International Journal of Gynecology and Obstetrics xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

International Journal of Gynecology and Obstetrics

journal homepage: www.elsevier.com/locate/ijgo

www.figo.org

CLINICAL ARTICLE

Analysis of first-trimester combined test results in preparation for a cell-free fetal DNA era

Semir Kose [a,*], Dilek Cımrın [b], Nuri Yıldırım [a], Ozge Aksel [c], Pembe Keskinoglu [d], Elcin Bora [c], Tufan Cankaya [c], Sabahattin Altunyurt [a]

[a] Department of Obstetrics and Gynecology, Dokuz Eylul University School of Medicine, Balcova, Izmir, Turkey
[b] Central Clinical Laboratory, University Hospital, Dokuz Eylul University School of Medicine, Balcova, Izmir, Turkey
[c] Department of Medical Genetics, Dokuz Eylul University School of Medicine, Balcova, Izmir, Turkey
[d] Department of Biostatistics, Dokuz Eylul University School of Medicine, Balcova, Izmir, Turkey

ARTICLE INFO

Article history:
Received 15 February 2016
Received in revised form 23 May 2016
Accepted 2 August 2016

Keywords:
Cell-free fetal DNA
First-trimester combined test
Invasive diagnostic test
Noninvasive prenatal testing
Trisomy 21

ABSTRACT

Objective: To survey experience with the first-trimester combined test (FCT) for trisomy 21 (T21) in different risk score groups to determine the most useful clinical application of cell-free fetal DNA (cffDNA) screening. *Methods:* In a retrospective study, the records of FCT results obtained at a center in Turkey between January 2009 and January 2014 were reviewed. The FCT results and rates of uptake of invasive diagnostic testing were compared among different risk score groups. *Results:* FCT results were available for 4804 pregnancies; 276 (5.7%) had IDT results. Ten (72.7%) of 11 cases of T21 had a risk score of 1:300 or more. The IDT uptake rates were 54.5%, 51.9%, and 47.4% at risk scores of 1:100 or more, 1:200 or more, and 1:300 or more, respectively. In the group at intermediate risk (1:1001–1:3000), no pregnancy had an FCT result of both low pregnancy-associated plasma protein A and high free β-human chorionic gonadotropin, but 30 (3.9%) of 766 pregnancies had both advanced maternal age and high β-human chorionic gonadotropin. *Conclusion:* cffDNA screening should be used to optimize IDT uptake in pregnancies with a risk score of 1:101–1:1000. The selective power of the FCT diminishes beyond the 1:1001 score and cffDNA screening cannot yet be recommended routinely.

1. Introduction

The introduction of the first-trimester combined test (FCT) has brought about one of the largest paradigm shifts in prenatal diagnostic studies in the 21st century. With a detection rate for aneuploidies of greater than 90% [1,2] and the innovative contribution of nuchal thickness (NT) measurement as a triage tool, the FCT at 11^{+0}–13^{+6} weeks of pregnancy has turned the practice of prenatal diagnosis into a robust scientific profession.

The FCT is essentially a tool to predict the fetal genotype from the phenotypic features of the fetus and the maternal serum biochemistry. The prototype FCT results indicative of trisomy 21 (T21) consist of an advanced maternal age (≥35 years), an increased NT (above the 95th percentile), an increased level of free β-human chorionic gonadotropin (fβ-hCG; at least 1.90 multiples of the median [MoM]), and a decreased level of pregnancy-associated plasma protein A (PAPPA; <0.40 MoM) [3]. However, only some of these indicators are present in a fairly high percentage of T21-affected pregnancies, which complicates the screening procedure.

Cell-free fetal DNA (cffDNA) testing has begun to reset the standards of obstetric care, and the effort to incorporate this new technology into conventional prenatal care is ongoing [4–7]. This new paradigm has given rise to new challenges in prenatal counselling by changing the way FCT results are interpreted [8].

Indeed, the definition of a cutoff risk for further testing to diagnose T21 is a periodic issue, and the previously widely accepted cutoff risk score of 1:300 [2,9] has risen to 1:3000 in the current literature [4]. Cutoff levels are useful in determining the performance of the FCT in terms of false-positive and detection rates, but individual reactions to a given risk level vary greatly beyond policy makers' expectations [8,10]. Pretest and post-test counselling aims to optimize the parental reaction to the test result [10], but wide differences remain in the uptake of invasive diagnostic testing (IDT).

