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done for IUD at 30 wks and cord blood was sent for karyotyping which revealed insertion in the above region.

Addl material on 15 p

46, XX, 15p + Female karyotype

This 23 yr old lady with G2P1L1 had a anomaly scan at 18 wks which showed hydrocephalus with thinned brain parenchyma and multiple dorsolumbar spina bifida. She underwent induced abortion at 19 wks and cord blood sample showed additional material on short arm of chromosome 15.

46, XY, 15p + Male karyotype

This karyotype was seen in newborn baby born to 25 yr old G3P2L2 mother. Anomaly scan at 30 wks showed cleft lip and palate.

46,XX, addl 1 (p36) female karyotype

Additional chromosome material was seen on short arm of chromosome 1 origin? partial Trisomy 13 and was advised to confirm by molecular tests FISH. This new born baby was born to 30 yr old G4P3L3 mother. Anomaly scan showed VSD and two soft markers (rocker bottom feet and clenched fist).

Downs syndrome

There were 5 cases of Trisomy 21. Two cases had Robertsonian translocation (The extra chromosome 21 is translocated to chromosome 22 in one and other chromosome 21 in one). The age ranged from 19 to 29 yrs. Only 2 cases had age more than 25 yrs. Five cases had free trisomy 21. Five of the seven cases had structural cardiac anomalies. Five had live babies at term. One had induced abortion at 19 wks and the other had IUD at 24 wks.

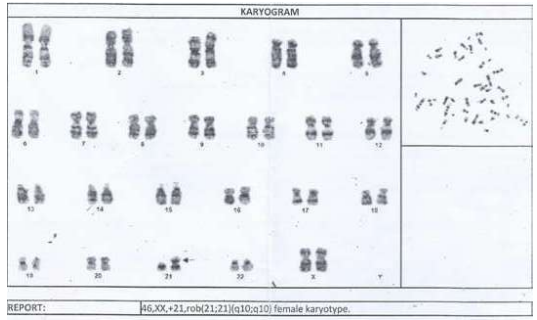

Fig. 2: Robertsonian translocation 46, XX, +21, rob (21; 21) q10; q10) indicating Downs syndrome

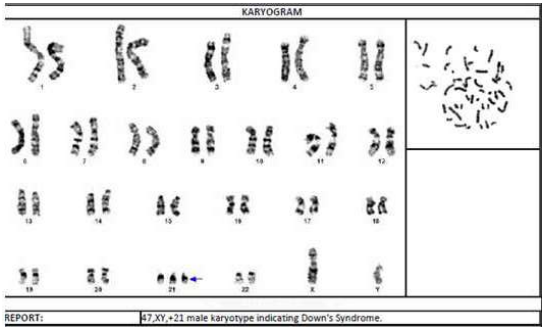

Fig. 3: Trisomy 21

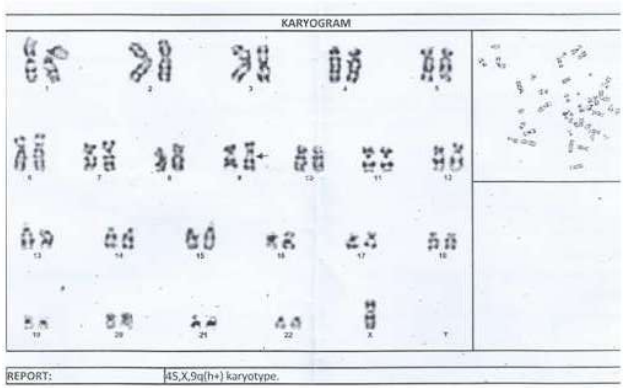

Fig. 4: Monosomy X. Turners syndrome

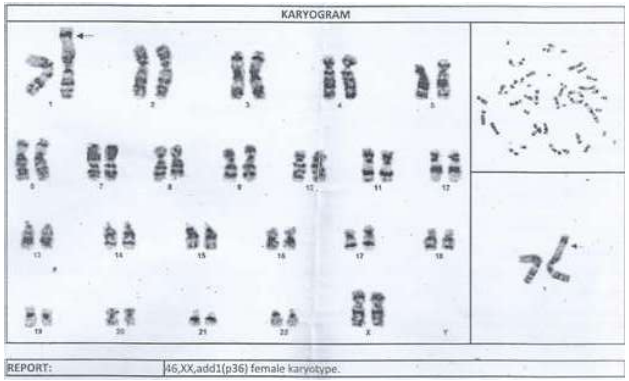

Fig. 5: Additional chromosomal material in 1p

Genetic Counselor's note: Additional chromosomal material is seen consistently on short arm of chromosome 1, the origin of which has to be confirmed by molecular tests.

