Karyotypic analysis in cases of multiple abortions

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Submitted by

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Under the guidance of

Dr. Sonal R. Bakshi

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DECLARATION

I declare that the thesis entitled karyotypic analysis in cases of multiple abortions has been prepared by me under the guidance of Dr. Sonal R. Bakshi, Professor of Institute of Science, Nirma University. No part of this thesis has formed the basis for the award of any degree or fellowship previously.

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ABSTRACT

PURPOSE: This study was conducted to determine the contribution of chromosomal abnormatilies in multiple abortions by means of karyotypic analysis.

MATERIALS & METHODS: A total of 6 female individuals were enrolled in the study. Two were having three spontaneous abortions, two were having first normal delivery followed by two spontaneous abortions, and two were having first normal delivery followed by five spontaneous abortions. Chromosomal analysis from short-term cultured peripheral blood lymphocytes was performed according to the standard cytogenetic methods using GTG banding techniques.

RESULT: Abnormal karyotype was found in one female (16.7%) with Robertsonian translocation t(13;14) and other five female were found to be having normal karyotype (83.3%).

CONCLUSION: The results indicated that chromosomal abnormalities can play a role in some cases of multiple abortions. Cytogenetic analysis could be valuable for these females when clinical data fail to clarify the cause.

INTRODUCTION

Multiple abortions are three or more consecutive, spontaneous, pregnancy losses under 20 weeks gestation from the last menstrual period. It is also known as miscarriage. Around 25% of all pregnancies end in miscarriage in the first trimester. The number of single miscarriages is actually higher, around 50% as women don't even know that they are pregnant when abortion occurs (Santiago Munne et al., 2005). Approximately 5% of couples experience two miscarriages in a row, while about 3% of couples experience three miscarriages in a row. Less than 2% of couples experience more than three consecutive miscarriages. The most common cause of single events of miscarriages is genetic abnormalities in the embryo. Such abnormalities also appear to play a role in between 3% and 5% of recurrent miscarriages. Many embryos that are conceived contain the abnormal number and structure of chromosomes. Majority of the chromosomal aberrations are lethal for the embryo, and are spontaneously aborted. Some of these chromosomal anomalies are inherited from the mother or father to the progeny. Structural chromosomal abnormalities are thought to contribute to at least 2% of all cases of recurrent pregnancy loss.

Other factors affecting multiple abortions are as listed below:

1. Endocrine factors:

Hormonal imbalances are believed to play a role in at least 20% of recurrent pregnancy loses. Many women suffer from disorders such as PCOS (polycystic ovarian syndrome),which disturbs the natural balance of hormones needed to ensure a healthy pregnancy. Women with high levels of luteinizing hormone and low levels of progesterone often experience miscarriage.

2. Blood clotting factors:

Blood clotting disorders appear to play a role in 15% of all cases of recurrent miscarriage. It prevent the placenta from getting a proper blood supply during pregnancy, causing the foetus to die. This is often due to the presence of high levels of antiphospholipid antibodies (APA) anticardiolipin antibodies (ACA).

3. Medical illness:

Systemic lupus erythematosus, congenital cardiac disease and renal disease are responsible for multiple abortions. High rate of foetal wastage among foetal wastage among patients with systemic lupus erythematosus is due to circulating immune complexes. Foetal wastage is 50% in cases of patients suffering from congenital cardiac disease. Foetal loss is extremely high in renal diseased patients. Diabetes mellitus also leads to pregnancy loss.

4. Immunological factors:

Due to the lacking of key serum-blocking antibody that protect the foetus from rejection by the mother. The blocking factor is related to the immunoglobulin class IgG class and acts to protect foetus from maternal antibodies and from the rejection via the coating of foetal antigens on the placenta. It is further believed that homozygocity in the HLA antigen system can prevent the mother from producing the blocking antibodies.

5. Environmental factors imparting physiological effect:

Radiation: Nuclear accident can cause radiation induced intrauterine demise.
Drugs and other substances: Cigarette smoking, abuse of alcohol, certain psychotropic drugs.
Environmental pollutants: Isotritinoin (accutane), Anaesthetics gases

6. Anatomical Causes:

In addition to genetic reasons, the anatomy of the uterus can impede upon the implantation of an embryo or prevent proper growth and development. Women with a particularly scarred uterus or divisions within the uterus can experience recurrent miscarriages (Srinivas et al., 2001).

a. Uterine septum (or other congenital problems)
b. Cervical insufficiency
c. Fibroids (controversial)
d. Uterine scarring

7. Genetic causes in terms of chromosomal abnormalities:

Chromosome abnormalities are believed to be the most common cause responsible for the spontaneous abortions. Random error occurring in sperms or in oocytes or during embryogenesis is responsible for the chromosomal damage (Bick, Rodger L et al., 2007).

A. Structural abnormalities:-

Translocations occur when chromosomes break and fragments rejoin to the other chromosome. A parent who carries a translocation is frequently normal; however there are increased chances of their embryo receiving abnormal chromosome complement leading to miscarriage. Couples with translocations or other specific chromosome defects may benefit from pre-implantation genetic diagnosis in conjunction with in vitro fertilization. Many early miscarriages are due to the random occurrence of a chromosomal abnormality in the embryo. In fact, 60% or more of early miscarriages may be caused by a random chromosomal abnormality, usually a missing or duplicated chromosome. Women (and men) older than 35 have a greater tendency to produce eggs or sperm with chromosomal abnormalities. In other cases, one or both partners may have a balanced translocation or other silent chromosomal

abnormality that gives the couple statistically increased odds of miscarriage in each pregnancy.

In reciprocal translocation, two non homologous chromosomes break and exchange fragments. Patients carrying such abnormalities still have a balanced complement of chromosomes and have a normal phenotype but with varying degrees of subnormal fertility. The subfertility is caused by problems in chromosome pairing and segregation during meiosis. Instead of having homologous chromosomes pair as bivalents, the translocation chromosomes and their homolog form quadrivalents, which affects segregation that leads to formation of a number of genetically unbalanced gametes and hence, offspring receive unbalanced genomes that are often lethal. The widely reported centric fusion is a translocation in which the centromeres of two acrocentric chromosomes fuse to generate one large metacentric chromosome. This is known as Robertsonian translocations, in which one of the two centromeres is lost. The karyotype of an individual carrying a centric fusion has one less than the normal diploid number of chromosomes. Chromosome translocations cannot be repaired. If a translocation has not resulted in any genetic material being lost, it is known as a balanced translocation. Balanced translocations do not usually cause any medical problems. This is because all the genetic material is present, even though it is in a slightly different arrangement. Robertsonian translocations only involve certain chromosomes i.e. acrocentric numbers 13, 14, 15, 21, 22 (Caroline Ogilvie et al., 2005).

In a Robertsonian translocation, the short arms of two of these chromosomes are lost and the remaining long arms join together. As the short arms of these chromosomes do not contain important genetic information, this translocation is described as balanced and has no effect on a person's health. Robertsonian translocations occur in about 1 in 1000 people. The most common Robertsonian translocation is between chromosomes 13 and 14. When the translocation is balanced, the person is called a Robertsonian translocation carrier. As carriers are healthy and have a normal lifespan, many never discover about their unusual chromosome rearrangement. In fact, the translocation can be passed down in families for many generations without anyone discovering. An unbalanced Robertsonian translocation may come to light

after a baby is born with a chromosome disorder. Most babies with unbalanced Robertsonian translocations have parents with normal chromosomes. A minority of babies have one parent who is a Robertsonian translocation carrier.

B. Numerical abnormalities:

Aneuploidy:

Embryos are usually aneuploid because of sporadic events, such as meiotic nondisjunction, or polyploid from fertilization abnormalities.

Autosomal trisomy:

Autosomal trisomy may arise de novo because of meiotic nondisjunction during gametogenesis in parents with a normal karyotype. Autosomal trisomy results from maternal meiosis I errors (either complete trisomies or monosomies).

Specific trisomies:

Trisomy 16 is the most common. Viable trisomies have been observed for chromosomes 13, 16, and 21.

Autosomal monosomies:

Autosomal monosomies are rarely, if ever, observed.

Monosomy X (Turner syndrome):

Turner syndrome is frequently observed and is the most common chromosomal abnormality observed in Spontaneous abortions. Turner syndrome accounts for 20-25% of cytogenetically abnormal abort uses.

Triploidy and tetraploidy:

Triploidy and tetraploidy are related to abnormal fertilization and are not compatible with life. Triploidy is found in 16% of abortions, with fertilization of a normal haploid ovum by two sperms (dispermy) as the primary pathogenic mechanism.

Tetraploidy occurs in approximately 8% of chromosomally abnormal abortions, resulting from failure of an early cleavage division in an otherwise normal diploid zygote.

Signs and Symptoms:

a) Bleeding, brown or bright red vaginal bleeding or spotting.

b) Passage of tissue from the vagina or a gush of clear or pink vaginal fluid

c) Signs of pregnancy, such as breast sensitivity and morning sickness, may go away

d) Dizziness, light-headedness, or feeling faint

Risk for having a miscarriage:

a) Previous miscarriages (two or more)

b) Age 35 or older

c) Smoking or drinking alcohol

d) Using cocaine or other illegal drugs

e) Environmental toxins -- excessive exposure to lead, mercury, organic solvents

f) Having chronic health problems

g) Low levels of folic acid

Management:

The standard of care is to offer genetic counseling. Amniocentesis is routinely offered for all pregnant women of advanced maternal age. A woman's risk of having an aneuploid fetus is 1 per 80 when she is older than 35 years; this is far greater than the risk of fetal loss after amniocentesis, which is 1 per 200.

