Combined detection of α-fetoprotein and free β-human chorionic gonadotropin in screening for trisomy 21 and management of cases in the moderate risk value range

YAHONG LI, XIAOJUAN ZHANG, YUN SUN, DONGYANG HONG, YANYUN WANG, ZHENGFENG XU and TAO JIANG

Center of Prenatal Diagnosis, Obstetrics and Gynecology Hospital Affiliated to Nanjing Medical University, Nanjing, Jiangsu 210004, P.R. China

Received February 7, 2017; Accepted July 22, 2017

DOI: 10.3892/mco.2017.1355

Abstract. Down syndrome is the most common cause of prenatal chromosomal abnormalities, and prenatal serum screening is an effective method for decreasing the birth prevalence of children with Down syndrome. The aim of the present study was to observe the effect of duplex screening and investigate the treatment of cases under specific conditions. The medians of free β-human chorionic gonadotropin (HCG) and α-fetoprotein (AFP) were calculated and compared with those embedded in the 2T software. The detection and false-positive rates were analyzed under different conditions, and the distribution of Down syndrome cases was investigated in different risk ranges. Finally, suitable recommendations for further diagnostic investigation were provided according to the status of each individual. The medians of free β-HCG and AFP were found to differ from the corresponding medians embedded in the 2T software ($P<0.01$), and on the basis of a 5% false-positive rate, the detection rate would increase from 63.6 to 67.8% when compared with medians embedded in the 2T software, indicating we should establish our own medians of free β-HCG and AFP. In addition, residual cases (risk value <1/300) with relevant Down syndrome indications mainly concentrated at risk values between 1/1,000 and 1/300, and partial residual screening cases were verified through diverse methods. These findings indicated that different laboratories should establish their own medians; furthermore, what is classed as moderate risk is extremely important in screening for Down syndrome and reasonable recommendations may be offered under different conditions.

Correspondence to: Dr Tao Jiang, Center of Prenatal Diagnosis, Obstetrics and Gynecology Hospital Affiliated to Nanjing Medical University, 123 Tianfei Road, Nanjing, Jiangsu 210004, P.R. China
E-mail: jiangzhang784@163.com

Key words: second trimester, Down syndrome, maternal serum screening, moderate risk, detection rate, false-positive rate

Introduction

Trisomy 21 (Down syndrome) is the most common cause of prenatal chromosomal abnormalities (1), with an incidence of 1/800-1/600 pregnancies (2). Thus, prenatal screening for trisomy 21 is extremely important in order to decrease the prevalence of Down syndrome births. Over that past few years, second-trimester maternal serum screening for trisomy 21 has further developed, and a number of serum biochemical markers have been reported in the screening for Down syndrome, such as α-fetoprotein (AFP), unconjugated oestriol (uE3), maternal serum human chorionic gonadotropin (HCG), free β-HCG and inhibin-A (3). Common dual serological indicators include AFP combined with total or free β-HCG (4-5), and the detection rate of AFP combined with total HCG is ~56%, with a 4.9% false-positive rate (6). The combination of AFP, uE3 and HCG is the common triple test (7). A recent report revealed that, with a 5% false-positive rate, the detection rate of the triple test was 66.7%, whereas it was only 50% with AFP and free β-HCG (8), demonstrating that the triple test achieved a higher detection rate compared with dual serum marker testing. Quadruple testing markers include AFP, β-HCG (total or free), uE3 and inhibin-A. The combined detection of different serum markers or different detection methods may yield different results. It has been demonstrated that second-trimester screening quadruple testing with AFP, HCG, uE3 and inhibin-A had a higher detection rate for trisomy 21 compared with a dual or triple test. The authors also reported that the detection of two or three indicators (AFP, uE3, total or free β-HCG) was better compared with that of any single indicator, and the detection rate was 60-70%, with a false-positive rate of 5%. In addition, it was demonstrated that the quadruple testing with AFP, HCG, uE3 and inhibin-A had the highest diagnostic yield, but there was no significant advantage when compared with the traditional triple test (AFP, uE3 and HCG) (3). It has been widely recognized that different serum markers, cut-off values of risks, or the determination of multiples of the median, may all affect the detection rate. For second-trimester screening, the detection of AFP and free β-HCG in maternal serum has been selected for several years in the Center of Prenatal Diagnosis, Obstetrics and Gynecology hospital Affiliated to

Nanjing Medical University, after balancing the benefit against the significantly higher cost of the triple screening test. The aim of the present study was to summarize our results on the in-depth screening for trisomy 21.

