Pregnancy Disorders
Brief on Cytogenetics
Introduction

- Chromosomes are thread like structures present in nucleus of the cell that carries DNA

- Cytogenetics is the study of chromosome
  - Structure
  - Pathology
  - Function
  - behavior

- Thus Cytogenetics is MACRO level study of DNA/genetics
Cytogenetics is the study of chromosomes. The behavior of the cell is determined by the chromosomes it carries.

E.g. the extra chromosome 21 present in the cells of a patient with Downs’ Syndrome is responsible for the clinical phenotype.

Study of chromosomes therefore becomes critical for diagnosis.
Chromosomes are the structures that hold our genes.
WHAT IS CELL?

- Building blocks of all living things
- Human body composed of trillion of cells
- Nucleus serves as the cell’s command centre
TJIO AND LEVAN from Sweden in 1956 found 46 chromosomes in human beings.
TOTAL NO. OF CHROMOSOMES : 46

- Mother
  23 chromosomes

- Father
  23 chromosomes

22 PAIRS OF AUTOSOMES + 1 PAIR OF SEX CHROMOSOME

23 chromosomes

egg

23

zygote

46

sperm

FERTILIZATION

TOTAL NO. OF CHROMOSOMES : 46
NORMAL MALE AND NORMAL FEMALE

46,XY

46,XX
Role of Cytogenetics in various medical fields

Oncology
   – Diagnosis and prognosis of tumors and hematological malignancies

Pediatrics
   – Diagnosis of syndromes and disorders in neonates

Gynecology
   – Diagnosis in infertility, BOH and Products of Conception
CHROMOSOMAL DISORDERS

CONSTITUTIONAL: EG. DOWN’S SYNDROME

ACQUIRED: EG. HAEMATOLOGICAL DISORDERS (CML, AML)
• **Lymphocyte Culture**
  Sodium heparinised peripheral blood sample

• **Leukemia**
  Sodium heparinised bone marrow / peripheral blood

• **Products of Conception**
  Chorionic / Placental Villi
CHROMOSOMAL ABNORMALITIES

- Numerical
  - Trisomy
    - e.g. Down’s
  - Monosomy
    - e.g. Turner

- Structural
  - Deletions
  - Duplications
  - Inversions
  - Translocations
Equivalent Terms

Test requested
Karyotyping
Chromosomal Analysis
Cytogenetic Investigation
WHO SHOULD UNDERGO THIS TEST?

• COUPLES
  – BOH and recurrent abortions
  – Infertility
  – Previous history of malformed child or mental retardation.

• INTERSEX / AMBIGUOUS GENITALIA

• TESTICULAR FEMINIZATION / ANDROGEN INSENSITIVITY HORMONE

• NEONATES WITH CONGENITAL ANOMALY
  EG. DOWN’S SYNDROME

• PRIMARY AMENORRHOEA

• TRIPLE MARKER TEST POSITIVE

• HEAMATOLOGICAL DISORDER
Chromosomal Analysis/ Karyotyping in cases of

- Bad Obstetric History
- Primary Amenorrhea
- Turner Syndrome
- Down Syndrome
- Klinefelter Syndrome
- Delayed milestones
- Family history of genetic disorders
- Mental Retardation
- Dysmorphism
- Multiple Congenital Abnormalities
Techniques For Chromosomal Studies

Karyotype Analysis/Conventional Cytogenetics

Fluorescent In Situ Hybridisation
KARYOTYPING
Karyotyping refers to the use of a microscope to examine the size, shape and number of chromosomes in a sample of body cells.

Steps:
- Tissue culture
- Harvesting
- Fixation
- Giemsa banding

A photographic representation of the stained preparation of chromosomes is then made and studied.

4 million base pairs need to be affected for detection.
Karyotype

- Photographic representation of chromosome

- 46 chromosomes
  - 22 homologous pairs of autosomes
  - 1 pair of sex chromosomes
    - XX female
    - XY male

- Chromosomes are studied at metaphase
- Fluorescent in situ hybridisation (FISH): on interphase cells
Banding

• Each chromosome has characteristic banding pattern
• Chromosome arms are p (short) & q (long)
• Regions are numbered from the centromere
  – Bands
  – Sub bands
• Computer assisted imaging
Karyotyping in cases of...

