

Cell-free DNA Results in Low Risk Pregnancy Screenings for Aneuploidies

Azadeh Mohammadpour Dehghani

Abstract

Introduction: Chromosomal aneuploidies are a common type of chromosomal defect that may impose heavy burdens on society and families. The most common chromosomal aneuploidies among the embryos are trisomies 21, 18, and 13. Prenatal screening and diagnosis are often used in prenatal care, and it seems to be a very useful tool for doctors and women to make informed decisions about the continuation of pregnancies involved with genetic diseases. Prenatal non-invasive tests using *cell-free fetal DNA* (cff DNA) are a method for testing trisomy in embryos using the peripheral blood sample of the pregnant mother. Considering the fact that most of the studies carried out on *cell-free DNA* have been in high-risk populations, this study attempted to examine the results of *cell-free DNA* in a population of low-risk aneuploidy pregnant women. **Materials and Methods:** In this retrospective study, the study population was all pregnant women (n= 410), who underwent aneuploidy screening during the first or second trimester (with a low-risk result in Quad test) in 2015- 2016 and were at the risk of trisomies 13, 18, and 21 evaluated using cell-free DNA test. **Results:** The mean age of pregnant women was 31.5 years and 92.0% of the subjects were naturally pregnant and only 8.1% had used Assisted Reproductive Techniques (ART). Of these, 5.9%

were fertilized by IVF and 2.2% by IUI method. Results of cf DNA in all pregnant women were negative. **Conclusion:** According to the results of this study, cf DNA testing results in all low risk pregnant women were negative and it is not recommended as a routine test in pregnant women reported as a low risk pregnancy in serum screening.

Keywords: Low-Risk Pregnancy; Aneuploidy Risk; Aneuploidy Screen; *Cell-free DNA*.

Introduction

Chromosomal aneuploidy is a common type of chromosomal defect that refers to the deviation from the *normal human chromosome complement* of 46. Fetal screening for chromosomal abnormalities is one of the mainstays of pregnancy care. Generally, women over the age of 35 are introduced for genetic counseling, while only 20% of Down's syndrome babies are born of women over the age of 30 years. With the advent of measuring the AFP maternal serum level in the mid-1980s, the pre-birth discriminant option for women younger than 35 years old was also created [1,2]. Common chromosomal aneuploidies are among the embryos of trisomy 21 (Down syndrome), trisomy 18 (Edward syndrome) and trisomy 13 (Patau syndrome). Trisomy 21 is the most common aneuploidy with an estimated prevalence of one in 700 births and its risk increases with maternal age. There is neither definitive treatment and nor effective treatment for these chromosomal aneuploidies. Therefore, identification of embryos with chromosomal aneuploidies is very important [3]. Prenatal noninvasive tests using *cell-free fetal DNA* (cff DNA) are a method for testing trisomy in embryos using

Resident, Department of
Obstetrics and
Gynecology, Student
Research Committee,
Ahvaz Jundishapur
University of Medical
Sciences, Ahvaz, Iran

Corresponding Author:
Azadeh Mohammadpour
Dehghani, Resident,
Department of Obstetrics
and Gynecology, Student
Research Committee,
Ahvaz Jundishapur
University of Medical
Sciences,
Ahvaz, Iran
E-mail:
azade1373@gmail.com

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a peripheral blood sample of a pregnant mother that have entered the market at present in 61 countries in Europe.

Fast measurements and non-invasive cff DNA can be used for many clinical applications, including gender identification, diagnosis of fetal aneuploidies, such as trisomies 13, 18 and 21, pregnancy complications, X-related diseases, cystic fibrosis, and several other single-gene diseases [5,6]. In *singleton* pregnancies, maternal blood- cff DNA testing can detect 99% of cases with trisomy 21, 97% of cases with trisomy 18, and 92% of cases with trisomy 13 with false positive rates of <0.1, <0.2 and 0.2%, respectively [7-9].