The karyotype analysis does not help in detecting abnormalities caused by single gene mutations or mutations at several loci (small structural deletions and rearrangements), hence techniques like fluorescence in situ hybridization (FISH), are being used to complement standard cytogenetics. If a parental chromosome

abnormality is found, this should be the starting point for familial testing, and proper family counseling is recommended. If an increased risk for future pregnancies is identified, all alternatives should be discussed, including foregoing any attempts at further conception, adopting, trying to conceive again with early prenatal testing, using donor gametes, or performing preimplantation genetic diagnosis (PGD).

The concept of preimplantation genetic screening (PGS) involves using FISH to screen the removed blastomeric for aneuploidy in older women and in those with recurrent spontaneous abortions. PGS and FISH can be used to accurately detect common aneuploidies accounting for 70% of aneuploidic first trimester losses (chromosomes 13, 15, 16, 17, 18, 21, X, and Y), but these methods are criticized for their inability to detect all chromosomal abnormalities. A randomized trial of 408 women of advanced maternal age undergoing a total of 836 cycles concluded that the ongoing pregnancy rate, as well as live birth rate, was significantly lower in the women assigned to the PGS group compared with those without PGS. Couples in whom pregnancy loss can be attributed to a balanced translocation may benefit from specific genetic testing by PGD (John C Petrozza and Inna Berin).

A karyotype is the characteristic chromosome complement of a eukaryotic species. The preparation and study of karyotypes is routine part of cytogenetic analysis in research and diagnostic set-up. Karyotype helps identify and evaluate the size, shape, and number of chromosomes in a sample of body cells. Extra, missing, or abnormal positions of chromosome pieces can cause problems with a person's growth, development, and body functions.

Karyotype analysis is carried out to rule out any abnormality in chromosome complement in atleast 20 cells, if mosaicism is observed more number of cells are counted. Mosaicism is the presence of two or more chromosomally distinct cell lines, each seen in two or more cells. It arise after fertilization through the inaccurate segregation of chromosomes at mitosis by the process called non- disjunction. In a patient having balanced translocation, there is a problem of forming meiotic figures and in preparing for disjunction. The level of mosaicism depends on the stage of development of organism when the error in division occurs:

a. If misdivision occurs at the first cell division after fertilization, it is possible for all tissues in the body to be affected, with mosaic lines of 50% each.

b. If the misdivision occurs after the three cell types (ectoderm, mesoderm and ectoderm) have been established, the abnormal cells could be localized to one cell type.

c. If it occurs later, abnormalities could appear in only one organ of the body.

The level of abnormal effect could be dependent on the percentage and location of abnormal cells present in the body. Mosaicism must also be considered as a result of the impact of viruses and chemicals on dividing cells during life. In prenatal diagnosis, each cell line must be observed in more than one independent culture. There is no way to totally eliminate the possibility of mosaicism in the patient under the study because the sample does not contain all of the patient's cells. The sample is the representative of the cells of the body from which it came, so it is possible to count enough cells to eliminate certain levels of mosaicism. Statistical tables have been developed to indicate the level of mosaicism by counting certain numbers of cells. This analysis is based on the assumption that the sample obtained is truly random and that all cells grow at the same rate and behave the same in the invitro culture flask. In counting 20 cells, if one cell is found with a different karyotype that may represent a clone of different cells, additional counting is indicated, usually to 30 cells. With lymphocyte cultures, if no further cells are found identical to the one different cell already noted, then the aberrant cell can be assumed to be artifactual, and mosaicism of greater than 10% can be excluded at a 95% confidence level.

Clinical applications of karyotype analysis:

a. To determine whether the chromosomes of an adult have an abnormality that can be passed on to a child.

b. To determine whether a chromosome defect is preventing a woman from becoming pregnant or causing miscarriages.

c. To determine whether a chromosome defect is present in a foetus, also to determine whether chromosomal problems may have caused a foetus to be stillborn.
d. To determine the cause of a baby's birth defects or disability.
e. To diagnosis and treatment monitoring of some types of cancer.
f. To identify the sex of a person by determining the presence of the Y chromosome. This may be done when a newborn's sex is not clear.

Chromosome analysis:

Giemsa banding stains the region rich in the adenine and thymine producing dark bands. It is specific for the phosphate group of DNA; this technique is called G-banding. This compares chromosomes for their length, the placement of centromere (areas where the chromatids are joined) and the location and size of G bands to identify chromosomal aberrations like translocation and interchanges.

G-banding –: It is obtained by trypsin followed by the Giemsa stain. It shows the light and dark bands. It is done by using trypsin and then by giemsa stain.

G bands have specific properties like:

a) Stain strongly with dyes that bind preferentially to AT-rich regions such as Giemsa and Quinicrine.
b) May be comparatively AT-rich.
c) DNase insensitive.
d) Condense early during the cell cycle but replicate late.
e) Gene poor.
f) Genes are large because exons are often separated by very large introns.

Banding pattern is related with:-

a) base composition
b) chromosome loop structure
c) repetitive elements

d) replication timing

e) Chromatin packaging.

Chromosome arrangement

1. According to the location of centromere:

a) Metacentric

b) Submetacentric and

c) Acrocentric

2. Size of chromosomes:

GROUP	SIZE AND TYPE	CHROMOSOME NUMBER
A	Largest metacentric	1 to 3
B	Largest submetacentric	4 to 5
C	Middle size submetacentric	6 to 12,X
D	Large acrocentric	13 to 15
E	Smallest submetacentric	16 to 18
F	Smallest metacentric	19 to 20
G	Smallest acrocentric	21,22,Y

Description and Landmarks of G-banded A Group Chromosomes

(Daniel G. Kuffel et al., 2007)**.** Training Guide for Chromosome Recognition

Chromosome 1:

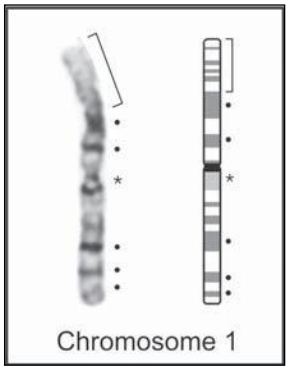
• Chromosome 1 is the largest human chromosome and is metacentric.

• The most distinctive feature of chromosome 1 is the large, light-stain region on the distal half of the p-arm.

• In the proximal half of the p-arm there are two distinct dark bands (DB's).

• Below the centromere on the q-arm is the qh region, which can vary in staining qualities: high-intensity dark stain, or dark and light. Varies in staining qualities more than the other qh regions.

• The distal end of the q-arm has three evenly spaced dark bands; the most proximal one has the highest stain density of the three.



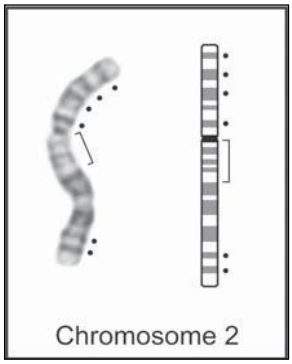
Chromosome 2:

• Largest submetacentric chromosome.

• The p-arm contains four distinct DB's that span the whole arm.

• The q-arm starts with a light stain region with three low-stain density DB's.

• The distal end of the q-arm has two evenly spaced DB's with equal stain density.



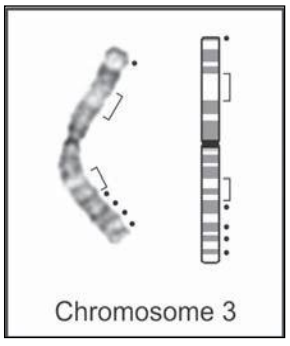
Chromosome 3:

• Second largest metacentric chromosome.

• It has a distinct DB cap at the distal end of the p-arm.

• There are two light band (LB) "windows": one is centrally located in the p-arm, one is located proximal to center in the q-arm.

•In the distal third of the q-arm there are 3-4 DB's (depending on band resolution)



Description and Landmarks of G-banded B Group Chromosomes

Chromosome 4:

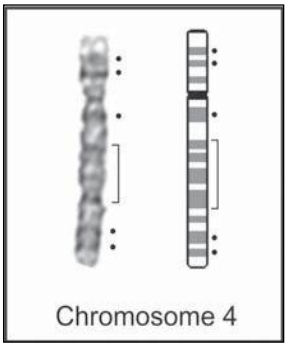
• Submetacentric chromosome: p: q-arm length ratio of 1:3.

• The p-arm has a broad "pure" LB followed by two medium stain density DB's.

• The proximal end of the q-arm contains a high density dark "shoulder" band.

• In the central q-arm there are four closely spaced, medium-stain density DB's, which may blend together.

• The distal end of the q-arm contains two DB's of similar stain density.