Through extensive research, it has been demonstrated that cell-free fetal DNA is present in the maternal serum (9); based on that finding, non-invasive prenatal testing (NIPT) for aneuploidy of chromosomes 21, 13, 18, X and Y rapidly developed (10) and has attracted significant attention. Regarding the difference between fetal and maternal DNA fragments, NIPT may detect the number of fetal DNA fragments; when trisomy 21 occurs, the difference between the normal and abnormal number of fetal DNA fragments may be significant and trisomy 21 may be identified based on that difference (11). NIPT has a high sensitivity (100%) for Down syndrome, and a 99.7% specificity by multiplexed massively parallel shotgun sequencing (12). NIPT may help avoid the risks associated with chorionic villus sampling and amniocentesis, such as abortion and premature delivery (13,14). Furthermore, NIPT may offer an opportunity for the prenatal treatment of Down syndrome (15). Although non-invasive prenatal diagnosis may have certain advantages, it cannot confirm the presence of chromosomal abnormalities, as possible chromosomal mosaicism may cause false-positive results (16); thus, for patients with positive results, amniocentesis is required.

Materials and methods

Population selection and gestational age calculation. A total of 221,288 normal singleton pregnancies who underwent second-trimester screening at the Center of Prenatal Diagnosis (Obstetrics and Gynecology Hospital Affiliated to Nanjing Medical University, Nanjing, China) between October 2004 and October 2013 were included in the present study, and blood samples were collected from each patient at 15^{+0} and 20^{+6} weeks of gestation. If the menstrual cycle was regular, gestational age was estimated from the date of the last menstruation; if not, gestational age was calculated by type B ultrasonic testing.

Quality control. Maternal venous blood samples were collected and centrifuged at 4,000 x g for 3 min, then stored at -20˚C until detection. AFP and free β-HCG in the serum were detected using a Wallac AutoDELFIA® hAFP/Free hCGβ Dual kit (PerkinElmer, Turku, Finland). In order to guarantee the reliability of the experiment, control serum samples (Hangzhou Biosan Biological Technology Co., Ltd., Hangzhou, China), including low, median and high concentrations, were processed along with the serum specimens.

Assessment of median and risk value. The calculation method of gestational age was as mentioned above. First, the original medians of each gestational week were obtained. Then, the optimal curve regression of this set of data was selected, and the best fitted curve was obtained. Finally, the regressive median for each week after regression was calculated. Comparisons between our median and the corresponding medians provided by 2T software of the same gestational week were performed.

For risk calculation, medians of AFP or free β-HCG were converted into multiple of the median (MoM) for gestational age, and adjusted by maternal weight. The 2T risk analysis software was applied (the medians were the original presented), and a risk value >1/300 was considered to be positive.

Confirmation of Down syndrome. For pregnant women with high risk (≥1/300), advanced age (≥35 years), an extremely high free β-HCG value (MoM ≥10), or abnormal ultrasound findings, the results were confirmed by chromosomal analysis via amniocentesis or umbilical cord blood sampling (cordocentesis). All Down syndrome cases were confirmed by amniocentesis or cordocentesis, and all screened subjects received telephone follow-ups; however, some of the subjects could not be contacted. The number of residual cases (low- and intermediate-risk cases that resulted in Down syndrome births) in 1/1,000-1/300 and <1/1,000 were compared, in order to demonstrate the significance of recommending intermediate-risk cases for further examination.

Statistical analysis. DataFit software (http://www.oakdaleengr.com/index.html) was used for curve regression analysis, and SPSS 17.0 software (SPSS, Inc., Chicago, IL, USA) was also applied to construct or compare charts. Mann-Whitney U tests were applied to compare the median difference of each gestational week (from week 15 to 20); Chi-square tests were also used and $P<0.05$ was considered to indicate statistically significant differences.

