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Analysis of Down syndrome failed to be diagnosed after prenatal screening

A multicenter study

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Abstract

To analyze the characters of Down syndrome (DS) who failed to be diagnosed after prenatal screening and hope to be able to improve the programs of prenatal screening and reduce the missed diagnosis of DS. In this multicenter study, we collected the missed cases from 3 prenatal diagnosis centers and analyzed their characters. A total of 126 DS babies failed to be diagnosed after prenatal screening. Their mothers accepted the prenatal screening in second trimester. We collected the mothers' blood and detected the levels of alpha-fetoprotein (AFP) and the free beta subunit of human chorionic gonadotropin (fβhCG) by time-resolved fluoroimmunoassay. The values were also presented as multiples of the median (MoM) and determined the risk of carrying a fetus with DS by Wallace LifeCycle Elipse analysis software. Compared with normal control group, the level of fβhCG and hCG MoM were dramatically increased, while AFP and AFP MoM were decreased. The area under the receiver-operating-characteristic curve of trisomy 21 was 0.8387 for hCG-MoM and AFP-MoM testing. The sensitivity, specificity, positive predictive value, and negative predictive value were 84.6%, 74.8%, 75.4%, and 83.6%, respectively. Meanwhile, the prediction mode was "0.39957 + 1.90897*HCG-MOM −3.32713*AFP-MOM". It was worthwhile noting that the risk of 65.9% DS missed diagnosis group were higher than 1/1000, 92.9% higher than 1/3000. However, 72.5% cases in normal control group were lower than 1/3000. Only 9.2% mothers would be higher than the value of risk in 1/1000. The prediction mode of hCG MoM and AFP MoM might be able to help us reduce the missed diagnosis. It is also necessary to adjust more reasonable range of noninvasive prenatal testing with further clinical researches.

Abbreviations: AFP = alpha-fetoprotein, DR = rate of detection, DS = Down syndrome, fβhCG = free beta subunit of human chorionic gonadotropin, FPR = false-positive rate, MoM = multiples of the median, NIPT = noninvasive prenatal testing, PAPP-A = pregnancy-associated plasma protein A, ROC curve = receiver-operating characteristic curve, uE3 = unconjugated estriol.

Keywords: biomarkers, Down syndrome, missed diagnosis, prenatal diagnosis, prenatal screening, serum

1. Introduction

Down syndrome (DS), also named as trisomy 21 syndrome, is one of the most common chromosomal diseases with inherited mental disability. It was first described by Lang Down in 1886, and it was confirmed that the disease was caused by 3 chromosome 21 until 1959. The main pathogenesis of DS was that the chromosome 21 does not separate during meiosis. According to epidemiological studies, the incidence of DS was about 1/700,[1] there were about 200 thousand cases increase in the global every year. Mental retardation was the most prominent and serious problem, which resulted in the loss of cognitive abilities and the development of

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TJ and JD have contributed equally to the article.

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Authors' contribution statement: BY, TW, and TJ carried out the assays and participated in designing the study. JD, X-QZ, X-JZ, and BZ carried out laboratory tests, participated in designing the study and performed the statistical analysis. BY, TW, and TJ conceived the study, participated in its design, and coordination and helped draft the manuscript.

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early onset Alzheimer's disease.[2] Most of the patients had mental retardation, and abstract thinking ability was the most serious injury. With the increase of age, low intelligence gradually was more obvious. Recently, the survival time of DS has been greatly improved due to the development of medical technology and the enhancement of social and humanistic care. However, the patient still could not take care of themselves, which bring heavy mental and economic burden to the family and the society. The problems concerning cognitive mental retardation have become more important than those symptoms with DS.

Until now, there is a lack of effective treatments for DS. Prenatal screening and diagnosis was an effective way to prevent the birth of children with DS. In the past 3 decades, it has been widely used worldwide[3,4] and achieved good achievement. The most common prenatal screening method is a combination of serum levels of alpha-fetoprotein (AFP), unconjugated estriol (uE3), and the free beta subunit of human chorionic gonadotropin (fβhCG) with maternal age in the second trimester.[5,6] According to most researches, the rate of detection (DR) was 75% along with a 5% false-positive rate (FPR).[7] In China, the DR was about 50% to 67% according to a multicenter study.[8–10] Recently, more scholars suggested a new screening method performed between 8 and 13 weeks of gestational age.[9] This new test consists of the concentration of fβhCG and pregnancy-associated plasma protein A (PAPP-A), ultrasound measurement of nuchal translucency, and maternal age. Using this screening program, 75% to 85% of fetuses of DS could be detected with a FPR of 5%.[11–13] However, both the DR and FPR of these methods were in need being improved.