Chromosome 5:

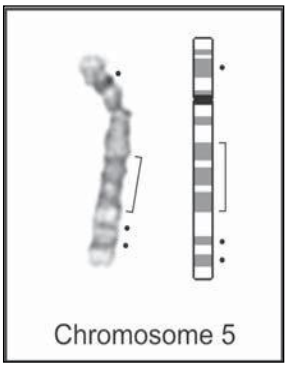
• Submetacentric chromosome: p: q-arm length ratio of 1:3.

• The p-arm has a distinct central DB.

• The proximal end of the q-arm contains a low-stain density "shoulder" DB.

• In the central q-arm there are three closely-spaced, medium stain density DB's, which may blend together.

• The distal end of the q-arm contains two DB's of different stain density; the lower band of two DB's is of higher stain density.



Strategy in distinguishing between the B Group chromosomes:

• Chromosome 4 has a characteristic light cap which distinguishes it from chromosome 5

• Chromosome 4 has two DB's in its p-arm. Chromosome 5 only has one.

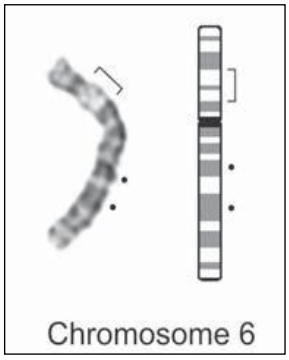
• Chromosome 4 has a high stain density "shoulder" DB.

• Chromosome 4's distal q-arm has two DB's of similar stain density; chromosome 5's distal q-arm contains two DB's of different stain density.

Description and Landmarks of G-banded C Group Chromosomes

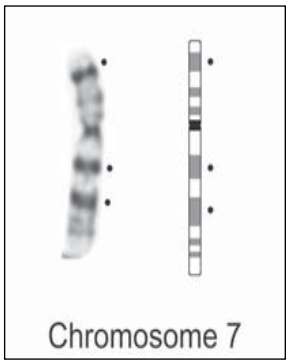
(All C-group chromosomes are submetacentric)

Chromosome 6:



- One of the three largest chromosomes in this group, the others being chromosomes 7 and X. It has a p: q-arm length ratio of 1:2.
- There is a characteristic broad LB "window" in the p-arm.
- The q-arm has several DB's, including two central high stain density DB's.

Chromosome 7:

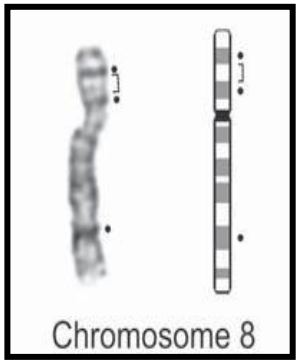


• Comparable in size and p: q-arm length ratio to chromosomes 6 and X.

• The p-arm has a prominent high stain density DB near the distal end of the arm.

• The q-arm has two prominent high stain density DB's, one located 1/3 and one located 2/3of the way down the arm.

Chromosome 8:

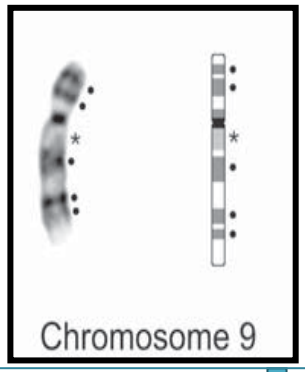


• Similar in size and p:q-arm length ratio to chromosome 10.

• The p-arm has a small LB "window" with two low stain density DB's on either side of the "window."

• The q-arm contains a prominent DB located about 2/3 of the way down the arm.

Chromosome 9:



• Similar in size and p: q-arm length ratio to chromosome 11.

• The p-arm has two DB's which are located in the upper 1/2-2/3 of the arm.

• There is a qh region commonly located right below the centromere in the q-arm. This region varies more in location on chromosome 9 than it does on chromosomes 1, 16, and Y.

Alternative locations are as follows:

1) The qh may be split into two sections, one above and one below the centromere

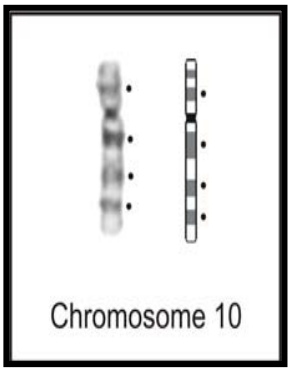
2) The entire region may be located right above the centromere.

• The qh region stains with a light to medium gray stain coloration.

• The q-arm has three distinct DB's: one DB is below the qh region followed by a broad LB; the other two DB's are distal to the broad LB.

• The q-arm finishes with a broad "pure" LB.

Chromosome 10:

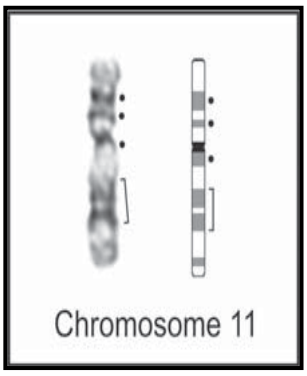


• This chromosome is similar in size and p:q-arm length ratio to chromosome 8.

• The p-arm has a distinct central DB.

• The q-arm has three evenly spaced DB's spread across the length of the arm; the first of the three has the highest stain density.

Chromosome 11:



• Similar in size and p: q-arm length ratio to chromosome 9.

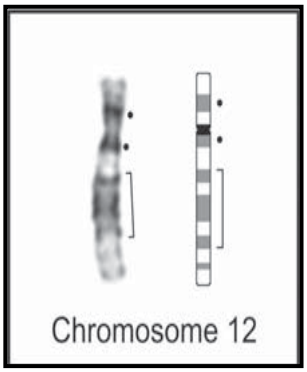
• The p-arm has two distinct DB's located in the lower ½ of the arm.

• The q-arm has a DB right below the centromere followed by a broad LB.

• There are two distinct DB's centrally located in the q-arm followed by a large light stain region with a low density gray DB.

• Distinctive features of chromosomes 11 and 12 are that they have broad light and dark staining regions in their q-arms.

Chromosome 12:

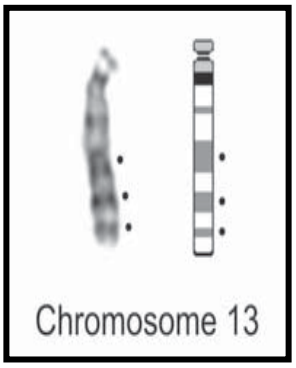


• p:q-arm length ratio of 1:3.
• Smallest p-arm of any C-group chromosome.
• The p-arm has a broad DB.
• The q-arm has a DB right below the centromere followed by a broad LB.
• The central portion of the q-arm has 3-4 DB's, depending on band resolution.
• The q-arm finishes with a large light stain region.

Description and Landmarks of G-banded D Group Chromosomes.

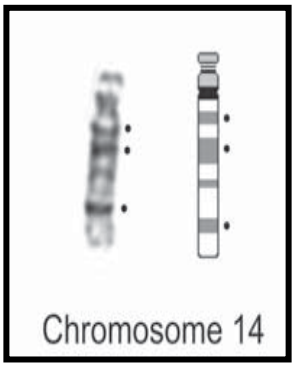
All chromosomes in this group are acrocentric and the p-arm/satellite region on these chromosomes is polymorphic. This group is made up of the three largest pairs of acrocentric chromosomes.

Chromosome 13:



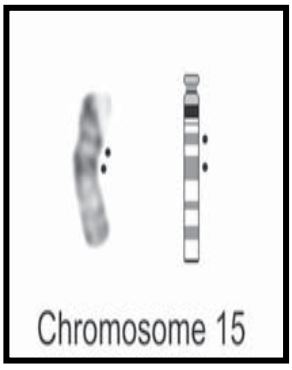
• The q-arm has its highest stain density DB's in the lower half of the arm.
• There are three distinctive DB's in lower half of the q-arm and one DB in upper half.

Chromosome 14:



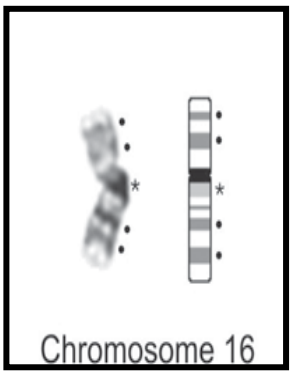
• The highest stain density DB's are located high and low in the q-arm.
• There are two DB's in the proximal end of the q-arm and one DB in the distal end of the q- arm

Chromosome 15:



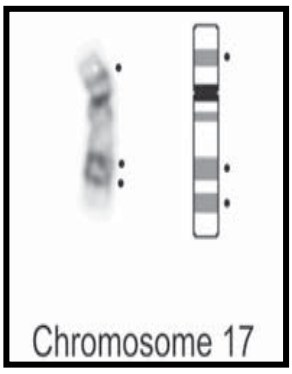
- The highest stain density DB's are located in the upper half of the q-arm.
- There are two distinctive DB's in the upper half of the q-arm
- The lower half of the q-arm is light stained.

Chromosome 16:



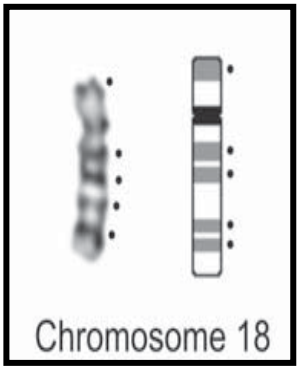
- The p-arm has two low density DB's.
- There is a qh region located right below the centromere in the q-arm. The qh region is a very high intensity dark staining area.
- The q-arm has three evenly spaced DB's spread across the length of the arm, the first of the three (the qh) has the highest stain density.