Two problematic aspects of FCT screening for T21 are the high number of false positives and the fairly wide—and seemingly ever increasing—range of risk scores that are interpreted as intermediate. The question of whether secondary screening using cffDNA testing

* Corresponding author at: Division of Perinatology, Department of Obstetrics and Gynecology, Dokuz Eylul University School of Medicine, Mithatpasa Caddesi, No:1606, Balcova, 35340, Izmir, Turkey. Tel.: +90 545 7668545; fax: +90 232 3901462.
E-mail address: semirkose@yahoo.com (S. Kose).

http://dx.doi.org/10.1016/j.ijgo.2016.05.014
0020-7292/

should be pursued for all pregnancies with an intermediate risk for T21 has triggered a discussion of the relevant science and ethics.

For these reasons, the present study analyzed the distribution of abnormal FCT markers in risk score groups (RSGs). The main objective was to examine T21 cases detected by FCT to find abnormal FCT markers that were common to all cases and to identify the outliers. A second goal was to assess the relationship between the risk score and the pregnant woman's decision to undergo an invasive genetic test. A third aim was to compare abnormal FCT markers in different RSGs and to discuss the efficacy of the FCT screening model and the rationale for secondary cffDNA screening in pregnancies with a risk for T21 of less than 1:1001. The findings might give clues as to how secondary cffDNA testing could be used to improve follow-up of the FCT results.

2. Materials and methods

The present study was conducted as a retrospective review of medical records at Dokuz Eylul University School of Medicine, Balcova, Izmir, Turkey. The study included singleton pregnancies for which an FCT was performed between January 1, 2009, and January 1, 2014. The start date was selected for the present study because, at that point, the pretest and post-test counselling program had become well established and was performed routinely. Any FCT results after January 1, 2014, were not included because, by this point, patients had begun to prefer noninvasive prenatal testing as a confirmatory test, and this complicated the analysis of the IDT uptake rates. The study included only pregnancies that were completely surveyed (sonographic measurements, laboratory analysis, pretest and post-test counselling, invasive procedure, and cytogenetic analysis) in the university hospital. The institutional ethics committee approved the present study. Given that this was a retrospective review of medical records, formal informed consent was not required.

The Central Laboratory of the Dokuz Eylul University School of Medicine provided the FCT results, and the Perinatology and Medical Genetics Departments provided the pregnancy follow-up records, which contained the counselling notes, the IDT proposals based on a cutoff level of 1:300, the final decision of the parents, and their written informed consent for invasive diagnostic testing.

Combined-test screening for T21 was performed between 11^{+0} and 13^{+6} weeks of pregnancy using the maternal age, fetal NT, and maternal serum concentrations of fβ-hCG and PAPPA for the risk calculation. The NT measurement was performed by certified physicians in accordance with the guidelines of the Fetal Medicine Foundation [11]. The pregnancy duration was determined on the basis of the fetal crown–rump length at the time of NT measurement. Maternal serum was sampled between 11^{+0} and 13^{+6} weeks of pregnancy, and the two serum markers were analyzed at the Endocrine Laboratory using the Immulite 2000 XPi (Siemens Healthcare Diagnostics, Deerfield, IL, USA) immunoassay system.

Cutoff levels were set to define the abnormal FCT components: advanced maternal age was defined as age 35 years or older, increased NT as 2.5 mm or higher, low PAPPA levels as 0.40 MoM or lower, and high fβ-hCG levels as 1.90 MoM or more. The risk for T21 was calculated using Prisca version 5.0 (Typolog Software, Tornesch, Germany). The risk scores were grouped as follows: 1:100 or more, 1:200 or more, 1:300 or more, 1:1000 or more, 1:1001–1:3000, and less than 1:3001.

The Student t test was used to compare the means between two independent groups, and the χ^2 test was used to compare the categorical variables and the IDT uptake rates. The analyses were performed using SPSS version 22 (IBM, Amonk, NY, USA). $P < 0.05$ was considered statistically significant.

3. Results

The study included 4804 pregnancies for which FCT results were available and 276 (5.7%) IDT results. The mean maternal age was 29.2 ± 5.1 years (range 15–48), the mean crown–rump length was 60.6 ± 8.9 mm (range 45–84), and the mean NT was 1.44 ± 0.44 mm (range 0.1–6.0). In total, 11 (0.2%) pregnancies were affected by T21. At a cutoff risk score of 1:300, the false-positive rate of the FCT was 1.9% and the detection rate (sensitivity) was 90.9%.