Materials and Methods

This retrospective study includes all pregnant women who underwent the first or second trimester (Quad test) aneuploidy screening during the years 2015 and 2016, and were reported as having low risk, as well as risk assessment of trisomy 13, 18, and 21 using the cff DNA test for the reasons of mother's high age, parents' request, history of chromosomal disorder in the family, and so on. The subjects were at least 18 years of age and at the time of collecting blood sample, had a pregnancy with a gestational age of 10-13 weeks and 6 days or the second trimester (20-14 weeks) in the quad test. The gestational age is based on the CRL of the NT measurement time. Patients who were outside the window of gestational

age did not have standard screening results, had known maternal aneuploidies, have been pregnant with the donor's ovule, or the empty gestational sack that was detected in ultrasound, were excluded from the study. All subjects underwent a standard screening (including PAPP serum measurements, Free or general β -hCG, and NT) or in the second trimester (UE3, Inhibin, Maternal AFP and β -hCG). *Aneuploidy risk* is estimated using a well-defined algorithm, including cfDNA chromosomal counts, cfDNA fetal fractions, and trisomy risk determined based on maternal and gestational age. A low-risk group is defined as pregnancies with a lower risk of less than 1 in 1500. If samples did not have lab quality control due to low cfDNA counts (<4%) or the inability to measure fetal cfDNA fractions, high variation in cfDNA counts, or failure of the test, they were not included in the analysis.

Results

In this study, 410 low risk pregnant women were examined. The demographic information about the subjects studied is presented in the table 1. The mean age of the women was 31.5 years. 75.4% of the women were in the first pregnancy. Furthermore, cfDNA results of all pregnant women were negative. In this study, the results of cfDNA in 100% of those who had negative results in the first trimester or quad test, were low risk.

Table 1: Demographic data of pregnant women studied

Variable		Number (percent)
Mother's age	18 to 30	154 (37.6%)
	31 to 35	132 (32.2%)
	Above 35	124 (30.2%)
Pregnancy frequency	1	175 (42.7%)
	2	134 (32.7%)
	3	68 (16.6%)
	4	22 (5.4%)
	5	9 (2.2%)
	6	2 (0.5%)
Delivery frequency	0	201 (49.0%)
	1	151 (36.8%)
	2	51 (12.4%)
	3	7 (1.7%)
Abortion frequency	0	325 (79.3%)
	1	64 (15.6%)
	2	16 (3.9%)
	3	2 (0.5%)
	4	3 (0.7%)

Variable		Number (percent)
Pregnancy method	Normal	377 (92.0%)
	IVF	24 (5.9%)
	IUI	9 (2.2%)
Number of fetal twins	1	401 (97.8%)
	2	9 (2.2%)
BMI	Lack of weight (less than 18.5)	5 (1.2%)
	Normal (18.5 to 24.9)	167 (40.7%)
	Overweight (25 to 29.9)	153 (37.3%)
	Obesity grade 1 (30 to 34.9)	69 (16.8%)
	Obesity grade 2 (35 to 39.9)	12 (2.9%)
	Excessive obesity (over 40)	4 (1.0%)

Table 2: Results of cfDNA by the results of first trimester screening and quad test

Routine screening tests	Results	Results of CF DNA	
		Low risk	High risk
First trimester	Negative	238	0
Quad test	Negative	172	0

Discussion

In this study, the mean age of pregnant women studied at the time of study was 31.5 years with a minimum of 18 years and a maximum of 40 years. Most of the subjects were aged 18 to 30 years old and 30.2% of women were over 35 years of age. The average weight of the subjects was 68.79 kg, with a minimum of 43 kg and a maximum of 115 kg, and the mean height of the subjects was 161.39 cm, with a minimum of 141 cm and a maximum of 186 cm. The average BMI of the subjects was 26.40 kg / m² with a minimum of 16.53 kg / m² and a maximum of 40.63 kg / m². Furthermore, 1.2% of the patients were underweight, and 1.0% of the patients had severe obesity.

In the study conducted by Radoi et al. [7] on Romanian women, the mean age of the studied women was 36.8 years with a minimum of 24 and a maximum of 44 years. The average weight of these people was 64.9 kg with a minimum of 45 and a maximum of 114 kg. In the study conducted by Norton et al., The mean age of the subjects was 31 years and 73.6% were younger than 35 years old. In the study conducted by Norton et al. [10], the average age was 30.7 years old, with a minimum of 18 and a maximum of 48 years. The average weight of mothers was 65.8 kg with a minimum of 31.8 and a maximum of 172.4 kg. In a study of pregnant women in Italy, conducted by Persica et al. [11], the average age of women examined was 36 years, with a minimum of 20 and a maximum of 46 years. The mean age of women in this study was lower than the mean age of women in the studies conducted in Romania and Italy and was

higher than the mean age in studies conducted by Norton and Wei-Lin Ke.