Fist with specific probes for the region in chromosome 13 is recommended to rule out partial trisomy 13.

Discussion

Abnormal karyotype

In our study abnormal karyotype was found in 28.5% (12/42) cases. Our study population included prenatally detected congenital abnormalities on ultrasound.The mean age of our population is 24. The incidence of abnormal karyotype in anomalous foetuses detected by ultrasound in table 10.

In our series one patient had IUD at 24 wks and one had stillbirth at 30 wks GA. In both cord blood was sent immediately after birth. An abnormal karyotype was found in both.

comfortably used for targeting diverse molecules.

Research applications of FISH are arduous and need expert manpower. The development of validated protocols is to be framed which are needed to be framed are laborious and require substantial fund for the establishment of set-up. In addition, it is equally important to ensure that the steps in the study protocol are specific to particular probe and for the sample as well [21]. This has to be taken careof individually for each set of conditions pragmatically. Moreover, it is recommended that by decreasing the span of hybridization cumulative mechanization of the assay should be done. It not only curtails use of expertise labor but also improves the comparability amongst specimens for investigations which can help lab set ups to considerably improve FISH ubiquity in clinical diagnostics.

Present status and recent advances

In present era, microfluidic technology have arisen as potentgear for evaluation of cells [22]. The researchers globally in the fraternity has developed micro FISH implementations, which is also known as μ FISH for evaluating cytological specimen. The peculiar physical characteristics of laminar flow and the persistent need of replacement of reagents can be leveraged to decipher few tailbacks of FISH technology by plummeting the cost per test, mechanizing the assay, or making the execution modest [23].

Another recent trend involves micro-scale FISH of suspended cells and adherent cytological substrates. Various μ FISH-based applications for the exploration of bacterial and mammalian cells in suspension have been formulated. In such cases the segregation attained by procedures such as flow cytometry and capillary electrophoresis etc.[24].

By the utilization of micro-scale FISH, spatial multiplexing of a range of probes on a solitary sample is done easily. This proposes the enhanced probability of perceiving multiple target sequences by the use of a single dye and detection pattern, enables the utility of FISH even in experimental set-ups with sparse microscopic facilities. Developments in detection and computer processing algorithms have consequently made possible the detection of the most specified minuscule targets.

The newly developed tissue microarray technology is another well-tested advancement for the overview of precise molecular profiling of various tumor specimens at the single cell level. These progressions has paved way for more astutemeans of minimally invasive biopsy procedures [25].

Conclusion

It is believed that forthcoming technologies in approaching times will comprise of progressively higher-order multiplexing, until the number of interesting nucleic acid targets is attained. The novel stream of molecular pathology is focussing on averting the obscurities of morphological features by reviewing the root cause of disease by depiction of genetic mutations and various gene products. FISH is therefore already a technique which has surfaced the path through which the researchers visualize and conceptualize genes, chromosomes, transcription and nucleic acid movements.

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History

Itstartedinitially withsimplerin-situhybridization which progressed to immunofluorescence-based protein detection procedures. In 1941 the pioneer to begin with immunofluorescence was Coons et al. [5]. Earlier the antibodies were conjugated to fluorochromes and this was completely without loss of their epitope-binding specificity. It took more than three complete decades to be used as regular application. Progression of technique then reached to obtain the first antibody-dependent fluorescent detection of nucleic acid hybrids [6]. It is noteworthy that this technology was shortly substituted with the induction of fluorescent nucleic acid probes. The preliminary in situ hybridizations, carried out in the late sixties were actually not fluorescent. In these procedures, the probes labeled with radioisotopes were utilized. The major reason to Curtail these initialtests were the truncated sensitivity and the inadequate availability of sequence-specific probes [7]. Even thoughin terms of detection the radioisotope labeling was deliberated the most sensitive method as it involved extensive exposure times in detection of H^3 (up to several weeks) and lower spatial resolution (in the range of Mega bases) [8]. These detriments steered to the progression towards the non-isotopic labelling approaches for in situ hybridisation probes.

FISH which was the assay of choice for localization of specific nucleic acids sequences was technologically advanced as a substitute to pre-existing procedures that used radiolabelled probes [9]. Conventional methods involved non-specific labelling strategies, such as the random integration of radioactive modified bases into emerging cells, trailed by autoradiography followed by development of novel procedures stimulated by fewsnags of isotopic hybridization.