Chromosome 17:



- Chromosome 17 is lighter than chromosomes 16 or 18. Its highest stain density DB's are in a distal area of the q-arm.
- The p-arm has a medium stain density central DB.
- The q-arm has a medium stain density "shoulder" DB.
- There are two high stain density DB's in the distal area of the q-arm followed by a broad "pure" LB on the telomeric end.

Chromosome 18:

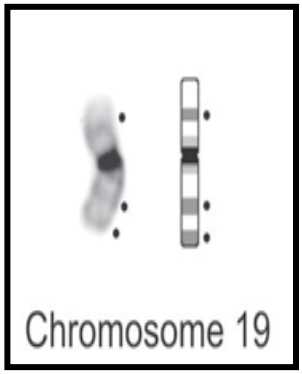


- The p-arm has a DB cap. There is a small LB on the telomeric end of the p-arm, but often this LB does not resolve. Instead the LB gives the DB cap a fuzzy edge.
- The p-arm is light after the DB cap and may give the arm an appearance similar to a satellite structure, but it is not a satellite.
- The q-arm has four DB's at higher levels of band resolution: there are two DB's in the proximal end of the arm and two DB's in the distal end of the arm.

Description and Landmarks of G-banded F Group Chromosomes

The chromosomes in this group are the smallest metacentric chromosomes found in humans.

Chromosome 19:



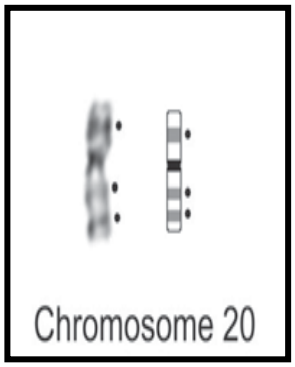
• This chromosome, in overall appearance, is very light with a dark pericentric area.

• The p-arm has a very low stain density central DB.

• The q-arm has a very low stain density central DB and a similar DB on the telomeric end that is hard to see.

• The p and q-arms at first glance look very similar, but the way to tell the difference between them is that the telomere of the p-arm fades into the background and the telomere of the q arm has a distinctive edge to it.

Chromosome 20:



• The p-arm has a broad, medium to high stain density DB in the middle to distal end(depending on resolution).

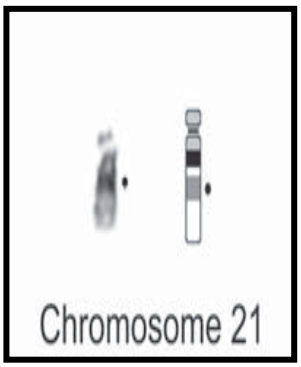
• The q-arm has two DB's evenly spaced down the arm with a LB at the telomeric end.

Description and Landmarks of G-banded G Group Chromosomes

The chromosomes in this group are the smallest human chromosomes and are acrocentric.

The p-arm/satellite region of chromosomes 21 and 22 are polymorphic.

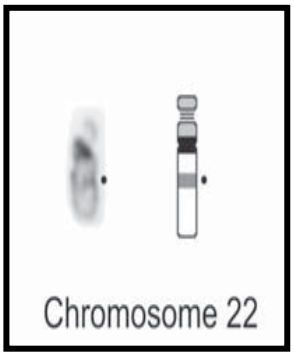
Chromosome 21:



• This is the smallest human chromosome and it is acrocentric.

• The q-arm has a broad, high stain density DB in the proximal end of the arm.

Chromosome 22:

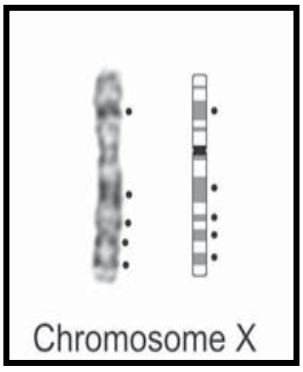


This acrocentric chromosome, in overall appearance, is very light with a dark pericentric area.

• The q-arm has a low stain density central DB.

Description and Landmarks of G-banded Sex Chromosomes

Chromosome X:



• A submetacentric chromosome.

•This chromosome is comparable in size and centromere position to chromosomes 6 and 7.

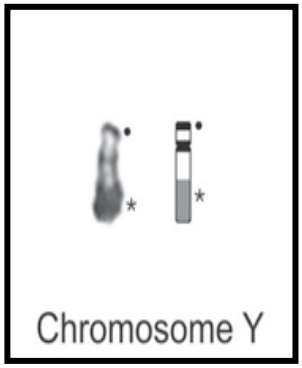
• The p-arm has a broad, high stain density mid-arm DB.

• The q-arm also has a broad, high stain density DB located about equal distance from the centromere as the prominent DB in the p-arm. The prominent DB's in the p and q-arms are of similar stain density, but the q-arm DB is broader.

• There are three DB's in the distal area of the q-arm and the third DB is the most distal high stain density DB of its size compared to the distal DB's of all other C group size chromosomes.

Chromosome Y:

- A submetacentric chromosome.
- The p-arm has a medium stain density DB at the end of the arm.
- The q-arm has a narrow, low stain density "shoulder" DB.
- There is a qh region located at the terminal end of the q-arm. The qh region is a very high stain density area typically either medium dark gray or very dark gray in coloration. Due to the variation of the qh region size, the overall size of a Y-chromosome can vary from smaller than a chromosome 21 to about the size of a chromosome 13.



Background Information

Genetic diseases can vary in severity, from being fatal before birth to requiring continuous management; their onset covers all life stages from infancy to old age. Globally, at least 7.6 million children are born annually with severe genetic or congenital malformations; 90% of these are born in mid and low income countries Genetic and congenital abnormality is the second most common cause of infant and childhood mortality and occurs with a prevalence of 25- 60 per 1000 births. Maternal age greater than 35 years is associated with higher frequencies of chromosomal abnormalities in the offspring (WHO 2005).

Women, especially those who have undergone multiple abortions, there are health risks that may arise including damage to the cervix, this should be explained in cases planning to have full term pregnancies in the future. The cervix is an important part of a woman's body that aids in conceiving and helps with hormonal balance. In healthy women who are trying to conceive, the cervix contains estrogen receptors on healthy tissue that assist in preparing a woman's body for the fertile phase of the menstrual cycle. In addition, in healthy women, the cervix also contains mucus glands which must provide healthy and normal secretions to promote pregnancies. When the cervix is damaged, as may occur in the case of multiple abortions, the estrogen receptors on the cervix may no longer receive estrogen signals and this will

lead to a hormonal imbalance in the cervical lining. In addition, and in the case of multiple abortions, the cervical tissue can be damaged which will lead to complications involving mucus secretions-a vital key to successful fertility in the future

Most studies demonstrate a spontaneous miscarriage rate of 10-15%. However, the true rate of early pregnancy loss is close to 50% because of the high number of chemical pregnancies that are not recognized in the 2-4 weeks after conception. Most of these pregnancy failures are due to gamete failure (eg, sperm or oocyte dysfunction). In a classic study by Wilcox et al in 1988, 221 women were followed up during 707 total menstrual cycles. A total of 198 pregnancies were achieved. Of these, 43 (22%) were lost before the onset of menses, and another 20 (10%) were clinically recognized losses (Wilcox et al., in 1988).

The likelihood for a spontaneous abortions increases with each successive miscarriage. Data from various studies indicate that after one spontaneous abortion, the baseline risk of a couple having another spontaneous abortion is approximately 15%. However, if two spontaneous abortions occur, the subsequent risk increases to approximately 30%. The rate is higher for women who have not had at least one live born infant. Several groups have estimated that the risk of pregnancy loss after three successive abortions is 30-45%, which is comparable to the risk in those who had two spontaneous abortions. This data prompted a controversy regarding the timing of diagnostic evaluation, with many specialists preferring to begin after two losses rather than three.

Sub fertile couples are those who require medical help to achieve pregnancy after trying unsuccessfully for a variable period of time. Although the term ‘sub fertility’ is also occasionally used in the context of women who can conceive but suffer recurrent miscarriages, this aspect is outside the scope of this review. Sub fertility can seriously affect mental and social well-being. It is not generally viewed as a disease that significantly causes physical ill health, though it may be an early manifestation of serious coexisting disease. Secondary infertility is the inability to conceive after one or more successful pregnancies. The medical causes are similar to

those of primary infertility, and include sperm problems, tubal factors, endometriosis, and ovulation difficulties. Over the last ten years it has become clear that the clinical expression of celiac disease is more heterogeneous than was thought in the past. Although celiac disease is a relatively frequent disease (1/170-200), it is only diagnosed in a small percentage of adult cases compared to the real situation because it is manifested with few symptoms or in an atypical form, or occasionally is completely silent. Gynaecological problems have been reported in women with celiac disease, in particular delayed menarche, early menopause, sterility, recurrent abortion and foetal intrauterine growth retardation.

