Diagnosis of Sex Chromosome Disorders and Prenatal Diagnosis of Down Syndrome using Interphase Fluorescent In-Situ Hyperidization Technique

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Abstract:

Background : Thousands of infants are born each year with chromosomal abnormalities that severely impact physical and mental development. Among common genetic disorders are Down syndrome (trisomy 21) and sex chromosomal disorders.

Objectives : Evaluation of guidelines used for prenatal diagnosis of Down syndrome (DS) as well as sex chromosomal disorders including interphase Fluorescent In Situ Hyperidization (FISH) technique.

Methods : Enrolled cases were among those presenting to Genetics and Neonatology Units, Mansoura and Ain-Shams University hospitals,(Egypt) during 2002 to 2004. These included: *Groups 1* comprised fifty pregnant women presenting for genetic counseling. They were subjected to complete history analysis, ultrasound examination in addition to triple screening test (for alpha feto protein (AFP), human chorionic goandotrophin (HCG) and unconjugated esteriol (E2). Results were confirmed by doing routine karyogram on cultured amniotic fluid. *Groups 2* comprised suspected cases with sex chromosomal disorders including neonates with ambiguous genitalia (64 cases) and adults with primary amenorrhea (69 cases) or infertility (38 cases). They were subjected to a diagnostic workup including

Results : Among the pregnant women group, seven were found to be at a high risk of having DS fetuses including 3 cases with a history of affected off-springs, 2 cases with age above 35 years, and 2 cases with high triple test. Only one case had positive trisomy 21 on interphase FISH confirmed by karyogram on cultured amniotic cells. The other 6 ladies had normal FISH confirmed by karyograms. Regarding the other group, 5 cases out of the 9 females were proved to be feminized males, one proved mosaic turner, one proved mixed gonadal dysgenesis and 2 normal females. On the other hand one out of three males were proved to be verilized female while the other one was a male with incomplete testicular feminization and the last one was a male with infertility diagnosed as Klinefelter syndrome at the age of 26 years.

Conclusion : Interphase FISH is a rapid, accurate and very sensitive method in sex chromosom and autosomal abnormalities. It adds to the diagnostic utility of routine cytogenetics and its use on interphase nuclei overcomes the difficulty of conventional cytogenetics. It could be used in the prenatal diagnosis of DS in addition to ultrasonography, and triple test.

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Introduction

Thousands of infants are born each year with chromosomal abnormalities that severely impact physical and mental development. A given abnormality may be present in all body cells or may be present in some cell lines, a situation termed mosaicism. The trisomic condition is one of chromosomal imbalance and can resunblt in abnormality or death. In chromosomally abnormal abortuses the most common abnormality observed is trisomy (30% of all losses). [(1)] Data on the chance of recurrence of Trisomy 21 is more frequent in the offspring of older mothers but the basis of this long-standing observation remains an enigma. Similar uncertainty surround the role, if any, of external or environmental factors that may act pre/or periconceptually to affect the chance of a Down syndrome conception. [(2)]

Also Sex chromosomes abnormalities are among the most common of all human genetic disorders, with an overall frequency of about 1 in 500 births**.** Although monosomics for all human autosomes die inutero, a sex-chromosome monosomic complement, produces a phenotype known as Turner Syndrome (XO). They are sterile females. Although their intelligence is near normal, some of their specific cognitive functions are defective**.** Several sex-chromosome trisomics can live to adulthood. Each of these types is found in the frequency range of about 1 in 1000 births of the relevant sex. The combination XXY results in Kleinfelter syndrome males who are mentally retarded and sterile.[(3)]

The mixed gonadal dysgenesis syndrome is characterized by the presence of testicular tissue and dysgenic ovarian "streak" tissue giving the individual either a male or female or an intermediate appearance. A decision on the sex of rearing is then made after cytogenetic, hormonal, and histologic investigations are carried out. The goal should be to achieve anatomic and physiologic sex (which are amenable to treatment) concordant with psychosocial sex.[(4)]

The use of biochemicaly modified DNA probes, by means of fluorescence in situ hybridization technique (FISH) also referred to as (NISH) (non isotopic in situ hybridization), interphase cytogenetics or molecular cytogenetics, it is possible to recognize targeted chromosomal abnormalities from metaphases as well as non metaphase cells. This technique gives much more rapid results and has higher resolution.[(5, 6)]

This work aims at the evaluation of using interphase FISH technique for prenatal diagnosis of Down syndrome as well as diagnosis of sex chromosome disorders.