Results

The medians of AFP and free β-HCG for each region were statistically significant when compared with medians embedded in the 2T software. In order to obtain the regressive medians of free β-HCG and AFP in Nanjing, curve regression analysis was performed, and the best curve regression equations among these were obtained. The equation for free β-HCG was $y=-0.039212963x^3 + 2.42734127x^2 - 50.90158069x + 369.7202778$ (Fig. 1A, $R^2=0.999$); and the curve equation of AFP was $y=0.065740741x^3 - 2.992460317x^2 + 48.4505291x - 247.344444$ (Fig. 1B, $R^2=0.999$). Then, the regressive medians of 15-20 weeks were obtained. The Mann-Whitney U test demonstrated that the medians of free β-HCG each week in our region were higher compared with the data provided by the 2T software ($P<0.01$). In terms of AFP, it was observed that from 15-17 weeks, the medians of AFP in our data were higher compared with those provided by the 2T software. However, after 18-20 weeks, an opposite trend was observed ($P<0.01$, Table I). All the abovementioned results revealed that the differences in the medians of free β-HCG and AFP between our region and the corresponding medians in the 2T software, which were obtained from Caucasians, were statistically significant, indicating that our own medians of free β-HCG and AFP must be set up.

Screening analysis of the combined detection of AFP and free β-HCG. In order to determine the detection rate, the cases with confirmed Down syndrome were analyzed. Among the 221,288 screened pregnancies, 118 had Down syndrome. The detection rate and false-positive rate varied with different cut-off values (Table II). When the cut-off value was set at 1/270, the detection rate was 59.3% and the false-positive

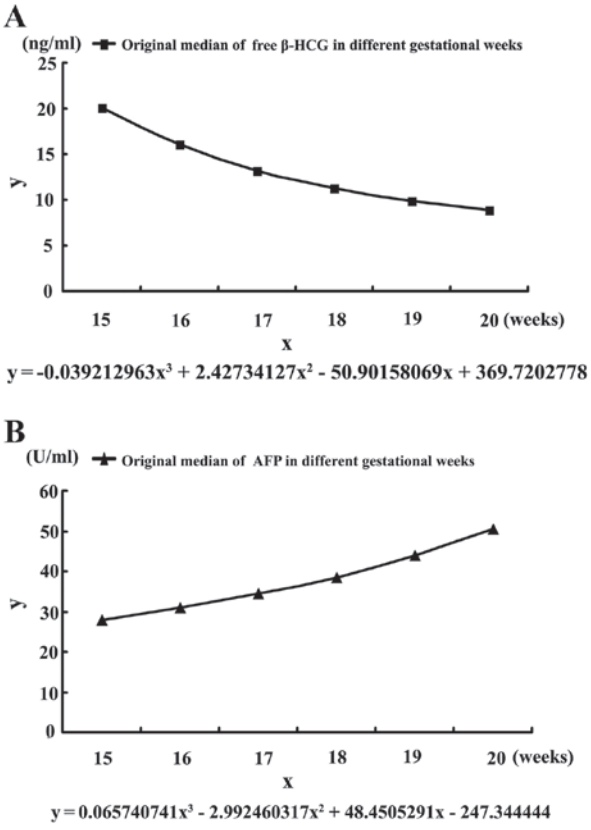

Figure 1. Curve regression for medians of free β-HCG and AFP. (A) Regressive curve of free β-HCG, using the third power of regression (R^2=0.999). (B) Regressive curve of AFP, using the third power of regression (R^2=0.999). HCG, human chorionic gonadotropin; AFP, α-fetoprotein.

rate was 4.43%. However, if the cut-off value was set at 1/300, the detection rate was 66.1% and the false-positive rate was 5.22%. Furthermore, when the cut-off value was set at 1/1,000, the detection rate and false-positive rate was 90.6 and 19.21%, respectively.

Analysis of the impact of different medians on the detection rate and false-positive rate of Down syndrome. Our previous conclusion revealed a significant difference in medians between those embedded in the 2T software and those of our laboratory. However, it remains unknown whether the difference may affect the detection rate or false-positive rate. In order to resolve these issues, 2,575 specimens within different batches were analyzed. First, the original Down syndrome risk values of each specimen were recorded. Then, the new medians were applied and a new risk value was obtained. Finally, the cut-off values with a 5% false-positive rate were obtained, and the original and new cut-off value was applied to analyze the detection rate for Down syndrome. Furthermore, the false-positive rate using the 2T medians and the medians from our laboratory was also analyzed at a cut-off value of 1/280.

