ORIGINAL ARTICLES

THE DETERMINATION OF MEDIANS OF BIOCHEMICAL MATERNAL SERUM MARKERS IN HEALTHY WOMEN GIVING BIRTH TO NORMAL BABIES

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ABSTRACT

Objective: To determine median values of biochemical maternal serum markers during second trimester maternal screening to rule out chromosomal anomaly, Down syndrome.

Study Design: Cross sectional study.

Place and Duration of Study: Department of Chemical Pathology and Endocrinology, Armed Forces Institute of Pathology (AFIP), from Nov 2016 to May 2017.

Patients and Methods: Non-probability consecutive sampling technique was used. All healthy pregnant women with single pregnancy were included. As non-parametric statistics was used, the sample size was collected to be 155. Blood sample for serum human chorionic gonadotropin (HCG) was analyzed on random access immulite 2000, alpha-fetoprotein (AFP) was analyzed on ADVIA Centaur, unconjugated estriol (uE3) and Inhibin A measured by Enzyme-Linked Immunosorbent Assay (ELISA) method by PR 4100 Micro plate Reader.

Results: Total 155 women were enrolled in this study. Mean maternal age was 33.46 ± 2.35 years and mean maternal body weight was 54.98 ± 2.88 kg. Median value of quadruple markers, calculated from 15-18th week of gestation, was used for calculation of multiple of median (MoM) for screening of trisomy21 in this gestational age. Median values at 15 week of gestation: hCG 36650 mIU/ml, AFP 23.3 IU/ml, uE3 3.5 nmol/l, Inh A 198 ng/l, at 16 week of gestation: hCG 29050 mIU/ml, AFP 35.4 IU/ml, uE3 4.1 nmol/l, Inhibin-A 179 ng/l; at 17 week of gestation: hCG 28450 mIU/ml, AFP 36.0 IU/ml, uE3 6.7 nmol/l, Inhibin-A 175 ng/l and at 18 week of gestation: HCG 25200 mIU/ml, AFP 38.2 IU/ml, uE3 8.2 nmol/l, Inhibin-A 190 ng/l.

Conclusion: In this study we were able to get median values of quadruple markers for regional population, which will be used in future to calculate MoM for the screening purpose of Down syndrome. It will help to rule out Down syndrome by non-invasive test and at early stage of pregnancy.

Keywords: Down syndrome, Median, Second trimester, Screening, Serum biomarker.

INTRODUCTION

Screening plays a major role to detect chromosomal abnormalities, neural tube defects and other inborn diseases of the newborn[1]. Some serum markers in second trimester of pregnancy are useful in determining risk of developmental and chromosomal anomalies; these include Alpha fetoprotein (AFP), Human chorionic gonadotrophin (HCG), Unconjugated estriol (uE3) and Inhibin A (Inh-A). These four along with other biochemical markers, carried out during second trimester, have been shown to have higher detection rates and higher performances[2]. In developed countries these makers play a key role in earlier diagnosis of stillbirth, intra uterine growth retardation, placenta-mediated poor pregnancy outcomes and other abnormalities, and help to avoid dangerous outcome[3]. The combination of these biomarkers has not only been used for screening but also help to identify adverse pregnancy outcome i.e. maternal and fetal survival[4]. The test values of these biomarkers have been shown to be influenced by maternal weight, age, race, ethnicity and presence of conditions like diabetes mellitus and smoking[5]. Chromosomal abnormalities like trisomy 21 and trisomy 18 are commonly screened by these biochemical

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markers, as these anomalies can be a cause of phenotypical diseases like down syndrome[6]. In live born infant most common chromosomal aneuploidy is Down syndrome and has the higher frequency of death in less than five years of age[7]. It is about 1 in 800 births in general population[8]. Double test screening i.e. combination of AFP and hCG has detection rate of about 67.8% with 5.0% false-positive rate[9]. Triple test screening, which include uE3, AFP and hCG, has a higher detection rate of about 77.0%[10]. While quadruple test include Inh A, uE3, AFP and Hcg has a detection rate of 81.8%[3]. Since the concentration of these markers show immense variations during various stages of pregnancy, it is neither possible nor useful to determine their pregnancy related reference ranges. Method of comparison used for these parameters is called Multiple of median (MoM). It is universally used in the screening programs all over the world (instead of reference values). Every country has to determine its own median values before starting this service. In Pakistan there is no authentic data regarding median values of these biochemical parameters. Quadruple serum biomarkers in second trimester have been introduced as an alternate to predict the risk of Down syndrome during antenatal period.