Because of the technical limitations of the prenatal screening, about 30% DS could not be found. In the present study, we have collected the missed cases from 3 prenatal diagnosis centers and analyzed their characters. With statistical analysis, we hope to be able to improve the programs of prenatal screening and reduce the missed diagnosis of DS.

2. Materials and methods

2.1. Patients and design

This multicenter retrospective study was conducted in the Changzhou Women and Children Health Hospital of Nanjing Medical University, Nanjing Women and Children Health Hospital of Nanjing Medical University, and Suzhou Municipal Hospital (China). From October 2002 to June 2015, we found a total of 126 babies of DS failed to be diagnosed in 3 centers after follow-up. Their mothers all accepted the programs of prenatal screening; however, we didn't found the abnormalities in the fetus. In present study, we took the 126 mothers as the research object, collected and analyzed their clinical parameters. Meanwhile, 131 mothers who had normal babies were selected as the normal control group. Both common parameters were shown in Table 1. Among 126 cases, 6 mothers were older than 35; however, they insisted on prenatal screening.

The study design and protocol were reviewed and approved by the ethics committee of Changzhou Woman and Children Health Hospital affiliated to Nanjing Medical University.

3. Methods

3.1. Samples collected

All of the subjects received the prenatal screening in second trimester after genetic counseling and informed consent. According to the operating program of prenatal screen, we collected the blood from every case in second trimester (15–20 weeks). Gestational age was calculated by each pregnant woman's last menstrual period or ultrasonography. An amount of 3 mL blood of all the cases were collected by simple needle aspiration. After being placed 0.5 to 2 hours at room temperature, the samples were centrifuged at 3000 rpm for 5 minutes to remove cells. The serums were stored at 4 °C and the levels of AFP, fβhCG were detected within 7 days, then they were long-stem stored at −80 °C.

3.2. Prenatal screening in second trimester

As Miao et al[14] described, the levels of AFP and fβhCG were quantified by time-resolved fluoroimmunoassay using Wallac 1235 AutoDELFIA (DELFIA1235: Perkin Elmer, Waltham, MA). The value were also presented as multiples of the median (MoM) and determined the risk of carrying a fetus with DS by Wallace LifeCycle Elipse analysis software (Perkin Elmer) in the second trimester.

High risk: T21 > 1/270, T18 > 1/350. Intermediate risk: T21 1/270 to 1/1000, T18 1/350 to 1/1000. Advanced age: maternal age ≥35.

3.3. Statistical analysis

The stratified analysis, the interaction test, covariate screening, curve fitting, and the receiver-operating characteristic curve (ROC curve) were performed using EmpowerStats ×64 software (X&Y solutions, Inc. Boston MA).[15] $P < .05$ was chosen to be statistically significant. Results of parameters were expressed as mean ± standard deviation for continuous variables with normal distribution median, 2.5th percentile, and 97.5th percentile for the data with abnormal distribution. *t* test and nonparametric test were employed to compare differences for continuous variables between 2 groups.

4. Results

In the past 13 years, a total of 126 DS babies have failed to be diagnosed in 3 prenatal diagnosis centers of Jiangsu Province. Their mothers accepted prenatal screening program. However, we did not discover their abnormal risk. Meanwhile, 131 mothers were selected as the normal control group after follow-up. There were no significant differences in maternal age, weight, and

Table 1

The baseline parameters of 2 groups in this study.

	DS missed diagnosis group	Normal control group	*P*
N	126	131	
Ethnicity (Chinese)	126	131	
Singleton pregnancy	126	131	
Maternal age, y	27.6 ± 4.2	27.0 ± 2.9	.132
Maternal weight, kg	57.2 ± 7.9	58.6 ± 8.7	.202
Gestational age, wk	17.0 (16.0–17.0)	17.2 (16.5–17.6)	.054
15–16 wk	11 (8.7%)	3 (2.3%)	
16–17 wk	40 (31.7%)	38 (29.0%)	
17–18 wk	48 (38.1%)	64 (48.9%)	
≥18 wk	27 (21.4)	26 (19.8%)	

The normal distribution data were expressed as mean ± SD. The abnormal distribution data were expressed as median (P2.5–P97.5). DS = Down syndrome, SD = standard deviation.

