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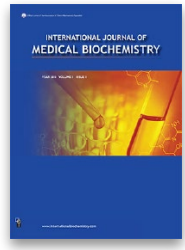

Research Article

Estimation of median second trimester screening test values at a single hospital

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Abstract

Objectives: The aim of this study was to determine the median values of the maternal serum triple screen test components, beta human chorionic gonadotropin (β-hCG), alpha-fetoprotein (AFP), and unconjugated estriol (uE3), at a single hospital in order to enhance prenatal diagnostic ability and to report fetal anomaly risks more accurately and reliably.

Methods: The triple test results of 692 pregnant women were evaluated retrospectively. The median values specific to a single laboratory were determined and compared with those of a fetal risk assessment software program.

Results: The laboratory estimated β-hCG medians according to gestational week were higher than those of the 14th and 16th week generated by the software program and lower in the other weeks. The serum estimated medians for AFP were higher than those of the program in all weeks except the 14th week. The difference between weeks 15, 16, and 17 was significant ($p<0.001$). For uE3, the estimated medians were lower than the software results and the difference was significant at weeks 15, 16, 17, and 18 ($p<0.001$).

Conclusion: The determination of regional median values would provide more accurate and reliable results for prenatal screening tests.

Keywords: Alpha-fetoprotein, beta human chorionic gonadotropin, median, second trimester, unconjugated estriol

Prenatal screening is very important to detect pregnancies at risk for Down syndrome. The results of an initial screening test guide the decision whether or not it is necessary to pursue interventional tests for a definitive diagnosis. The use of a combination of screening tests and diagnostic tests ensures that more patients can obtain accurate information about their personal risk status [1]. Among genetic disorders, such as Down (trisomy 21), Edwards (trisomy 18), and Patau (trisomy 13) syndromes, as well as neural tube defects, the most common chromosomal anomaly in newborns is Down syndrome, with a prevalence of 1/800 [2]. When only maternal age was used in the prenatal screening of genetic disorders, all mothers over 35 were considered at risk. Chromosomal anomalies could be detected in only one-third of those referred for amniocentesis [1]. Maternal age was found to be an inadequate screening method as the mean gestational age increased. In second trimester screening methods developed since the 1980s, various analytes detected in maternal serum are used in combination with maternal age.

The continued progress in the development of screening tests has demonstrated that the combination of ultrasonographic data and the maternal serum parameters of beta human chorionic gonadotropin (β-hCG), alpha-fetoprotein (AFP), and unconjugated estriol (uE3) at 15-20 weeks of gestation can detect Down syndrome at a rate of 74%. The rate may increase up to 81% in a quadruple test that includes evaluation of inhibin-A in addition to the other parameters. There is a 5% false positive detection rate [3].

The development of prenatal screening tests has reduced the need for interventional procedures such as a chorionic villus biopsy or amniocentesis. Interventional diagnostic pro-

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cedures can have serious consequences, including bleeding, preterm labor, and fetal loss [4, 5]. The psychological dimension of tests and interventional procedures performed during pregnancy, which can affect both the mother and the fetus, is also important [4]. A risk of Down syndrome of 1/270 or more in a second trimester screening test is typically used to recommend interventional procedures [6]. Multiples of median (MoM) calculated for each gestational week are used to standardize the biochemical parameter values used in prenatal screening tests and make them more understandable and easier to evaluate. The MoM value is calculated by dividing the result of the analysis by the median value of the analyte previously determined for the gestational week [7]. Other factors, such as age, weight, and race of the mother can also be used to adjust MoM values. It has been reported in various studies that MoM values vary according to race, ethnicity, and geographical region of origin of the women [8, 9]. For second trimester screening test results to be interpreted properly, the analytes must first be measured precisely. Then, the median values used in the MoM calculation should be determined for the region of origin of the pregnant women and for each laboratory performing the analysis. The aim of this study was to estimate the median values of the parameters of the triple screening tests at our hospital for the most accurate prenatal diagnostic evaluation of the fetus and to report fetal anomaly risks more accurately and reliably.

Materials and Methods

The second trimester screening tests of pregnant women (n=692) performed between 2017 and 2018 were examined retrospectively. Diabetics, cigarette smokers, twin pregnancies, and those who became pregnant via in vitro fertilization were excluded from the study.