a) Bad obstetric history
b) Primary Amenorrhea
c) Family history of genetic disorders
d) Down syndrome
e) Turner syndrome
f) Short stature
g) Klinefelter syndrome
h) Other autosomal and sex chromosomal disorders
i) Mental retardation
j) Dysmorphism
k) Multiple congenital abnormalities
Down’s Syndrome

- Most common chromosomal disorder
- There are three chromosome 21 instead of normal two
Indications for Cytogenetic Analysis in Obstetric Practice

• Multiple congenital abnormalities

• Mental retardation or developmental delay

• Ambiguous external genitalia

• Infertility – Both partners (to rule out sex chromosome abnormality)

• Multiple spontaneous abortions (parents as carriers of balanced translocations i.e. defect in their chromosomes)

• Abortus (POC)
Chorionic villi or Placental villi or skin tissue should be first washed in sterile normal saline with procaine penicillin for two three times and then transferred in fresh sterile normal saline with procaine penicillin or gentamycin (2-3 drops) in red top vacutainers or sterile medium provided by us in red top vacutainers.

Send samples within 12-hrs minimum, 24-hrs max in sterile condition.
BAD OBSTETRIC HISTORY
Bad Obstetric History

• Recurrent pregnancy loss
• Congenital abnormalities in the fetus
• Still Birth
• History of Neonatal deaths

Primary Amenorrhea

• Menses not commencing in the reproductive age
Dysmorphic Features

- Hare-lip/Cleft Palate
- Flat Nasal Bridge
- Low set ears
- Mongolian fold
- Micrognathia
- Dysmorphic facies
Cytogenetics: Blood Lymphocyte Culture: 5814B

In this Category
Bad Obstetric History, Infertility, Recurrent Abortions
Patients with Suspected Down’s, Edward’s, Patau, Klinefelter’s, Turner, Testicular Feminization
Dysmorphic Features
Idiopathic Mental Retardation

Sample Type
Peripheral Blood in Sodium Heparin

Turn Around Time
3 weeks

Clinical History is mandatory
Cytogenetics: Blood Lymphocyte Culture: DT5102B (AUTOGEN)

This test is part of a panel including karyotyping of the couple and Antiphospholipid disorders.

In this Category
Bad Obstetric History, Recurrent Abortions

Sample Type
Peripheral Blood in Sodium Heparin

Turn Around Time
2 weeks

Clinical History is mandatory
Balanced reciprocal translocation
Robertsonian translocation - 45,XX,robt(13q;14q)
MCA child inherited due to balanced translocation in father

t(6;12)

der(6), t(6;12) pat

d(6), t(6;12) pat

Father

Son
Pedigree Charts Showing t(1;15) in mother and her two sons
Pedigree chart showing t(2;21)(p23;q22) as a result of consanguinous marriage.

t(2;21)(p23;q22)
In this Category

Patients with Suspected Down’s, Edward’s, Patau, Klinefelter’s, Turner, Dysmorphic Features

Patient age

< 1 month

Sample Type

Peripheral Blood in Sodium Heparin

Turn Around Time

2 weeks

Clinical History is mandatory
• Sample quantity
• Sample quality
• For Neonatal Karyotyping the cultures are setup in triplicates to ensure sufficient mitotic index
• Turn Around Time (quicker report)

Clinical History is mandatory
47,XY,+21 (Down’s Syndrome)
Baby with Dysmorphic features showing 49,XXXY
FLUORESCENT IN SITU HYBRIDIZATION (FISH)
Fluorescent in situ hybridisation (FISH)

- Molecular cytogenetic technique
- Detects and localises DNA sequences on chromosomes
- Fluorescent probes bind to specific part of chromosome
- Fluorescence microscope
Advantages of FISH

• Highly sensitive and specific
• Cell culture is not required
• Reduced TAT
• Ease of use-longer stability, rapid detection
• Performed on interphase cells
• Confirmatory test
• Preferred to detect minimal residual disease in leukemia
• More reliable for detection of gene amplification
FISH on Products of Conception

Cell showing three aqua, two green and one orange signal indicating triploidy

Cell showing three green and three orange signals indicating triploidy
Uses of FISH

- Prenatal diagnosis and screening
- Individuals with birth defects and mental retardation
- Infertility or reproductive failure
- Male infertility
- Genetic diagnosis of preimplantation embryos
- Identification and monitoring of acquired chromosomal abnormalities in leukemia/lymphoma-breast cancer
Whom to advise prenatal chromosomal analysis?

