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STUDY OF APPLICATIONS OF FLUORESCENCE IN SITU HYBRIDIZATION (FISH) IN PRE-NATAL AND POST-NATAL DIAGNOSIS

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ABSTRACT : Genetic testing focuses on DNA molecules that are packaged into thread-like structures called chromosomes. Gene mutations occur due to changes to the DNA sequence, chromosomal structure, or number of chromosomes. Genetic aberrations play an essential role in many genetic disorders and can be inherited from parents or occur spontaneously during embryonic development. With advances in human genetic research and analysis technologies, various types of causative genetic aberrations associated with disorders can be detected prenatal and postnatal thus providing valuable information to aid parents, physicians, and genetic counsellors in making the best decisions before and after birth. Although conventional cytogenetic remains the 'gold standard' for whole genome screening from a variety of prenatal and postnatal tissues, its use is restricted entirely to dividing cells. Problems are encountered while dealing with tissues like amniotic fluid, bone marrow etc. that yield a low mitotic index with poor quality metaphases. The advent of Fluorescent In Situ Hybridization (FISH) has been a boon in such cases, as it offers an unprecedented opportunity for analysis of non-dividing cells (interphase cells). Apart from this, FISH has the power to detect sub-microscopic rearrangements and abnormal clone of small size and can be used on various tissues like buccal mucosa, blood and bone marrow slides or fixed pellets usually available can be used for FISH analysis.

Key words: Chromosomes, Aberrations, Prenatal, Postnatal, FISH.

Introduction

Conventional cytogenetics plays an important role in the identification of chromosomal aberrations associated with human disease, particularly in prenatal and postnatal diagnosis and in malignancies (Balwan and Saba, 2020). Chromosomal changes often reflect events occurring at the molecular level within the cell and provide important clues about the location of genes involved in these events (Rabbits, 1994; DeVita e.al., 1997). Although traditional chromosomal banding techniques are critical in the assessment of karyotypic changes, these techniques have certain inherent limitations that complicate accurate characterization of genomes (Heim and Mitelman, 1995, Balwan and Gupta, 2012). These limitation which apply particularly to prenatal diagnosis are (a) difficulty in culturing of fetal tissues (amniotic fluid, chorionic villus and fetal blood) which typically produce chromosomes of poor quality (b) maternal cell contamination (in case of fetal tissues) that makes subsequent analyses problematic (c) time consuming (d) labor intensive and (e) the presence of complex karyotypes which often precludes reliable, comprehensive identification and characterization of chromosomal abnormalities.

Since the discovery, by Zech and Caspersson (Caspersson et al. 1968, 1970), that appropriate staining results in a banded appearance of chromosomes, various methods for banding of metaphase chromosomes have been used as standard techniques in pre- and postnatal diagnostic applications. Giemsa bands obtained by digestion of the chromosomes

by the proteolytic enzyme trypsin (GTG-bands) are the bands most widely used for routine chromosome analysis in clinical laboratories. However, GTG-banding can achieve a resolution to only the single-band level—that is, ~5–10 million bp. Thus, it is not surprising that another option for karyotype analysis, FISH, has become very popular in diagnostic applications. Although conventional cytogenetic remains the gold standard for whole genome screening from a variety of prenatal and postnatal tissues, its use is restricted entirely to dividing cells. Problems are encountered while dealing with tissues like amniotic fluid, bone marrow etc. that yield a low mitotic index with poor quality metaphases. The advent of Fluorescent In Situ Hybridization (FISH) has been a boon in such cases, as it offers an unprecedented opportunity for analysis of non-dividing cells (interphase cells). Apart from this, FISH has the power to detect sub-microscopic rearrangements and abnormal clone of small size and can be used on various tissues like buccal mucosa, blood and bone marrow slides or fixed pellets usually available can be used for FISH analysis. FISH technique can also be used on previously banded slides that can not only yield immediate results but can also be used to correlating FISH results with those of conventional cytogenetics. The molecular cytogenetic technique that bridges the gap in resolution between conventional cytogenetics and molecular cytogenetics is based on the ability of single stranded DNA to anneal to complimentary DNA. The applications of this technique include aneuploidy detection, translocation and structural breakpoint analysis, microdeletion detection, gene mapping, identification of marker chromosomes and diagnosis and prognosis of various cancers.

Delineation of Numeric Chromosomal Abnormalities

Numeric chromosomal abnormalities like Trisomy 21, Trisomy 13, trisomy 18, etc. can be detected readily using chromosome enumeration probes. For example, alpha satellite probe specific for chromosome 21can be used to detect trisomy 21 or Down's syndrome. This is particularly helpful in cases with low level of mosaicism. A variety of tissues that are not amenable to conventional cytogenetic analysis can be analysed using FISH for aneuploidies.