Analysis of the triple test included the serum β-HCG, AFP, and uE3 data of pregnant women with a gestational age of 15-19 weeks. Ultrasonographic biparietal diameter (BPD) measurements were performed on the date of collection. The biochemical parameters of β-HCG and AFP were assessed using a Roche Cobas e 601 (Roche Diagnostics GmbH, Risch-Rotkreuz, Switzerland) and uE3 was evaluated with an Immulite 2000 immunoassay system using the electrochemiluminescence immunoassay method (Siemens Healthcare Diagnostics Products GmbH, Marburg, Germany). The SsdwLab 5 program (SBP Soft 2007 S.L., Girona, Spain) was used to determine comparable risk values. The median values estimated from patient results were compared with those of the SsdwLab 5 software program for the same gestational week.

Descriptive analyses were conducted to provide information about the general characteristics of the study groups. The data of continuous variables were expressed as mean±SD or median and interquartile range; categorical variables were given as n (%). For the comparison of the averages of the quantitative variables between groups, an independent samples t-test and one-way analysis of variance were used. For non-normal continuous data, the Kruskal-Wallis test was used. Age risk, and the risk of trisomy 21 and 18 were the only tests that were not parametric. This assessment was taken into account when performing the statistical analysis. A p value of <0.05 was considered statistically significant. IBM SPSS Statistics for Windows, Version 19.0 (IBM Corp., Armonk, NY, USA) was used to perform the statistical analyses.

The Clinical Research Ethics Committee of Tokat Gaziosmanpasa University granted approval for the study on 19.02.2019 (No: 19-KAEK-037), and recognized ethical guidelines were observed.

Table 1. Distribution of quantitative variables

Variables	Mean±SD	Minimum	Maximum
BPD (mm)	37.02±3.41	29.00	51.00
Days of gestation	120.29±7.18	104.00	151.00
Weight (Kg)	67.37±14.82	36.0	129.00
β-hCG (mIU/mL)	25295.22±16564.1	2264.00	115569.00
AFP (IU/mL)	40.44±18.98	10.94	211.20
EU3 (ng/mL)	0.87±0.36	.15	2.37
AFP MoM	1.33±0.66	.35	8.67
β-hCG MoM	1.15±0.69	.00	4.61
uE3MoM	1.06±0.38	.32	3.04
Age (years)	27.46±5.85	16.00	44.00
Age risk	0.0009 [0.0007-0.0015]	.0006	.0284
Trisomy 21 risk	0.0002 [0.0001-0.0005]	.0000	.0492
Trisomy 18 risk	0 [0-0]	.0000	.0141

Data are presented as mean±SD or median [interquartile range]; AFP: Alpha-fetoprotein; β-hCG: Beta human chorionic gonadotropin; BPD: Biparietal diameter; MoM: Multiples of median; uE3: Unconjugated estriol.

Table 2. Distribution of quantitative variables according to gestational weeks

Variables	Week of Gestation					p
	15	16	17	18	19	
BDP	31.43±0.83 (a)	34.73±1.07(b)	37.9±0.83 (c)	40.87±0.85 (d)	44.38±1.77 (e)	<0.001
Gestational days	108.87±1.67 (a)	115.48±2.15 (b)	121.81±1.65 (c)	128.72±1.7 (d)	135.98±3.96 (e)	<0.001
Weight	70.11±14.53	66.9±14.59	66.81±14.1	68.11±16.19	67.43±16.31	0.598
β-hCG (mIU/mL)	32077.57±21120.52 (a)	28950.34±18179.71 (a)	23569.87±14225.66 (c)	19981.35±12126.6 (cd)	15719.62±8754.5 (d)	<0.001
AFP (IU/mL)	30.77±2.99 (a)	37.26±18.16 (ab)	40.91±16.67 (bc)	46.47±22.8 (cd)	55.02±22.77 (d)	<0.001
uE3 (ng/mL)	0.54±0.22 (a)	0.72±0.27 (b)	0.95±0.33 (c)	1.07±0.34 (d)	1.33±0.32 (e)	<0.001
AFP MoM	1.45±1.01	1.34±0.68	1.31±0.56	1.3±0.63	1.34±0.48	0.683
β-hCG MoM	1.17±0.76	1.21±0.75	1.11±0.63	1.12±0.66	1.04±0.53	0.392
uE3MoM	1.15±0.43 (a)	1.07±0.41 (ab)	1.1±0.39 (ab)	0.98±0.3 (b)	1.02±0.24 (ab)	0.026
Age (years)	27.39±6.44	27.77±5.62	27.07±5.83	26.95±6.03	28.93±5.92	0.267
Age risk	0.0008	0.0009	0.0008	0.0008	0.0009	0.251*
	[0.0007-0.0019]	[0.0007-0.0015]	[0.0007-0.0014]	[0.0007-0.0015]	[0.0007-0.0017]	
Trisomy 21 risk	0.0002	0.0002	0.0002	0.0002	0.0002	0.488*
	[0.0001-0.0006]	[0.0001-0.0006]	[0.0001-0.0004]	[0.0001-0.0005]	[0.0001-0.0004]	
Trisomy18 risk	0 [0-0]	0 [0-0]	0 [0-0]	0 [0-0]	0 [0-0]	0.458*

