

Amniocytes cytogenetical Study of 100 high risk pregnant women with chromosomal abnormalities by using GTG banding high resolution method

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Abstract

Background and Objectives: Early detection of abnormalities in early pregnancy and they can be terminated once the baby was born with a disability that economic, social and cultural rights, there are a lot of families and community and overhead families and community prevent, so far none of the known human chromosomal abnormalities are treatable and the only way to deal with these diseases limit to prenatal diagnosis and abortion of affected. The purpose of this study is examine amniocytes cytogenetic of pregnant women with high-risk study of chromosomal anomalies to determine the number of disorders and structural chromosome in the study population to identify and information to families and early pregnancies as a result of such termination and the effects of these abnormalities and prevent families from the burden of economic, social and cultural kept safe.

Methods: In this functional - practical study fetal chromosomal abnormalities in 100 high risk of chromosomal anomaly screening and identification of numerical and structural chromosome abnormalities were studied. Amniotic fluid was cultured for this purpose to prepare karyotype and high resolution Gtg method Bndyng metaphase cells were studied. Results of amniotic fluid culture and chromosome typing and GTG bonding High resolution trisomy 21 most frequent in the trisomy (2 percent) and seven per cent of all fetal abnormalities construction have been as follows four percent of those with inv 9, one percent of Atypic y additional band on chromosome 15 and two percent, respectively.

Conclusion: The results suggest that detection of fetal chromosomal anomalies mothers with high risk cytogenetic testing amniotic fluid can be effective in detecting chromosomal abnormalities as well as the results show the effectiveness of this method for prenatal diagnosis timely notification to the families of the birth of chromosomally abnormal embryos prevented.

Keywords: cytogenetic test, chromosomal abnormalities, GTG bonding, amniotic fluid

Introduction

The birth of children with congenital anomalies in addition to the emotional stress on the family, for the individual and the health system is costly. According to Centers for Disease Control and Prevention estimated that in recent years the annual America was born out of every 33 children who had a child with congenital abnormalities (15). Now one of the main causes of congenital malformations in the infant mortality rate is 20 percent of the deaths are neonatal period (7). Most miscarriages are due to genetic defects and chromosomal abnormalities, one of the important genetic

disorders that cause seven to five percent of infant death, six to eleven percent of stillbirths and neonatal deaths, and at 0.9 percent of infants are seen with live births (6, 10). Routine ultrasound screening in pregnancy is 22-18 weeks is a standard method of prenatal in many countries. The anomaly detected before birth depending on the population studied and the experience of the person performing 21 to 84 percent is variable. The incidence of chromosomal disorders associated with abnormal ultrasound findings and 65 percent depending on the type of anomaly is different. Detecting a major flaw or two small defects in chromosomal ultrasound examination in the fetus considered. In a study in 30.5% of fetuses with major defects and 4.5 percent of embryos with small defect ultrasound, chromosomal abnormalities were seen (5). Screening for fetal chromosomal abnormalities is a measure of prenatal care is essential. Old age was a risk factor. Due to the loss of the fetus as a result of diagnostic procedures, amniocentesis and genetic counseling and childbirth for women who were older than 35 years was recommended.

However, only 20 percent of infants with trisomy are 21 common women were born age over 35 years. Alpha-fetoprotein (AFP) test developed (AFP) serum mother in the mid-1980s, for women under the age of 35 years provided the options for prenatal diagnosis. Over the past two decades, other tests can increase the detection of chromosomal abnormalities, while maintaining a false positive rate is low. This is an opportunity for women in all age groups-invasive screening or diagnostic test before 20 weeks of gestation provided (5). Due to the high incidence of chromosomal abnormalities in embryos with chromosomal abnormalities risk as well as the capabilities of standard cytogenetic methods for detection and prevent the occurrence of these disorders in infants the aim of this study was to determine the types of chromosomal abnormalities in embryos visible chromosomal anomalies and a platform for the implementation of a strategy for the care of pregnancy for prenatal diagnosis and prevention of birth of children who will followed. Most studies show that about 1-0.5% of infants are with chromosomal disorders. Autosomal trisomy of chromosome abnormalities accounted for over a third of them (4). About 15 to 20 percent of pregnancies end in abortion, more than 50 percent of repeated miscarriages in pregnant women 8 to 15 weeks due to genetic abnormalities of which approximately 95% related to disruptions number and 5% of the abnormality of chromosomes (11). Much care about the health of the fetus during pregnancy, during 9 months of pregnancy.

