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Original Article

Screening of Fetal Chromosome Aneuploidies in the First and Second Trimester of 125,170 Iranian Pregnant Women

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Abstract

Background: Aneuploidy is one of the main causes of congenital anomalies, mental and physical disabilities, in newborns. The aim of this study was to determine various chromosomal aneuploidies in the first and second trimester screening of pregnant women, in Iran.

Methods: A descriptive retrospective study was conducted on 125,170 pregnant women referred to a major referral medical diagnostic laboratory (Niloo Laboratory, Tehran) for prenatal screening tests (2010-2013). Patients were divided into 3 groups: first trimester screening (FTS), second trimester screening (STS), and combined screening groups. In positive and borderline cases, and amniocentesis and cytogenetic analysis were carried out.

Results: Total prevalence of aneuploidy in 125,170 pregnant women was one in 491, (Detection Rate=82.7% for Down syndrome). The DR for DS in three groups was as follow: 87.5% for FTS (25783 women), 80.9% for STS (91345 women), and 94.7% for combined tests (8042 women). Total number of cases with Edward's syndrome was 18, Patau's syndrome six, Klinefelter syndrome six, triploidy three, and Cri-du-chat syndrome one.

Conclusion: The present study shows the frequency of aneuploidy in the first and second trimester screenings in a major medical laboratory in Tehran. The prevalence of aneuploidies grows with increased maternal age. The rate of aneuploidy in first trimester is higher than second.

Keywords: Prenatal diagnosis, Chromosomal aberration, Aneuploidy, First and second trimester screening, Iran

Introduction

Spontaneous abortions of the first trimester occur in 15 to 20% of all clinically recognized pregnancies, and chromosomal anomalies are responsible for more than 50% of all of these cases (1). Chromosomal aberrations is one of the most common and serious pregnancy complications in human (2, 3). Most of these abnormalities are numerical (86%), and a minority of the cases is caused by structural chromosome abnormalities (6%) and mosaicism (8%) (4). Trisomy 21 was first described in 1866 by John Langdon Down, which its birth incidence is 1/700 live births (5). Down syndrome is the most frequent chromosomal aneuploidy in liveborns and the most prevalent genetic cause of intellectual disability (6). About 95% of the Down syndrome is a result of conventional 21 trisomy or maternal nondisjunction, 4% translocation and 1% mosaisism (7-10). Trisomy 13 was first described by Thomas Bartholin in 1657 and was cytogenetically identified by Klaus Patau in 1960. It was referred as Patau syndrome. This syndrome has an incidence of

1/5000 live births (1). There is a high fetal loss of 97% for trisomy 13 conceptions and in the post-natal period nearly all trisomies 13 die within 4 months. Trisomy 13 is most commonly caused by a maternal meiotic nondisjunction but a minority of trisomy 13 cases is caused by an unbalanced robertsonian translocation (1). The Trisomy 18 syndrome (Edwards syndrome) is a common autosomal chromosomal disorder due to the presence of an extra chromosome 18. The first reported infants were described in 1960 by Edwards et al. and Smith et al. (11). Trisomy 18 represents the second most common autosomal trisomy syndrome after trisomy 21 (11).

The rate of fetal abnormalities in central Iran is 5.2% (12), the rate of fetal abnormalities in Tehran is 3.1% (13) and in north of Iran this rate is 1.5-1.7% (14). These abnormalities are the main cause of newborn mortality and more than 1/4 of admittance in children hospitals (15-17). Based on this information, physicians try to offer appropriate counseling in this regard and to improve the outcomes of pregnancies (18). The risk of trisomy augments significantly with the mother's age; this increasement becomes higher after the age 35. Traditionally, this age is known as the mother's oldness threshold (3, 19). Until the mid-1980s, prenatal diagnostic tests were only recommended to women who were older than 35 at delivery, but about 80% of Down syndrome children are born from mothers younger than 35 (3). The search for high-risk mothers giving birth to Down babies has become an objective for many prenatal researches, so that in early 1980s, screening test became available for pregnant women. Following abnormal screening test, the patients are referred to medical genetic labs to perform diagnostic tests (amniocentesis) (3, 9, 20, 21).

The aim of this study was to determine the aneuploidy frequency in first and second trimester screening of pregnant women in Iran.