Table 2

Compared the value of fβhCG and AFP between 2 groups.

	DS missed diagnosis group	Normal control group	*P*
N	126	131	
fβhCG, ng/mL	21.9 (15.5–30.6)	11.1 (7.8–16.6)	<.001
hCG MoM	1.7 (1.2–2.1)	0.9 (0.6–1.2)	<.001
AFP, U/mL	30.9 (23.1–40.2)	38.5 (31.8–50.4)	<.001
AFP MoM	0.8 (0.6–1.0)	1.0 (0.8–1.2)	<.001

The abnormal distribution data were expressed as median (P2.5–P97.5). AFP = alpha-fetoprotein, DS = Down syndrome, fβhCG = free beta subunit of human chorionic gonadotropin, MoM = multiples of the median.

gestational age between 2 groups, and their parameters were shown in Table 1.

Compared with normal control group, the level of fβhCG and hCG MoM were significant increased, while AFP and AFP MoM were decreased (Table 2). By regression analyzed after adjusted for maternal age and weight, the odds ratios (ORs) and 95% confidence intervals were shown in Table 3. hCG-MoM was one of the dangerous factors in DS missed diagnosis (OR = 5.35). While the OR of AFP-MoM was 0.14. Because the fβhCG and AFP were closely related to the gestational age, we divided the cases into 2 groups according to the gestational week (17 weeks). Regardless of gestational age less than 17 weeks or more than 17 weeks, we got the same results as above. The data of every group were shown in Table 4. Meanwhile, Fig. 1 showed the scatter diagram of hCG-MoM and AFP-MoM according to the gestational week.

Table 3

Association of fβhCG and AFP levels with Down's syndrome missed diagnosis events.

	Total	Odds ratios*	95% CI	*P*
fβhCG, ng/mL	15.78 (10.24–25.20)	1.11	1.08–1.15	<.0001
hCG MoM	1.19 (0.75–1.76)	5.35	3.24–8.83	<.0001
AFP, U/mL	34.51 (27.11–44.70)	0.94	0.92–0.97	<.0001
AFP MoM	0.88 (0.69–1.12)	0.14	0.06–0.34	<.0001

AFP = alpha-fetoprotein, CI = confidence interval, fβhCG = free beta subunit of human chorionic gonadotropin, MoM = multiples of the median.

* Adjust for: Age; weight. Odds ratios and 95% confidence intervals are presented to show the risk of Down's syndrome.

Table 4

Compared the value of fβhCG and AFP according to gestational age.

	DS missed diagnosis group	Normal control group	*P*
<17 wk	51	41	
fβhCG, ng/mL	25.6 (18.7–35.4)	13.7 (8.0–19.3)	<.001
hCG MoM	1.7 (1.1–2.1)	0.9 (0.5–1.2)	<.001
AFP, U/mL	25.9 (21.0–34.2)	34.9 (30.8–43.2)	<.001
AFP MoM	0.8 (0.6–1.0)	1.0 (0.8–1.1)	.002
≥17 wk	75	90	
fβhCG, ng/mL	19.2 (14.2–26.3)	10.8 (7.6–14.1)	<.001
hCG MoM	1.5 (1.2–2.1)	0.9 (0.6–1.2)	<.001
AFP, U/mL	32.8 (26.0–42.5)	41.0 (32.5–53.5)	<.001
AFP MoM	0.8 (0.6–1.0)	1.0 (0.8–1.2)	<.001

The abnormal distribution data were expressed as median (P2.5–P97.5). AFP = alpha-fetoprotein, DS = Down syndrome, fβhCG = free beta subunit of human chorionic gonadotropin, MoM = multiples of the median.