Identification of Sex Chromosome Anomalies

FISH technique using probes specific for X and Y chromosome plays an important role in assessing chromosome copy number in interphase as well as metaphase cells. This is particularly useful in sex chromosome aneuploidies like Turner's syndrome (45,X), Klinefelter's syndrome (47,XXY) etc. the importance of FISH lies in the characterisation of sex chromosome compliment in cases with ambiguous genitalia. FISH using specific probe for SRY or the sex determining region on Y chromosome in cases with ambiguous genitalia is essential. Sex chromosome aneuploidies can also be detected using FISH on non-invasive tissues like fibroblasts or buccal mucosa.

Detection of Duplications

When structural rearrangements or duplications are detected, the identity of the chromosomes involved allows appropriate counselling. However it has been difficult to determine the chromosomal origin of the extra material and exact breakpoints of the duplicated segments using routine banding techniques. Both conventional and molecular cytogenetic methodologies should be used to characterise the duplicated chromosomal material in these de novo rearrangements and allow for the appropriate counselling. It has been recommended that FISH be used to study all chromosome abnormalities (Neumann, et al., 1992, Siffroi et al., 1994).

Detection of Subtle/Cryptic Rearrangements

The detection of subtle chromosomal rearrangements with standard banding analysis can often be difficult. This is especially true for prenatal diagnostic studies in which the specimens cannot be analysed easily with high resolution procedures. However, even high resolution analysis is not always sufficient for the interpretation of small structural

rearrangements or complex karyotypes. Over the past several years, several studies have demonstrated the effectiveness of FISH with chromosomal libraries or single copy probes for confirming or clarifying the G-banded interpretation of subtle or cryptic constitutional translocations (Bernstein et al., 1993).

Before the development of FISH, precise characterisation of subtle rearrangements was tedious. Additional work involving multiple cell harvests and additional chromosome banding techniques together with a high degree of analytical skill at the microscope was necessary for interpretation of these subtle rearrangements. This work is time consuming and laborious. These obstacles were especially formidable in the area of prenatal diagnosis, in which time is of the essence. Thus, the advent of FISH was especially advantageous in the analysis of subtle rearrangements. When a carrier of a subtle translocation decides to have prenatal testing, application of FISH provides a definite advantage for determining whether the fetus has unbalanced karyotypes.

Detection of Microdeletion

One of the most common uses of FISH over the last several years has been in the detection of microdeletions associated with contiguous gene syndromes. FISH is the most effective in detecting these syndromes in postnatal populations in which the clinical phenotype dictates which probes should be tested. For prenatal diagnostic studies, FISH probes are often used if there is a question posed by the G-banding pattern, affecting the regions involved in the microdeletions. Additionally, an increased number of foetuses identified on prenatal diagnosis with congenital heart defect are referred for FISH with a probe to detect a 22q deletion. FISH analysis can also be used to study microdeletions that have resulted from cryptic rearrangements. Such cryptic rearrangements have been identified both in Prader-Willi syndrome, Angelman syndromes and Miller-Dieker syndrome (Kuwano et al., 1991, Burke et al, 1996).

Identification of Marker Chromosomes

Determining the origin of chromosomal material that cannot be identified by conventional banding (i.e. marker chromosome) has been greatly facilitated by molecular cytogenetic studies. Classification of such marker chromosomes is important for phenotype/karyotype correlations, which is imperative for proper counseling. FISH analysis using repetitive alpha satellite DNA probes is less complicated and an effective technique for identification of origin of marker chromosomes.

Although marker chromosomes have been identified prenatally also, the majority of this work has been done in postnatal studies. The frequency of marker chromosomes identified at birth is 0.14-0.72/1000 births, whereas their frequency in prenatal diagnostic studies is slightly elevated to 0.65-1.5/1000 (Ferguson-Smith and Yates, 1994; Hook and Cross, 1987). The elevated frequency seen in prenatal studies is most likely associated with the advanced maternal age seen in the prenatal population. Approximately 40% of detected markers are inherited and thought to be heterochromatic, approximately 60% are *de novo*.

Diagnosis and Prognosis of Cancers

FISH analysis is a useful adjunct to conventional cytogenetics in the analysis of various cancers like leukemias, lymphomas etc. The use of FISH analysis on non-dividing cells is important in cancer tissues especially solid tumors and leukemic patients on therapy (e.g., CML patients on Interferon therapy) where it is difficult to yield good quality well spread metaphases. This molecular cytogenetic technique helps not only in diagnosis of molecular rearrangements in cancers but also in evaluation of minimal residual disease and prognosis.