Our results revealed that, if a 5% false-positive rate was used to select a cut-off value, the original cut-off value would be 1/280, and the new cut-off value would be 1/310. For the 118 Down syndrome cases, if the cut-off value was 1/280, a total of 75 cases would be detected (63.6%). Furthermore, if a cut-off value of 1/310 was applied, 80 of 118 cases would be detected (67.8%). However, there was no significant difference on the detection rate (P>0.05, Table III). If 1/280 was used as the cut-off value, the original false-positive rate would be 5.05% (130/2,575), and taking into consideration the new risk values calculated by our own medians, the false-positive rate was 4.38% (113/2,575). Although the latter false-positive rate was lower compared with the former, the difference was not statistically significant (P>0.05, Table IV). Our results suggested that the difference in medians between our laboratory and those embedded in 2T, did not statistically significantly affect either the detection rate or false-positive rate. However, considering the increase in detection rate and decrease in false-positive rate, we recommend the use of our own medians of AFP and free β-HCG.

Residual Down syndrome cases are mainly concentrated in a certain risk range. After a long period of research, it was found that residual Down syndrome cases were primarily concentrated at risk values between 1/1,000 and 1/300 (Table V). A total of 221,288 screened cases were counted in our hospital, and the value at risk within 1/300 were 11,636 cases, accounting for 5.3% of the total number of screened cases; the number of cases with risk values within 1/1,000-1/300 was 30,997, accounting for 14.0%; and the number of cases with risk values <1/1,000 was 178,655 (80.7%). It may be concluded that Down syndrome cases were mainly concentrated at risk values ≥1/300 (66.95%), suggesting that our screening was efficient. However, there were certain residual cases: For example, there were 39 residual cases and 28 had a risk value within 1/1000-1/300 (23.73%); in addition, only 9.32% (11 residual cases) had a risk value of <1/1,000, indicating that residual cases were mainly concentrated at risk values between 1/1,000 and 1/300 (P<0.01, Fig. 2). In summary, close attention should be paid to cases with a risk value 1/1,000-1/300 to significantly reduce the number of Down syndrome births.

Further diagnosis for the different ranges of risk value. According to the abovementioned findings, the results were divided into three categories. First, a risk value ≥1/300 was considered as high-risk. For such cases, amniocentesis or cordocentesis would be advisable. Second, a risk value of 1/1,000-1/300 was considered to be in the extenuation range, and it would require a rational suggestion. If the risk value was within 1/1,000-1/300, but the value of free β-HCG was extremely high (MoM>10), or the ultrasound revealed abnormal results that may be associated with chromosomal abnormalities, amniocentesis or cordocentesis is recommended. If the correction of MoM of free β-HCG was in the normal range, massive parallel sequencing of maternal plasma DNA may be applied. Finally, if the risk value was <1/1,000 and there were abnormal ultrasound results possibly associated with chromosomal abnormalities, amniocentesis or cordocentesis would be recommended. Through this approach, leak cases may significantly decrease (Fig. 3). For risk values <1/300, there were 18 Down syndrome births, as these subjects did not undergo further examinations; 7 Down syndrome cases were detected by massive parallel sequencing; amniocentesis detected the majority of the cases (12 cases); only 2 cases underwent cordocentesis and terminated their pregnancies. It may be concluded that amniocentesis remains the primary method for confirming Down syndrome, and NIPT is a new

Table I. Median comparisons of free β-HCG and AFP between data of our hospital and data embedded in the 2T software.

		Free β-HCG (ng/ml)			AFP (U/ml)		
Gestational week	Cases (n)	Original median	Median of the original median predicted	Median embedded in the 2T software	Original median	Median of the original median predicted	Median embedded in the 2T software
15	5,955	20.0	20.0	19.0	28.0	28.0	27.6
16	42,410	16.1	16.1	15.4	31.0	31.0	30.6
17	80,045	13.2	13.2	13.0	34.6	34.5	34.2
18	58,758	11.3	11.2	10.8	38.5	38.6	39.3
19	23,966	9.9	9.9	8.9	43.9	43.9	45.8
20	10,036	8.9	8.9	8.0	50.6	50.6	50.8

A total of 221,288 normal singleton pregnancies from 15 to 20 weeks were analyzed. The values of AFP or free β-HCG were assessed at different gestational weeks, and an original median was obtained. Then, the curve regression was established according to these medians, and the predicted medians were calculated. The medians embedded in the 2T software were consistent with the instructions of the Wallac Auto DELFIA® hAFP/Free hCGβ Dual kit. The specimen numbers at different gestational weeks (15-20) for the kit were 8,113; 12,457; 7,688; 2,182; 936 and 533, respectively. HCG, human chorionic gonadotropin; AFP, α-fetoprotein.