Methods

The present research is a retrospective study, on 125,170 pregnant women referred to a major referral medical laboratory (Niloo Laboratory) from 2010 until 2013 to perform screening tests of the first and second trimesters as well as sequential tests.

In the first trimester, the mothers with gestational ages were between 11-14 weeks referred to the medical diagnostic laboratory. At first, the patients filled out a questionnaire including age, weight, number of previous deliveries, number of abortions, twin pregnancy, in vitro fertilization (IVF), smoking history, inter-family marriage, human chorionic gonadotropin (hCG) drugs consumption, infertility history, and hypertension.

Furthermore, the informed consent was signed by the subjects. Then they were tested for pregnancy-associated plasma protein A (PAPP-A) and free-βhCG biochemical tests. During the interval to carry out these tests the patients were referred to trusty sonographic centers to measure nuchal translucency (NT) and nasal bone (NB). In addition to NT, other biometric markers that have been studied were Crown-Rump Length (CRL), Biparietal Diameter (BPD), Head Circumference (HC), Femur Length (FL), and average ultra sound gestational age. The appearance of skull/brain, spine, abdomen, stomach, bladder, and upper and lower limbs were also evaluated. The obtained results of NT and biochemical markers were then entered in software and final Multiple of Medians (MoM), Corrected MoM, and Risk assessment were calculated based on normal medians of the Iranian population.

In the second trimester, (15-22 week) in terms of 21, 18 and 13 trisomies, neural tube defects (NTDs) and Smith Lemli Optiz Syndrome (SLOS) evaluation, were attended in the lab to perform biochemical tests of Alpha-fetoprotein (AFP), βhCG, unconjugated estriol (uE3 or free estriol), and Inhibin A (Quad Marker).

The following methods were used to measure biochemical markers; PAPP-A: Swiss made Roche kits with Electro Chemi Lumincene (ECL), AFP: ELISA using CanAg kit, uE_3: ELISA using Dimeditec kit, and Inhibin A: ELISA using Beckman-Coulter kit.

All pregnant women in this study, were monitored up to delivery and if possible, their newborns were

studied in terms of Down syndrome and other chromosomal abnormalities. Pregnancy complications such as miscarriage, premature delivery and low birth weight were also monitored.

According to the results, the mothers were classified into two groups of high risk and low risk. The high risk mothers were referred (by their physicians) to genetic centers for performing amniocentesis or chorionic villus sampling (CVS) diagnostic tests. To collect the data of the fetus and mother, all patients were contacted after 6 months of their referral to the lab. Then false positive rate (FPR), false negative rate (FNR), detection rate (DR), and odds of being affected given a positive result (OAPR), which indicate an affected baby, were also calculated.

Results

60.2% of total patients were less than 30 yr old; of them 61.2%, 60.1%, and 59.3% were in the first trimester (FTS), second trimester (STS), sequential and integrated protocols, respectively. About 26.4% of patients were 30-35 years old; of them 26.7%, 27.3%, and 25.2% were in the FTS, STS, sequential and integrated protocols, respectively. In addition, 12.5% of total patients were 36-40 years old; of them 11.2%, 11.9%, and 14.5% were in the FTS, STS, sequential and integrated protocols, respectively. Finally, 0.9% of total mothers were over 40 years old; of them 0.9%, 0.7%, and 1% were in the FTS, STS, sequential and integrated protocols, respectively (Table1). A brief medical of history of mothers, are given in Table 2. Total number of patients referred to perform FTS tests was 25,783 persons. Total number of false positive screening cases in first trimester was 1,332 persons (5.17%) (Table 3).

Table 1: The age category of mothers in three groups (%)

Age	All mothers (%)	FTS (%)	STS (%)	Sequential and Integrated (%)
30>	60.2	61.2	60.1	59.3
30-35	26.4	26.7	27.3	25.2
36-40	12.5	11.2	11.9	14.5
40<	0.9	0.9	0.7	1
Total	100	100	100	100

Table 2: A brief medical history of mothers in three groups (% from the total of 125,170)

History	FTS (%)	STS (%)	Sequential and Integrated (%)
IVF pregnancy	0.39	0.14	1.1
Smoking	0.28	0.16	0.9
Preeclampsia	1.2	1.7	1.9
Infertility	2.3	2.6	2.5
Consanguineous marriages	8.5	24.6	12.9
Drug consumption	7.4	19.0	13.1