In eighties, the first application of fluorescent in situ detection was introduced. Here a fluorophore was used as a probe for specific DNA sequences on 3′ end of the RNA [10]. Fluorescent probes were prepared by enzymatic integration of fluorophore-modified bases all the way through the length of the probe which produced one color at single instance [11]. Then came theusage of amino-allyl modified bases [12]. With a feature that it could be conjugated to any genus of fluorophore. This was a turning point in the field of in situ expertise because it permitted development of arange of low key probes by simpler chemical interactions. Approaches of indirect detection permitted signal productivity to be augmented artificially by the practice of ancillary reporters that fix to the hybridization probes.In the same decade, evaluations including nick-translated, biotinylated probes and secondary detection started to be utilized for detection of specific nucleic acid [13,14] targets. In 1993, upgraded labelling of synthetic, single-stranded DNA probes allowed the chemical preparation of hybridization probes carrying enough fluorescent molecules to allow direct detection [15]. Several alterations of indirect and direct labelling being discovered so far, provided an eclectic continuum of detection schemes to be picked up from.

Advancing technology

There are many probes and diverse probe labelling methods followed for producing closely labelled FISH probes such as digoxigenin labelling, cis-platinum complex-mediated labelling or PCR [16] etc. This aided in effectual cohort of probes containing several labels for multiplex FISH. It paved the way for other novel means such as comparative genomic hybridization for cytogenetic analysis of tumors, or spectral karyotyping by chromosome painting [17]. New-fangled approaches embraces the use of quantum dots and click chemistry [18]. Initially the progression of FISH involved development of different varieties of probes and number of evident targets. It is now said that the outlook for prospective advancement of fluorescence techniques will comprise of expansion of the focuses of investigation.

Clinical Scenario and the various challenges

In clinical set up, use of fluorescence imaging necessitates more development in programming that permits the probes to be delivered to the target specifically, photographed, and analysed spontaneously, thus plunging individual variability. Specimen thickness plays a crucial role, until lately microstructure investigation based on means such as confocal microscopy etc. was confined to samples of not more then 1-2 mm thickness. The novel technological enhancement of optical projection tomography has made the image-reconstruction possible even in the samples having seven folds thickness. This in turn has widened the platform for more elaborative application ranging from biological to clinical specimens [19]. Advanced RNA detecting novel techniques involving fluorophores or probes that fluoresce only when hybridized dodge the limitation of high backgrounds [20]. These methods are quite

Karyotype abnormalities varies from 7% to 50% depending on sample population. In a population which included 42 anomalous foetuses (15 samples between 13 to 20 wks, 3 between 21 to 27 wks and 24 samples between 28 wks till birth) we found abnormal karyotype in 28.5% samples. Most of the patients had multiple abnormalities on ultrasound.

Karyotype sample

In our study 10.7% (5/47) of samples failed to show culture due to contamination or improper sampling. 38 of 39 cord blood samples and 3 of 3 venous blood samples yielded culture. However when tissue samples were collected (3 chorionc villi and 1 umbilical cord), none of them showed the culture. Interestingly from 1 IUD and 1 stillborn we could collect cord blood immediately after birth and both of them showed the culture.

In our series 41 of 42 samples which were successfully cultured were obtained after abortion or delivery. Immediately after delivery cord blood was aspirated and sent for culture. In cases where cord blood sample was not possible, blood was aspirated from heart. If it was not possible tissue sample was sent. None of the tissue samples showed positive culture. Conventional G-band cytogenetic studies can be performed on the stillborn's blood or tissue as long as the cells are viable.

