PRENATAL DIAGNOSIS AND SCREENING OF GENETIC ABNORMALITIES IN EARLY PREGNANCY

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ABSTRACT

BACKGROUND

Genetic diseases are one of the major causes of hospital admissions due to disability and mortality particularly among children (1:5 children of hospital admission either partially/completely) as distribution of genetic diseases is not related to socioeconomic background, which implies that developing world has a large number of genetic diseases largely left uncared for, i.e. overall incidence of foetal/neonatal loss due to genetic/genetic environmental causes are as follows: 1:50 newborns have major congenital abnormality, 1:100 have a unifactorial disorder, 1:200 have a major chromosomal abnormality before birth. Diagnosis of chromosomal anomalies in foetus is one of the most important challenges in modern perinatology as invasive or noninvasive methods.

The aim of the study is to review on cytogenetic evaluation of CVS obtained (transcervically) during first trimester of pregnancy by direct karyotyping of tissue.

MATERIALS AND METHODS

This study was conducted in 2001 in Department of Anatomy along with Obstetrics and Gynaecology Department, LNJP Hospital. 37 healthy cases with 6-12 weeks of gestational age coming for medical termination of pregnancy were included in the study. After written informed consent for procedure, ultrasound-guided transcervical chorionic villus sampling was done (Brambati's method). Tissue procured was then processed for direct karyotyping and studied. Metaphase spreads were photographed and karyotypes prepared and studied.

RESULTS

Out of 37 pregnant females, 30 samples were successfully prepared and processed by Direct method out of which 23 were normal female (46, XX) and 7 were normal male (46, XY). No normal anomaly was detected. Best biopsies were obtained with 8-12 weeks gestation. G Banding could not be performed as chromosome obtained were found to be resistant to banding.

CONCLUSIONS

To summarise chromosome preparations obtained from CVS by Direct method has advantage of providing sufficient number of suitable metaphases, foetal karyotype can be determined in few hours of sampling. No maternal cell contamination. But, Direct method is not good for diagnosing structural chromosomal aberration, although it's fast, technically simple and reliable method and could be used as a routine procedure for first trimester foetal diagnosis in peripheral hospitals and thus can decrease load on referral centres.

KEYWORDS

Prenatal Diagnosis, Direct Karyotyping, Early Pregnancy, Chorionic Villus.

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BACKGROUND

In developed countries, the advances in medical sciences and healthcare system have caused minimisation of diseases due to nutritional and infectious causes. Therefore, genetic diseases have become relatively more important. The last few decades have witnessed the growth of a number of prenatal diagnostic methods from applied research to being routine components of genetic counselling and obstetric management. Methods of prenatal diagnosis can be divided into invasive and noninvasive techniques. Diagnosis of chromosomal anomalies in foetus is one of most important challenges in modern perinatology. The most common chromosomal abnormalities in newborns are trisomies 21, 18, 13 monopsony X and other sex aneuploidies.[1,2,3] These aneuploidies can account up to 95% of liveborn abnormalities.[4] This study was conducted few years back in MAMC, Delhi, (transcervical CVS tissue was procured in early pregnancy patients going for medical termination of pregnancy and processed for direct karyotyping) as prenatal diagnosis and screening of genetic abnormalities in early pregnancy in 2001 by author herself. Although, conventional cytogenetics is accurate and reliable, but carries a disadvantage of prenatal tissue to be cultured for several days prior to analysis. It takes 10 days to obtain results and