In humans, natural killer cells are present in abundance in the uterus and appear to play an important role in early pregnancy. Women with reproductive failure have been shown to have higher levels of natural killer cell numbers and activity in peripheral blood. The evidence relating to uterine natural killer cells, however, is contradictory. While earlier studies suggested an increase of uterine natural killer cells in women with recurrent pregnancy loss, more recent investigations have not confirmed this trend. Uterine natural killer cell number or activity appear to be higher in spontaneous abortions with normal chromosomes compared with chromosomally abnormal pregnancies.

Studies of Gianaroli et al., 1987 showed that infertile patients with poor prognosis have an increased risk of having embyos with chromosomal abnormality, which could be one of the main reasons of implantation failure or recurrent spontaneous abortions (Gianaroli et al., 1987). With the development of Assisted Reproductive Technologies, genetic counselling and screening of couples assume greater importance. Karyotyping of every person attending the infertility clinic would be necessary to identify those with genetic defects. Approximately 15% of all clinically recognised pregnancies are spontaneously aborted before 20 weeks of gestation, and approximately half of these are attributable to detectable chromosome abnormalities (Hassold & Jacobs 1984). The most common abnormality observed is trisomy (~ 30% of all loses), although sex chromosome monosomy and polyploidy account for the majority of the remaining chromosome abnormalities found (each contributing to

~10% of total losses). Risk of most trisomies increase dramatically with increasing age of the mother, whereas sex chromosome monosomy and polyploidy do not (Hassold & Chiu 1985). From the latter study, it is estimated that >40% of clinically detected pregnancies end in spontaneous abortion among women >40 years of age, with autosomal trisomy accounting for > 60% of the total spontaneous abortions in this group. Increasing age is the overwhelming risk factor for trisomy & any evidence of greater recurrence risk of a trisomic spontaneous abortion after a first trisomic spontaneous abortion is generally found to be weak (Morton et al., 1987) or nonsignificant (Warburton et al., 1987).

Recently, reduced total follicular number in the ovaries has been associated with increased risk for trisomy (Freeman & Kline et al., 2000). An altered risk for trisomy is due to DNA methylation or chromosome segregation during meiosis.

Several developments in the late 1950s and early 1960s led to the first explosion of cytogenetics as a clinical science. The use of colchicine to block mitosis (Levan, 1938) and the accidental discovery of hypotonic shock (Hsu, 1952) resulted in the correct determination of the human chromosome number (Tijo and Levan, 1956). Improvements in cell-culture technology and the observation that phytohemaglutinin stimulates mitosis in leukocytes (Nowell, 1960; Moorehead et al., 1960) then provided a reliable source of mitotic cells. This combination of improved culture and harvest techniques and consistent slide making methodology are the foundation upon which cytogenetics rests today (Hungerford, 1965).

Well-spread metaphase chromosomes are fundamental substrates to cytogenetic studies. The success of complex molecular cytogenetic studies depends on having properly spread chromosomes. However, inconsistency of optimum chromosome spreading remains a major problem in cytogenetic studies. Slide making is very important and can be optimized according to the environment and surroundings. Spurbeck et al., 1996 emphasized that chromosome spreading depends on the natural drying time of the fixative and that approximately 90 seconds of drying time is needed for best spreading under the ambient condition of 20°C and relative humidity of 55%. In contrast, Henegariu et al., 2001 specified no ambient conditions and used hot steam to moisten the slides and then put them on a 65–75°C metal plate, which,

according to our own tests, dries the slides in 10 s. Whereas some researchers dropped cells on dry slides Spurbeck et al., 1996, Barch MJ et al., 1997, others suggested that having a thin layer of water on the slides immediately before adding the cells improves spreading Barch MJ et al.,1997, Henegariu et al.,2001, McFee AF et al.,1997. Dropping cells from a height reportedly enhanced chromosome spreading (Barch MJ et al.,1997).

MATERIALS AND METHODS

Requirements

Reagent	Company
RPMI 1640	Himedia
Trypsin	sd fine chem. Limited
KCl	MERCK specialist Pvt. Limited
Phosphate buffer saline (PBS)	MERCK specialist Pvt. Limited
DPX	sd Fine Chem. Limited
Giemsa stain	Sigma
Methanol	MERCK specialist Pvt. Limited
Acetic acid	sd Fine Chem. Limited
Colchicine	Himedia
Phytohemaglutinin(PHA)	Gibco
Chromic acid	MERCK specialist Pvt. Limited

Materials	Company
Glass wares	Borosil
Needles	Greiner bio-one
Aluminium foil	Fresh wrap
Micropipette	Eppendorf
Vacuatte (sodium heparin)	C.D. RICH
Chromic acid	MERCK specialist Pvt. Limited

Instruments	Company
Laminar air flow	Nova
Incubator	Sturdy, Elegant & Reliable EIE Instrument
Centrifuge	Nuve
Digital pH meter	E1 products
Electronic balance	Unibloc
Karyotype imaging system	Metasystem Ikaros
Water bath	WISWD Instruments
Microscope	Nikon-eclipse E100
Freezer	Samsung

METHOD

Blood collection and culturing of cells:

a) 5 ml of venous blood was taken in a sodium heparin Vacuatte with a sterile syringe needle.

b) 8 ml media was added with 300 µl of phytohemaglutinin (PHA) reconstituted in sterile deionized water (store at 4°C)

c) 1 ml of whole blood was expelled into a sterile culture tube which contained 8 ml RPMI 1640 media supplemented with L-alanyl-L-Glutamine, HEPES buffer, Penicillin (60 mg/litre), streptomycin (100 mg/litre), 15% FBS and $NaHCO_3$.

d) The culture was incubated for 69-70 hours at 37° C. Then agitate the culture once or twice daily during the incubation period.

Cell Harvesting:

a) 3 µl of colchicine was added to the culture tubes at 69^{th} hours and incubated for additional 2-3 hours.

b) Then centrifuge at 2000 rpm for 10 minutes.

c) All the supernatant was aspirated and discarded. Then gently tap the bottom of the centrifuge tube to resuspend the cells.

d) 7 ml of 0.56% KCl was added at 37^{o}C, drop wise at first, and then with gentle agitation to the culture tube.

e) The cells were allowed to stand exactly 20 minutes in the hypotonic KCl at 37^{o}C previously in water bath.

f) Then add 4-5 drops of fixative to the tube till it turns dark brown.

g) The cells were centifuged at 2000 rpm for 10 minutes. Then discard the supernatant and gently resuspend the cells.

h) Thereafter, add 1 ml of fixative to tube and keep for 1 min and discard it without disturbing the pellet.

i) This step was repeated two times, which is called still washing.

j) 7 ml freshly prepared and chilled fixative was added drop wise and then agitated it.

k) Then centrifuge at 2000 rpm for 10 minutes and remove the supernatant.

l) The cells were resuspended in fresh fixative.

m)The above step was repeated 2 to 3 times until clear pellet is obtained.

n) Again resuspend the pellet of cells in fixative to give just turbid appearance.

Slide Preparation:

a) Few drops of the suspended cells were drawn using pipette (Pasteur) and dropped it on the surface of the clean, grease free, chilled and wet slide from a height of a few inches in the centre.

b) The slide was allowed to dry first by keeping on a steaming water bath.

c) The slide was kept at a slight angle.

d) The slide was dried by steam on boiling water bath.

e) The slide was air dried and observed under the microscope using 10X and later with high resolution.

GIEMSA-BANDING TECHNIQUE USING TRYPSIN (GTG)

a) Slides were dipped in trypsin solution for 10 -12 seconds.

b) Then dip it in phosphate saline buffer to stop action of trypsin (protein igestion).

c) Slide was immersed in fresh PBS.

d) Then drain it well and dip the slide in distilled water.

e) It was stained with Giemsa for 7 minutes.

f) Then rinse the slide in distilled water.

g) The slide was allowed to dry completely.

h) Then the DPX was placed and mounted, then cover slip was kept on the slide.

i) Then was observed under the microscope.

Karyotype analysis

Per patient 20 cells were counted and around 10 metaphase plate were selected for image capture and analysis using microscope attached with a CCD camera and karyotyping software.

Representative karyotyping images with metaphase plate are depicted in the next chapter.

After the collection of blood sample in sterile heparinised vacutainer it is kept at room temperature is marked with patient's name, culture tube number, date and time of culture setting. The culture is set within 24 hrs of drawing blood from the patient.

Culturing of blood sample:

Short term culture is performed in strict aseptic condition, using aseptic techniques. In short term peripheral blood culture the blood is suspended in growth medium.

The pH of culture is maintained by the bicarbonate buffer present in media by:

a) Allowing the CO_2 and other metabolites in the culture to adjust the pH

b) Gassing the culture flask and tubes with the desired gas mixture and closing it tightly to prevent gas exchange

c) Providing the incubators with a constant flow of desired gas from external gas tanks.

The usual gas mixture is 5% CO_2 in ambient air, which is about 15-18% oxygen which adjust pH around 7.25 – 7.40. The culture from the human tissues is best grown at a physiological body temperature of 37-37.5˚C.

Phytohemaglutinin (PHA) is a mitogen, derived from extracts of Phaseolus vulgaris seeds, on account of its twin properties of causing erythroagglutination and of stimulating progressive lymphocyte mitosis is used as mitotic inducer in the culture.

Harvesting of cells:

The cells that are growing in culture are processed (harvest) to obtain metaphase cells. The three constant features for harvest protocol are mitotic arrest, hypotonic treatment and fixation.