Table II. DR and FPR for DS at different cut-off values.

Cut-off value	DR for DS (%)	FPR for DS (%)
1/200	51.7	2.72
1/250	55.9	3.94
1/270	59.3	4.43
1/280	63.6	5.05
1/300	66.1	5.22
1/350	73.7	6.36
1/500	78.8	9.61
1/1,000	90.6	19.21
1/1,500	91.5	26.74

The data reflected detection rates and false-positive rates when different cut-off values were used, and the original medians embedded in the 2T software were applied. DS, Down syndrome; DR, detection rate; FPR, false-positive rate.

Table III. Comparison of different medians for detection rate for DS.

Median	Cut-off value of 5% FPR	DS cases detected	P-value
Original	1/280	75/118	>0.05
New	1/310	80/118	

On the basis of a 5% FPR, if the original medians embedded in the 2T software were used, the cut-off value was 1/280 and 75 Down syndrome cases would be detected. When the new medians were applied, the regressed medians were calculated, the cut-off value was 1/310, and 80 Down syndrome cases would be detected. The differences were not statistically significant (P>0.05). However, the detection rate increased to a certain extent. DS, Down syndrome; FPR, false-positive rate.

Table IV. Effect of different medians on false-positive rate for DS.

Median	Positive cases ratio	FPR for DS at 1/280 (%)	P-value
Original	130/2575	5.05	>0.05
New	113/2575	4.38	

In order to investigate the effect of changes of medians on false-positive rates, 2,575 cases of specimens within different batches were analyzed. The new risk values were first calculated using the new medians (the regressed medians were calculated), using 1/280 as the cut-off value, and obtained a new false-positive rate (4.38%). Upon comparison, there was no statistically significant difference with the original false-positive rate (5.05%). However, there was a decrease in the false-positive rate. DS, Down syndrome; FPR, false-positive rate.

method developing rapidly, as it may be used to avoid the risk of intrauterine infection or abortion caused by amniocentesis or cordocentesis. Appropriate advice must be offered to subjects with extenuation risk values, in order to reduce the number of Down syndrome births.

Discussion

Trisomy 21 is associated with relatively common chromosomal abnormalities, which affect human health and the quality of life. Hence, scholars have been searching for more accurate screening methods to prevent Down syndrome births. Serological screening is a simple and relatively risk-free method, and has been attracting increasing attention. A good screening index may increase positive detection rate and

Table V. Composition of DS pregnancy in different ranges of risk value.

Risk value range of DS	Number of screenings	Proportion of the total sample (%)	Number of pregnancies with DS	Proportion of pregnancies with DS (%)
≥1/300	11,636	5.3	79	66.95
1/1,000-1/300	30,997	14.0	28	23.73
<1/1,000	178,655	80.7	11	9.32

Down syndrome cases were mainly concentrated at high risk values (≥1/300). This contained the least percentage of the overall population (5.3%), but the majority of positive cases (66.95%). As regards risk values <1/300, it was observed that Down syndrome cases were primarily located in the range of 1/1,000-1/300 (23.73%), when compared with risk values <1/1,000 (9.32%) (P<0.01). This clearly demonstrates that the former only comprised 14.0% of the total sample, whereas the latter comprised 80.7%. Thus, residual screening cases were primarily concentrated at risk values 1/1,000-1/300. DS, Down syndrome.

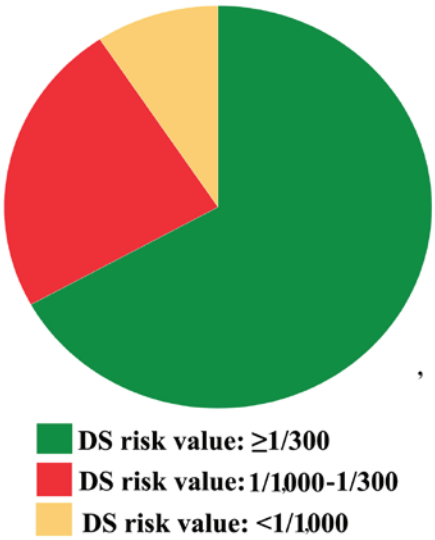

Figure 2. Proportion of Down syndrome in different risk value ranges. This demonstrates that the number of Down syndrome cases in the high-risk value range comprised the majority (66.95%). Of the remaining cases, most pregnancies resulting in Down syndrome birth were concentrated in the moderate risk value range (1/1,000-1/300), constituting 23.73% of the total; and cases with risk values <1/1,000 included 11 Down syndrome births, constituting 9.32% of the total. DS, Down syndrome.

