Serological prenatal screening and diagnosis for Down syndrome

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Summary

Objective: This study aimed to review and summarize the recent first-trimester and second-trimester prenatal screening and sequential prenatal screening to analyze the role of the existing prenatal screening system in the secondary prevention of birth defects. *Materials and Methods:* This study included 3,665 cases of 14~20-week pregnant women that underwent prenatal screening using double serum alpha-fetoprotein (AFP) and beta-human chorionic gonadotropin (β-hCG) and ultrasound screening; 512 cases of 9~12-week pregnant women underwent triple serological detection of serum β-hCG, pregnancy-associated plasma protein A (PAPP-A), and nuchal translucency (NT) for early screening. *Results:* The overall screening was with a high-risk rate of 8.52%. Among 356 cases of high-risk pregnant women, a total of 308 cases underwent karyotype analysis of fetal amniotic fluid cells. Of these, five cases of trisomy 21, one case of trisomy 18 and one case of "47, XXY" were diagnosed; among 37 cases of neural tube defect (NTD)-affected high-risk pregnant women, one case of anencephalus, and one case of open spina bifida were diagnosed. *Conclusion:* The overall detection rate for chromosome abnormalities was about 3.25% in the existing screening system, which could effectively prevent these seriously teratogenic fetuses from being born.

Key words: Down syndrome; Prenatal screening; Prenatal diagnosis; Chromosome abnormality.

Introduction

Among about 20 million newborns in China every year, the increased congenital deformity and mentally disabled children reach up to 1.2 million. The birth defects account for about four to six percent of total born population every year. As one of the main means of the secondary prevention of birth defects, prenatal screening and diagnosis play an important role in the prevention of birth defects in the country at present. Down syndrome (DS) had become one of the main objects of prenatal intervention in various countries due to its high morbidity and incurable characteristics. The direct prenatal diagnosis for DS fetuses requires much human and material resources with higher risk, but cost-effective prenatal screening could be one of the schemes in solution of this problem. In the 1950s, Penrose *et al.* [1] found that DS was positively correlated to the ages of pregnant women [2, 3]. In 1977, Hook and Chambers firstly reported that pregnant women over 35 years old were considered high-risk pregnancies [4], so that age became the earliest indicator used in prenatal screening. In 1984, Cuckle *et al.* [5] firstly discovered that the maternal serum alpha-fetoprotein (AFP) levels were decreased in those with DS fetus, so that AFP was suggested to be a screening indicator for detection of fetal DS in pregnant women under 35 years of age. In 1987 and 1988, researchers successively found that the serum human chorionic gonadotropin (hCG) levels and unconjugated estriol (uE3) levels in pregnant women were correlated to DS. In 1988, Wald *et al.* from St Bartholomew's Hospital in London put forward a triple screening test using AFP, beta-hCG (β-hCG) and uE3 [6]. In 1994, the American College of Obstetricians and Gynecologists officially recommended this method to the nation's medical community, which was been applied ever since. With the in-depth study, it was gradually found that maternal age alone as a indicator was not comprehensive enough, which should be associated with the combined screening diagnosis of maternal serum marker or ultrasonic morphology. The combined free β-hCG and pregnancy-associated plasma protein A (PAPP-A) screening based on ages was the most certainly serological screening programme during early pregnancy but with a low DS detection rate, which was only 60% - 65% when the false positive rate was five percent [7, 8]. The development of genetics ultrasound technology and the determination of fetal nuchal translucency (NT), the first ultrasound screening indicator, were included in the most significant progresses in the prenatal screening in the 21st century, which directly promoted the development of prenatal screening during DS early pregnancy (maternal age + NT + free β-hCG + PAPP-A) and increased the detection rate to 85% (when the false positive rate was 5%) [9, 10]. However, the ultrasound indicator was with poor accuracy and repeatability compared to the maternal serum indicator for the influence of various factors on the accuracy of NT screening, including fetal position, body shapes of pregnant women, physicians' experience and technology, etc [11, 12]. Therefore, the high technical requirement and low detected flux of NT screening limited its large-scale screening applications, which was difficult to spread even in the developed countries. However, some studies had shown differences in marker levels between races [13-15]. In this study, the recent first-trimester and second-trimester prenatal screening and sequential prenatal screening in the present hospital were reviewed and summarized to analyze the role of the existing

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prenatal screening system in the secondary prevention of birth defects and establish standard screening criteria adapted to Central China.