Subjects and Methods

Cases enrolled in the current study were among those presenting to Genetics Units, Mansoura and Ain-Shams University hospitals, Egypt during the period from 2002 to 2004. These included two main groups:

Group 1: Fifty pregnant women presenting for genetic counseling. Their age ranged from 25 to 42 years. They were subjected to complete history analysis, ultrasound examination in addition to triple screen test for alpha feto protein (AFP), human chorionic goandotrophin (HCG) and unconjugated esteriol (E2). Triple screen risk factor is calculated via a risk-calculation software that calculates multiples of the median (MoM) risk . [(7)] Furthermore, interphase FISH was done on amniotic fluid for 7 high risk cases including 3 cases with a history of having an affected off-springs with DS, 2 cases with advanced age above 35 years, and 2 cases with high risk value of triple test. Amniotic fluid samples were collected in a manner that minimizes the risk of maternal cell contamination .[(8)] Interphase cells prepared from uncultured amniotic fluid samples were hybridized to 21 specific probe (Ls121 DNA probe). The Ls121 DNA probe is a spectrum orange directly labeled fluorescent DNA probe that contain unique DNA sequences corresponding to the D21S259, D21S341 and D21S342 loci located in the 21q22.13 to 21q22.2 region on chromosome 21. The probe is pre-denatured in hybridization buffer for ease of use. The assay is designed for the detection and quantification of chromosome 21 by (FISH). Results were confirmed by doing routine karyogram on cultured amniotic fluid.

Group 2: include cases suspected to have sex chromosomal disorders as neonates and children with ambiguous genitalia (64 cases) and adults with primary amenorrhea (69 cases) or infertility (38 cases). They were subjected to a diagnostic workup including history taking, physical examination in addition to pelvi-abdominal ultrasonography, gonadal biopsy with or without endoscopy and hormonal profile (Testosterone, LH, FSH, E2, Cortisol and 17OH Progesterone) in addition to routine cytogenetic analysis. Interphase FISH) was applied on blood smears of selected 12 cases. Of them 9 were presented phenotypically as females and 3 as males. These included 8 cases with ambiguous genitalia (phenotypicaly 2 males and 6 females aged less than 1year), 1 female aged 5 years with inguinal hernia, 2 female cases with primary amenorrhea and delayed puberty (aged 23 and 15 years respectively) and one male case with infertility (male aged 26 years).

7-10 ml of heparinized peripheral blood was collected for doing both smears of interphase cells as well as metaphase preparation after lymphocyte culture. Both preparations were hybridized to X, Y centromeric probe (CEP X,Y) in addition to an

autosomal probe as a control. The CEP X/Y DNA probe is a mixture of spectrum Green and spectrum Orange directly labeled fluorescent DNA probes specific for the alpha satellite DNA sequences at the DXZ1 and DYZ3 regions of chromosome X and Y, respectively. Conventional cytogenetic analysis as well as FISH signal analysis were done using imaging system (Cytovision 4.4 U.K) and viewed using a fluorescence microscope equipped with appropriate excitation and emission filters allowing visualization of the intense orange and green fluorescent signals and the blue counterstained nuclei. . FISH technique was done according to what was previously desscribed in literature**.** [9, 10, 11]

Results

Regarding the first group of pregnant women, interphase FISH showed only one case having 3 positve signals of chromosome 21 confirmed by karyogram on cultured amniotic fluid cells (Fig. 1). The expected diagnosis of of getting a baby with Down syndrome was explained to that lady for further decision. On the other hand all the other 6 ladies had 2 normal signals of chromosome 21 on interphase FISH that was confirmed by karyograms after amniotic fluid culture. Diagnosis was confirmed after delivery and follow up of normal pregnancy outcome.