FISH is also a useful diagnostic tool for detecting premalignant lesions or secondary tumors in bladder washes. Identification of gene amplifications (c-myc, N-myc, HER-2/neu etc.) and gene deletions (p53, Rb etc.) has important implications in diagnosis and prognosis of various cancers like breast cancer, prostrate cancer, Retinoblastoma etc.

Interphase FISH for Prenatal Diagnosis

Both repetitive and locus specific probes have to be used to determine chromosome number for aneuploidy. The obvious advantage of interphase analysis or direct analysis is that it provides a result more quickly because it obviates the need to wait for the growth of cells. When applied to prenatal diagnosis, this can reduce the necessary time from 7-10 days for metaphase analysis to just 48 hours. Two basic approaches for rapid interphase analysis used are:

i. Hybridization of probes onto cells after attachment to coverslips and
ii. Hybridization of probes directly onto fixed non-cultured cells.

Most studies have indicated that the later approach is more successful and yields better results.

Interphase FISH analysis has been successfully applied in diagnosis of chromosome aberrations in uncultured or short term cultured amniocytes and chorionic villus cells (Eiben et al., 1999; Pergament et al., 2000). For women with advanced maternal age (>35 years), FISH analysis could identify 845 of all chromosome aberrations. FISH was shown to be very reliable for diagnosing 60% of cases with Down syndrome when maternal serum screening indicated a risk. However, it detected upto 94% of the chromosome aberrations present in women whose second trimester ultrasound revealed structural changes in the fetus (Pergament et al., 2000). The majority of laboratories found there were no false positives or false negatives and except for rare cases of chromosome mosaicism, the number of cells with abnormal signals consistently exceeded 95% for monosomy of the X chromosome, Trisomies of 13, 18 or 21, triploidy or aneuploidy for X or Y chromosome. Furthermore, maternal contamination was not clinically significant complication in these studies, although the expertise of the obstetrician performing amniocentesis or CVS was critical (Hockstein et al., 1998). There was upto 20-30% of the cases, however, wherein FISH was not designed to identify the chromosome aberration, of which 8% would potentially be responsible for an adverse pregnancy outcome i.e., congenital malformation and delayed development (Evans et al., 1999; Pergament et al., 2000).

Confined Placental Mosaicism

This is a phenomenon described in infants born with unexplained intrauterine growth retardation. Unlike mosaicism, which is characterised by the presence of two or more karyotypically different cell lines within both the fetus and placenta, confined placental mosaicism represents tissue specific chromosomal mosaicism affecting only the placenta. Such situations have been observed in cases where chorionic villus sampling karyotypes are mosaicism but follow-up amniocentesis or fetal blood samplings show normal diploid results. FISH analysis can be used for screening for various aneuploidies in such cases.

FISH in Pre-implantation Genetic Diagnosis

The major cause of pregnancy failure following in-vitro fertilization (IVF) and embryo transfer is a chromosome aberration. Using standard FISH analysis for as few as three chromosomes, estimates of chromosome aberrations in fertilized oocytes and Preimplantation embryos have exceeded 60% (Delhanty, 1997). Studies are currently in progress to determine whether selecting Preimplantation embryos prior to transfer on the basis of FISH analysis for seven chromosomes i.e. 13, 16, 18, 21, 22, X and Y will enhance the rate of implantation, decrease the rate of spontaneous abortion which is characteristically higher in women undergoing IVF and increase the so called ‘take home baby rate’. Moreover this same approach is being applied, in particular, to women of advanced maternal age, 37 years of age and older, since this group has significantly lower pregnancy rates following IVF compared to their younger counterparts. The potential of these studies lies in determining whether molecular techniques for chromosome analysis of Preimplantation embryos will be applied on a regular basis to all women undergoing IVF (Neumann et al., 1992; Pergament, 2000).

For parents at high reproductive risk for chromosomally unbalanced gametes because they carry structural rearrangements, such as reciprocal translocations, Preimplantation genetic diagnosis offers the opportunity to enhance pregnancy outcomes. By using a combination of sub-telomeric probes, it is possible to identify all possible segregation products in parents who are balanced translocation carriers.