- Advanced maternal age (>34yrs)
- Parental consanguinity
- Parent with a previous child with a chromosomal abnormality
- Parent carrier of balanced reciprocal translocation
- Parent is a carrier of X-linked genetic disorder, to determine sex
- Elevated or low maternal serum AFP or positive Triple test
- Abnormal ultrasound findings
Fluorescent in situ Hybridization: Principle

**Probe:** Complementary to the specific region of interest
**FISH: 5814F**

**Interphase nuclei:** independent of cell culture  
**Mosaicism:** larger cell number increases sensitivity  
**Rapid:** reduces anxiety

**Applications:**  
Downs Syndrome  
Ambiguous Genitalia
Interphase cell showing two green signals for chromosome 13 and three orange signals for chromosome 21, indicating Presence of Trisomy 21 (Down’s Syndrome).
Interphase cell showing two aqua signals for chromosome 18, **two** green signals for the X chromosome and **one** orange signal for the Y chromosome, indicating presence of three sex chromosomes (XXY) and confirming Klinefelter’s Syndrome.
• Detects only numerical aneuploidies of chromosomes 13, 18, 21 and the sex chromosomes.

• Structural abnormalities (Isochromosome X) and anomalies of other chromosomes cannot be detected.

• Therefore, unlike Karyotyping, FISH cannot provide analysis of the entire genome.
PRODUCT OF CONCEPTION
Cytogenetics in Gynecology: Products of Conception

- Chromosomal abnormalities are a leading cause of abortions.
- Genetic Diagnosis crucial for diagnosis and further management.
Cytogenetics in Gynecology: Products of Conception

Test Code

5818F: FISH (TAT: 2 weeks)
9219 RFX: Karyotyping reflex to FISH (TAT: 3 weeks)

Sample collection and transport

- Chorionic villi (Gestation age <12 weeks)
- Placental villi (Gestation age >12 weeks)

• Collected in normal saline with antibiotic under sterile conditions
Chorionic villi or Placental villi or skin tissue should be first washed in sterile normal saline with procaine penicillin for two three times and then transferred in fresh sterile normal saline with procaine penicillin or gentamycin (2-3 drops) in red top vacutainer or sterile medium provided by us in red top vacutainer.

*Send samples within 12-hrs minimum, 24-hrs max in sterile condition.*
Triploidy in a case of Products of Conception

Cell showing three aqua signals for chromosome 18, two green signals for the X chromosome and one orange signal for the Y chromosome, indicating Triploidy.

Cell showing three green signals for chromosome 13 and three orange signals for chromosome 21, indicating Triploidy.
Tetraploidy in a case of Products of Conception

Cell showing four aqua signals for chromosome 18, two green signals for the X chromosome and two orange signals for the Y chromosome, indicating Tetraploidy.

Cell showing four green signals for chromosome 13 and four orange signals for chromosome 21, indicating Tetraploidy.
### FISH

- Almost 100% success rate.
- Results available within 72 hours after receipt of specimen.
- Polyploidy chromosome complement accurately detected (13,18,21,X,Y)
- Translocations and deletions **not detected**

### Karyotyping

- Full chromosome complement analyzed
- Translocations and deletions identified
- But, high failure rate as a result of difficult growth and infection
- Long turn around time
- Maternal cells dominate clonal growth
Cytogenetics in Gynecology: Prenatal Diagnosis

Test Code

Chorionic Villus Sampling

5833B: Karyotyping and/or 5833F: FISH

Amniotic Fluid

5832B: Karyotyping and/or 5832F: FISH

Fetal Cord Blood

5831B: Karyotyping and/or 5831F: FISH

Turn Around Time:

Karyotyping: 3 weeks

Study of all 46 chromosomes

FISH: 5 days

Study of only chrs 13, 18, 21, X and Y
Amniotic fluid cell showing two green signals for chromosome 13 and three orange signals for chromosome 21, indicating Presence of **Trisomy 21** (Down’s Syndrome).
PND Registration Number is mandatory.

Patient Consent is mandatory.

Clinical History is mandatory
Chromosomal analysis is an important component in diagnosis and evaluation of genetic disorders.

It clarifies and refines the clinical diagnosis.

Chromosome analysis is mandatory in women with advanced maternal age and couples with bad obstetric history and repeated abortion.

FISH is a rapid and sensitive technique and is preferred to detect low level mosaicism.
Gynaecologist, Obstetrician, Paediatrician, Endocrinologist, Physicians
THANK YOU