Overall, 88 (1.8%) pregnancies had a risk score of 1:100 or more, and 4165 (86.7%) pregnancies had a risk score of 1:1001 or less (Table 1). These figures show that the study population overall had a low risk [2]. With regard to the NT, there was an accumulation of data points between 1.01 mm and 2.00 mm, and six of the 11 fetuses with T21 were in this category. In total, 4042 (84.1%) pregnant women were younger than 35 years, and 123 (44.6%) women who underwent IDT were in this group. By comparison, 762 (15.9%) pregnant women were aged 35 years or more, and 153 (55.4%) women who underwent IDT were in this group. Within the group with a risk score of 1:300 or more, the frequency of IDT uptake did not differ between pregnant women less than 35 years and those 35 years and older (46.4% and 48.9%, respectively; $P = 0.705$). Of the 11 pregnancies affected by T21, 4 (36.4%) were in women who were younger than 35 years and 7 (63.6%) were in women who were aged 35 years or more.

The FCT characteristics of the pregnancies affected by T21 are presented in Table 2. In these pregnancies, the median PAPPA level was 0.41 MoM, the median fβ-hCG level—after correction for one woman with a very high fβ-hCG level of 6.32 MoM—was 1.92 MoM, the median NT was 1.89 mm, and the mean maternal age was 34.4 years.

The highest frequency of IDT uptake (54.5%) was observed in the group with a risk score of 1:100 or more, and this frequency decreased with declining risk (Table 1). When the frequency of IDT uptake was compared between the groups with risk scores of 1:100 or more, 1:200 or more, and 1:300 or more, the difference was statistically significant ($P = 0.016$); this result was attributable to the difference between the groups with risk scores of 1:100 or more and 1:300 or more ($P = 0.03$).

The IDT results and the distribution of abnormal FCT markers were assessed in two RSGs: the group with the traditional cutoff of 1:300 or more and the group with an intermediate risk (1:1001–1:3000). In the group with a risk score of 1:300 or more, 109 (47.3%) women underwent IDT, and 10 cases of T21 and 6 cases of other cytogenetic abnormalities were detected. In the group with a risk score of 1:1001–1:3000, 56 (7.3%) women underwent IDT. No cases of T21 were detected in this group, but two pregnancies were affected by other cytogenetic abnormalities (Table 1).

The frequency of abnormal markers was higher in the group with a risk score of 1:300 or more than in the group with an intermediate risk score, in terms of both individual markers (Table 3) and marker pairs (Table 4). In the group with a risk score of 1:1001–1:3001, free β-hCG was the most common abnormal marker, both when single markers were compared and when paired markers were compared. In this group, there were no pregnancies with a combination of low PAPPA (less than 0.40 MoM) and high fβ-hCG (more than 1.90 MoM), but there were many pregnancies with a combination of advanced

Table 1
Uptake and results of IDT by risk score from the first-trimester combined test (n = 4804).[a]

Risk score group	Frequency	IDT accepted or requested	Cytogenetic result		
			Normal karyotype	T21	OCA
≥1:100	88 (1.8)	48 (54.5)	37 (77.0)	7 (14.6)	4 (8.3)
≥1:200	156 (3.2)	81 (51.9)	68 (84.0)	8 (9.9)	5 (6.2)
≥1:300	230 (4.8)	109 (47.4)	93 (85.3)	10 (9.2)	6 (5.5)
≥1:1000	639 (13.3)	181 (28.3)	164 (90.6)	11 (6.1)	6 (3.3)
1:1001–1:3000	766 (15.9)	56 (7.3)	54 (96.4)	0	2 (3.6)
<1:3001	3399 (70.8)	39 (1.1)	39 (100.0)	0	0

Abbreviations: IDT, invasive diagnostic testing; T21, trisomy 21; OCA, other cytogenetic abnormalities.

[a] Values are given as number (percentage).

Please cite this article as: Kose S, et al, Analysis of first-trimester combined test results in preparation for a cell-free fetal DNA era, Int J Gynecol Obstet (2016), http://dx.doi.org/10.1016/j.ijgo.2016.05.014