Regarding the second group of sex chromosome disorders, interphase FISH coupled with karyotyping in addition to the above mentioned workup revealed that 5 out of the 9 phenotyped females were proved to be feminized males (46, XY) with testicular feminization (Fig. 2), one proved to be mixed gonadal dysgenesis (45,XO / 46,XY),

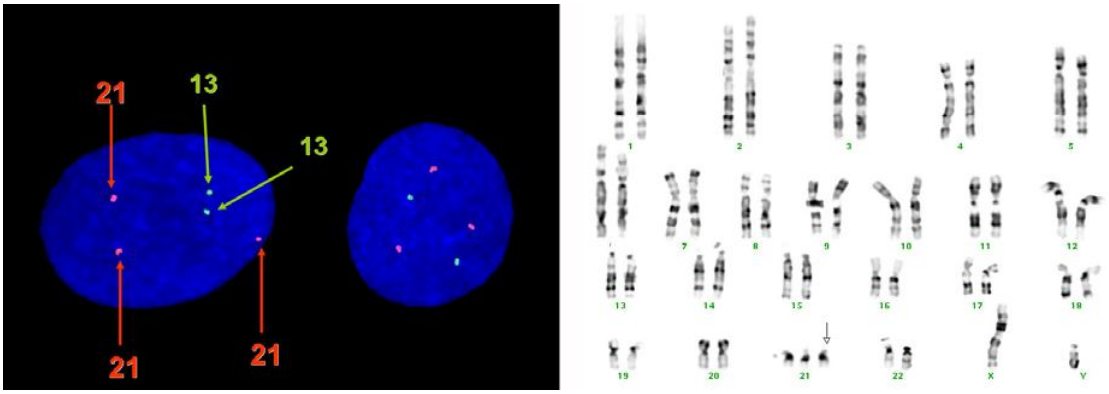

Fig. (1). Interphase FISH for uncultured amniocytes (right) using a fluorescent probe for chromosome 21 (orange), and chromosome 13 (green) showing 3 signals of chromosome 21 giving the diagnosis of trisomy 21 or Down syndrome. The karyogram (left) done on cultured amniotic fluid cells confirms the FISH diagnosis.

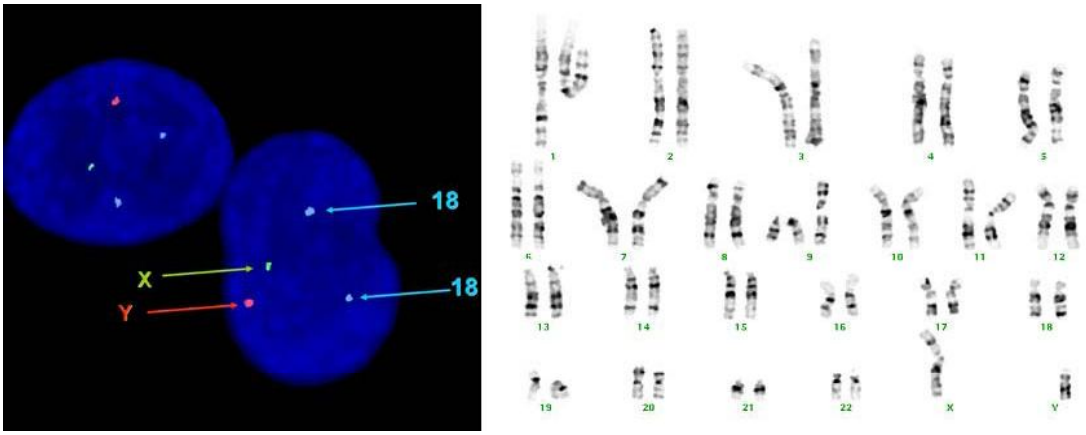

Fig. (2). Interphase FISH for blood smear leucocytic cells (right) using a fluorescent probe for chromosome X (green), Y (orange), and chromosome 18 (violet) showing one signal for each of the X and Y chromosomes in addition to 2 normal signals for chromosome 18 giving the diagnosis of a male pattern. The karyogram (left) done on cultured blood lymphocytes confirms the FISH diagnosis.

one with mosaic turner (45, XO / 46, XX) and one female with agenesis of internal genitalia (46, XX, pure gonadal dysgenesis) presenting with amenorrhea at the age of 23 years, the other one aged 5 years was presenting with inguinal hernia to rule out testicular feminization and was found a true normal female (46, XX) Fig. (3).