Table: Percentile values of quadruple markers.

Gestational week	Percentile	hCG	AFP	uE3	Inh A
15th weeks	25th	33850	18.0	1.9	147
	50th	36650	23.3	3.5	198
	75th	45150	37.1	3.7	222
16th weeks	25th	23650	26.8	3.7	161
	50th	29050	35.4	4.1	179
	75th	36875	42.1	5.6	196
17th weeks	25th	18950	28.5	6.2	151
	50th	28450	36.0	6.7	175
	75th	35950	38.1	8.5	186
18th weeks	25th	18300	34.5	7.4	171
	50th	25200	38.2	8.2	190
	75th	31750	42.1	9.1	251

PATIENTS AND METHODS

A cross sectional study was carried out in Department of Chemical Pathology & Endocrinology AFIP Rawalpindi from Nov 2016 to May 2017. Total sample size was 155 calculated by using formula (n=Z2α/2 (SD)2/(MOE)2). All subjects were in 15th-18th week of gestation with no co morbidity or any significant chronic disease. Study setting was ante-natal clinics of CMH and MH Rawalpindi. Registered pakistani women were counseled and informed consent was obtained from the subjects after explaining them the non-invasive nature of the study. Non-probability consecutive sampling technique was used after approval from Institutional Review Board (IRB), AFIP Rawalpindi. Particulars of the subjects were recorded, about 3.0 ml venous blood was collected in plain gel tube for analysis of biochemical markers. Gestational weeks were estimated by ultrasonography based on biparietal diameter and also matched with last menstrual period (LMP). Subjects with discordant in gestational week, multiple pregnancies, lack of antenatal follow-up, feto placental and chromosomal anomaly, diabetes mellitus, and hypertension were excluded from study. Biochemical maternal serum markers in second trimester were obtained from 155 pregnant women with single pregnancy for hCG, AFP, Inh A and uE3. The hCG was measured by two sites chemiluminescence method on random access Immulite 2000®, automated immunoassay system by Siemens Health care Diagnostics USA. AFP was measured by two sites chemiluminescence on ADVIA Centaur®, Immunoassay

system by Siemens diagnostics USA. uE3 was measured by ELISA method by PR 4100 Micro plate Reader® Biorad and Inh-A measured by ELISA method by PR4100 Microplate Reader® Biorad. All data were entered and analyzed using SPSS version 21. Visual inspection of data generated for each biochemical marker was carried for gaussian distribution. Kurtosis and skewness of the data was also determined. Outliers were removed by using Dixie` test. Then test of normality i.e. Kolmogorov-Smirnov was applied to confirm the nature of the data. For heterogeneity of median values, Mann Whitney test was carried out. Non-parametric method i.e. percentile was used to determine the 50th percentile (median) values. The 95% confidence interval was determined for each median value. Median of serum biomarkers were calculated by 25th, 50th and 75th centile value in ranked data arranged in the descending manner for rounded weeks (like 15th rounded week includes 14 ± 4 to 15 ± 3 gestational ages) using simple statistical analysis. Median values at 15-18 weeks of gestation were also compared with other countries. Mann Whitney test and Kolmogorov Smirnov test were applied to calculate *p*-value and to test the significance.

RESULTS

After exclusion of unsuitable samples, total of 155 women were enrolled in this study. Out of which 5% non-responded in the study. Finally, 150 patients were included. Mean maternal age was 33.46 ± 2.35 years and body weight was 54.98 ± 2.88 kg. Quadruple biomarkers (Inh A, hCG, AFP and uE3) results were used to assess median