1. Colchicine

It arrests the cells in metaphase stage and causes mitotic arrest is to prevent spindle fibre formation. Colcemid is a synthetic analogue of colchicines, a derivative of autumn crocus which causes chromosome condensation. Colchicine is more toxic, affects cell cycle speed and effectively yields longer chromosomes because of its slowing effect.

Inhibition by colchicine occurs by means of:

Direct effect: Involve binding of colchicine to spindle fibres causing them to dissociate into protein subunits.

Indirect effect: By the activation of an enzyme which attacks the spindle fibre.

2. Hypotonic treatment:

It increases the cell volume so the chromosomes can find the space to spread out. It work by creating a concentration gradient across the cytoplasmic membrane and water rushing inside the cell. If the potassium pump poisoned, the cell do not swell.

Prewarming: Prewarming of hypotonic solution to 37˚C increases effectiveness by speeding up water transport across the cell membrane and possibly by softening the cytoplasmic membrane, which has a lipid component, giving it more stretching capability. The type of salts used in hypotonic solution can affect the width and sometimes the length of the chromatids. Most cells seem to respond well in the first 10-20 minutes of exposure. Problems due to large volumes of hypotonic solution are serious: loss of mitotic cells due to increased fragility during centrifugation as well as incomplete migration to the pellet. Long exposure to hypotonic may cause weak areas in the cytoplasmic membrane which, if they burst at any point, will allow some or all of the chromosomes to escape. This lead to the low mitotic index, scattered chromosomes, partial metaphase.

Addition of few drops to millilitres of fixative to the hypotonic at the end of incubation period is called the prefixative step. It helps to prevent the mitotic cell from becoming dislodged during the addition of first fixation. This prefixation begins the process of hardening the cells and preserving the chromosomes and make the cells more resistant to damage from centrifugation and the shock of pure fixative. It also encourages the lysing of any red blood cells present, and since the lysed cells are not removed as debries, a cleaner cell preparation is obtained after centrifugation

3. Fixation

Fixative removes water from the cells, kills and preserves them, hardens membrane and chromatin and prepares chromosome for the banding procedure. Fixative used in cytogenetic is methanol: acetic acid in 3:1 ratio. It affects very less on chromatin structure and gives very good banding quality. It creates turbulence at first when added to the remnant hypotonic solution. During this the fixative is added slowly, or metaphase cells may be lost to breakage. Then the fixative is added more quickly. The alcohol in the fixative is a cell hardening agent and the acetic acid is cell softening agent. Methanol-acetic acid fixative changes with time (at first becoming more acid and then contaminated with acetates). Fixative also absorbs so absorbs water from air. Fixation extracts most of the histones and some non histones proteins (50,000-70,000 Dalton).

4. Slide making

Slide making is done after the cells are kept in fixative at 4ºC overnight. Factors like relative humidity, room temperature, drying time and number of cells per slide affects the slide making. Increased height is often used to increase spreading.

Cells are dropped onto a clean, wet slide at a 20-30º lengthwise on a paper towel, drain the excess water off, and may flood the slide with a little fixative to improve water removal and make the slide dry consistently and dry the slide flat or at an angle to get good preparations. In dry air, fixative evaporates fast, while in moist air, evaporation occurs slowly, this method ensures complete removal of water from the slide and uniform drying across the slide. Neither too fast nor slow evaporation is ideal because it can result in poor spreading, scattered chromosomes, or visible cytoplasmic background around the metaphase. Drying time should be roughly 30 – 45 seconds. Cold wet slide will slow drying, increase spreading, whereas higher than room temperature or warm slide will accelerate drying time. The thickness of the water film may be varied by draining the slide on a paper towel more or less thoroughly and this can help control spreading by using a thicker film of water on dry slides.

5. Slide aging

Slide aging process is very important as it gives better contrast and crispness to banded chromosomes, and also it removes water content. For performing G-banding, slides are allowed to kept for 3 or 4 days. Aging is also done by heating the chromosomes on the slides in an oven or a hot plate for 12-24 hours at 40-60˚C or 20 minutes 90-95˚C. The major change in chromosome from aging may be the oxidation of protein sulfhydral groups and degradation of chromosomal DNA.

6. GTG banding (G- banding by trypsin with Giemsa)

GTG-banding uses the proteolytic enzyme trypsin for pre-treatment followed by staining with Giemsa. However, as standard cytogenetic analysis using G banded technique cannot detect chromosomal rearrangement.

Trypsin is a serine protease which hydrolyzes the protein component of the chromatin; hence it allows Giemsa to react with DNA. Histones are present in extremely high concentration in the cell nucleus; their total mass is approximately same as that of DNA. The histones are basic proteins with strong positive charge at neutral pH because they contain a high proportion of the positively charged amino acid lysine and arginine. Histones bind tightly to the negatively charged DNA.

Giemsa stain is a complex mixture of dyes. The main components are the basic aminophenothiazin dye- azure A, azure B, azure C, thionin, and methylene blue- and the acidic dye, eosin. The thiazin dyes vary in number of methyl groups attached to a core of two benzene rings bound together by nitrogen and sulphur atom. The selectivity of G banding is largely due to hydrophobic bonding, which is enhanced by the loss of hydrophilic histones.

RESULTS

Clinical history of patients

Patient #1: 31/F

Occupation: Housewife

Husband:

Age: 34 years.

Familial occurrence of Bad Obstetric History: N.A.

History of Consanguineous marriages: N.A.

No. of FTND (No. of Children): 3

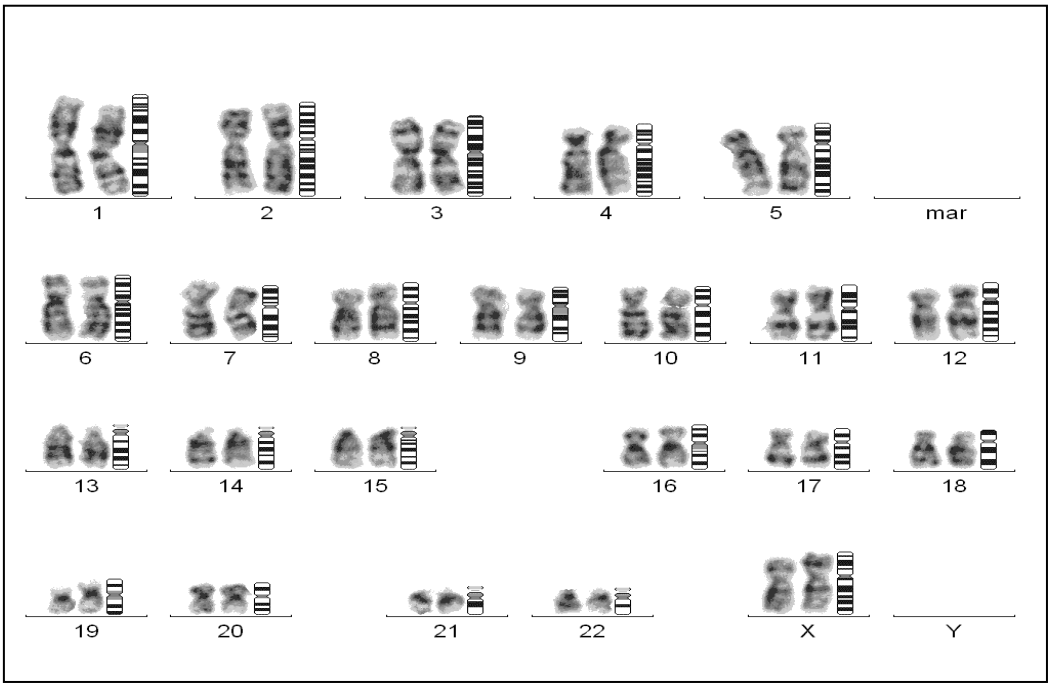
Age at which 1st menstruation cycle occurred: N.A.

Regular/Irregular (with/without pain): N.A.

Miscarriage details: First normal delivery then two spontaneous abortions and now pregnancy of 1 month and 10 days

Clinical History: N.A.

Patient#1, GTG BANDING METAPHASE & KARYOTYPE



Karyotype: - 46,XX

Inference: - Normal karyotype

Remarks: - Possibility of mosaicism cannot be ruled out as one tissue is being studied. Submicroscopy and microdeletions cannot be ruled out due to karyotype analysis.

Patient # 2: 31/F

Occupation: Housewife

Husband's name:

Age: 32 years.

Familial occurrence of Bad Obstetric History: N.A.

History of Consanguineous marriages: N.A.

No. of FTND (No. Of Children): 6

Age at which 1st menstruation cycle occurred: 17 years

Regular/Irregular (with/without pain): Regular with no pain

Miscarriage details: First normal delivery and then five spontaneous abortions at 1.5 months.

Clinical History: No

Patient # 3: 31/F

Occupation: Housewife

Husband's name:

Age: 35 years.

Familial occurrence of Bad Obstetric History: No

History of Consanguineous marriages: No

No. of FTND (No. Of Children): 3

Age at which 1st menstruation cycle occurred: 16

Regular/Irregular (with/without pain): Regular with no pain

Miscarriage details: First normal delivery (5 years girl child) then two spontaneous abortions at 2.5 and 1.5 months respectively.