Table 10: Karyotype abnormalities in abnormal foetuses detected by ultrasound

Author	Study population	Abnormal karyotype
Karyotype abnormalities in abnormal foetuses detected by ultrasound		
Staebler 2005 [3]	428 fetal malformation on ultrasound. Mean maternal age 28.5 years (16–36)	11.2% (48/428) - abnormal karyotype 9.3% with isolated malformation and 18.8% with multiple malformations had abnormal karyotype
Nicolaides 1992 [4]	2086 Fetuses with malformations on ultrasound or IUGR or both.	14% (301/2086) had chromosomal abnormality 72.7% (222/305) had maternal age <35 yrs
ZalenSprock et al. 1991 [5]	N=210 ultrasound detected fetal malformations. GA 11 to 38 wks,	14.7% (41/288) had chromosomal abnormality 25/149 17% with single abnormality and 26% with multiple abnormalities had abnormal karyotype
Halliday et al. 1994 [6]	n=306 before 20 wks and n=241 after 20 wks ultrasound abnormalities. Maternal age <35 yrs	with isolated malformation<20 wks 18%, >20 wks 31% abnormal karyotype
Shimada S 2009 [7]	n=417 fetuses with anomaly or IUGR or amniotic fluid volume	Abnormal karyotype 17.7%
Gagnon et al. 1992 [8]	N=117 fetuses with congenital anomaly or IUGR or amniotic	19/117 (16.2%)
Present study	47 cases of prenatly detected congenital malformations. 42 had successful karyotype	Abnormal karyotype 28.5% (12/42) 2nd trimester 23.5% (4/17) 3rd trimester 32% (8/25)
Karyotype abnormalities in various study populations		
Sheth et al. 2015 [9]	n=1728 (1,324 amniotic fluids, 366 chorionic villi and 38 cord blood samples). Mean maternal age 31.6 years (19 to 53 years)	7.2% (125/1,728)
Yashwanth et al. 2010 [1]	176 cases of congenitally malformed children (1 day to 14 yrs).	17% had abnormal karyotype 32 (37.6%) with multiple system malformations
Pinar et al. 2009 [10]	Still births of more than 20 wks GA	25.8% (60/232) had abnormal karyotype
Korteweg et al. 2008 [11]	n= 508 fetal deaths median maternal age 31 years (range 17–46 years)	13% (32/246)
Pylyp 2018 [12]	1000 products of conception in first trimester miscarriages after spontaneous conception and IVF chorionic villi was assessed in 944 cases mesodermal cells—in 66. Mean maternal age 33.7 ± 5.6 years	50.1% (501/1000) had chromosomal abnormalities
Success of karyotype samples in various series		
Gagnonetal 1992 [8]	N=117 fetuses with congenital anomaly or IUGR or amniotic fluid	karyotype failed in 12.2% cases
Pinar etal 2009 [10]	Still births of morethan 20 wks GA	Karyotyping attempted in 342 cases, failed in 66 (16)%
Kortewegetal 2008 [11]	n= 508 fetal deaths	n= 508 fetal deaths, karyotyping successful in 246 (48.4%) Success - 85% - invasive (amnio, CVS), 15%, Post partum tissue analysis, 35% success in severely macerated fetus

Maternal age

The mean maternal age in our series was 24 ± 3.1 (18 to 32 yrs). None of our patients had advanced maternal age >35 yrs. In a series by Nicolaides 1992 [4] 72.7% (222/305) of abnormal karyotype had maternal age <35 yrs. Risk of fetal trisomy increases with maternal age. This is particularly well documented for trisomy 21 but has also been demonstrated for trisomies 13 and 18. Staebler et al. [3], Halliday et al. [6] and Sheth et al. [9] have shown significant chromosomal anomalies in mothers aged less than 35 years. Our series is also consistent with the literature.

Consanguinity

Consanguinity (n=5) had no relation to abnormal karyotype in our study. Comparison between genetic diseases with different modes of inheritance showed that recessive disorders, multifactorial disorders, autosomal dominant had higher correlation with consanguinity and chromosomal disorders had the lowest one.

Miscarriage

In couples with two or more spontaneous miscarriages, the frequency of chromosome anomalies in one parent is 4% to 5% [4]. Balanced chromosome rearrangements can lead to the production of gametes with unbalanced karyotypes. In our series 42% (5/12) of cases with abnormal karyotype had history of abortion.

Types of chromosomal abnormalities

In our series the commonest anomaly was Trisomy 21 (5/12, 41% of patients). In published literature Trsimoy 21 or Trisomy 18 is the commonest anomaly. There was no case of Trisomy 18 or 13 in our series. There was one case of monosomy X in our study. This may be attributed to the small sample size of our population. There were no cases of Triploidy or tetraploidy as we didn't have any cases in first trimester. Also we didn't include molar pregnancy in our study.

In structural abnormalities we found Translocation (2), insertion (1), additional material on 15p(2) and additional material on 1p(1). The rarer anomalies have to be further confirmed by FISH or PCR.

