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ORIGINAL ARTICLE

First-Trimester Contingent Screening for Trisomy 21 by Fetal Nuchal Translucency and Maternal Serum Biomarkers and Maternal Blood Cell-Free DNA Testing

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Abstract Analysis of cell-free DNA in maternal blood has been proposed as a novel screening method for evaluation of aneuploidies. The higher performance of this technique in screening of trisomies compared to all currently available methods would lead to widespread use of this technique in clinical settings. In total, 1,066,829 singleton pregnancies referred to Nilou Clinical Laboratory were screened for chromosomal trisomies during a period of 12 years. First-trimester screening (FTS), Triple and Quad markers of second-trimester screening (STS) as well as integrated results have been obtained from 444,515, 34,984, 560,857 and 26,473 singleton pregnancies respectively. Non-invasive prenatal test (NIPT) using cfDNA was applied in 3500 pregnant women. Risk cutoffs, detection rates (DRs) and false positive rates (FPRs) were assessed for combinations of screening strategies to identify the most efficient strategy for contingent cfDNA testing. Contingent screening including FTS and NIPT offer to 20% of cases would lead to detection of 98% of fetuses with trisomy 21 at a total invasive testing rate of 1.1%. Contingent screening including STS and NIPT offer to 9.0% of cases would lead to detection of 95.5% of fetuses with trisomy 21 at a total invasive testing rate of 4.5%. Contingent screening including FTS or STS and cfDNA testing are efficient strategies for screening of trisomy 21.

Keywords Cell free DNA · Trisomy · First trimester screening · Second trimester screening · NIPT · Detection rate

Introduction

Considering the role of chromosomal abnormalities including trisomies 21, 18 and 13 and sex chromosome aberrations in developmental delay in children, definite parental diagnosis should be available to prospective parents. However, procedure-related complications following invasive diagnostic tests have led to establishment of screening strategies with the potential of lowering invasive test offers [1]. Combination of maternal age, maternal serum-freeβ-human chorionic gonadotropin (fβ-hCG), pregnancy-associated plasma protein-A (PAPP-A) and fetal nuchal translucency thickness (NT) at 11 to 13 + 6 weeks, is the main strategy for first-trimester screening (FTS) of frequent chromosomal aneuploidies leading to detection of 79–96% of abnormal cases for a false-positive rate (FPR) of 3–5% based on the timing of serum biochemistry testing and measurement of NT [2]. Second-trimester screening (STS) which was introduced earlier than FTS exploits maternal serum markers hCG or free β-hCG, alpha-fetoprotein (AFP) and unconjugated oestriol (uE3) ("Triple" test) or plus inhibin A ("Quad" test) [3]. In STS, the risks for neural tube defects (NTDs) and Smith Lemli Optiz Syndrome (SLOS) are assessed in addition to common trisomies [4]. A sequential strategy has also been suggested that has both FTS and STS. This approach includes PAPP-A and NT measurement in the

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first trimester together with the second trimester Quad test markers ("Integrated" test) [3]. On the other hand, analysis of cell-free DNA in maternal blood has been proposed as a novel screening method for evaluation of aneuploidies. The higher performance of this technique in screening of trisomies 21 and 18 compared to all currently available methods would lead to widespread use of this technique in clinical settings. The only obstacle in this regard is the high price for this test [5] which limits its application as a first-line method [6]. Consequently, cfDNA testing contingent on the results of the combined test at 11–13 weeks' gestation has been suggested as a strategy to yield a very high DR and very low invasive testing rate at a noticeably lower cost compared to first line cfDNA testing [6]. Preserving the benefits of FTS by ultrasound such as precise pregnancy dating and early identification of numerous major fetal abnormalities is another advantage of such contingent strategy [6]. Here, we report the result of a 12-year retrospective study to evaluate the performance of available screening strategies for chromosomal abnormalities and assess risk cutoffs, DRs and FPRs for combinations of biomarkers to identify the best strategy for contingent cfDNA testing.