On the other hand, one out of three phenotyped males were proved to be verilized female (XX, adrenogenital syndrome) while the other two were in the form of 46, XY, partial testicular feminization and 47,XXY, Klinefelter syndrome presenting by infertility Fig. (4).

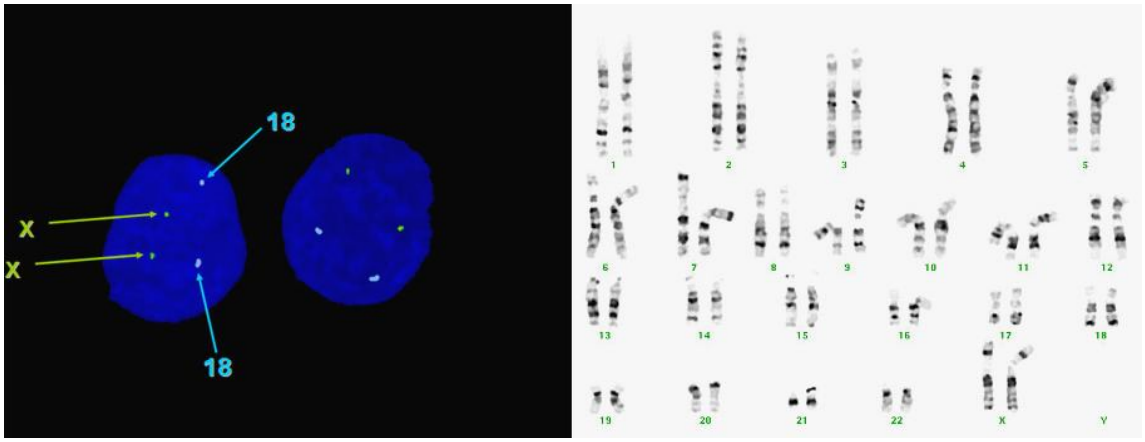

Fig. (3). Interphase FISH for blood smear leucocytic cells (right) using a fluorescent probe for chromosome X (green), Y (orange), and chromosome 18 (violet) showing 2 signals for each of the X chromosome in addition to 2 normal signals for chromosome 18 giving the diagnosis of a female pattern. The karyogram (left) done on cultured blood lymphocytes confirms the FISH diagnosis.