Interphase FISH on various Tissues

Sex chromatin (Barr body) analysis of buccal mucosa is an inexpensive, non-invasive and rapid means for sex determination. Due to its lack of reliability and inability to detect mosaicism, it is not used as a routine test. However, with the advent of FISH technique, the value of this sample, albeit obsolete test is being re-evaluate. Buccal smears, in absence of a more suitable sample can be used for identification of various sex chromosome aneuploidies by FISH analysis.

Similarly FISH can be performed on cervical smears for screening of cervical cancers. Apart from smears, FISH analysis can also be done on formalin-fixed or paraffin embedded tissues. This is important for correlating genetic findings with pathological results. Further FISH analysis can be applied on sperms for infertility and genotoxicology studies.

Conclusion

This field of molecular cytogenetics marks the ‘colour age’ in cytogenetics that brings with it enormous potential to detect gene and chromosomal alterations in cells at the highest level of resolution. With the ready access of commercially available probes, FISH has become an integral part of genetic testing in prenatal as well as postnatal diagnosis.

Reverences

1. Balwan W.K and Saba N. (2020). Prenatal Diagnosis in service of mankind. A Review. *International Journal of Science and Research, 9(7):631-634.* ISSN-2319-7069.
2. Balwan W.K and Gupta S. (2012). Karyotypic Detection of Chromosomal Abnormalities in Referred Cases with Suspected Genetic Disorders. *Bulletin of Environment, Pharmacology and Life Sciences (BEPLS)*, 2(1):16-19.
3. Bernstein R., Bocain M.E. and Cain M.J. (1993). Identification of a cryptic t(5;7) reciprocal translocation by fluorescent in-situ hybridisation. American Journal of Medical genetics, 46:77-82.
4. Burke L.w., Wiley J.E. and Glenn C.C. (1996). Familial cryptic translocation resulting in Angelman syndrome implications for imprinting or localization of the Angelman gene. American Journal of Medical Genetics, 58:777-784.
5. Caspersson T., Farber S., Foley G.E., Kudynowski J., Modest E.J., Simonsson E and Wagh U. (1968). Chemical differentiation along metaphase chromosomes. Exp Cell res, 49: 219-222.
6. Caspersson T., Zech L and Johansson C. (1970). Differential banding of alkylating fluorochromes in human chromosomes. Exp Cell Res, 60:315-319.
7. Delhanty J.D. (1997). Chromosome analysis by FISH in human Preimplantation genetics. Human reproduction, 12:153-155.
8. DeVita V.T., Hellman S. and Rosenberg S.A. (1997). Cancer principles and practice of oncology. 3rd edition Phildelphia, New York: Lippincott-Raven.
9. Eiben B., Trawicki W., Hammans W., Gebel R., Pruggmeyer M and Epplen J.T. (1999). Rapid prenatal diagnosis of aneuploidies in uncultured amniocytes by fluorescence in situ hybridization. Fetal Diagn Therapy, 14:193-197.
10. Evans M.I., Henry G.P. and Miller W.H. (1999). International collaborative assessment of 146,000 prenatal karyotypes: expected limitations if only chromosome specific probes and fluorescent in-situ hybridization are used. Human reproduction, 14:1213-1216.
11. Ferguson-Smith M.A and Yates J.R.W. (1984). Maternal age specific rates for chromosome aberrations and factors influencing them; report of a collaborative European study on 52,965 amniocentesis. Prenatal Diagnosis, 4:5-44.
12. Heim S and Mitelman f. (1995). Cancer Cytogenetics. 2nd Edition, New York: Wiley-Liss.

13. Hockstein S., Chen P.X and Thangavelu M. (1998). Factors associated with maternal cell contamination in amniocentesis samples as evaluated by Fluorescence In Situ Hybridization. Obstetrics and Gynecology, 92:551-556.
14. Hook E and Cross P. (1987). Rates of mutant and inherited structural cytogenetic abnormalities detected at amniocentesis, result on about 63,000 fetuses. Ann Hum Genet., 51:27-55.
15. Kuwano A., Ledbetter S.A and Dobyns W.B. (1991). Detection of deletions and cryptic translocations in Miller-Dieker syndrome by in-situ hybridization. American Journal of Human Genetics, 49: 707-714.
16. Neumann A.A., Robson L.G and Smith A. (1992). A $15p^{+}$ variant shown to be a t(Y;15) with fluorescence in-situ hybridisation. Ann. Genet., 4:227-230.
17. Pergament E., Chen P.X., Thangavelu M and Fiddler M. (2000). The clinical application of interphase FISH in prenatal diagnosis. Prenatal Diagnosis, 20:215-220.
18. Rabbits T.H. (1994). Chromosomal translocations in human cancer. Nature, 372:143-149.