Materials and Methods

In the present retrospective study, we collected the results of FTS, Triple and Quad markers of STS as well as integrated results from 444,515, 34,984, 560,857 and 26,473 singleton pregnancies undergoing screening for aneuploidies in Nilou Clinical Laboratory, Tehran, Iran, between September 2004 to May 2016 respectively. In addition, non-invasive prenatal test (NIPT) using cfDNA was applied in 3500 pregnant women based on American college of obstetricians and gynecologists (ACOG) recommendations [7]. In brief, women over the age of 35, women with a positive results in any screening tests (FTS, STS, integrated or sequential screening test), pregnancies with a marker in solography indicative of aneuploidies, positive personal or family history of trisomic pregnancy or pregnancies that had not been screened for trisomies till 22 weeks of gestation were offered for NIPT. Singleton pregnancies with gestational age of at least 10 weeks entered the study. Exclusion criteria were history of blood transfusion in recent years, bone marrow transplantation or pregnancy with donated ovum. Informed consent was obtained from all participants. Approximately one-third of women had the results of FTS with the remaining having STS results before application of NIPT. All clinical and demographic data including maternal weight and height at the time of blood sampling, ultrasonographic parameters and biochemical results were collected by trained personnel and recorded. Maternal age, race, method of conception (natural, the use of ovulation drugs or in vitro fertilization), cigarette smoking during pregnancy (yes or no), pre-existing maternal illnesses and obstetric history were recorded in questionnaires and then reviewed by a doctor as a part of counseling session. The results of serum free β-hCG, PAPP-A, AFP, uE3 and inhibin A which were assessed by automated machines were also collected from previously available database. In addition, ultrasound parameters including measurement of fetal crown–rump length (CRL) as well as fetal NT thickness were also collected. The patient-specific risks for trisomies 21 were calculated from a combination of maternal age, fetal NT and serum free β-hCG and PAPP-A for FTS; β-hCG, AFP and uE3 ("Triple" test) or β-hCG, AFP and uE3 and inhibin A ("Quad" test) for STS. Furthermore, for a subset of patients the sequential strategy was applied that included PAPP-A and NT measurement in the first trimester together with the second trimester Quad test markers ("Integrated" test). When the screening results proposed a high risk pregnancy, women were offered chorionic villus sampling (CVS) or amniocentesis for fetal karyotyping. The final screening strategy was based on a combination of FTS and cfDNA testing. cfDNA testing was performed using next-generation sequencing on ion semiconductor platforms by using IONA kits from Premaitha Co., Ltd., Manchester, England. In this method we have 6 steps: Plasma separation, DNA extraction, automated library construction, library amplification and enrichment, library DNA sequencing and finally automated data bioinformatics analysis. In brief, in this strategy all women underwent screening according to a combination of maternal age, fetal NT and maternal serum biochemistry. Those with a risk above a high cut-off were regarded as screen positive and those with a risk below a low cut-off were considered as screen negative. For patients with an intermediate risk (between the upper- and lower-risk cut-offs) cfDNA testing was applied. The cut offs used to determine intermediate risk for trisomy 21 in FTS and STS were 1:51–1:2500 and 1:251–1:800 respectively. They were considered as screen positive if the result was abnormal or uninformative and screen negative if the result was normal. All pregnancies were followed-up until the delivery and the outcome of the pregnancy was recorded. All relevant complications including miscarriage, premature delivery and low birth weight were also reported. FPR, false negative rate (FNR), detection rate (DR), and odds of being affected given a positive result (OAPR) were determined after the follow-up period. True positive cases were detected through amniocentesis in the presence of positive screening results, while false negative cases were detected through karyotype analysis of infants.

In addition, we analyzed the data of STS and integrated protocol to identify the cut-offs and FPR of contingent cfDNA testing strategies based on the results of these protocols.