Check the status of the fetus as having different chromosomal abnormalities in the first trimester of pregnancy test done. The most important considerations during the second 3 months of pregnancy is done, is Triple Test. The risk of miscarriage increases with the number of births and increases with high maternal and paternal age. Genetic factors, especially thrombotic syndrome, chromosomal abnormalities and complications parent antibody anti-phosphoinositide lipid abnormalities are the major

causes of abortion and, though the proportion of patients diagnosed with the disorder is variable in the study population. Other factors include anatomical abnormalities (15 percent) endocrine problems (20%), infections (about 5-0.5%) and immunological factors (20 to 50 percent) also are effective in spontaneous abortion and fetal abnormalities. More than 80 percent of abortions occur in the first 12 weeks of pregnancy and chromosomal disorders are responsible for 50% after the first 3 months of the pregnancy and the incidence of chromosomal abnormalities is reduced (4).

A number of studies in which a large number of products have been investigated abortion culture and karyotype observed 50 percent of all first trimester abortions, 30% second quarter and 30 percent of natural stillbirth of chromosomal in these studies, the incidence of chromosomal abnormalities in aborted products is likely lower than the actual needs (5). Considering that so far none of the known human chromosomal abnormalities are treatable the only way to prevent the birth of children with prenatal diagnosis of chromosomal abnormalities and therapeutic abortion is performed. These abnormalities used cytogenetic methods. Using examples such as amniotic fluid and chorionic villi can be detected with high accuracy (5).

Chromosomes can be from various sources, including peripheral blood lymphocytes, umbilical cord blood, fibroblasts of the skin, amniotic fluid, chorionic villi with peripheral blood and bone marrow were obtained and analyzed. Amniotic fluid cells, the first and best source for prenatal chromosomal analysis are considered. More than 99 percent of all chromosomal abnormalities numerical synthesis detected (10). Amniocentesis is a prenatal diagnosis technique in which a small amount of amniotic fluid (20 ml) and the genetic study done on it. Amniocentesis is one of the most common methods of invasive prenatal diagnosis in the second 3 months of pregnancy (14). Amniocentesis can be important factors for maternal age over 35 years, abnormal levels of biochemical markers, abnormal ultrasound findings, positive family history of genetic disorders, and pregnancy at risk of chromosomal abnormalities noted. In early pregnancy, fetal problems amino synthesis to detect chromosomal abnormalities such as trisomy 21, trisomy 13, trisomy 18 and fragile X syndrome is used. Triple test is a test to measure the combination of three hormones produced during pregnancy is important at 20-15 weeks of pregnancy at the request of physicians and risk to the fetus trisomy 21 (Down syndrome), trisomy 18 (Edwards Syndrome), trisomy 13 (Patau Syndrome) and fetal neural tube defects (encephalopathy) statistically measured.

Amniocentesis is done between weeks 15 and 20 of pregnancy. If the test ahead of time in weeks is low failed to grow due to the low number of cells in metaphase and if you reduce the amount of amniotic fluid is higher in the weeks and damage to the fetus and also spent time therapeutic abortion is legal. Amniocentesis means to do it early in the week of 11 to 13 is the final results will be ready 2-3 weeks after amniocentesis (8).

Chromosomal abnormalities Down syndrome are born with a frequency of about one out of every 700-650 and the abnormal gene determines the phenotype of the syndrome is located in the 21q22 region. Several risk factors for the occurrence of this syndrome is necessary, but it seems the most important risk factor for the high maternal gestational age, as well as other factors such as chromosomal abnormalities in parents will be considered (4). Two other relatively common autosomal trisomies are: Edward syndrome (trisomy 18) and Patau Syndrome (trisomy 13). In patients with this syndrome, disorders severe physical - and mental retardation are common phenotype can be seen. Average life expectancy of people with the type of trisomy few weeks but about 10 percent of them live more than a year (16).