Table3: Data of first and the second trimesters in different years

Year	FTS	STS	False Positive FTS (%)	False Positive STS (%)
2010	2447	12387	129 (5.27)	710 (5.73)
2011	5952	20987	302 (5.07)	1147 (5.46)
2012	7861	26041	409 (5.20)	1502 (5.78)
2013	9523	31930	492 (5.16)	1743 (5.45)
Total*	25783	91345	1332 (5.17)	5102 (5.58)

Total number of mothers referred to perform STS tests was 91,345 persons and total number of false positive screening cases in second trimester was 5,102 persons (5.58%) (Table 3). The total number of positive Down syndrome cases was 49 persons in the FTS tests. Total number of false negatives in the range of 1:251-1:500 was 5 persons, total number of false negatives in the range of 1:501-1:1000 was 8 persons and finally, the total number of false negatives less than 1:1000 was one person (Table 4). The detection rate of Down syndrome in the first trimester was 84.2. Odds of being affected given a positive result (OAPR) in the first trimester was 1:26.9 (Table 4).

Table 4: The data of the first trimester tests of Down syndrome cases in different years

Year	Positive Cases	False Negative Cases 1:251-1:500	1:501-1:1000	<1:1000	DR (%)	OAPR	Down Prevalence
2010	5	1	5	0	83.3	1:25.8	1:407
2011	11	1	1	0	84.6	1:27.4	1:458
2012	15	1	2	0	83.3	1:27.3	1:437
2013	18	2	0	1	85.7	1:27.3	1:453
Total	49	5	8	1	84.2	1:26.9	1:438

In total, the prevalence of Down syndrome was 1 per 438 persons in FTS (Table 4). As given in Table 5, total number of Down syndrome reported in the second trimester tests of this study was 144 persons. Total number of false negative cases in the second trimester tests in the range of 1:251-1:500 was 14 persons, in the range of 1:501-1:1000 was 15 persons, and finally, total number of false negative less than 1:1000 was 5 persons. The detection rate of Down syndrome in the whole studied community was 81.1 in the second trimester. OAPR in total individuals was 1:36.6 in the second trimester. In total, the prevalence of Down syndrome was 1 per 536 persons in the second trimester (Table 5). The total number of Down syndrome cases in sequential protocol was 18 persons. The total number of false negative cases in this protocol in the range of 1:251-1:500 was zero. Total number of false negatives in this protocol in the range of 1:501-1:1000 was 2 persons. Finally the total number of false negatives less than 1:1000 was zero. The detection rate of Down syndrome in total people of this protocol was 89.0. OAPR in whole people was 1:14.1 (Table 6). The additional findings were obtained in the study, including 18 cases with Edward's syndrome (trisomy 18, ~1:7000), 6 cases with Patau's syndrome (trisomy 13, ~1:20000), 6 cases with Klinefelter's syndrome (XXY, ~1:20000), 3 cases with Triploidy (~1:40000), 4 cases with chromosome 4 deletion, 1 cases with Cri du chat syndrome.

Table 5: The data of the second trimester tests of Down syndrome cases in different years

Year	Positive Cases	False Negative Cases 1:251-1:500	1:501-1:1000	<1:1000	DR (%)	OAPR	Down Prevalence
2010	18	1	2	1	81.8	1:39.4	1:590
2011	31	3	3	2	81.6	1:37.0	1:552
2012	38	3	4	1	80.8	1:39.5	1:554
2013	57	7	6	1	80.3	1:30.5	1:450
Total	144	14	15	5	81.1	1:36.6	1:536

Table 6: The data of the sequential tests of Down syndrome cases in different years

Year	Positive Cases	False Negative Cases 1:251-1:500	1:501-1:1000	<1:1000	DR (%)	OAPR	Down Prevalence
2012	6	0	1	0	85.7	1:12.5	1:322
2013	12	0	1	0	92.3	1:15.7	1:445
Total	18	0	2	0	89.0	1:14.1	1:383

Discussion

In the present study, 125,170 pregnant women who were referred to a major referral medical lab in Tehran from 2010 to 2013 were undergone the first trimester screening tests (25,783 persons), the second trimester screening tests (91,345 persons), and the first and second trimester sequential tests (8,042 persons). This number of subjects were 2.4% of total number of pregnant women who

were 5,200,000 persons, based on birth rate during 2010-2013 in Iran.