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has a culture failure rate of 1%.[5] Trophoblastic sampling during first trimester of pregnancy has been a good source of foetal material for prenatal diagnosis. CVS is performed best in 10-13 wks. gestation while amniocentesis after 15 weeks gestation. Foetal chromosome analysis has been traditionally performed using Giemsa (G-banding) of cultured cells in metaphase and is considered the gold detection method.[4] This technique is accurate and reliable allowing detection of a variety of numerical and structural aberrations. The diagnostic accuracy of karyotyping and amniocentesis is 99.4%-99.8% and for CVS 97.5-99.6%.[1] The direct karyotyping method by Simoni et al in 1984 was based on direct analysis of spontaneous mitosis normally present in placental villi during first trimester of pregnancy. It being a fast and reliable method so became quiet acceptable (mitotic activity in trophoblastic tissues is a well-known phenomenon and histologic examination frequently reveals dividing cells in Langhans cells). On these grounds, we perform direct chromosome preparation on the spontaneous mitosis of placental tissues using a short treatment with 60% aqueous acetic and solution.[5,6] Thus, direct karyotyping of CVS tissue still carries importance for screening of genetic diseases in Peripheral Hospital (PHC) where minimal facility is available and there setting a lab can give us lot of information on genetic problems antenatally. This can also decrease load on referral centres (after explaining prospective parents about limitations and usefulness of CVS in detecting abnormalities). With advances in molecular genetics using Fluorescence in Situ Hybridisation (FISH) or Quantitative Fluorescence-Polymerase Chain Reaction (QF-PCR) can be applied to karyotype results within one or two days (with their own limitations).

AIM AND OBJECTIVES

This study was conducted few years back in MAMC, Delhi, (transcervical CVS tissue was procured in early pregnancy patients going for medical termination of pregnancy and processed for direct karyotyping) as prenatal diagnosis and screening of genetic abnormalities in early pregnancy in 2001 by author herself. Aim of this study was to review on cytogenetic evaluation of CVS obtained during first trimester of pregnancy by direct method of karyotyping of tissue, which can be used as a routine screening procedure for screening genetic abnormalities in peripheral hospitals and thus decreasing load on referral centres.

MATERIALS AND METHODS

Study conducted earlier in 2001 in Department of Anatomy, MAMC, in collaboration with Department of Obstetrics and Gynaecology, LNJP Hospital. 37 healthy cases with 6-12 weeks of gestational age coming for medical termination of pregnancy were included in the study. The patients were all meticulously evaluated through detailed history and examination and then subjected to routine and special investigations. Patients with following history were excluded from study- vaginal infection, vaginal bleeding and Rh-negative pregnancy. Written informed consent was taken prior to procedure and then each of subject was subjected to ultrasound-guided transcervical chorionic villus sampling by Brambati's method. Tissue was then immediately transported for direct karyotyping in a sterile container filled with RPMI (nutrient medium) to the Department of Anatomy for further processing.

DIRECT METHOD

(Simoni's method) the specimen was cleaned immediately to remove maternal blood and cervical mucus. Villus material was then separated from other tissue pieces under microscope to avoid maternal cell contamination. 10-20 mg of villus material was then incubated in 35 mm Petri dish containing 3 mL of RPMI 1640 medium substituted with 5% foetal calf serum and 1% Garamycin 0.5 micro gm/mL, colcemid was added at the final concentration and incubated for 4 hrs. Incubated specimen was then treated with hypotonic solution of 1% sodium citrate for 30 mins. at 37°C. Carnoy's fixative was then added. 0.4 to 0.8 mL of freshly prepared 60% acetic acid was added for 1-2 mins. and then dish was agitated the release of cells from villi was observed under microscope. The slides were prepared and allowed to air dry. They then were stained with Giemsa and subjected to banding (using Seabright's method). The metaphase spreads of each case was screened under high resolution microscope and at least 2 well spread metaphase chromosomes from each case was photographed. Paired chromosomes were cut out and arranged in groups and karyotypes were prepared. Thus, although, this method is fast, technically simple and reliable, chromosome preparation are available within a few hours of sampling for declaration of results. This is of great psychological stress of women requiring foetal diagnosis, but few technical problem like of obtaining high number of incomplete metaphases, unsatisfactory quality of banding remains at times still remain to be solved.

Therefore, Direct method is not good for diagnosing structural chromosomal aberrations, but still remained being successfully used as a part of diagnosis of genetic diseases in several centres of different countries. In first trimester, several hundred diagnosis have been made during past 10 yrs. by direct technique as preferential technique for chromosomal analysis.