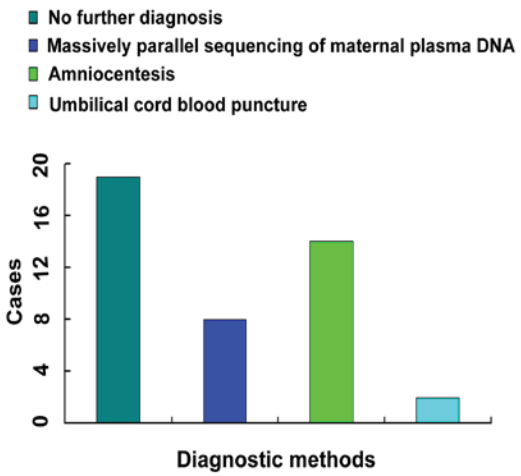

Figure 3. Analysis of the further diagnostic methods for Down syndrome cases with low risk values. In the 39 positive Down syndrome cases with low risk values, 18 cases were born due to disregard of the doctors' recommendation for further diagnostic investigation. Amniocentesis was the major method applied to confirm Down syndrome, and 12 cases were confirmed by this method. Massive parallel sequencing of maternal plasma DNA is a non-invasive method, and 7 cases of Down syndrome were diagnosed through this method, whereas only 2 cases underwent umbilical cord puncture.

reduce missed Down syndrome pregnancies; therefore, close attention should be given to these serological markers.

There were differences in the medians of AFP and free β-HCG. Data provided by Wang *et al* (8) revealed that the medians of AFP were higher compared with those in Caucasian women for 15-20 weeks, which was different from our results; however, the medians of free β-HCG were similar, which was higher compared with the medians of the 2T software. They also reported that, using the total β-HCG (HCG) and AFP, the detection rate for Down syndrome was 50%, and that of triple screening was 66.7%, with a 5% false-positive rate. It was reported that, when using AFP and free β-HCG, the detection rate for Down syndrome was 56.25% by Lifecycle software (17). In our data, the detection rate is 63.6%, with a false-positive rate of 5.05%. It may be concluded that our detection rate was higher compared with the previously reported detection rate of double screenings. Although our laboratory medians differed from the medians embedded in the 2T software, there was no significant statistical impact on the detection rate or the false-positive rate; however, there was an increase in the detection rate and a decrease in the false-positive rate. Taking this finding into consideration, we recommend the use of our own medians of AFP and free β-HCG.

If risk values are >1/1,000, further diagnostic investigation is crucial. It is recommended that high-risk cases undergo amniocentesis or cordocentesis. Our results revealed that a risk value between 1/1,000-1/300 was the main range of residual cases. Thus, providing more rational suggestions may lead to fewer residual cases. Amniocentesis or cordocentesis are effective diagnostic methods, but are associated with a risk of miscarriage (~1%); however, NIPT is considered as a safe method (18). Subjects with results raising clinical suspicion who decline further diagnostic examinations constitute a major cause of Down syndrome births. Thus, if the results are abnormal, a doctor should be consulted as soon as possible.

Thorough screening and prenatal diagnosis of Down syndrome is crucial, as it may help reduce the financial burden

on families, reduce stress, and exert an overall beneficial effect on society. Further in-depth studies are required on this subject to design screening or diagnostic methods that are more accurate and cost-effective.