The only way to prevent the birth of children with this syndrome can be diagnosed before birth. For a better interpretation of the test results, in addition to measuring parameters in the information age, weight during breast biopsies and clinical conditions such as diabetes are taken into account. Fetal Anomaly Screening results will show only risk. Amniocentesis or CVS test results and diagnosis as the criterion for termination of pregnancy. If the screening indicates a low risk, the need for invasive diagnostic procedures because of the risk of Down syndrome is low. If the screening indicates high risk is positive, since it means having a fetus with Down syndrome is a positive result, for a definitive diagnosis requires invasive methods such as CVS (chorionic villus sampling tissue) and Amino synthesis (sampling the amniotic fluid) (8).

In this study, researchers sought to determine due to the sensitive nature of the issue of structural disturbances (displacement and inversion) and numerical abnormalities (Anopoloeedi and polyploid) and mosaicism in patients with cytogenetic methods GTG method Binding hereby High resolution is to prevent and reduce hospitalizations for infants with chromosomal abnormalities. Pregnant women at any age before the 20th week of pregnancy by invasive diagnostic testing and screening for chromosomal abnormalities are subject to review. New advances in screening methods, the number of options available for patients has increased. Diagnostic options include chorionic villus sampling in the first 3 months and 3 amino syntheses in the second quarter. Diagnostic options in the first quarter of transparency test back of the neck (nuchal translucency) is measured with Protein A associated with pregnancy and human chorionic Tropic Gnado. Transparency neck tests alone, as much as the previous option is not effective. Options include screening in the second trimester serum screening using the screening and ultrasound are three or four. It is also possible with a combination of first and second trimester screening tests with a focus on the sequence of steps or sequence of conditional pick. These options include:

A plasma protein analysis related to pregnancy or no test transparency of neck back with four screening. Screening for fetal chromosomal abnormalities is a measure of prenatal care is essential. Maternal age is a risk factor. Genetic counseling for women

in childbirth and amino synthesis of over 35 years of age is recommended. However, only 20 percent of infants with trisomy 21 are born to women who are older than 35 years. Alpha-fetoprotein (AFP) test developed (AFP) serum mother in the mid-1980s for women aged less than 35 years to provide options for prenatal diagnosis. Over the past two decades, other tests can increase the detection of chromosomal abnormalities, while maintaining a low false positive rate (11). This is especially true for pregnant women in all age groups and the opportunity to perform screening or invasive diagnostic testing before 20 weeks of gestation. Genetic factors, chromosomal abnormalities, especially parents and side effects of anti-thrombotic syndrome phosphoinositide lipid antibodies main causes of spontaneous abortion are chromosomal abnormalities of the fetus. Though the proportion of patients diagnosed with the disorder is variable in the study population.

Because of the necessary research so far none of the known human chromosomal abnormalities are treatable and the only way to deal with these diseases limit to prenatal diagnosis and abortion is affected and the aim of the research for the researcher to determine the types of chromosomal abnormalities using cytogenetic methods and strengthening the implementation of a strategy for the maintenance of pregnancy for prenatal diagnosis and prevention of birth of children infected. The desired objectives for the study are as follows:

A) Study of 100 pregnant women with high risk cytogenetic amniocytes chromosomal abnormalities GTG method Binding High resolution and determination of chromosomal abnormalities in fetuses with abnormalities visible.

B) Strengthening the scientific strategy for the maintenance of pregnancy for prenatal diagnosis

and prevention of birth of children with chromosomal disorders.

The hypothesis of this study is as follows:

1) Embryos chromosomally abnormal in the second trimester by cytogenetic methods (GTG Binding High resolution) are recognizable.

2) Pregnant women with a positive triple marker compared with mothers of fetal chromosomal anomalies higher percentage of negative tests or their babies.

3) Positive triple marker test cannot be decisive because the abnormality of the fetus or newborn.

The definition of variables

In the following table the dependent and independent variables were examined in this study have been presented.