RESULTS

A complete assessment in all 37 pregnant females was done according to preset proforma and for each patient 3 generations were covered (Picture A). One case no. 20 had a previous child with meningomyelocele. One case no. 29 had a previous child with history of delayed milestones. Out of 37 samples obtained, chromosomal analysis was performed on 30 samples metaphase spreads, which were processed by Direct method (Simoni's method) and following observations were made (Table 1, 2). Out of 37 samples processed, the biopsies gave positive result in 30 samples out of which 23 were normal female karyotypes, i.e. 46 XX and 7 were normal male karyotypes, i.e. 46 XY as studied by chromosome patterns (Pic B). No chromosomal

anomalies were detected on any of these karyotypes. Best biopsies were obtained with 8-12 weeks of gestation. G Banding could not be performed as chromosome obtained by above processing were found to be resistant to banding.

Sl. No.	Duration of Pregnancy	Transcervical CVS Done in Cases	Adequate CVS Tissue Obtained in Cases
1	6-7 wks.	4	Nil
2	7-8 wks.	3	Nil
3	8-9 wks.	16	16
4	9-10 wks.	12	12
5	10-11 wks.	1	1
6	11-12 wks.	1	1
Total	**6-12 wks.**	**37**	**30**

Table 1. Duration of Pregnancy Versus Adequate CVS Tissue

Age	Primi Gravida	2nd Gravida	3rd Gravida	4th Gravida	5th Gravida	6th Gravida
20-25 yrs.	Nil	2	10	0	0	0
26-30 yrs.	Nil	1	5	2	2	1
31-35 yrs.	Nil	2	6	2	1	1
>35 yrs.	Nil	1	1	0	0	0
Total	**0**	**6**	**22**	**4**	**3**	**2**

Table 2. Age Versus Gravidas

DISCUSSION

Genetic diseases are one of the major causes of hospital admissions due to disability and mortality particularly among children (1:5 children of hospital admission either partially/completely) as distribution of genetic diseases is not related to socioeconomic background, which implies that developing world has a large number of genetic diseases largely left uncared for, i.e. overall incidence of foetal/neonatal loss due to genetic/genetic environmental causes are as follows: 1:50 newborns have major congenital abnormality, 1:100 have a unifactorial disorder and 1:200 have a major chromosomal abnormality before birth. It is manifested as spontaneous abortion in early pregnancy, 60% have chromosomal abnormality and 50% cases of mental retardation, but most of the birth defects are preventable by appropriate screening, invasive and noninvasive prenatal diagnostic measures and accurate genetic counselling. Thus, fundamental philosophy and aim of foetal diagnosis is giving assurance to patients at risk of having a defective offspring or selectively having children free of serious genetic diseases.[7] Prenatal diagnosis employs a variety of techniques to determine the health and condition of an unborn foetus. Noninvasive methods include ultrasound, biochemical screening from maternal blood. Maternal serum screening in the second trimester has now been available for over two decades. More recently, first trimester screening tests offer women the opportunity of early screening for foetal aneuploidy and the option of early diagnosis. Invasive tests (amniocentesis and chorionic villus sampling) is advised for pregnancies that bear a high risk of being affected by a chromosomal aberration from family and individual history.

In 1968, first prenatal diagnosis of Down's syndrome was made by amniocentesis and foetal karyotyping. Although, CVS has been in vogue in India in selected centres since 1984.

Physicians prefer this method for easier management of cases, especially if undesirable results are obtained, while for patients, it is more acceptable for social and psychological reasons. Since, syncytiotrophoblast contains non-proliferating cells, the cytotrophoblast contains actively dividing cells. The mesenchyme core contain cells, which can be released by different dissociation protocols and released cells will proliferate in culture. So, advantage of direct preparation (Simon's method) is that a karyotype obtained in a relatively short time (within 24-48 hrs.) after CVS and risk of maternal cell contamination.