Materials and Methods

Among a total of 4,177 pregnant women with single pregnancy for regular checks-ups from 2008 to 2011, there were 3,665 cases of second-trimester screening (14~20 weeks) and 512 cases of first-trimester screening (9~12 weeks), who were aged from 19 to 34 years old. This study was conducted in accordance with the Declaration of Helsinki and with approval from the Ethics Committee of the Third Affiliated Hospital of Xinxiang Medical University. Written informed consent was also obtained from all participants.

Second-trimester screening (14~20 weeks) was performed in 3,665 cases. The maternal serological screening in the second trimester used the AFP + β-hCG serum biochemical marker screening programme. Since chromosome aneuploidy was often associated with other malformations at the same time, such as the hygroma on the back of the neck, duodenal atresia, pyelectasia, cardiac abnormalities, choroidal cyst, polyhydramnios, etc., in this research multiple ultrasound markers were selected for screening based on double serum biochemical markers screening to improve the positive detection rate; 512 cases underwent the first-trimester screening (9~12 weeks) using triple screening test using β-hCG, PAPP-A, and NT.

The pregnant women undergoing screening had two ml venous blood drawn, fully under the principle of informed consent. After natural coagulation, the serum was separated and the specimen was stored at -20°C for specialist detection. The timed-resolved fluoroimmunoassay (TRFIA) detector and prenatal risk assessment system were used; The used kits included the PAPP-A quantitative determination kit, human alpha-fetoprotein (hAFP) quantitative determination kit, free β-human chorionic gonadotropin (free-β-hCG) quantitative determination kit, and free estriol (FE3) quantitative determination kit. The quality control was strictly performed according to the operating procedure to measure the coefficient of variation, which was repeated twice if there were abnormal results. The cutting value to distinguish standard DS from high-risk trisomy 18 was 1:275, and the cutting value to distinguish high-risk neural tube defects (NTD) was AFP MoM > 2.5. Pregnant women with regular menstruation before pregnancy (menstrual cycle of 28~30 days) had their gestational age age calculated according to the last menstrual period; those with irregular menstruation were calculated the risk according to B-gestational week. The screened high-risk pregnant women underwent amniocentesis at 18~24 weeks for conventional culture and karyotype analysis of fetal amniotic fluid cells, followed by the future prenatal diagnosis.

Results

Screening for prenatal diagnosis results of high-risk pregnant women

Among 4,177 pregnant women, there were 238 cases of high-risk DS, 81 cases of high-risk trisomy 18, and 37 cases of high-risk NTD, with an overall high-risk rate of 8.52%. Among 356 high-risk pregnant women, 308 cases underwent amniocentesis for karyotype analysis of fetal amniotic fluid cells and molecular genetic analysis at 18~24 weeks. Eventually, among 308 high-risk pregnant women underwent prenatal diagnosis, there were seven cases of chromosome aneuploidy diagnosed, including five cases of trisomy 21, one case of trisomy 18, and one case of "47, XXY". In addition, two cases of chromosome structural abnormalities (polymorphism) were found. Among 37 pregnant women with high-risk NTD, there was one case of anencephalus and one case of open spina bifida diagnosed by ultrasound.