Statistical Analysis

Bayes theorem was applied for the computing FTS and STS-based risks, by combining the likelihoods of trisomy with the maternal age-specific prior risk of each trisomy at certain gestational age [8]. The subsequent risks were compared with the risk cut-off to acquire an age-specific DR for each year of maternal age, from 12 to 50 years. Standardized FPRs were calculated by finding the likelihoods in unaffected pregnancies and then applying these to each year of maternal age, from 12 to 50 years, to appraise the age-specific FPRs. The risk distributions were based on prospective screening rather than modeling. OAPR was calculated from the numbers predicted after applying the cfDNA rates to the observed numbers. Statistical analyses were performed using DIANASoft (BioChem, ImmunoSystemes, France) and SPSS Statistics software version 21 (IBM Corporation, Armonk, NY, USA).

Results

We collected values of NT and serum biomarkers of first trimester screenings from 444,515 singleton pregnancies, Triple and Quad markers of second trimester screenings of 34,984 and 560,857 singleton pregnancies as well as sequential and integrated results of 26,473 singleton pregnancies. The total of 1,066,829 study population included 1337 cases of trisomy 21. The mean age and weight of study participants was 28.9 ± 4.7 years and 65 ± 6.7 kg respectively. About 17.8% of participants were over 35 years old. The NT and CRL values of the participants were 1.9 ± 0.3 and 65 ± 9.8 mm respectively.

After first-line screening based on FTS, STS and integrated tests, the patients were divided into a high-risk requiring invasive testing, an intermediate-risk group that went through cfDNA testing and a low-risk group that had no additional testing. Among total number of pregnant women who underwent NIPT (3500), 32 were identified as being trisomy 21. After exclusion of the six failed samples, the false-positive rate for local NIPT was 2/3464 (0.058%). Table 1 shows the prospective distribution of risks for FTS, quad and integrated protocols, and predicted DR, IR and OAPR for a contingent cfDNA protocol based on the local cfDNA results (detection rate = 100%, false-positive rate = 0.058%).

Invasive tests confirmed the presence of trisomy 21 in 30 cases. In FTS, at the upper- and lower-risk cut-offs of 1:50 and 1:2500 respectively, the high-risk group (> 1:50) comprised 0.93 and 71.8% of euploid and trisomy 21 cases respectively. In contingent screening including FTS and NIPT offer to 20% of cases would lead to detection of 98% of fetuses with trisomy 21 at a total invasive testing rate of 1.1%.

Discussion

In this study, we have outlined risk cut-offs, DRs and FPRs for first-line screening for trisomy 21, using FTS, STS and integrated tests as the basis of identification of cases needing cfDNA testing. We have shown that in contingent screening, a DR of 98% in fetuses with trisomy 21, at an overall invasive testing rate of 1.1%, could be achieved by offering the cfDNA test to about 20% of cases detected by first-line screening using the FTS. Using STS as the first-line screening, if the intermediate risk group is defined at the upper- and lower-risk cut-offs of 1:251 and 1:800, respectively; with offering the cfDNA test to 9.3% of total cases, the DR of 95.5% of trisomy 21 cases can be achieved. Finally, in the integrated protocol, by defining the intermediate risk group at the upper- and lower-risk cut-offs of 1:101 and 1:1000, with offering the cfDNA test to 8.8% of total cases, the DR of 98% of trisomy 21 cases can be attained.

The substitute strategy of first-line cfDNA testing for all cases is expected to identify 99% of cases of trisomies 21 with a total FPR of 0.7% and invasive test rate of 1%. In the current study, we outlined the intermediate-risk group based on the cut-offs of 1:51 and 1:2500. Screening for trisomies 21 based on FTS protocol described above in all pregnancies, subsequent invasive testing in the high-risk group (≥ 1:50) and cfDNA testing in the intermediate-risk group (1:51–1:2500) can practically identify about 98% of cases, respectively, with a total FPR of 1.1%.

The current study was a retrospective study performed in a single center which might affect the validity of generalization of its results. In addition, as in the recent decades prediction of trisomy 21 risk has been the main factor in establishment of strategies of screening for aneuploidies, in the current study the results of first trimester contingent screening were provided for trisomy 21 solely in order to lessen the complexity of statistical analyses.