*: Kruskal-Wallis test; one-way analysis of variance was used for the remainder. (abcde): The common letter in a row indicates statistical insignificance. For BDP, Gestational days, and uE3 variables, all pairwise comparisons are significant; AFP: Alpha-fetoprotein; β-hCG: Beta human chorionic gonadotropin; BPD: Biparietal diameter; MoM: Multiples of median; uE3: Unconjugated estriol.

Table 3. Distribution of qualitative variables

	Count (n)	Percentage (%)
Gestational week		
14	2	0.3
15	54	7.8
16	272	39.3
17	206	29.8
18	116	16.8
19	34	4.9
20	7	1.0
21	1	0.1
Age risk		
Risk-free	627	90.6
At risk	65	9.4
Trisomy 21 risk		
Risk-free	664	96.0
At risk	28	4.0
Trisomy 18 risk		
Risk-free	691	99.9
At risk	1	0.1

Results

The demographic data of the study participants, the results of biochemical tests, and the MoM values of these tests are summarized in Table 1. Table 2 summarizes the distribution of quantitative variables according to gestational weeks. The distribution of qualitative variables is summarized in Table 3. The reports given to all of the women included in the study were evaluated and the reported risks were examined. Of the 692 pregnant women who underwent a triple screening test, 4.0% (n=28) were reported to be at risk of Down syndrome using the median values of the program. The determination of a risk of trisomy 18 was 0.1% (n=1). A very strong positive correlation(r=0.999; p=0.000) was found between BPD and gestational week. There was a weak negative correlation (r= -0.296; p=0.000) between BPD and β-hCG, a weak positive correlation (r=0.293; p=0.000) with AFP and a moderate positive correlation (r=0.584; p=0.000) with uE3. There was a negative correlation (r= -0.294; p=0.000) between gestational week and β-hCG, a weak positive correlation (r=0.294; p=0.000) with AFP and a moderate positive correlation (r=0.581; p=0.000) with uE3. A weak negative correlation (r= -0.221; p=0.000) was found between weight and AFP and a weak positive correlation (r=0.201; p=0.000) with gestational age. There was a very strong positive correlation (r=0.945; p=0.000) between β-hCG and β-hCG MoM and a moderate positive correlation (r=0.423; p=0.000) with trisomy 21 risk. There was a weak positive correlation (r=0.251; p=0.000) between AFP and uE3 and a strong positive correlation (r=0.744; p=0.000) with AFP MoM. There was a positive correlation (r=0.403; p=0.000) between trisomy 21 risk and β-hCG MoM and a weak positive correlation (r=0.296; p=0.000) with age.

Table 4. Comparison of serum estimated median values and those of the SsdwLab 5 program

Gestational week		β-hCG (mIU/mL)			AFP (IU/mL)			UE3 (ng/mL)		
	Case number	Estimated median	Program median	p	Estimated median	Program median	p	Estimated median	Program median	p
14	2 (0.3)	54888	34440	0.180	15.37	23.2	0.157	0.15	1.80	0.157
15	54 (7.8)	25757	28962	0.510	31.34	25.6	<0.001	0.50	2.33	<0.001
16	272 (39.3)	24712	23930	0.011	33.95	30.0	<0.001	0.69	2.97	<0.001
17	206 (29.8)	19103	20860	0.306	38.58	33.5	<0.001	0.95	3.44	<0.001
18	116 (16.8)	17708	19817	0.227	43.73	40.1	0.006	1.02	4.20	<0.001
19	34 (4.9)				46.59	43.5	0.031			
20	7 (1)									
21	1 (0.1)									

AFP: Alpha-fetoprotein; β-hCG: Beta human chorionic gonadotropin; uE3: Unconjugated estriol; (SsdwLab 5: SBP Soft 2007 S.L., Girona, Spain).

