Prenatal detection of aneuploidies using fluorescence in situ hybridization: A preliminary experience in an Indian set up

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Fluorescence in situ hybridization (FISH) is a powerful molecular cytogenetic technique which allows rapid detection of aneuploidies on interphase cells and metaphase spreads. The aim of the present study was to evaluate FISH as a tool in prenatal diagnosis of aneuploidies in high risk pregnancies in an Indian set up. Prenatal diagnosis was carried out in 88 high-risk pregnancies using FISH and cytogenetic analysis. Multicolour commercially available FISH probes specific for chromosomes 13, 18, 21, X and Y were used. Interphase FISH was done on uncultured cells from chorionic villus and amniotic fluid samples. FISH on metaphase spreads was done from cord blood samples. The results of FISH were in conformity with the results of cytogenetic analysis in all the normal and aneuploid cases except in one case of structural chromosomal abnormality. The hybridization efficiency of the 5 probes used for the detection of aneuploidies was 100%. Using these probes FISH assay yielded discrete differences in the signal profiles between cytogenetically normal and abnormal samples. The overall mean interphase disomic signal patterns of chromosomes 13, 18, 21, X and Y were 94.45%; for interphase trisomic signal pattern of chromosome 21 was 97.3%. Interphase FISH is very useful in urgent high risk cases. The use of FISH overcomes the difficulties of conventional banding on metaphase spreads and reduces the time of reporting. However, with the limited number of probes used, the conventional cytogenetic analysis serves as a gold standard at present. It should be employed as an adjunctive tool to conventional cytogenetics.


1. Introduction

The most common chromosomal abnormalities in new-borns are trisomies 21, 18, 13, monosomy X and other sex chromosome aneuploidies (Divane et al 1994). These aneuploidies can account for up to 95% of liveborn chromosomal abnormalities (Whiteman and Klinger 1991). Chromosomal abnormalities lead to a significant genetic disease burden on the society. Fluorescence in situ hybridization (FISH) introduced more than a decade ago, as a potentially powerful tool in clinical cytogenetics (Cremer et al 1986; Julien et al 1986) can provide a rapid and relatively reliable detection of aneuploidy of these chromosomes (Jalal et al 1998).

Prenatal diagnosis is accomplished by conventional cytogenetic banding of metaphase chromosomes, obtained from fetal trophoblast tissue (from chorionic villus biopsy), amniocytes (from amniotic fluid) or fetal lymphocytes (from cord blood). This technique is accurate and reliable allowing the detection of a variety of numerical

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Abbreviations used: βhCG, β-human chorionic gonadotropin; CVS, chorionic villus sampling; FISH, fluorescence in situ hybridization; MSAFP, maternal serum alpha-feto protein.
and structural aberrations (Ferguson-Smith and Yates 1984; Hook and Cross 1987). The primary disadvantage of the conventional cytogenetics is that the prenatal tissue must be cultured for several days prior to analysis. It takes 10 days–3 weeks to obtain results and has a culture failure rate of about 1% (Thein et al 2000). In certain clinical situations the time required to complete the chromosomal analysis might place a significant clinical or emotional burden on the patient and/or health-care provider. In such cases a method which provides rapid and accurate identification of aneuploidies would be a useful adjunctive diagnostic test to conventional cytogenetics (Ward et al 1993). FISH has been found to be highly effective for rapidly determining the number of specified chromosomes in interphase cells (Cremer et al 1988; Lichter et al 1988). FISH technique allows identification of specific nucleic acid sequences from chromosomes, even when the nuclei are in non-dividing interphase stage.

During late 1980s and early 1990s technical issues were the focus of research. Specific probes, determination of cell types suitable for use with FISH, and more effective techniques for cell preparation and signal detection were intensively studied (Philip et al 1994). The results of these efforts were technical advances such as commercially available highly specific and reliable probes, direct labelling, and multicolour computerized signal detection systems (Divane et al 1994; Jalal et al 1998). Such improvements have only increased the demand for immediate answers, and have made clinicians and their patients even more intolerant to delays in receiving results (Evans et al 1999). Advances in molecular techniques, including chromosome-specific probes and in situ hybridization techniques have generated considerable demand for extremely rapid results, particularly as they can be applied to uncultured cells (Martin et al 1996). These have been applied to common trisomies and monosomies with a rapid acceptance in the early 1990s to use FISH in high risk situation. However there has also been pressure towards its utilization in low risk situations (Evans et al 1992). Despite these advances, concerns on the sensitivity, specificity and predictive values of the test and lack of uniform laboratory methods produced profound scepticism in the genetics community. The singular benefit of FISH is the rapid detection of aneuploidy by chromosome specific probes applied to interphase cells. A FISH assay can easily be completed in less than 48 h compared to the 10–14 days usually required with conventional cytogenetic analysis. FISH analysis for detection of aneuploidies of chromosome 13, 18, 21, X and Y has been successfully performed with a high degree of concordance with cytogenetic results (Nederlof et al 1990; Ward et al 1993; Philip et al 1994; Eiben et al 1999). Not all chromosome abnormalities particularly structural rearrangements can be routinely identified by FISH when compared with conventional cytogenetic analysis (Evans et al 1999). Guidelines have been established for the use of FISH in prenatal diagnosis (American College of Medical Genetics 1993). These guidelines stated that FISH for clinical cytogenetic studies should be considered as an investigational technique.

There are extensive data available for prenatal diagnosis of chromosomal abnormalities using FISH from the western countries (Bryndorf et al 1996, 1997; Cacheux et al 1994; Cooper et al 1998; Eiben et al 1998; Klinger et al 1992; Mercier and Bresson 1995; Jalal et al 1998; Verlinsky et al 1998; Ward et al 1993) but there are no reported studies from an Indian set up. The policy statement of the American College of Medical Genetics (1993) also states that among the foremost unresolved issues in the utilization of FISH for interphase analysis in prenatal cytogenetics is to expand and bring this technology to a large number of prenatal diagnostic centres via independent FISH trials.

The aim of the present study was to evaluate FISH as a tool in prenatal diagnosis of high risk pregnancies in an Indian set up. Prenatal diagnosis was carried out in high-risk pregnancies i.e. advanced maternal age, previous child with chromosomal abnormality, fetal malformation detected through ultrasound examination, abnormal values of biochemical markers in the maternal serum and in parents with chromosomal rearrangements. These high risk patients can benefit from fast results since they suffer from substantial anxieties. It was anticipated that this study would allow cytogenetic evaluation of a significant number of normal as well as abnormal cases as the incidence of chromosomal abnormalities is expected to be relatively high in this group. These cases were analysed using FISH on interphase cells and metaphase spreads and conventional chromosomal analysis in order to test the efficiency and utility of FISH for the detection of common aneuploidies. For prenatal diagnosis, aneuploidies of chromosomes 13, 18, 21, X and Y were screened using FISH. The present study was based on the use of multicolour commercially available probes.

2. Materials and methods

During the years 1997–2000, prenatal diagnosis was performed in 88 high risk pregnancies presenting at the antenatal clinic of the Department of Obstetrics and Gynecology, All India Institute of Medical Sciences, New Delhi. Indications used to classify the pregnant patients as high-risk pregnancies for prenatal diagnosis were as follows: advanced maternal age (age > 35 years) (n = 22), previous child with chromosomal abnormality (n = 31), fetal abnormality detected through ultrasound.