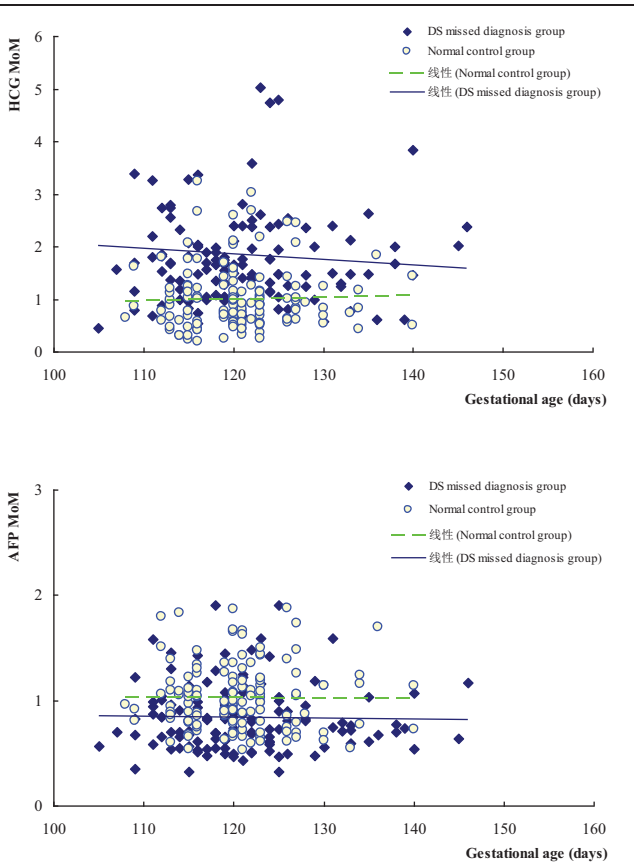

Figure 1. Compared the value of human chorionic gonadotropin-multiples of the median (MoM) and alpha-fetoprotein-MoM between 2 groups.

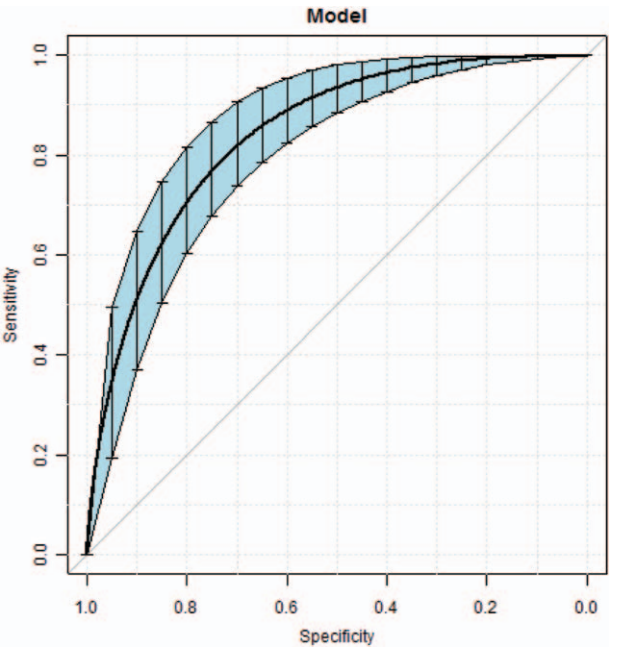

Figure 2. Prediction performance of human chorionic gonadotropin-multiples of the median (MoM) and alpha-fetoprotein-MoM for down syndrome screening.

Table 5

Compared the distribution of 2 groups according to DS-risk.

	DS missed diagnosis group	Normal control group
N	126 (100%)	131 (100%)
<300	5 (4.0%)	0 (0%)
300–500	34 (27.0%)	5 (3.8%)
500–1000	44 (34.9%)	7 (5.3%)
Total (<1000)	83 (65.9%)	12 (9.2%)
1000–2000	23 (18.3%)	15 (11.5%)
2000–3000	11 (8.7%)	9 (6.9%)
Total (<3000)	117 (92.9%)	36 (27.5%)
>3000	9 (7.1%)	95 (72.5%)

DS = Down syndrome.

Based on the changes of hCG-MoM and AFP-MoM, we calculated their primary outcome for trisomy 21 screening by the area under the ROC curve (AUC). As shown in Fig. 2, the AUC for trisomy 21 was 0.8387 by hCG-MoM and AFP-MoM testing. The sensitivity, specificity, positive predictive value, and negative predictive one were 84.6%, 74.8%, 75.4%, and 83.6%, respectively. Meanwhile, the prediction mode was "0.39957 + 1.90897*HCG-MOM −3.32713*AFP-MOM."

The value of risk is the most important index in prenatal screening program, so we analyzed the distribution of 2 groups according to DS-risk. It was worthwhile noting that the risk of 65.9% DS in missed diagnosis group were higher than 1/1000, 92.9% higher than 1/3000. However, 72.5% cases in normal control group were lower than 1/3000. Only 9.2% mothers would be higher than the value of risk in 1/1000. Table 5 and Fig. 3 showed the characteristic of DS-risk clearly.