References

1. Smith M and Visootsak J: Noninvasive screening tools for Down syndrome: A review. Int J Womens Health 5: 125-131, 2013.
2. Avent ND: Maternal plasma biomarkers for down syndrome: Present and future. Drugs Today (Barc) 49: 145-152, 2013.
3. Alldred SK, Deeks JJ, Guo B, Neilson JP and Alfirevic Z: Second trimester serum tests for Down's Syndrome screening. Cochrane Database Syst Rev 13: CD009925, 2012.
4. Androutsopoulos G, Gkogkos P and Decavalas G: Mid-trimester maternal serum HCG and alpha fetal protein levels: Clinical significance and prediction of adverse pregnancy outcome. Int J Endocrinol Metab 11: 102-106, 2013.
5. Extermann P, Bischof P, Marguerat P and Mermillod B: Second-trimester maternal serum screening for Down's syndrome: Free beta-human chorionic gonadotrophin (HCG) and alpha-fetoprotein, with or without unconjugated oestriol, compared with total HCG, alpha-fetoprotein and unconjugated oestriol. Hum Reprod 13: 220-223, 1998.
6. Shaw SW, Hsu JJ, Lee CN, Hsiao CH, Chen CP, Hsieh TT and Cheng PJ: First- and second-trimester Down syndrome screening: Current strategies and clinical guidelines. Taiwan J Obstet Gynecol 47: 157-162, 2008.
7. Sayin NC, Canda MT, Ahmet N, Arda S, Süt N and Varol FG: The association of triple-marker test results with adverse pregnancy outcomes in low-risk pregnancies with healthy newborns. Arch Gynecol Obstet 277: 47-53, 2008.
8. Wang YY, Luo J, Zhu MW, Liu LN and Ma X: Second-trimester double or triple screening for Down syndrome: A comparison of Chinese and Caucasian populations. Int J Gynaecol Obstet 94: 67-72, 2006.
9. Sifakis S, Papantoniou N, Kappou D and Antsaklis A: Noninvasive prenatal diagnosis of Down syndrome: Current knowledge and novel insights. J Perinat Med 40: 319-327, 2012.
10. Nicolaides KH, Syngelaki A, Gil M, Atanasova V and Markova D: Validation of targeted sequencing of single-nucleotide polymorphisms for non-invasive prenatal detection of aneuploidy of chromosomes 13, 18, 21, X and Y. Prenat Diagn 33: 575-579, 2013.
11. Shaw SW, Chen CP and Cheng PJ: From Down syndrome screening to noninvasive prenatal testing: 20 years' experience in Taiwan. Taiwan J Obstet Gynecol 52: 470-474, 2013.
12. Ehrich M, Deciu C, Zwiefelhofer T, Tynan JA, Cagasan L, Tim R, Lu V, McCullough R, McCarthy E, Nygren AO, *et al*: Noninvasive detection of fetal trisomy 21 by sequencing of DNA in maternal blood: A study in a clinical setting. Am J Obstet Gynecol 204: 205.e1-11, 2011.
13. Baffero GM, Somigliana E, Crovetto F, Paffoni A, Persico N, Guerneri S, Lalatta F, Fogliani R and Fedele L: Confined placental mosaicism at chorionic villous sampling: Risk factors and pregnancy outcome. Prenat Diagn 32: 1102-1108, 2012.
14. Bamberg C, Fotopoulou C, Thiem D, Roehr CC, Dudenhausen JW and Kalache KD: Correlation of midtrimester amniotic fluid cytokine concentrations with adverse pregnancy outcome in terms of spontaneous abortion, preterm birth and preeclampsia. J Matern Fetal Neonatal Med 25: 812-817, 2012.
15. Guedj F and Bianchi DW: Noninvasive prenatal testing creates an opportunity for antenatal treatment of Down syndrome. Prenat Diagn 33: 614-618, 2013.
16. Van Opstal D and Srebniak MI: Cytogenetic confirmation of a positive NIPT result: Evidence-based choice between chorionic villus sampling and amniocentesis depending on chromosome aberration. Expert Rev Mol Diagn 16: 513-520, 2016.
17. Yu DY, Fu P, Zhang ZH, Wang F, Han MY, Ren HY, Zhao W, Zhang K, Li S and Jiang N: Evaluation of Down's syndrome screening methods using maternal serum biochemistry in the second trimester pregnancy. Zhonghua Yi Xue Yi Chuan Xue Za Zhi 28: 332-335, 2011 (In Chinese).
18. Lim JH, Park SY and Ryu HM: Non-invasive prenatal diagnosis of fetal trisomy 21 using cell-free fetal DNA in maternal blood. Obstet Gynecol Sci 56: 58-66, 2013.