Clinical History: No

Patient # 4: 32/F

Occupation: Housewife

Husband's name:

Age: - N.A

Familial occurrence of Bad Obstetric History: N.A.

History of Consanguineous marriages: N.A.

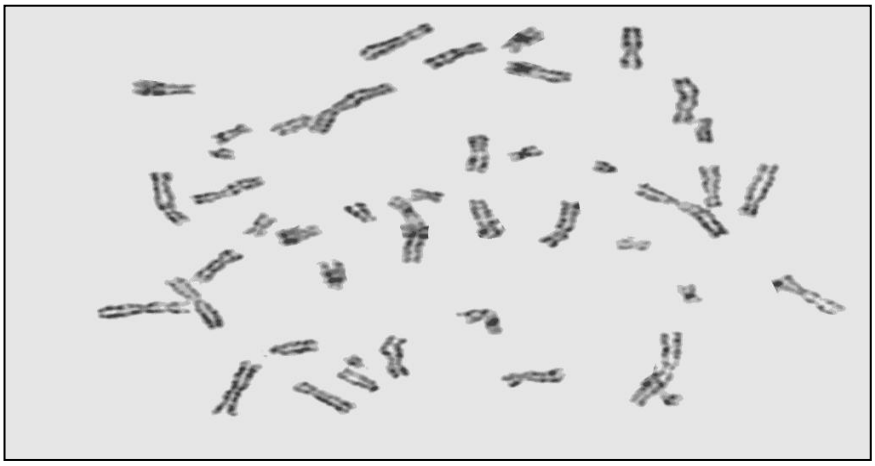
No. of FTND (No. of Children): 6

Age at which 1st menstruation cycle occurred: N.A.

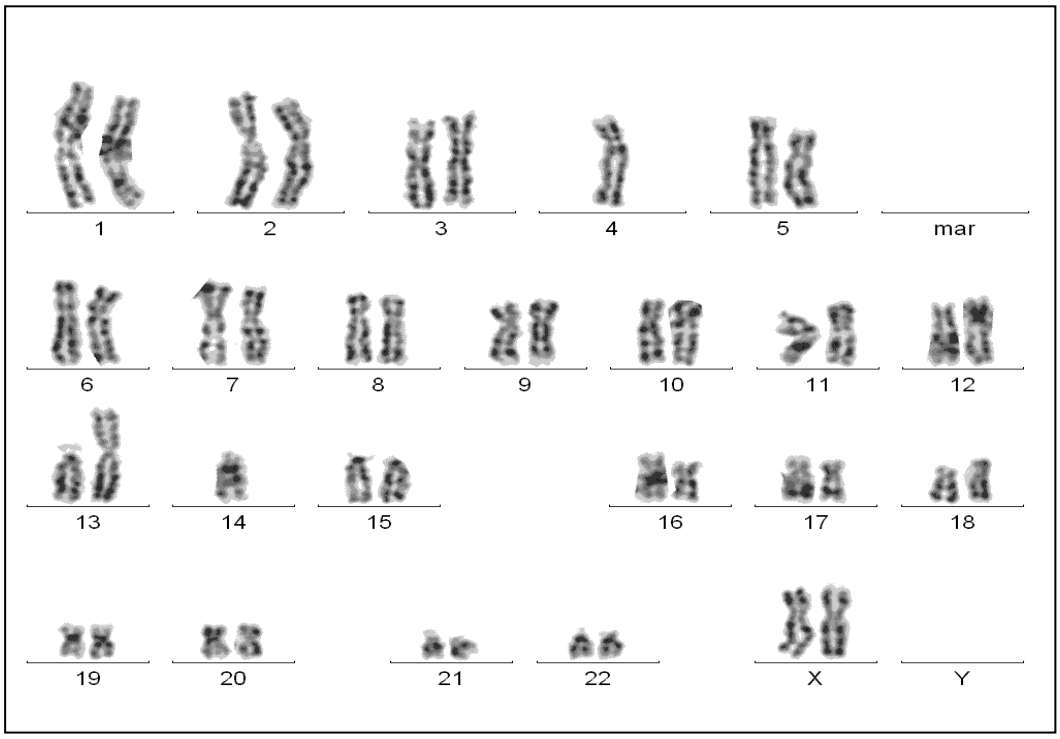
Regular/Irregular (with/without pain): N.A

Miscarriage details: One normal delivery (10 years old male at present) and 5 times miscarriages

Clinical History: Rubella, Cytomegalo and Herpes are positive



GTG BANDING METAPHASE AND KARYOTYPE OF PATIENT#4 DEPICTING ROBERTSONIAN TRANSLOCATION t(13;14)



Karyotype: 45,XX,rob(13;14)(p10;q10),inc[4]

Inference: Abnormal karyotype

Remarks: Female is having balanced Robertsonian Translocation but submicroscopy and microdeletions cannot be ruled out by using karyotypic analysis.

Patient # 5: 27/F

Occupation: Housewife

Husband's name:

Age: 31 years.

Familial History of Bad Obstetric History: No

History of Consanguineous marriages: No

No. of FTND (No. Of Children): 3

Age at which 1st menstruation cycle occurred: 16 years

Regular/Irregular (with/without pain): Yes

Miscarriage details: miscarriages 3 times

Clinical History: treated for Tuberculosis

Patient # 6 : 24/F

Occupation: Housewife

Husband's name:

Age: 30 years

Blood Group: N.A.

Familial occurrence of Obstetric History: A sister with history of infertility

History of Consanguineous marriages: No

No. of FTND (No. of Children): 3

Age at which 1st menstruation cycle occurred: 14 years.

Regular/Irregular (with/without pain): Regular but with pain

Miscarriage details: 3 spontaneous abortions

Clinical History: No

Diagnosis: Toxoplasma positive was treated; rubella and cytomegalo are positive

The type of translocation for one patient and other normal patient is listed in the below table, along with the patient's age, the number of spontaneous abortions, the number of normal children, and/or the number of abnormal offspring. The mean age for these patients was 29.3 years.

BALANCED CHROMOSOMAL TRANSLOCATIONS IN SIX PATIENTS WITH SUMMARY OF REPRODUCTIVE HISTORY

PATIENT INFORMATION

Chromosomal abnormality	Age.	No. SABs	No. healthy children	No. abnormal
46, XX	31	2	1	0
46, XX	31	5	1	0
46, XX	31	2	1	0
45, XX~rob (13; 14)	32	5	1	0
46, XX	27	3	0	0
46, XX	24	3	0	0
Mean	29.33	3.33	---	---

The rest of the five females with normal karyotype and multiple abortions, three were monoparous and two were nuliparous.

Occurence of the translocation type can be due to parental inheritance or de novo. It is possible that trisomy is due to Robertsonian translocation. Cases of trisomy 13 and trisomy 21 have both been reported with these rearrangements. When an inherited Robertsonian chromosome is associated with an otherwise normal karyotype, one of the normal chromosomes involved in the trisomy is considered to be extra. The Robertsonian translocations are equivqlent to isochromosomes being formed from two homologous chromosomes, which has been reported for both chromosomes 13 and 21. (Jenderny J et al., 2010)

DISCUSSION

The frequency of balanced chromosomal translocations in the general population is 0.3%. The 16.7% incidence of balanced chromosomal translocation found in female in present study who had five spontaneous abortions is little higher than that reported by Ward et al. in which no balanced translocations were found in 100 couples(Ward et.,). The reasons for these different results are not clear; however, several differences between these two studies may be relevant. In our study, of the six females on whom specific information was available, two had only two spontaneous abortions also had one healthy offspring, two with three spontaneous abortions also had no healthy offspring and two had five spontaneous abortions and had one healthy offspring.

Other factors that can influence the outcome of these studies are the type and extent of obstetrical-gynecologic evaluation performed prior to referral of patients for chromosome analyses. Factors in the family history such as miscarriages in the couples' parents may also influence the chromosome analyses for the female with multiple miscarriages. These types of considerations may have influenced the rate of translocation carriers found in the female with five spontaneous abortions. We cannot claim that the incidence of balanced translocation would be this high in all females with five spontaneous abortions. Rather, we suggest that for our current clinical population a history of five spontaneous abortions is a reasonable criterion for performing chromosome analysis. Several other studies using the criterion of two or more spontaneous abortions found similar incidences of balanced translocations. In many of these studies, the females are not analyzed separately on the basis of number of spontaneous abortions however. Our study also showed that the percentage of balanced translocation carriers among the group with multiple spontaneous abortions is lower than in many other studies (Kim HJ et al., 1975). It is known that the chances of achieving pregnancy for a translocation carrier are directly linked to the number of normal or balanced embryos available for replacement.