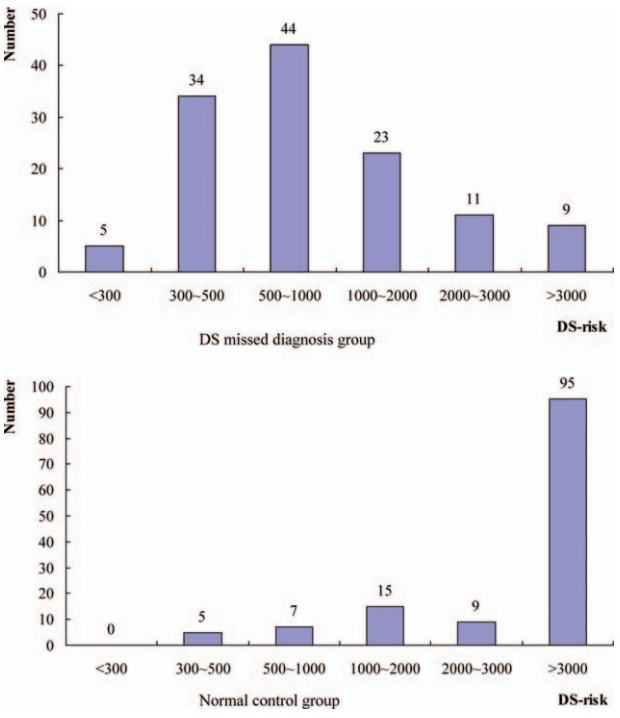

Figure 3. The distribution of 2 groups according to Down syndrome-risk.

5. Discussion

It is well known that prenatal screening can contribute to avoidance the birth of DS. However, it needs improving greatly. Because of the technical limitations of the prenatal screening, about 30% DS would failed to be diagnosed. The error source of prenatal screening test was related to many factors, including the affected factors of the indexes of AFP, fβhCG, uE3, PAPP-A, the population data which we used to calculated the risk, the establishment of MoM, methodology selection, experimental error, and so on. A local mathematical algorithm has been calculated in order to improve the second trimester strategy of prenatal screening for fetal aneuploidies. Currently, we calculated the risk by lifecycle4.0, which used the data of Caucasian population because we still do not have the data of Chinese pregnant women. However we still got the DR was 62.0% for trisomies 21 in the second trimester, which was similar to other studies.

In present study, we collected the DS whose mothers received the prenatal screening program from 3 centers. A total of 126 DS babies were unfortunately born after prenatal screening. Maybe it helps to improve the effectiveness of prenatal screening by analyzing the data of these cases better.

At first, we found that the level of fβhCG and hCG MoM were dramatically increased, while AFP and AFP MoM were decreased in DS missed diagnosis group. Although 2 indexes related to the gestational week, we did not found these changes above were due to the gestational age. In the common prenatal screening program, we calculated the DS-risk combined with maternal age, gestational week, the concentration of fβhCG, and AFP. We have already considered the effectuation of the value hCG MoM and AFP MoM to DS-risk. However, maybe we could make use of them better to improve prenatal screening program. As you can see, the area under the ROC for primary outcome of trisomy 21 screening was 0.8479. The sensitivity, specificity, positive predictive value, and negative predictive value were 84.6%, 74.8%, 75.4%, and 83.6%, respectively. Meanwhile, we also got a prediction mode which might be able to help us reduce the DS's missed diagnosis. We thought we should used the hCG-MoM, AFP-MoM and the prediction model better to improve the effectiveness of screening. However, whether it could be 1 index for prenatal diagnosis still needs more validation by clinical data.

Second, the characteristic of the distribution according to DS-risk was worthwhile noting. We found the risk of 65.9% in DS missed diagnosis group were higher than 1/1000, 92.9% higher than 1/3000. Only 9.2% normal mothers would be higher than the value of risk in 1/1000. So in theory, if we adjust the cutoff of prenatal diagnosis in 1/3000, we can find about 93% of the DS fetus, but it will also make 27% of the normal mothers receive unnecessary test.