Measuring instruments and measuring	Variable name	Variable type
Optical microscope	Trisomy 21 - trisomy 18 - trisomy 13	Independent variable (qualitative and discrete)
Triple marker test Double-marker test Quadrant test marker	AFP – B – HCG – UE3 Inhibin A	The dependent variable (quantitative and continuous)

Materials and methods

This research study is practical. The research study is pregnant women admitted to hospital in Tabriz for sampling the amniotic fluid formed. The Research Laboratory of Medical Genetics doctor Seyed Ali Rahmani is in the city of Tabriz. Amniocytes sample of one hundred pregnant women were admitted to hospital in Tabriz. Sampling and sample preparation for the study, so that pregnant women who go to gynecologists and screening for pregnant women with high risk for chromosomal abnormalities are detected Al Zahra Hospital presented the following method to check sample fluid karyotype and study of chromosomal anomalies is done. Amniocentesis is a procedure this way: Before the start of the process, to reduce pain associated with needle which is used to draw amniotic fluid from the mother used local anesthesia. After the desired location anesthetic, the needle through the abdominal wall and then the wall of the uterus and amniotic sac finally arrived. Using ultrasound guidance, away from baby's amniotic sac local doctor in the whole and about 20 ml of amniotic fluid is the patient's name and date of sampling syringe written and karyotype and genetic study will be sent to the genetic laboratory (3-1).

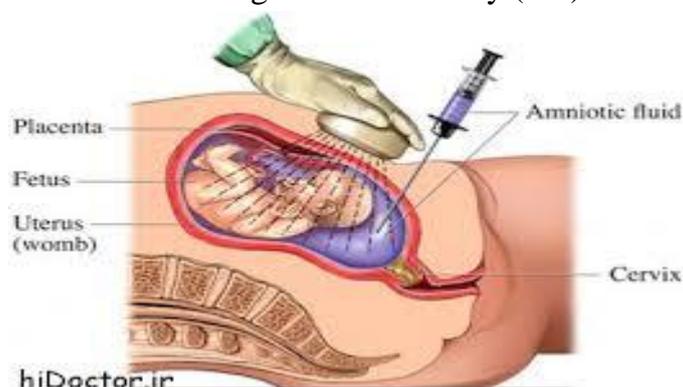




Figure (3-1): sampling the amniotic fluid

In the genetic laboratory following steps to obtain a karyotype is done.

Providing 15 to 20 cc of liquid Amnion pregnant women with high risk for chromosomal anomalies were explained.

1- Phase liquid culture of the Amnion

- All grown in sterile conditions inside laminar hood done.
- Medium contains a mixture of Aminomax and F10 in PH = 7-7.5. The medium should be fresh (not more than 4 days to prepare) and cultured in an incubator at 37 ° placed before, all cultures in terms humidity 88% of 5% Co2 and at 37 ° C is incubated.
- Amniocytes samples to a laboratory centrifuges are usually within a conical tubes. Rpm1600 centrifuge tubes are 8-6 minutes.
- The deposits within the tube 2 with 5 ml culture medium are done in the Flask.
- Czech growth medium of the fourth day, the individual colonies are scattered on the cover of Sleep.

On 6 and 7 depending on the number and size of the colonies, the medium should be replaced.

On the sixth or seventh game environment is done in accordance with the following procedure.

At this point must be sterile laminar replaced under the hood to make the surface area of each flask to provide backup in the Falcon Foundry and enrollment will then be added to each flask 5 ml Amniomax environment and the significant CO2 incubator for 24 hours and then harvesting we do.

Backup for the Falcons in a centrifuge for 8 minutes tops rpm 1600 after completion of centrifuge Falcons slowly bring the machine and pulled supernatant sterile pipette and stay away till cc2 and then shaking the Falcon so that the cells can be separated and then 5 ml Amniomax environment in Falcon poured into the flask contents bleed and put in the incubator.

Backup for the Falcons in a centrifuge for 8 minutes tops 1600rpm after completion of centrifuge Falcons slowly bring the machine and pulled supernatant sterile pipette and stay away till cc2 and then shaking the Falcon so that the cells can be separated and then 5 ml Amniomax environment in Falcon poured into the flask contents bleed and put in the incubator.

1. Harvest Stages

- Are examined under a microscope and inverted flasks are harvested checked. Several colonies of cells to divide and gather around any slip cover. If the cell density is greater in this case would be appropriate to play their cytoplasm and chromosome bands will be clearly seen, if very small colonies may be seen metaphase inadequate.