Table 1. — *The range of normal values of three kinds of first-trimester screening markers.*

Gestaion weeks	NT (mm)	PAPP-A (∝g/ml)	β-hCG (∝g/ml)
9		9.17 ± 3.97	191 ± 14
10	2.28 ± 0.35	12.24 ± 0.79	177 ± 27
11	2.74 ± 0.37	16.78 ± 3.42	157 ± 19
12	3.09 ± 0.29	18.49 ± 3.77	119 ± 19
13	2.86 ± 0.39	22.03 ± 1.67	98 ± 21

Forty-eight pregnant women that refused prenatal diagnosis were successfully followed up. There was an unexplained stillbirth in one case, termination of pregnancy for merged with oligohydramnios and broaden bilateral fetal paraceles in one case, and no anomaly in other cases. In addition, it was also found that except for the incidence rate of chromosomal abnormalities, the incidence rate of merged ultrasound abnormalities in the high-risk screening group was also obviously higher than that in the control group, indicating the important and supplementary role of ultrasound in the screening of chromosome abnormalities. Among 3,821 low-risk pregnant women, there were a total of 3,808 cases (99.65%) successfully followed up. There were neonatal cleft lip and palate deformities in one case, simple congenital heart disease in one case, and odinopoeia for ultrasonic tips of fetal malformation in two cases. Until the end of statistics, the highest age of follow-up children was 2.4 years old. There was no missed case that had been found.

Due to the less domestic data of first-trimester screening accumulated, in this study, the range of normal values of three kinds of first-trimester screening markers are summarized in Table 1.

Discussion

The prenatal screening of DS was a very special group of preventive engineering [16]. The present study synthesized the current situation in the region of study, 4,177 pregnant women that underwent screening using the first-trimester triple indicators β-hCG, PAPP-A and NT or the second-trimester double indicators AFP and β-hCG serological indicators. High-risk pregnant women were 356, with an overall high-risk rate of 8.52%. The high-risk rate for DS was 5.70%, and close to reports in literatures [5, 17]. Forty-eight pregnant women refused the prenatal diagnosis and the other 308 high-risk pregnant women underwent amniocentesis for karyotype analysis of fetal amniotic fluid cells at 18~24 weeks, in which five cases of trisomy 21, one case of trisomy 18, one case of open neural tube defects (anencephalus), one case of sex-chromosome anomaly, and two cases of chromosome structural abnormalities were diagnosed, with an overall anomaly detection rate of 3.25%. In was indicated that the first-

trimester triple indicators β-hCG, PAPP-A, and NT or the second-trimester double indicators AFP and β-hCG serological indicators could effectively prevent the seriously teratogenic fetus from being born to achieve the desired purpose.

Recent researches indicated that the first-trimester and second-trimester combined sequential screening might have a higher efficiency compared to the traditional second-trimester screening [18], but the sequential screening had been attempted in the present study. Specifically, pregnant women underwent the first-trimester prenatal screening to give the first-trimester risk value. The high-risk ones were advised for prenatal diagnosis, while the low-risk and middle-risk ones were advised for second-trimester screening, which were followed according to prenatal diagnosis or not. However, patients were found with poor acceptability and mostly low-risk pregnant women in first-trimester prenatal screening did not accept or refused second-trimester screening. Therefore, there was a low second-trimester returning rate. Even some pregnant women refused the first-trimester screening for "not heard", which was also the common problem encountered in the implementation of sequential screening at home and abroad [19]. Finally, the statistical summary for sequential screening was abandoned because of the poor returning rate of first-trimester screening (less than 70%). It was indicated that the implementation of sequential screening in Henan region still needed to enhance the propaganda and education, as well as strengthen the learning and propaganda consciousness of new technologies and methods to medical staff. Therefore, expanded sample size was more expected in the early sequential studies to find out the more accurate standards in early screening compared to this study.

It was of great significance to popularize the prenatal screening for improvement of the quality of Chinese population. The result in the existing screening system was likely to be affected with screening method, screening programme, different races, different quality control, etc [20]. In addition, the false positives and negatives were also the major problems to be overcome. In recent years, some studies had also successively found that the inhibin A, cancer antigen 125 (CA-125), etc., were related to women pregnant with DS fetus, which might also be gradually applied to the prenatal screening for DS. In recent two years, in addition, the new DS screening using of free fetal DNA in maternal plasma was a comprehensive innovation from principle to technology, which would be the important direction in the authors' future researches. It was believed that as the continuous progresses of various technologies, the occurrence of DS would be better controlled.

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