A comparison of the estimated median values with those generated by the Ssdw Lab 5 program is shown in Table 4. The median values we estimated for AFP in the triple screen test were higher than those of the program in all gestational weeks except the 14th week, and the difference between the results for the 15th, 16th, and 17th weeks was significant (p<0.001). The median values estimated for uE3 were lower than those of the program and the difference was significant at 15, 16, 17, and 18 weeks (p<0.001). The serum median values estimated for β-hCG were higher than the median values generated by the program at 14 and 16 weeks, but the differences were not significant.

Discussion

Prenatal screening tests developed for prenatal detection of genetic disorders have high diagnostic value. However, if the result is uncertain, the patient may face further unnecessary invasive procedures that can cause serious complications. Prenatal screening tests with greater predictive ability would facilitate more accurate family counseling. Risk analysis based on prenatal screening tests must be sensitive and specific.

In the literature, there are many studies demonstrating the performance of triple screening tests to determine Down syndrome and other prenatal risks. Our results shared both similarities and differences with previous research.

Atak et al. [10] reported that 5.9% (n=353) of those who underwent triple screening tests were reported to be at risk for Down syndrome. In our study, 4.0% (n=28) of the pregnant women who underwent the triple screening test were reported to be at risk for Down syndrome, and 0.1% (n=1) at risk for trisomy 18.

The accuracy of biochemical analysis, the calculation of gestational age, BPD measurement, the entry of USG date of the ultrasound and the algorithms used by the software program affect the results of screening tests [11]. In addition to all these factors, variations in race, geographical region, and laboratories may also affect the results.

Vranken et al. [12] compared the median values estimated in their study in Belgium with the median values of different countries (Canada, Germany, England, and USA) using the same chemiluminescent immunoassay system. Their results determined a statistically significant difference based on geographic region of origin as well as preanalytical factors.

Each laboratory studies the biochemical markers with the screening protocol of its choice and different computer programs are used for the risk assessment. There are many reports in the literature comparing the median values estimated in different geographical regions with different devices.

Atak et al. [10] conducted a retrospective study of 5820 women with a singleton pregnancy using triple screening data obtained with a Unicel DxI 800 device (Beckman Coulter, Inc., Brea, CA, USA) and a comparison was made with the median values produced by Benetech PRA software (Benetech Inc., Toronto, Canada). The AFP median values were lower than those of the prenatal risk assessment program for all gestational weeks and the difference was significant (p<0.05) in all but the 20th week. The median values of uE3 were significantly higher than those of the program with the exception of week 20 (p<0.05). The median β-hCG values were statistically significantly different at weeks 16, 19, and 20 (p<0.05), but at weeks 15, 17, and 18, the difference was not significant.

In a retrospective study reported by Şanlı and Kartkaya [13] that included 5410 women with a singleton pregnancy, triple screening data obtained using the Immulite 2000 immunoassay analyzer were compared with the median values determined using Prisca 4.0 risk calculation software (Typolog Software Ltd. & Co KG, Tornesch, Germany). The median estimated for the 17th to 20th gestational weeks was significantly different from that of the program.

Akalın and Arıkan [14] examined the triple screening data of 1130 women with a singleton pregnancy from the Antalya region of Turkey in a retrospective study. The screening data were obtained using an Immulite One analyzer (Siemens Healthcare Diagnostics Products GmbH, Marburg, Germany)

and compared with the median values of Prisca 4.0 Typolog software. The estimated median value of AFP according to the screening tests for the 16th-19th weeks was significantly lower than that of software program ($p<0.05$), which is consistent with our results. There was a significant decrease in the β-hCG median value at the 17th week, which is also similar to our results, while a significant increase was seen at weeks 16, 18, and 19 ($p<0.05$).The median value of uE3 determined using the risk assessment tools was significantly lower at the 17th week ($p<0.05$), and lower at weeks 16, 18, and 19 without significance. Our results for AFP were also significantly lower.