The first trimester screening of 25,783 pregnant women detected 49 Down syndrome, although 9 cases remained undetected. Thus the detection rate of Down syndrome was 84.2% and the false positive rate was 5.17%. This finding is consistent with other investigation which reported the detection rate of 83.3% in 9,730 patients in 2007 (22).

This finding is also consistent with a review study of 2011 which reports a detection rate of 85% and a false positive rate of 5%, regarding extensive studies in different countries (23). In addition, according to screening protocols of the Society of Obstetricians and Gynecologists and Genetics Society of Canada guidelines in 2011, the detection rate of Down syndrome in the first trimester was 83%, the false positive rate was 5%, and OAPR was 1:27 which are consistent with the findings of the present study (24).

Performance of the second trimester screening, 91,345 pregnant women studied, in whom 144 Down syndrome were detected through a diagnostic tests, although 34 cases remained undetected. The detection rate of the second trimester screening tests and the false positive rate were 81.1% and 5.58%, respectively. This finding is slightly higher than the study, published in 2011 (detection rate of 70-75% and false positive rate of 5%); this may be due to the age distribution of pregnant women in our sample, that is the ratio of over 35 years women to total women was 12.6% while it was 5% in Nicolaides report, which resulted in increase of age risk and ultimately increase of false positive cases and detection rate (23).

In addition, according to guidelines of Genetics Society of Canada and the Society of Obstetricians and Gynecologists of Canada published in 2011, the detection rate was 77%, the false positive rate was 5.2%, and OAPR was 1:50 which are consistent with the findings of the present study (24).

Performance of sequential protocol of the first and second trimester 8042 pregnant women was referred to the referral lab; in which 18 Down's syndrome cases were detected while one case remained undetected. Therefore, the detection rate and the false positive rate were obtained 94.7% and 3.28%, respectively. This finding is consistent with Nicolaides study with detection rate of 90-94% and false positive rate of 5% (23), although it is slightly higher than the guidelines published by Genetics Society of Canada and the Society of Obstetricians and Gynecologists of Canada which have reported the detection rate of 87%, false positive rate of 1.9%, and OAPR of 1:15; this difference is due to age distribution of referred patients (24).

Down syndrome is the most common chromosomal disorder in the population and an important cause of children with mental retardation. The age of 35 years is considered as the threshold of high age; hence mothers over the age of 35 undergo amniocentesis to be sure of the fetal health. This invasive intervention detects 20-30% of trisomy 21 and the remaining cases remain unidentified (25, 26). Therefore, some non-invasive and non-expensive tests that can be performed on every pregnant woman seem essential.

Conclusion

Although the global prevalence of Down's syndrome is 1 per 700, it was obtained in this study as 1 per 491. This difference in prevalence may be due to mothers' age distribution that is not consistent with the community of pregnant women. On the other hand, positive screening cases of other laboratories are referred to a major referral medical lab and physicians perform screening tests on mothers over 35 years old.