45, X, 9q(h+) with single X chromosome

This indicates that fetus has monosmy X indicating Turners syndrome. There is increase in length of the heterochromatin on the long arm of chromosome 9, indicating that material of unknown origin has been added to the long arm of chromosome 9, but the exact location the added material is unknown. This 19 weeks fetus had large cystic hygroma in occipitocervical region and nonimmune fetal hydrops. In a large European registry of Turners syndrome the most frequent anomalies were cystic hygroma (59.5%) and hydrops fetalis (19%). The most frequent karyotype was 45, X (81.6%) followed by different types of mosaicism (16.8%). Our case also had the same anomalies. Ninety-nine percent of 45, X conceptuses result in spontaneous loss, usually by 28 weeks. Cystic hygroma with nonimmune fetal hydrops during pregnancy is the most severe form. Although 45, X is quite lethal in the fetus, those that survive to term have relatively minor problems. The reasons for this are that all

Table 11: Types of chromosomal anomalies in various series

Author	abnormal karyotype (%/ No of cases	Trisomy 21	Trisomy 18	Trisomy 13	Translocation	Mono somy X	Tripolidy	Others
Sheth et al. [9] 2015	125(7.2%)/ 1728	46/125 (36.8%)	11/125 (8.8%)	2/125 (1.6%)	20/125 [16%]	7/125 (5.6%)		Supernumary marker chromosomes 6/125 (4.8%)
Nicolaides [4] 1992	14%(301)/ 2086	69/301 (22.9%)	83/301 (27.5%)	31/301 (10.2%)			42/301 (13.9%)	
ZalenSprock et al. [5] 1991	41 (14.7%)/288.	11//41 (27%)	13/41 (32%)	3/41 (7%)	5/41 (12%)	4/41 (10%)	5/41(12%)	
Shimada S [7] 2009	74(17.7%)/417	24/74 (32.4%)	21/74 (28.3%)	3/74 (4%)		8/74 (10.8%)		Deletion/ duplication 5(6.7%) Others 6/74(8.1%)
Present study	n=42	5/12 (41.6%)			2/12 (16.7%)	1/42 (2.3%)		Addlmaterial on 15p-2 cases,insertion 3p;3q -1 Partial trisomy 13 -1

Review Article

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Fluorescence in Situ Hybridization (FISH): A Review on its Contemporary Implementations

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Abstract

In the present article, an imperative cytogenetic technique - fluorescence in situ hybridization (FISH) is reviewed- which is a substantial tool of choice for localization of specific nucleic acids sequences in native context. This technology is extensively utilized in genomic and cell biological research as well as for diagnostic applications in various fields. Over the period, several procedures and alterations have been instated to optimize the detection of nucleic acids. It is the definitive standard practice for the detection of chromosomal aberrations. Even though the FISH has high specificity and shows the capability of direct quantitative imaging, certain limitations still masksits systematic practise in diagnostic purposes. To stimulate the widespread use of FISH in diagnostic and clinical practices for these applications, restrictions suchas prolonged assay period and expensive probe consumption is to be addressed. In the study the prime focus is to highlight the selected historical outlooks on FISH, review its contemporary implementations, challenges faced in diagnostic and clinical scenario and to provide a perspective on the future progression.

Keywords: FISH; Cytogenetics; DNA; Probes.

Introduction

Fluorescence in situ hybridization (FISH) is a technique which utilizes DNA fragments combined with fluorophore-coupled nucleotides as probes to look for the presence or absence of complementary sequences in fixed cells or tissues with the help of a fluorescent microscope. Gene mapping is done effectively by this hybridization-based macromolecule recognition technique. It helps in locating polymorphic loci onto metaphase chromosomes. Both of these are basically for assembling a physical map of the human genome [1,2]. Probes can be targeted at DNA both at metaphase and interphase chromosomes, as well as RNA. As a result it makes the study of genomic sequences and transcriptomic expression profiles of specific cells feasible [3]. The key advantages of this technology over its counterparts includes high sensitivity and specificityin identifying targeted nucleic acid sequences, direct application to both metaphase chromosomes and interphase nuclei, and visualization of hybridization signals straight at the single-cell level. All these in total, has upgraded the analytic resolution from Giemsa bands to the transcriptome and genome level. Not only this, it has facilitated prompt detection of various chromosomal aberrations [4]. In clinical scenario FISH technology hasmade a shift from conventional cytogenetics to the molecular level. With the furtherance in the efficiency of probe labelling and the deployment of the advanced resolution imaging system, FISH has been refurbished for research explorations of nuclear structures and various functions accomplished by genes. This article therefore presents the contemporary progress in FISH technology and recapitulates it's diagnostic and research applications in existing era.

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