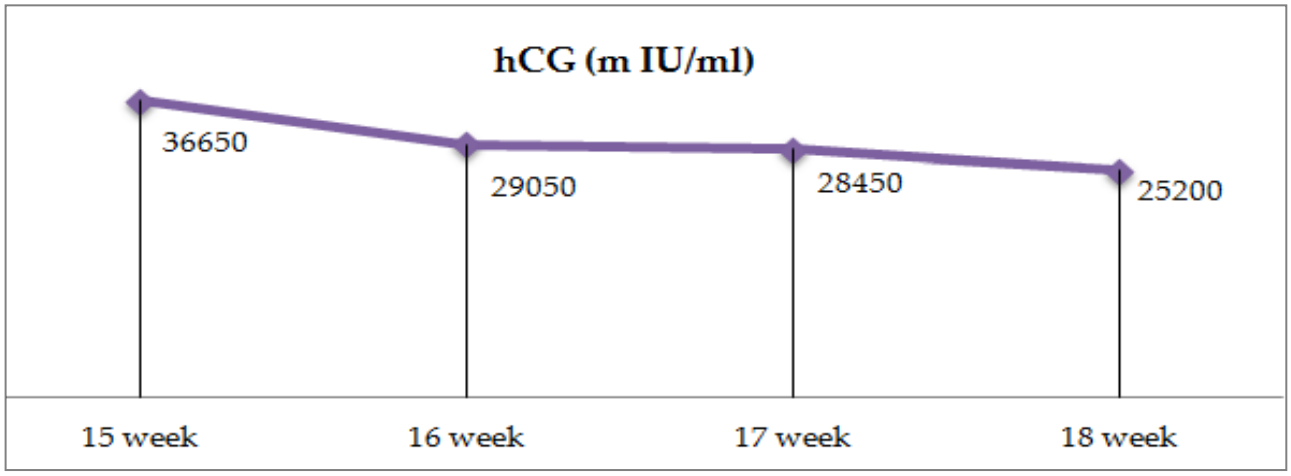

Figure-1: Median value of hCG (IU/ML) in 15, 16, 17 and 18 gestational week.

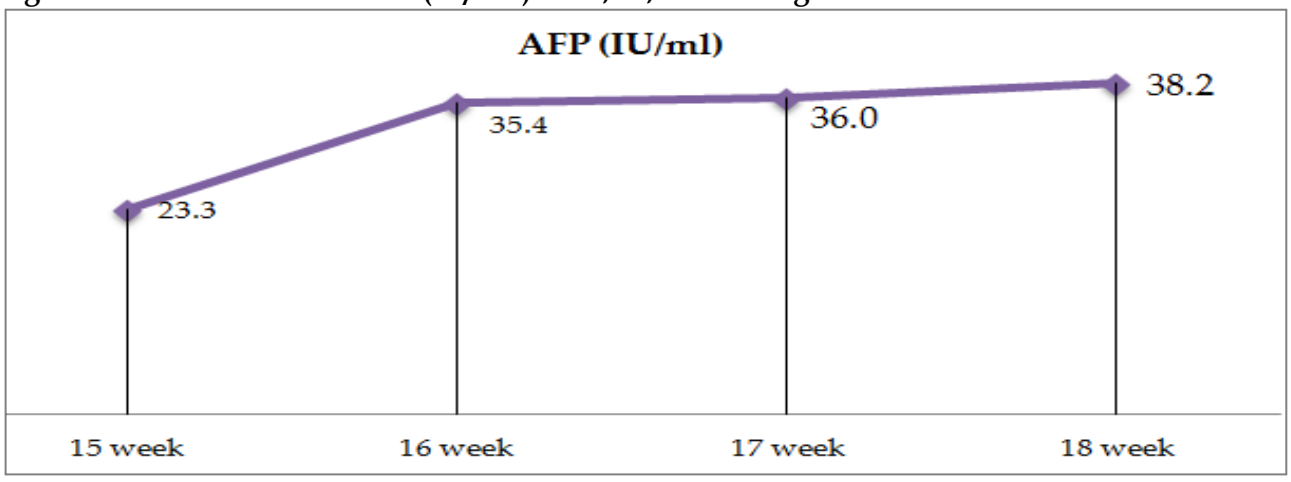

Figure-2: Median value of AFP (IU/ml) in 15, 16, 17 and 18 gestational week.

values for normal and healthy population, which would be used in future for calculation of MoM (Multiple of Median) by using formula MoM=Patient result/Lab median, to predict the risk of Down Syndrome. Median values (standard curves) of hCG, AFP, uE3 and Inh A were created in normal healthy pregnant women at 15-18 weeks of gestation (figure-1-4) (table). For estimation of medians for the gestation. For comparison of gestational age groups related to AFP, hCG, uE3 and Inh A, which showed significant results (p-value=0.016) for hCG, (p-value=0.005) for AFP, (p-value=0.001) uE3 and (p-value = 0.133) Inh A.