Chorionic Villus Sampling

Indications for CVS; advanced maternal age, bad obstetric history, thalassaemia, sickle cell anaemia, inborn errors of metabolism, etc. Advantages as it is first trimester procedure, so less disturbing to mother and family. It is comparatively safe in expert hands and abortion risk being as low as 0.1%. The material obtained can be used for karyotyping, DNA analysis, enzyme studies and other biochemical tests. Disadvantages- maternal cell contamination, miscarriage/abortion following CVS, infection/bleeding after procedure, vanishing twins being commonest disadvantages of CVS sampling, besides all these many of the malformations cannot be diagnosed before 2nd trimester, thus CVS cannot be used to diagnose such disorders.

Risk of transverse limb deficiency (0.03-1.00%) according to a study conducted in 1995. Risk of limb deficiency appear to be associated with if done in <10 weeks gestation (0.20%). This study can't be ignored and most important an experienced hand is required for doing it.

As sufficient number of suitable metaphases obtained, foetal karyotype is determined within few hours of sampling. No problem of maternal cell contamination are advantages of direct karyotyping. So, it could be used as a routine procedure for first trimester foetal diagnosis to obtain wealth of information in a short time on the characteristics of the tissue and abnormal chromosomal constitution. While high number of incomplete metaphases obtained and unsatisfactory quality of banding remains as disadvantages of direct karyotyping.

In 1989, a multicentric trial was done, which concluded that CVS if done in early pregnancy or first trimester is relatively safe. No evidence of IUGR or preterm birth weight was noticed.

In 1993,[8,9] possible association with foetal limb defects in 1991 especially in late pregnancy was reported. Transabdominal CVS was preferred to amniocentesis in a study done in 1994. In 1994 and 1997, prenatal ultrasonic and molecular diagnosis on Apert syndrome was made for advanced maternal age and 46 XY dup(10q) in direct CVS preparation and mosaic 48 XXY dup(10q) in CVS long-term culture foetal tissue was found.

Hence, recent availability of high-resolution ultrasound and molecular approaches to analysis has made CVS for first trimester prenatal diagnosis, a timely development in 1983, 1984,[2,8,9] and biochemical DNA analysis was also made possible.[10,11,12,13] Thus, direct karyotype technique allows one to do a chromosome count and to detect any major structural derangement within a short period.[14,15,16] Although, Chorionic Villus Sampling (CVS) and amniocentesis are prenatal diagnostic procedures that are performed to detect foetal abnormalities (0.03%-0.10%), but concerns about relative safety of these procedures arose in 1991 and after CVS reports were published about a possible association between CVS and birth defects in infants, i.e. either digital/limb defects after CVS is one of important factor and for this it should be done in early pregnancy. Second factor was miscarriage, which was attributed to (0.5%-1%) of CVS procedures and 0.25%-0.50% of amniocentesis.[4] Besides all these, possibility of this procedure being misused for sex determination can't be ruled out.

SUMMARY AND CONCLUSION

Thus, to summarise chromosome preparation, which were obtained from CVS by Direct method (Simoni's method) had advantage of providing sufficient number of suitable metaphases, foetal karyotype can be determined within few hours of sampling, no maternal cell contamination and thus could be used as a routine procedure for first trimester foetal diagnosis. It can be used to obtain wealth of information in a short time on the characteristics of the tissue and abnormal chromosomal constitution. But, in our study, metaphase spreads of 30 out of 37 cases prepared showed low mitotic index, less no. of metaphases and best transcervical CVS should be done at 8-12 weeks of gestational age.

Therefore, direct method is not good for diagnosing structural chromosomal aberrations, but still remained being successfully used as a part of diagnosis of genetic diseases in several centres of different countries. This method is fast, technically simple and reliable chromosome preparation are available within a few hours of sampling for declaration of results. This is of great psychological stress of women requiring foetal diagnosis. So, can be used as a routine screening procedure for screening genetic abnormalities in peripheral hospitals and thus decreasing load on referral centres. Thus, main aim of prenatal diagnosis to secure accurate diagnosis of suspected abnormality as early as possible with minimal disturbance to pregnancy so that adequate preventive measures can be taken is achieved.

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