- Then 80 Landa from Kelsmaid solution added to the flask and put in incubator with CO₂ for an hour.

- The top of the flask into a Falcon throw to each flask cc2, EDTA are added to the cells relax and then picked off the flask walls we wait 30 seconds.

- Top liquid pour again into the flask to neutralize EDTA and throw back into the Falcon.

- 2 cc Tripson and Resin added to the flask and 3 minutes putting CO₂ into the incubator.

m- After 3 minutes, look under invert microscope to make sure that all the cells have been dug out of the flask. If the cells were not dug up a few blows to the flask into our cells are completely off.

- Top liquid of the Falcon again throw into the flask to neutralize Tripson and Resin many times we shake and throw the contents of the flask into a Falcon and in 1600 the Falcons into a centrifuge for 8 minutes tops.

- Remove Falcon from the centrifuge and the top environment by pipette 2 ml remain empty and we move to precipitate dissolved.

- Kcl (0.37 percent) inside the incubator heated to about 37 degrees drop in Falcon added and the contents of the Falcon reach 10 cc and 15 minutes in a conventional incubator.

- The Falcon 1600 inside the centrifuge for 8 minutes tops.

- Falcon removed from centrifuge and the supernatant environment empty by pipette remain 2 ml.

- We washed four times with fixative and methanol. Washed 2 times with methanol and 2 operation is done with fixative and wash and as such is done ad Falcon, first with the wave, so that all the sediments settle and then throw drops of methanol or fixative and the contents of the Falcon 1600 cc10 and around the centrifuge for 8 minutes tops.

- Remove the supernatant environment 2 ml remain three times longer to stay and do the same steps.

- In the last round Falcon centrifuge remove and put in the refrigerator for 24 hours and then we prepared slides. The temperature and slide the humidity is very important, temperature 20 to 23 ° C and humidity 50 to 60 percent.

1. After harvesting slide preparation is done as follows:

Empty Falcon supernatant environment to 5.1 cc remain pipette contents of the Falcon by several rounds and we mix, slide over a distance of 50 cm steamers and the cells on the slide Falcon bleed. Pour 3 drops on each slide and a total of 15 slides prepared for each patient and then put to dry in the air laboratory. And then incubator 37 ° C for 4 hours in order to put Aging, and then we painted.

1-High Resolution

For High resolution flasks that at harvest step on 8 or 9 with cells metaphase well selected and 20 Landa solution of ethidium bromide to each flask was added for 40 minutes, incubated, and then the rest of the harvest as above done

Step 1 Binding chromosomes

To Binding chromosomal Trispin was used.

2 painting step slides with Giemsa (GTG Binding)

Slides for one or two minutes and then ten percent within banding with water slides and check do.

Phase 1 study of chromosomes

Slides under the light microscope with a magnification of 10 and 100 studied. The method of determining the sample size in one year from December 2013 to December 2014, one hundred samples from amniocytes of pregnant women with high risk for chromosomal abnormalities were referred to the Al Zahra hospital in Tabriz were selected. Data was collected as the information concept as field and library (books, articles, theses and reputable sites) were collected. And to study and obtain information on the samples obtained from the Women's Hospital and inverted optical microscope was used. The data were obtained by studying the cell under the microscope and analyzed using SPSS statistical software was developed.

Results

The results of this study indicate that maternal fetal chromosomal anomaly risk with cytogenetic testing the amniotic fluid can be effective in detecting chromosomal abnormalities of the samples in this study, cytogenetic tests, 2% of fetal chromosomal anomaly risk mothers were diagnosed with Down syndrome among the whole sample of Pato and Edward syndrome was observed but of the 100 cases studied, 7% of fetal chromosomal anomalies have been as follows 4% of them inv9, 1 percent and 2 percent Atypic Y with additional band were on chromosome 15. The results of the efficiency of this method for prenatal diagnosis shows the families when the fetus is born with chromosomal abnormalities economic times, social, cultural, family and society that these births can be prevented.