DISCUSSION

Quadruple test is very useful in detecting chromosomal abnormality. In the second half of

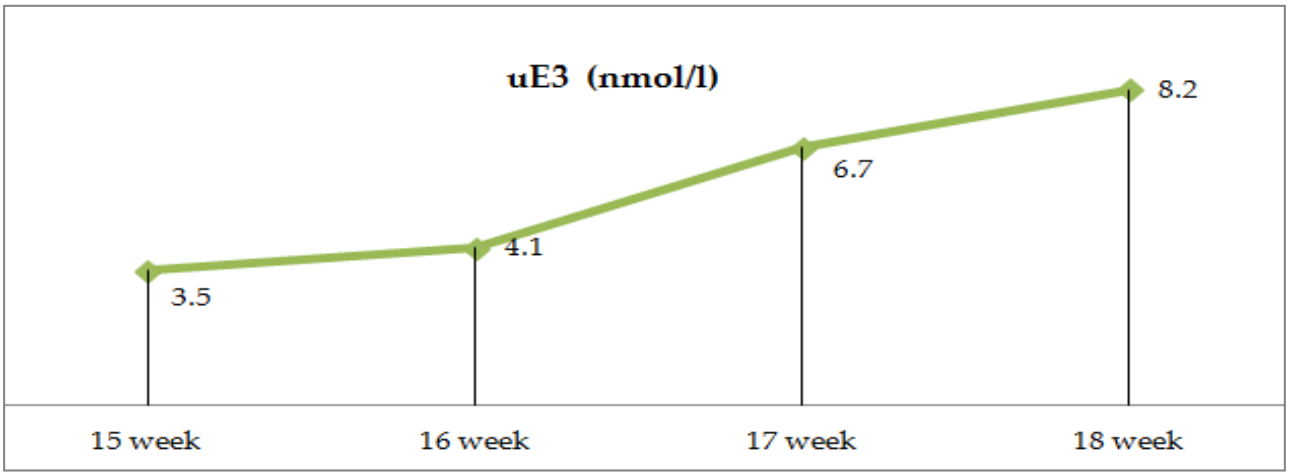

Figure-3: Median value of uE3 (nmol/l) in 15, 16, 17 and 18 gestational week.

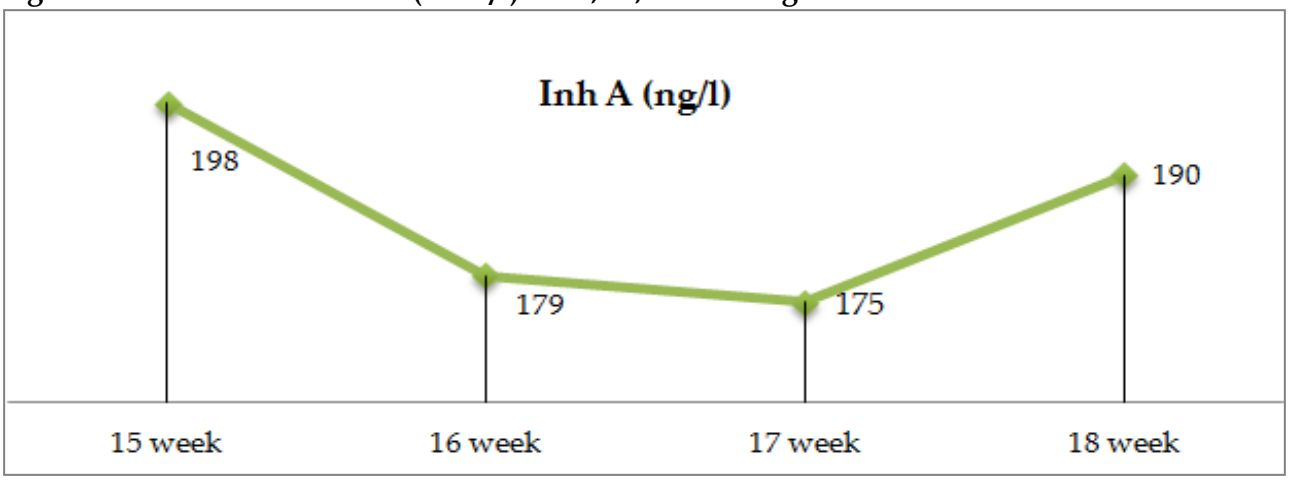

Figure-4: Median value of Inh A (ng/l) in 15, 16, 17 and 18 gestational week.