William Rees Brebner Robertson (1881-1941), an American insect geneticist was the first to describe Robertsonian translocation in grasshopper in 1916. Carriers of Robertsonian translocations are at increased risk for infertility, repeated miscarriage and aneuploid offspring. Robertsonian translocations 13/14 are the most common chromosome rearrangements in humans (Keymolen K et al., 2009). However, most studies aimed at determining risk figures are more than 20 years old. Here, a study on a sample of 101 previously unreported pedigrees of der (13;14)(q10;q10) is presented (Engels H et al., 2008). In order to minimize problems of partial ascertainment, families with a wide range of reasons of ascertainment such as birth of a child with congenital anomalies, prenatal diagnosis due to advanced maternal age, fertility problems and recurrent pregnancy loss were included. No evidence of increased infertility rates of female and male carriers was found. The detected miscarriage frequency of female carriers was higher than previously reported (27.6 $\pm$ 4.0% of all spontaneous pregnancies). This may be explained by an over-correction of earlier studies, which excluded all unkaryotyped miscarriages. In three out of 42 amniocenteses, translocation trisomies 13 were diagnosed (7.1 $\pm$ 4.0% of all amniocenteses). The frequency of stillbirths was 3.3 $\pm$ 1.6% for female carriers and 1.4 $\pm$ 1.4% for male carriers. A low risk for the live birth of translocation trisomy 13 children was confirmed since no live born children with trisomy 13 or Patau syndrome were detected in the ascertainment-corrected sample.

Robertsonian translocation induces a high frequency of mosaicism specifically for the chromosomes implicated in the translocation; the analysis by FISH of two blastomeres is strongly recommended for these patients (Emiliani S et al., 2003). Rearrangements of the acrocentric chromosomes (Robertsonian translocations and isochromosomes) are associated with an increased risk of aneuploidy (Berend SA et al., 2000). It may be worth taking up analysis for Unipaternal Disomy (UPD) in our patient as Robertsonian translocation has been correlated with UPD. Low level of trisomy 13 mosaicism is a rare condition (Jenderny J et al., 2010). In a case of a 19-month-old boy with poor feeding, poor weight gain, mild dysmorphic features, mild muscular hypotonia, and speech delay trisomy 13 was identified. Cytogenetic analysis on metaphases of lymphocytes revealed an 8% mosaic Robertsonian

translocation with trisomy 13 in the boy and a balanced Robertsonian translocation, 45,XX,der(13;14)(q10;q10), in his normal mother. Fluorescence in situ hybridization (FISH) on patient lymphocytes disclosed 4% of metaphases with a trisomy 13. The trisomy 13 mosaicism in metaphases could not be identified by interphase FISH. The detailed report of the patient described the infrequent occurrence of a low mosaic Robertsonian translocation trisomy 13 (Robinson WP et al., 1996).

Robertsonian translocations carry reproductive risks that are dependent on the chromosomes involved and the sex of the carrier. Preimplantation Genetic Diagnosis (PGD) was carried out using cleavage-stage embryo biopsy, fluorescence in-situ hybridization (FISH) with locus-specific probes, and day 4 embryo transfer.

In one case, female (45,XX,der(13;14)(q10;q10)) had four miscarriages, two with translocation trisomy 14. One cycle of Preimplantation Genetic Diagnosis (PGD) resulted in triplets. Other female (45,XX,der(13;14)(q10;q10)) had four years of infertility; two cycles were unsuccessful. One case (45,XY,der(13;14)(q10;q10)) presented with oligozoospermia. A singleton pregnancy followed two cycles of PGD. This female (45,XY,der(13;14)(q10;q10)) had a sperm count within the normal range and low levels of aneuploid spermatozoa. PGD was therefore not recommended. No evidence for a high incidence of embryos with chaotic or mosaic chromosome complements was found. For fertile couples, careful risk assessment and genetic counselling should precede consideration for PGD. Where translocation couples need assisted conception for sub fertility, PGD is a valuable screen for imbalance, even when the risk of viable chromosome abnormality is low.

Robertsonian translocations can affect fertility, with various degrees of sperm alterations in men; or the pregnancy outcome of the carriers. The studies on meiotic segregation of chromosomes in sperm of Robertsonian translocation males found a majority of normal or balanced spermatozoa for the chromosomes related to the translocation (mean 85.42%; range 60-96.60%).

In translocation carriers, the presence of aneuploidy for the chromosomes unrelated to the rearrangement may lead to an additional risk of abnormal pregnancy or implantation failure (Gutierrez-Mateo C et al., 2005). The application of CGH for the

study of Robertsonian translocations of maternal origin will be useful to study imbalances of the chromosomes involved in the rearrangement, as well as alterations in the copy number of any other chromosome. The combination of PGD for translocations with aneuploidy screening could help to reduce the replacement of chromosomally abnormal embryos.

The meiotic segregation patterns found in female Robertsonian translocations are different from those described in male carriers, with higher rates of unbalanced gametes in females than in males (Munne S et al., 2000).

Cytogenetic analysis on metaphases of lymphocytes revealed an 8% mosaic Robertsonian translocation trisomy 13 in the boy and a balanced Robertsonian translocation, 45,XX,der(13;14)(q10;q10), in his normal mother. Unipaternal disomy of chromosomes 13 and 14 were excluded in the boy, and therefore, his phenotypic abnormalities most likely were caused by the low level of trisomy 13 mosaicism (Jenderny J et al., 2010).

The first case of maternal uniparental disomy of chromosome 14 in humans is reported. The male proband inherited a balanced 13;14 Robertsonian translocation from his mother. Molecular studies showed that neither chromosome 14 was of paternal origin. The proband is of above average intelligence, but he has hydrocephalus, a bifid uvula, premature puberty, short stature, and small testes. It is not known if the clinical findings are related or coincidental to the uniparental disomy (I K Temple et al., 1991).

Marriages involving partners both of whom have abnormal karyotypes are rare and are usually ascertained because of a history of infertility, repeated abortions, or the birth of a balanced translocation carrier or chromosomally abnormal offspring. Abnormalities which have been noted include sex chromosome aberrations in both parents or a sex chromosome abnormality in one parent and an autosomal abnormality in the other. Four papers have reported balanced reciprocal autosomal translocations in both parents, two couples representing a first cousin marriage. A case of a paternal 13; 14 Robertsonian translocation and a maternal reciprocal

translocation t(7p;13q) in a couple with repeated fetal loss was presented (P R Scarbrough et al., 1984).

Preimplantation genetic diagnosis (PGD) is an alternative option for couples with chromosome abnormalities. A 34-year-old woman with balanced Robertsonian translocation (45,XX,der(13;14)(q10;q10)] requested PGD due to recurrent spontaneous abortion. Embryos of good quality were biopsied on day three post-oocyte retrieval. The aspirated blastomeres were fixed and analyzed using fluorescence in situ hybridization. In the first cycle, two unaffected embryos were transferred back without success. No unaffected embryo was available in the second cycle. On day 5 in the third cycle, two unaffected embryos were transferred resulting in a twin pregnancy. Amniocentesis confirmed the diagnosis. At the gestational age of 35 weeks, two healthy girls were born via cesarean section. Postnatal physical examination found no evidence of major abnormalities (Wu YC et al., 2004).

Segregation of Robertsonian chromosomes has been examined in many families, there is little consensus on whether inheritance in the balanced progeny conforms to Mendelian ratios. To address this question, segregation data was compiled in one case study, by sex of the parent, for 677 balanced offspring of Robertsonian carriers from 82 informative families and from a prenatal diagnosis study on the risk of unbalanced offspring in carriers of chromosome rearrangements. Analysis leads to the following conclusions:

(1) The transmission ratio is not independent of the sex of the carrier;

(2) The transmission ratio distortion is observed consistently only among the offspring of carrier females;

(3) The transmission ratio distortion does not appear to be dependent on the presence of a specific acrocentric chromosome in the rearrangement. The sex-of-parent-specific origin of the non-Mendelian inheritance, the finding that the rearranged ("mutant") chromosomes are recovered at significantly higher frequency than the acrocentric ("normal") chromosomes, and the similarities between these observations and the segregation of analogous rearrangements through female meiosis in other vertebrates strongly support the hypothesis that the transmission ratio distortion in

favor of Robertsonian translocations in the human results from the preferential segregation of chromosomes during the first meiotic division. This non-Mendelian inheritance will result in increased overall risk of aneuploidies in the families of Robertsonian translocation carriers, independently of the origin of the transmission ratio distortion (Pardo-Manuel de Villena F et al., 2001).

A case report is described of a female t(13q;14q) Robertsonian translocation carrier. Her firstborn daughter appeared to be a carrier of the same translocation. Chromosomal investigation of the third of three subsequent spontaneous abortions revealed a triploidy (69,XXX). Literature shows discrepancies in the reported abortion rate in the reproductive performance of t(13q;14q) carriers. It is concluded that these could in part be explained by heterogenicity of the study groups due to variable presence of other factors known to influence the abortion risk. In this case report the treatment for epilepsy, a luteal phase defect and high numbers of spermatozoa in the sperm count of the husband were considered as contributory factors. It is recommended to perform systematic investigations in all cases of recurrent abortion even when a chromosomal anomaly is found in one of the partners (Kwikkel HJ et al., 1982).

SUMMARY

The reported incidence of balanced chromosomal translocation in females with multiple spontaneous abortions (SABs) ranges from 0% to 31%. Because our experience has suggested that spontaneous abortions are are the candidates for ascertaining balanced translocation, we report the results of chromosome analyses performed on blood specimens from six females.

We have enrolled females with two or more spontaneous abortions. Balanced translocation was found in one of these six females. The detailed reproductive history was available in five of these six females. Out of two females with five spontaneous abortions, have a healthy child each with no abnormal child. Only one of them had balanced translocation whereas in case of other two females with two spontaneous abortions and two females with three spontaneous abortions, there was no balanced translocation observed.

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