Ethical consideration

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

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References

1. Witters G, Van Robays J, Willekes C, Coumans A, Peeters H, Gyselaers W, et al. (2011). Trisomy 13, 18, 21, Triploidy and Turner syndrome: the 5T's. Look at the hands. *Facts, Views & Vision Obstet Gyn*, 3 (1): 15-21.
2. Cohen MM, Rosenblum-Vos LS, Prabhakar G (1993). Human cytogenetics. *Am J Dis Child*, 147 (11): 1159-66.
3. Hawk AF, Saller DN (2012). Screening for fetal aneuploidy: is maternal age relevant? *Clin Obstet Gyneco,* 55 (1): 217-25.
4. Goddijn M, Leschot NJ (2000). Genetic aspects of miscarriage. *Baillieres Best Pract Res Clin Obstet Gynaecol*, 14 (5): 855-65.
5. Verma IC, Mathew S, Elango R, Shukla A (1991). Cytogenetic studies in Down syndrome. *Indian Pediatr*, 28 (9): 991-6.
6. Leonard H, Wen X (2002). The epidemiology of mental retardation: challenges and opportunities in the new millennium. *Ment Retard Dev Disabil Res Rev,* 8 (3): 117-34.
7. Farhud DD, Ameli H, Bagheri A, Hosseini Mazhari Z (1996). Cytogenetic study of 366 affected children with Downs Syndrome in Iran. *Iran J Public Health,* 25 (3-4): 1-4.
8. Dent KM, Carey JC (2006). Breaking difficult news in a newborn setting: Down syndrome as a paradigm. *Am J Med Genet C Semin Med Genet,* 142C(3):173-9.
9. Gilbert RE, Augood C, Gupta R, Ades AE, Logan S, Sculpher M, et al. (2001). Screening for Down's syndrome: effects, safety, and cost effectiveness of first and second trimester strategies. *BMJ (Clinical research ed),* 323 (7310): 423-5.
10. Hulten MA, Jonasson J, Nordgren A, Iwarsson E (2010). Germinal and Somatic Trisomy 21 Mosaicism: How Common is it, What are the Implications for Individual Carriers and How Does it Come About? *Curr Genomics,* 11 (6): 409-19.
11. Cereda A, Carey JC (2012). The trisomy 18 syndrome. *Orphanet J Rare Dis,* Oct 23;7:81.
12. Sereshti M, Kazemeyan A (2008). Prevalence of apparent major congenital malformations and some associated factors, in terminated pregnancies in Hajar hospital of Shahrekord, 2005-2006. *J Shahrekord Univ Med Sci,* 10 (1): 36-43.
13. Shajari H, Karbalai Aghai M (2006). Prevalence of congenital malformations observed in neonates in Shariati Hospital (1381-1383). *Iran J Public Health*, 16 (3): 308-12.
14. Golalipour M.J, Vakili M.A (2002). Gross congenital malformations in 10000 births (Gorgan Dezyani Hospital 1997-99). *J Gorgan Uni Med Sci*, 4(2):42-7.
15. Antonarakis SE, Epstein CJ (2006). The challenge of Down syndrome. *Trends Mol Med,* 12 (10): 473-9.
16. Roubertoux PL, Kerdelhue B (2006). Trisomy 21: from chromosomes to mental retardation. *Behav Genet,* 36 (3): 346-54.
17. Schreinemachers DM, Cross PK, Hook EB (1982). Rates of trisomies 21, 18, 13 and other chromosome abnormalities in about 20 000 prenatal studies compared with estimated rates in live births. *Hum Genet,* 61 (4): 318-24.
18. Sheridan E, Williams J, Caine A, Morgan R, Mason G, Mueller RF (1997). Counselling implications of chromosomal abnormalities other than trisomy 21 detected through a maternal serum screening programme. *British BJOG*, 104 (1): 42-5.
19. Kurahashi H, Tsutsumi M, Nishiyama S, Kogo H, Inagaki H, Ohye T (2012). Molecular basis of maternal age-related increase in oocyte aneuploidy. *Congenit Anom (Kyoto),* 52 (1): 8-15.
20. Spencer K (2014). Screening for Down syndrome. *Scand J Clin Lab Invest Suppl*, 244: 41-7.
21. Saridogan E, Djahanbakhch O, Naftalin AA (1996). Screening for Down's syndrome: experience in an inner city health district. *Br J Obstet Gynaecol,* 103 (12): 1205-11.
22. Hwa HL, Ko TM, Hsieh FJ, Yen MF, Chou KP, Chen TH (2007). Risk prediction for Down's syndrome in young pregnant women using maternal serum biomarkers: determination of cut-off risk from receiver operating characteristic curve analysis. *J Eval Clin Pract,* 13 (2): 254-8.
23. Nicolaides KH (2011). Screening for fetal aneuploidies at 11 to 13 weeks. *Prenat Diagn,* 31 (1): 7-15.
24. Chitayat D, Langlois S, Wilson RD (2011). Prenatal screening for fetal aneuploidy in singleton pregnancies, *J Obstet Gynaecol Can,* 33 (7): 736-50.
25. Farhud DD, Walizadeh GR, Kamali MS (1986). Congenital malformations and genetic diseases in Iranian infants. *Hum Genet*, 74 (4): 382-5.
26. Korn ES, Schwanitz G, Baur M.P, Farhud D.D (1987). Comparative Studies Of The Chromosomal Arrangement In The C-Metaphase Between Normal Karyotype And Trisomy-21. *Iran J Public Health,* 16 (1-4): 25-36.