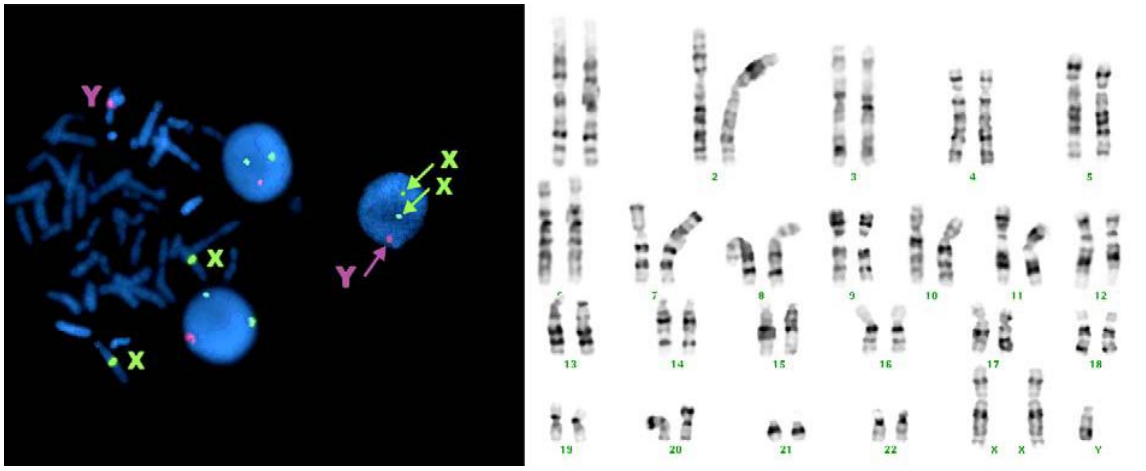

Fig. (4). Interphase FISH for cultured blood leucocytic cells in addition to a metaphase using a fluorescent probe for chromosome X (green), Y (orange) showing 2 signals for the X chromosome and only one for the Y chromosomes giving the diagnosis of XXY (Klinefelter syndrome). The karyogram (left) done on cultured blood lymphocytes confirms the FISH diagnosis.

Discussion

Down syndrome (trisomy 21) is the most prevalent unbalanced chromosomal aberration seen in live births, with an incidence of 1 per 600 live births or 1 per 150 conceptions. It is one of the common genetic disorders that have an impact on the Arab World including Egypt. [12, 13]

Prenatal diagnosis of amniotic fluid for analyzing chromosomal disorder has become standard under certain indications in highly developed countries world wide by using fluorescence in situ hybridization (FISH) with chromosome specific DNA probes on uncultured amniotic cells. [14]

In this study, diagnostic guidelines for prenatal detection of Down Syndrome was applied on all pregnant women seeking genetic counseling. These have included prenatal ultrasound, triple test screening in addition to interphase FISH for high risk cases. These guidelines have proved effective for prenatal diagnosis of Down syndrome. In addition, using interphase FISH on uncultured amniocytes is a relatively simple and rapid procedure that can give results in one or two days time, thus avoiding lengthy culture days and allows taking a rapid action regarding the current pregnancy.

Other authors have also reported that using FISH for the detection of a few common chromosome aneuploidies on interphase nuclei of uncultured amniotic fluid cells is a rapid, accurate and very sensitive method**.** [8, 15, 14]

Sex chromosome disorders, a relatively common problem in our community. Thus among all cases presenting to Genetics Unit during the study years (2002-2004) 1.9 % presented with primary Amonerrhea , 1.14% with primary Infertility and 1.82% with ambiguous genitalia (data not shown).

Using the above mentioned approach including interphase FISH using probes specific for sex chromosomes, we could characterize among 9 phenotyped females, five with testicular feminization (XY) one with mixed gonadal dysgenesis (XO, XY), one with mosaic turner (XO, XX), one with dysgenetic overy (XX), and one normal with an inguinal hernia (XX). On the other hand, among 3 phenotyped males, one diagnosed as a true female with adrenogental syndrome (XX), one with partial testicular feminization (XY) and third with Klinefelter syndrome (XXY).

Using these guidelines to reach to these diagnoses are actually important for further management. Thus, testicular feminization and mixed gonadal dysgenesis should be reared as females but still they have to undergo gonadectomy as a prophylactic step against potential malignancy. [16] Cases with mosaic turner and overian dysgenesis needs supplementation with growth and sex hormonal therapy for adequate physical growth and sexual development. Cases with adrenogenital syndrome need supplementation with mineralo /glucocorticoid therapy for life. Cases with partial testicular feminization and mixed gonadal dysgenesis can undergo corrective surgery and reared as males or females. [17, 18]

in a study for sex identification of normal persons and sex reverse cases from blood stains using FISH and PCR stated that FISH techniques using an X/Y cocktail probe had demonstrated that FISH technique is fast, easy to perform, reliable and efficient for sex identification [19] as a result using FISH, low-level mosaicism could be identified in some cases of primary amenorrhea and suspected Klinefelter Syndrome. Submicroscopic gene rearrangements could be detected using FISH in cases of ambiguous genitalia and cancers. [4, 10, 20]

We can safely come to the conclusion that FISH is a rapid, accurate and very sensitive method in sex chromosom and autosomal abnormalities, It adds to the diagnostic utility of routine cytogenetics and its use on interphase nuclei overcomes the difficulty of conventional cytogenetics. It could be used in the prenatal laboratory especially for diagnosis of DS coupled with ultrasonography and triple test.

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