In the current study we have also compared prevalence, DR, FPR and OAPR of trisomy 21 with previous studies [9–12]. The higher prevalence of trisomy 21 in the current study compared with the previous studies might be due to the referral status of our laboratory which leads to referral of high risk pregnancies detected in other labs or the

Table 1 Prospective distribution of risks for FTS, quad and integrated protocols, and predicted DR, IR and OAPR for a contingent cfDNA protocol

Protocol (total)	Risk group (intermediate)	Prospective		Predicted: invasive			Predicted: rates
		T21	Unaffected	T21	Unaffected	All	
FTS (444,515)	> 1:50	419	3715	419	3715	4134	DR = 98%
	1:51–1:2500 (20%)	253	86,561	253*	50**	303	IR = 1.0%
	< 1:2500	14	353,553	–	–	–	OAPR = 1:6
	All	686	443,829	672	3765	4437	
Quad (560,857)	> 1:250	669	24,065	669	24,065	24,734	DR = 95%
	1:251-1:800 (9.0%)	115	50,306	115*	29**	144	IR = 4.4%
	< 1:800	37	485,665	–	–	–	OAPR = 1:31
	All	821	560,036	784	24,094	24,878	
Integrated (44,179)	> 1:100	27	264	27	264	291	DR = 98%
	1:101–1:1000 (4.5%)	14	20,014	14*	12**	26	IR = 0.7%
	< 1:1000	1	23,859	–	–	–	OAPR = 1:7
	All	42	44,137	41	276	317	

Invasive prenatal diagnosis in high risk group and following a positive cfDNA result in the intermediate group

DR detection rate, *IR* invasive prenatal diagnosis rate, *OAPR* odds of being affected given a positive result

*Based on 100% cfDNA detection rate in 30 T21 pregnancies

**Based on 0.058% cfDNA false-positive rate in 3464 unaffected pregnancies

Table 2 The comparisons between the current study and mentioned studies in terms of DR, invasive test rate and cfDNA test referral rate for trisomy 21

Criteria	Present study	Nicolaeides et al.	Kagan et al.
Cut off for intermediate group	1:51–1:2500	1:101–1:5000	1:11–1:3000
Invasive tests rate (%)	1.1	1.0	0.8
cfDNA test referral rate (%)	19.7	35.0	19.2
Detection rate (%)	98.02	98	96.5

observed difference in age distribution of pregnant women in our study compared with the previous ones (17.8% of pregnant women aged more than 35 years compared with 5–15% with the same age reported in previous studies [13]). The latter would lead to higher observed FPR in our study.

Several studies have noted the superiority of cfDNA testing for the screening of the most prevalent trisomies compared to previous methods of screening [14, 15]. Implementation of this test as the first-line screening test is hampered by its high cost as well as the possibility of deprivation from benefits of fetus ultrasound examination. Accordingly, cfDNA testing has been suggested to be kept for a certain subgroup of pregnant women [5]. In contingent screening, the group that would profit most from cfDNA testing are those with an intermediate risk. Two recent studies have aimed at identification of appropriate cut-offs for referral to cfDNA testing [5, 6]. Table 2 shows the comparisons between the current study and mentioned studies in terms of DR, invasive test rate and cfDNA test referral rate. The present study shows that with modulating cut-offs and referral of only 20% of patients for NIPT we can achieve a similar DR with comparable invasive test rate.

Screening for trisomy 21 by cfDNA testing contingent on the results of FTS and STS is an effective strategy for keeping the benefits of FTS and STS, but with a concurrent improvement in DR and reduction of invasive testing.

Authors' Contribution SY, MHM and SS designed the study and supervised it. SGF contributed in data analysis and wrote the manuscript. MMT, PS, SJ, PB, SD, FN, FA and SA carried out the study and provided patients' counseling.

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no competing interests.

Ethics Approval The procedures of the study received ethics approval from the institutional review board. All procedures followed were in accordance with the ethical standards of the responsible

committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000. Informed consent was obtained from all patients for being included in the study.

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