Conclusion

In this study, 100 samples of liquid Amnion with high risk of chromosomal anomalies were Cytomegalovirus genetic karyotype studies were conducted that examined embryos of 2% of fetuses with Down syndrome were diagnosed Pato and Edward syndrome were observed and 7% of fetal chromosomal abnormalities have been

described as 4 percent of those with inv 9, 1% and 2% Atypic Y with additional band were on chromosome 15. The lowest among pregnant women age 17 and maximum age was 45 years and the average age of mothers in this study was 33.5. Age, pregnant women fetus with Down syndrome, respectively 37 and 41 years of age and pregnant women have been reported in seven percent due to chromosomal anomalies between 31 to 39 years of age involved in the formation of fetal malformation Down syndrome and chromosomal abnormalities are confirmed and in 2 cases of embryos typing them in Cairo 9 inv observed have grade 3 relatives.

Yoon et al in 1996 among the 170 fetuses were studied the expansion of trisomy 21 by maternal origin about 86 percent (75 percent meiosis I and 25 percent meiosis II) and the rest of the parents (50 percent and 50 percent meiotic two early country music). The results of this study were consistent with the study (17). Fitch et al, 3419 in New York in 2003 study the births in the years (1983-1997), and the study lasted 15 years. In this period the number of embryos that parents were born more than 35 years were examined and 110 per cent of pregnant women older than 35 years and 60% at the age of fathers and in the role of paternal age and the incidence of Down syndrome was found effective in creating syndrome. But when the mother's age, especially over 40 years was associated with an increased paternal age the results of this study are consistent with the result of the involvement of maternal age on the risk of Down's syndrome (9).

Stein et al (1986) in a study examine the association between maternal age and the risk of trisomy 21 on 258 mothers of aborted fetuses with trisomy 21 karyotype fetus due to their diagnosis and concluded that 54% of mothers under 30 years of aborted fetuses and 67% of the embryos were their mothers at the age of 30 and the results showed a significant association between maternal age and the incidence of trisomy and corresponded with a study (13). Vranekovic et al in 2012 examine the relation between aging parents in establishing Trisomy 21. The review was carried out on 102 Down syndrome population of Croatia. The genetic analysis based on polymerizes chain reactions on q21 duplications were conducted with 11 markers. The results show the highest maternal origin trisomy 21 (93%) and paternal origin (3%) and the origin of the mitotic (2 percent). Frequency meiosis error and meiosis of one parent, 86% of 14%. A large portion of telomeric changes in cases where the mother's age was young, but it has seen the error of early country meiosis error but it was not statistically significant. The results show the trisomy 21 etiology of various human populations is unique; these results clearly confirm the results obtained in this study (15).

Lutfi also investigated in a study in 91-92 Cytomegalovirus genetic of pregnant women tested with positive triple marker screening for chromosomal anomalies in the North West of Iran in this study, which was carried out on 100 samples of suspected fetal type was found after Cairo 4 cases of Down syndrome fetuses whose mothers

were aged 32, 37, 41 and 44 years old the results also indicate that contribute to high maternal age was confirmed in the formation of fetal malformation Down syndrome (3). In terms of the average age of mothers with suspected fetal chromosomal abnormalities also present results that show the average age of 33.54 years, with the results often matched internal and external researchers. Among these studies, amniocentesis tests that Zahra Shahshahan and Nahal Azimi in 2013 on 136 pregnant women with high risk were performed. In this study, the mean age of women over 33 years and the results indicate that the risk of women over 30 years showed (1).

Tabatabai et al., in 2009 in a study on 62 samples of aborted fetuses did in East Azarbaijan province these samples were evaluated in terms of chromosomal abnormalities and fetal karyotype after an investigation concluded that the average age of mothers was thus tested over 30 years, the study also reveals that contribute to high maternal age in fetuses with chromosomal abnormalities is formed. The results of these studies are consistent with the direction relatives and Seyed Mahmoud Tabatabai et al in the study because about 62 percent of couples with miscarriage due to genetic abnormalities were studied were with relatives (2). The aim of this study was to determine the rate of fetal chromosomal abnormalities in patients with high risk of chromosomal anomalies, which according to previous studies conducted in other countries and provinces, the rate of abnormality was observed in 7% which is consistent with previous studies in patients with abnormalities of chromosomal karyotype is therefore necessary so that the birth of children they have three with congenital abnormalities of social and economic pressures on families and the community can be prevented.

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