Recently, noninvasive prenatal testing (NIPT) for common fetal aneuploidies was proved to be a better prenatal screening program, which was detected cell-free Deoxyribonucleic acid obtained from maternal plasma by massively parallel sequencing. Nowadays, NIPT was widely used in prenatal screen for T21, T18, T13, and presented good accuracy.[16] The American College of Obstetricians and Gynecologists,[17] International Society for Prenatal Diagnosis[18] have issued the committee opinions and guidelines about the clinical application of NIPT, and they also recommend the patients of high risk group to accept NIPT. In 2015, Chinese scholars firstly suggested the mothers whose DS screening results were intermediate risk (1/300–1/1000) to accept NIPT in order to reduce the missed diagnosis. The

views and our results are consistent. If the mothers whose DS-risk are higher than 1/1000 could accept NIPT, it could reduce the birth of 65% DS cases. We can get higher DR by adjusting the cut-off value to 1/3000, but it also brings more false positive results. Therefore, the results of the study could contribute to select the population of NIPT more reasonably and it is necessary to adjust more reasonable range of NIPT with further clinical researches.

In conclusion, we collected the missed DS cases from 3 prenatal diagnosis center and analyzed their characters and found the prediction mode of hCG MoM and AFP MoM might be able to help us reduce the missed diagnosis. It is also necessary to adjust more reasonable range of NIPT with further clinical researches.

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References

[1] Roizen NJ, Patterson D. Down's syndrome. Lancet 2003;361:1281–9.
[2] Nieuwenhuis-Mark RE. Diagnosing Alzheimer's dementia in Down syndrome: problems and possible solutions. Res Dev Disabil 2009;30: 827–38.
[3] Wald NJ. Prenatal screening for open neural tube defects and Down syndrome: three decades of progress. Prenat Diagn 2010;30:619–21.
[4] Maxwell S, Bower C, O'Leary P. Impact of prenatal screening and diagnostic testing on trends in Down syndrome births and terminations in Western Australia 1980 to 2013. Prenat Diagn 2015;35:1324–30.
[5] Cuckle HS, Wald NJ, Thompson SG. Estimating a woman's risk of having a pregnancy associated with Down's syndrome using her age and serum alphafetoprotein level. BJOG 1987;94:387–402.
[6] Wald NJ, Cuckle HS, Densem JW, et al. Maternal serum screening for Down's syndrome in early pregnancy. BMJ 1988;297:883–7.
[7] Spencer K. Second trimester prenatal screening for Down's syndrome using alpha-fetoprotein and free beta hCG: a seven year review. BJOG 1999;106:1287–93.
[8] Wang YY, Luo J, Zhu MW. Second-trimester double or triple screening for Down syndrome: a comparison of Chinese and Caucasian populations. Int J Gynaecol Obstet 2006;94:67–72.
[9] Liu QL, Hu YL, Xu ZF, et al. A study on population-based prenatal screening and diagnosis of Down's syndrome in Jiangsu Province. Zhonghua Yi Xue Yi Chuan Xue Za Zhi 2010;27:340–2.
[10] Hu YL. Serum screening of fetal chromosome abnormality during second pregnancy trimester: results of 26,803 pregnant women in Jiangsu Province. Zhonghua Yi Xue Za Zhi 2007;87:2476–80.
[11] Cuckle H, Benn P, Wright D. Down syndrome screening in the first and/or second trimester: model predicted performance using meta-analysis parameters. Semin Perinatol 2005;29:252–7.
[12] Schielen PC, van Leeuwen-Spruijt M, Belmouden I, et al. Multi-centre first-trimester screening for Down syndrome in the Netherlands in routine clinical practice. Prenat Diagn 2006;26:711–8.
[13] Wright D, Spencer K, Nix B. First trimester screening for Down syndrome using free beta hCG, total hCG and PAPP-A: an exploratory study. Prenat Diagn 2007;27:1118–22.
[14] Miao ZY, Liu X, Shi TK, et al. First trimester, second trimester, and integrated screening for Down's syndrome in China. J Med Screen 2012; 19:68–71.
[15] Wu J, Geng J, Liu L, et al. The relationship between estimated glomerular filtration rate and diabetic retinopathy. J Ophthalmol 2015;2015: 326209.
[16] Gil MM, Quezada MS, Revello R, et al. Analysis of cell-free DNA in maternal blood in screening for fetal aneuploidies: updated meta-analysis. Ultrasound Obstet Gynecol 2015;45:249–66.
[17] [No authors listed].Committee Opinion No. 640: cell-free DNA screening for fetal aneuploidy. Obstet Gynecol 2015;126:e31–7.
[18] Benn P, Borrell A, Chiu RW, et al. Position statement from the Chromosome Abnormality Screening Committee on behalf of the Board of the International Society for Prenatal Diagnosis. Prenat Diagn 2015; 35:725–34.