Quadruple biomarkers simple median values were calculated for every week of gestation. Trend of median values of hCG was decreasing with increasing gestational age. AFP trend progressively increased with gestation age. uE3 trend showed increase with gestational age, while Inhibin. A showed initially increase followed by decrease in 16th and 17th week of 20th century the screening for Down syndrome was started, which played a vital role in categorizing the women as a high risk for carrying fetus affected with Down syndrome. In our study work has been carried out in determining the medians of serum biomarkers for healthy pregnant women in Pakistan. Biochemical maternal serum markers in second

trimester (15th to 18th week of gestation) were obtained from 155 pregnant women with single pregnancy for hCG, AFP, Inh A and uE3. Result of the study showed that median values of hCG gradually decreased with gestational age, whereas median values of AFP and uE3 progressively increased. It was surprisingly seen in pattern of inh A which was initially decreased from 15th week to 16th as well as in 17th week and again increased in 18th week (figure 1, 2, 3 and 4).

One of the study by Vranken et al[11] in Belgium showed the same set of trends but different values for median, most likely due to large sample size and varied instrumentation used. The median values for Belgium population for hCG (IU/ml) were 30.2 at 15th week, 24.5 at 16th week, 19.9 at 17th week and 16.1 at 18th week of gestation. Median value of AFP (IU/ml) was 25.8 at 15th week, 30.2 at 16th week, 35.3 at 17th week, and 41.2 at 18th week of gestation. Similarly, median values of uE3 (ng/ml) was 2.8 at 15th week, 3.6 at 16th week, 4.7 at 17th week and 6.2 at 18th week of gestation. There was some documented variation in biomarkers throughout different tropics of world with region and ethnicity[12-14]. Another study was performed by Kaur et al[15] in India. The calculated values of median in this study for maternal serum biomarker hCGβ (ng/ml) were 15.2 at 15th week, 12.1 at 16th week, 10.2 at 17th week and 8.4 at 18th week of gestation, median values of AFP (IU/ml) were 27.7 at 15th week, 30.9 at 16th week, 34.6 at 17th week and 38.5 at 18th week of gestation and median values of uE3 (nmol/l) was 4.1 at 15th week, 4.9 at 16th week, 5.8 at 17th week and 6.6 at 18th week of gestation. These calculated values of AFP and uE3 are comparable with the study in hand but the values of hCG are different as they have used β hCG and study in hand focused on total hCG where as the set of increasing trend of different maternal serum marker shown is same as shown in our study.

Similar type of study was performed by Abou-Youssef et al[16] in Egypt, which showed median values of maternal serum markers AFP (IU/ml) around 33.3 at 15th week, 35.5 at 16th week, 37.2 at 17th week and 43.3 at 18th week of gestation, median values of hCG (mIU/ml) were 30700 at 15th week, 26900 at 16th week, 23500 at 17th week and 21500 at 18th week of gestation and median values of uE3 (ng/ml) were 0.48 at 15th week, 0.65 at 16th week, 0.81 at 17th week and 1.18 at 18th week of gestation. It showed same set of trends of AFP and hCG but uE3 median results were discordant with our study. Another study conducted by Shaw et al[3] in Taiwan reflects the same picture of values and trends of median like hCG (mIU/ml) were 38163 at 15th week, 30921 at 16th week, 26237 at 17th week and 23206 at 18th week of gestation, median values of AFP were 31.3 at 15th week, 37.3 at 16th week, 45.5 at 17th week and 54.8 at 18th week of gestation. All results of AFP were expressed in ng/ml (conversion factor to IU/ml = 0.826) and also uE3 were expressed in ng/ml (conversion factor to nmol/l = 3.47), median values of uE3 were 0.69 at 15th week, 0.86 at 16th week, 1.08 at 17th week and 1.35 at 18th week of gestation but there is a slight difference in trends of median of Inh-A (pg/ml) which were 212 at 15th week, 182 at 16th week, 168 at 17th week and 167 at 18th week of gestation[3]. In this study hCG and AFP median values are comparable with our study but uE3 were slightly different and inh A median values result in our study decreased in 16th and 17th weeks and again increased in 18th week of gestation but in Taiwan study it remained decreased till 19th week of gestation. Steps taken to calculate population-specific median values for the biomarkers (AFP, hCG, uE3, Inh-A) may be used as reference values during antenatal screening in Pakistani pregnant women. Slight variation present in the median values may be due to different tropics[12]. Our study had few limitations. Firstly, our sample size was less. Secondly, there is a need to conduct multicentric study to determine the median values of these four biochemical markers.

CONCLUSION

In this study we were able to get median values of quadruple markers for regional

population, which will be used in future to calculate MoM for the screening purpose of Down syndrome. It will help to rule out Down syndrome by non-invasive test and at early stage of pregnancy.

CONFLICT OF INTEREST

This study has no conflict of interest to declare by any author.

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