Duran [15] performed a retrospective study in the Bingöl region of Turkey using the triple screening data of 480 singleton pregnancies. An Immulite 2000 XPi device (Siemens Healthcare Diagnostics Products GmbH, Marburg, Germany) was used and the results were compared with the median values of Prisca 5.0 Typolog software. The median serum marker AFP result for the 18th week was lower than that of the program ($p=0.0219$), which is in contrast to our results. As in our study, the median values of uE3 in the 15th-19th weeks were lower than those of the program ($p<0.0001$). The β-HCG median value was not statistically different from those of the program at any interval ($p>0.05$).

In a retrospective study conducted by Yılmaz [16] of 5820 women with a singleton pregnancy in the Erzurum region of Turkey, triple screening data were obtained using an Immulite 2000 device and compared with the median values of Prisca 7.0 Typolog software. The AFP medians were lower than program results values, unlike our results. The uE3 medians based on the screening tests were higher, which is contrary to our results, and β -HCG values were higher, with the exception of the 18th week ($p<0.05$).

Sucu et al. [17] retrospectively evaluated the triple screening data of 513 singleton pregnancies in the Okmeydani district of Istanbul, Turkey, gathered with the Immulite 2000 device and compared the results with the median values of the Prisca 4.0 Typolog software. The difference in medians for 16th and 17th weeks was found to be significant for both uE3 and β-hCG. For AFP, the median was only statistically significant for the 16th week.

In a retrospective study conducted by Akarsu et al. [18] using a Hitachi E 170 system (Hitachi Ltd., Tokyo, Japan) for AFP and β-HCG and Dynex Magellan Biosciences devices for uE3 (Dynex Technologies Inc., Chantilly, VA, USA), median values of SsdwLab 5 software were compared with the data of 711 women with a singleton pregnancy. At weeks 16, 17, and 18, the serum median values of uE3 were significantly lower than those of the program ($p<0.05$), which is consistent with our results.

These study findings have led to the need for each laboratory to determine its own median values. This was a shared opinion of the authors of the studies mentioned above.

The determination of regional median values would help to improve the performance of prenatal screening tests and provide more accurate and reliable results.

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Ethics Committee Approval: The Clinical Research Ethics Committee of Tokat Gaziosmanpasa University granted approval for the study on 19.02.2019 (No: 19-KAEK-037), and recognized ethical guidelines were observed.

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Multiple comparisons for Table 2.

Tukey honestly significant difference test		Multiple comparisons			
Dependent variable	**(I) Week of gestation**	**(J) Week of gestation**	**Mean difference (I-J)**	**Std. error**	**p**
BDP	15	16	-3.305*	.148	<0.001
		17	-6.471*	.152	<0.001
		18	-9.437*	.164	<0.001
		19	-12.952*	.206	<0.001
	16	15	3.305*	.148	<0.001
		17	-3.167*	.093	<0.001
		18	-6.132*	.112	<0.001
		19	-9.647*	.168	<0.001
	17	15	6.471*	.152	<0.001
		16	3.167*	.093	<0.001
		18	-2.966*	.117	<0.001
		19	-6.481*	.171	<0.001
	18	15	9.437*	.164	<0.001
		16	6.132*	.112	<0.001
		17	2.966*	.117	<0.001
		19	-3.515*	.182	<0.001
	19	15	12.952*	.206	<0.001
		16	9.647*	.168	<0.001
		17	6.481*	.171	<0.001
		18	3.515*	.182	<0.001
Gestational days	15	16	-6.603*	.304	<0.001
		17	-12.936*	.312	<0.001
		18	-19.841*	.337	<0.001
		19	-27.101*	.422	<0.001
	16	15	6.603*	.304	<0.001
		17	-6.333*	.191	<0.001
		18	-13.238*	.229	<0.001
		19	-20.498*	.343	<0.001
	17	15	12.936*	.312	<0.001
		16	6.333*	.191	<0.001
		18	-6.905*	.240	<0.001
		19	-14.166*	.350	<0.001
	18	15	19.841*	.337	<0.001
		16	13.238*	.229	<0.001
		17	6.905*	.240	<0.001
		19	-7.261*	.373	<0.001
	19	15	27.101*	.422	<0.001
		16	20.498*	.343	<0.001
		17	14.166*	.350	<0.001
		18	7.261*	.373	<0.001
β-hCG	15	16	3127.230	2346.341	0.671
		17	8507.702*	2409.660	0.004
		18	12096.218*	2601.800	<0.001
		19	16357.952*	3263.826	<0.001
	16	15	-3127.230	2346.341	0.671
		17	5380.473*	1476.823	0.003
		18	8968.988*	1773.108	<0.001

Cont.

