biochemical marker levels and pregnancy outcomes.

LIMITATIONS OF STUDY

We did not determine NT in our study. Further studies are recommended for that.

CONCLUSION

Median values of PAPP-A and HCGβ can be used to calculate (Multiple of Medians) MoM during the first trimester in tertiary care setup.

Conflict of Interest: None.

Authors' Contribution

SK: Study design, patience, proforma, data collection, NA: Literature search, article writing, Conception of work, Drafting, SB: Final approval, study design, AI: Conception, Design, KB: Interpretation of data, SN: Literature review.

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