In this study, the percentage of women over 35 years was higher than in other studies, which highlighted the percentage of women above 35 years of age. Considering that the increase in the age of mothers is also a risk factor for pregnancy with a fetus with a chromosomal disorder, this happens with mothers over the age of 35 years, and in these cases, it is necessary to carry out more screening tests during pregnancy, most women in this study and other studies are women older than 35 years of age.

The mean weight of the women examined in this study is greater than in studies conducted in Romania and Norton. Given that maternal weight gain increases the probability that the exact results of the cfDNA test are not diagnostic, as well as the results of investigations done in various studies in cases where the cfDNA test is not diagnostic, have shown that the weight of these subjects was greater than that of the women who have been tested using a diagnostic cfDNA test. According to the results of previous studies, more precision in this screening test is recommended in high-weight women.

On average, frequency of pregnancy of the women was 1.93 with at least 1 time and up to 6 times of pregnancy, and only 24.6% of women had experienced more than 2 pregnancy periods. On average, delivery frequency in the subjects was 0.67 times, with a minimum of 0 and no more than 3 times. 49% of the subjects were nulliparous, and 20.7% of the subjects reported at least one incidence of abortion.

About 92.0% of the subjects were naturally pregnant and only 8.1% had used Assisted

Reproductive Techniques (ART), of which 5.9% were fertilized by IVF and 2.2% by IUI method. 97.8% of the subjects had singleton pregnancies and only 2.2% had a twin pregnancy. In the study conducted by Norton, 3.0% of pregnancies were by ART. This prevalence of using ART in the present study has been due to the unwillingness to carry out screening by the families who were naturally pregnant.

This study was performed on 410 pregnant women, 100% of whom in routine screenings were reported as low risk in the first or second trimester. In the study of women in Romania, conducted by Radoi et al., 13.93% of the subjects were at high risk for trisomy 21 in the first or second trimester screenings, and the rest (86.07%) were low risk due to their high maternal age or signs of chromosomal disorder in sonography or due to doing the cfDNA test for them on their own request. In the study conducted by Norton et al., 5% of the subjects were reported as high risk in the first or second trimester screenings, and 95% were low risk in the first or second trimester screenings. In the study conducted by Persico et al., all women were reported as high risk in the first or second trimester screenings.

In this study, negative cfDNA results were reported in 100% of pregnant women. In the study conducted by Radoi et al., cfDNA results of 94.2% of patients was negative and 2.48% of patients had positive cfDNA reports that were confirmed in amniocentesis and post-abortion examinations. In 3.48% of patients, cfDNA was not reported as a result of which the weight of mothers was higher than others [5]. In the study conducted by Norton et al., the diagnostic power of cfDNA testing in trisomies 21, 18, and 13 was 99.2%, 96.3%, and 91.0%, respectively [4,8]. In the study conducted by Wei-Lin Ke et al., 17 pregnancies were reported in the cfDNA diagnostic tests for trisomy 21, and also 6 pregnancies were reported for trisomy 18, and 1 pregnancy for high-risk trisomy 13, which 100% of these subjects were confirmed using karyotype analysis after birth or abortion. In addition, in the 2316 pregnancies, the results of the lower risk cfDNA testing were reported, which were confirmed in the upcoming evaluations [9]. In a study of low-risk individuals for trisomy 21, conducted by Norton et al., cfDNA test diagnosed 100% of cases of non-infected pregnancies, and also 100% of the affected cases, and sensitivity, specificity, positive predictive value and negative predictive value calculated of the test for low risk subjects in trisomy 21 diagnosis was reported to be 100%, 99.9%, 50%, and 100%, respectively. In the study conducted by Persico et al., CfDNA results

were positive in 97.2% of pregnancies with trisomy 21 and in 100% of cases, trisomy 18 and 13 were positive. Also, in 100% of low-risk pregnancies, the reported cfDNA test results were negative [8]. The results of this study were consistent with earlier studies that reported the results for low risk pregnant women and also, in all pregnant women who were reported as low risk in the first or second trimester screenings, the reported cfDNA test results were negative.

Conclusion

According to the results of this study, cfDNA testing results in all low risk pregnant women were negative and it is not recommended as a routine test in pregnant women reported as a low risk pregnancy in serum screening.

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