Tukey honestly significant difference test		Multiple comparisons			
Dependent variable	**(I) Week of gestation**	**(J) Week of gestation**	**Mean difference (I-J)**	**Std. error**	**p**
		19	13230.723*	2650.870	<0.001
	17	15	-8507.702*	2409.660	0.004
		16	-5380.473*	1476.823	0.003
		18	3588.515	1856.086	0.301
		19	7850.250*	2707.075	0.031
	18	15	-12096.218*	2601.800	<0.001
		16	-8968.988*	1773.108	<0.001
		17	-3588.515	1856.086	0.301
		19	4261.734	2879.437	0.576
	19	15	-16357.952*	3263.826	<0.001
		16	-13230.723*	2650.870	<0.001
		17	-7850.250*	2707.075	0.031
		18	-4261.734	2879.437	0.576
AFP	15	16	-6.494	2.673	0.109
		17	-10.139*	2.745	0.002
		18	-15.704*	2.964	<0.001
		19	-24.253*	3.718	<0.001
	16	15	6.494	2.673	0.109
		17	-3.645	1.682	0.194
		18	-9.210*	2.020	<0.001
		19	-17.759*	3.019	<0.001
	17	15	10.139*	2.745	0.002
		16	3.645	1.682	0.194
		18	-5.565	2.114	0.066
		19	-14.114*	3.083	<0.001
	18	15	15.704*	2.964	<0.001
		16	9.210*	2.020	<0.001
		17	5.565	2.114	0.066
		19	-8.549	3.280	0.070
	19	15	24.253*	3.718	<0.001
		16	17.759*	3.019	<0.001
		17	14.114*	3.083	<0.001
		18	8.549	3.280	0.070
uE3	15	16	-.183*	.044	<0.001
		17	-.408*	.045	<0.001
		18	-.525*	.049	<0.001
		19	-.787*	.061	<0.001
	16	15	.183*	.044	<0.001
		17	-.225*	.028	<0.001
		18	-.343*	.033	<0.001
		19	-.605*	.050	<0.001
	17	15	.408*	.045	<0.001
		16	.225*	.028	<0.001
		18	-.117*	.035	0.007
		19	-.379*	.051	<0.001
	18	15	.525*	.049	<0.001
		16	.343*	.033	<0.001

Cont.

Tukey honestly significant difference test	Multiple comparisons				
Dependent variable	**(I) Week of gestation**	**(J) Week of gestation**	**Mean difference (I-J)**	**Std. error**	**p**
		17	.117*	.035	0.007
		19	-.262*	.054	<0.001
	19	15	.787*	.061	<0.001
		16	.605*	.050	<0.001
		17	.379*	.051	<0.001
		18	.262*	.054	<0.001
uE3 MoM	15	16	.084	.056	0.562
		17	.054	.057	0.878
		18	.174*	.062	0.041
		19	.132	.078	00.432
	16	15	-.084	.056	0.562
		17	-.030	.035	0.918
		18	.090	.042	0.205
		19	.048	.063	0.939
	17	15	-.054	.057	0.878
		16	.030	.035	0.918
		18	.120	.044	0.053
		19	.078	.064	0.745
	18	15	-.174*	.062	0.041
		16	-.090	.042	0.205
		17	-.120	.044	0.053
		19	-.042	.068	0.974
	19	15	-.132	.078	0.432
		16	-.048	.063	0.939
		17	-.078	.064	0.745
		18	.042	.068	0.974

*. The mean difference is significant at the level of 0.05; AFP: Alpha-fetoprotein; β-hCG: Beta human chorionic gonadotropin; MoM: Multiples of median; uE3: Unconjugated estriol.