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KARYOTYPING AND ITS RECENT ADVANCES- A REVIEW

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ABSTRACT:

Living beings have variegated species that are distinct from one another. In the same species itself, every member has his/her genetic constitution. The majority of the genetic abnormalities are related to chromosomal aberrations. The chromosomal aberrations are studied by cytogenetics. In cytogenetics one of the methods is karyotyping. Karyotyping is the photographic representation of the chromosomes. Karyotyping is made to visualize undetected chromosomal anomalies. The chromosomal anomalies are determined by the length of the chromosomes, position of centromeres, banding pattern, and the difference in sex chromosomes. It is also used to identify chromosomal aberrations, cellular functions, taxonomic relationships, and to find out medicine for diseases or disorders and to gain information about the past evolutionary events. There are different techniques used for karyotyping. Banding technique, Fluorescent in situ hybridization (FISH), Spectral karyotyping, and Comparative genomic hybridization (CGH). Karyotyping determines chromosomal abnormalities in both dividing and non-dividing cells. Fluorescent in situ hybridization (FISH) is used to detect chromosomal anomalies in non-dividing cells. Comparative genomic hybridization (CGH) and spectral karyotyping detected by fluorescent color. The methods involved are arresting the cell divisions at metaphase using colchicine. The advantages of karyotyping are, it is used to determine the sex of the fetus, and chromosomal anomalies in a tiny part of the chromosome. The disadvantages are, fluorescent in

situ hybridization (FISH) is probes specific to a subregion. The banding technique has a low mitotic index. A literature survey was carried out from Scopus and Pubmed database articles to date. The chromosomal anomalies are detected using karyotyping. Medicines can be found. It enables one to distinguish chromosomes from each pair individually.

INTRODUCTION

Living beings have variegated **species** that are distinct from one another. In the same species itself, every member has his/her genetic constitution. The genetic constitution is made up of chromosomes, genes, and DNA (1). The blueprint for the formation and maintenance of the organism is provided by DNA which is packaged in chromosomes. Chromosomes are distinguished from one species to another. Chromosomes are the factor that enables the transmission of genetic information from one generation to another. If a father or mother has diseases or disorders for example if a father has diabetic Mellitus it will be inherited to children (2). Hematological diseases like sickle cell anemia, the parent of autosomal recessive conditions, carry one copy of the mutated gene that will be inherited to the next generation (3). Chromosomes are the vehicles that facilitate the reproduction and maintenance of species (4).

The number of genetic abnormalities is related to chromosomal patterns. The characterization of chromosomes is of diagnostic importance which is done by cytogenetics. Cytogenetics is the diagnostic study of structure and properties of chromosomes and cell division, which employs various methods one of them is karyotyping. When we view as such the abnormalities and the defects in a tissue are not visible. So a microscope is used to view deeper (5) and the details are recorded in a photograph (6). Karyotyping is also similar to this. Karyotyping is the procedure that involves the photographic representation of stained chromosomes which are arranged in a standard manner (Figure 1). A karyotype is the individual constitution of the chromosome (7). Karyotyping made it possible to visualize undetected chromosomal anomalies such as deletion, translocation of tiny parts from one chromosome to another chromosome.

In 1956 Tijo and Levan, Ford and Hamerton found that a normal human somatic cell contains 46 chromosomes (8). Chromosomal anomalies are detected by the length of the chromosomes, the position of the centromere, banding pattern, and physical characteristics (9). Maleness is determined by the Y chromosome, regardless of the number of X chromosomes present in each cell (10). The collection of cells from the skin (fibroblast), diseases, and disorders can be detected (11). Methods used by Tijo and Levan are modified and now being used in cytogenetic laboratories to analyze karyotypes. In 1960, fibroblast was first used for karyotyping (12). Karyotyping is also used to identify chromosomal aberrations, taxonomic relationships, past evolutionary events, and sex chromosomes (13). There are a variety of diseases and disorders. Some people urinate often at night (14). Some bacteria can affect eye and eyelashes (15).

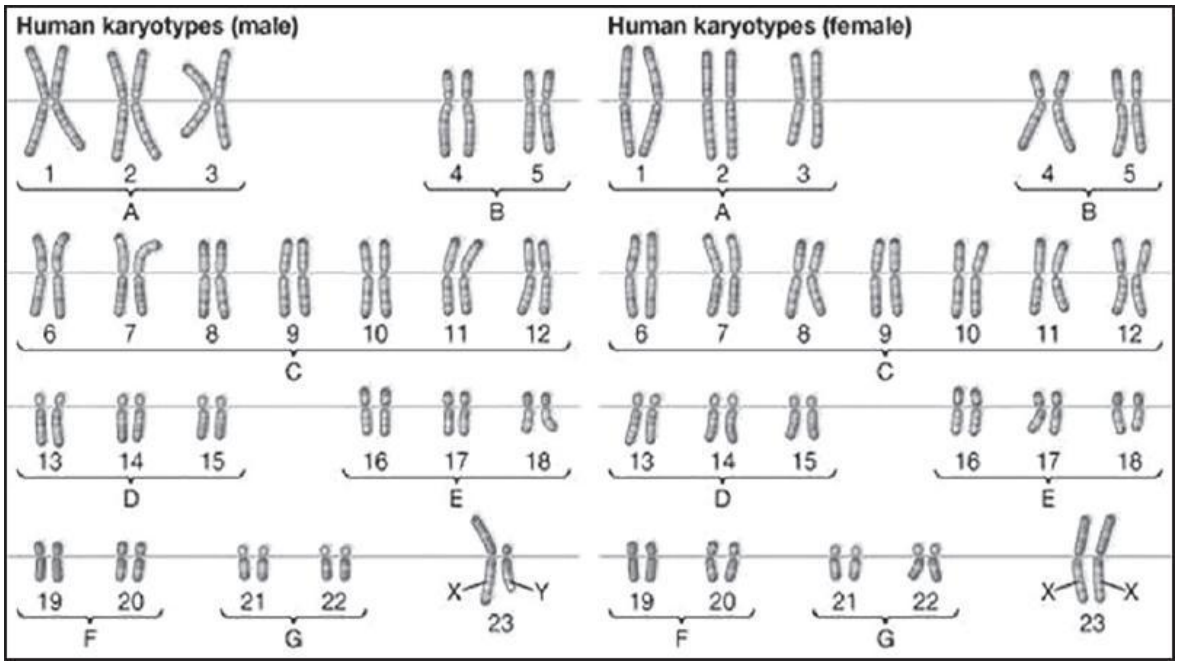

Figure 1: Photograph of chromosomes by karyotyping(16).

Medicines to be found to help them. Karyotyping helps in finding medicines (17) and plays a major role in finding appropriate treatment for cancer and other health problems (18). Liquid paraffin can be used for hydrating the mouth for the person of dried mouth (19). Chromosomes are classified into seven groups based on size and centromere location. Centromeres are found in the middle, near one end (acrocentric), and in between (submedian) (20). Group A chromosomes 1-3, are longest with a median centromere. Group B chromosomes 4-5, with submedian centromere. Group C chromosomes 6-12, median sized with submedian centromere. Group D chromosomes 13-15, medium-sized with acrocentric centromere. Group E chromosomes 16-18, short with median or submedian centromere. Group F chromosomes 21-22, very short with acrocentric centromere. Chromosome X is similar to group C and chromosome Y is similar to group G (21). Karyotyping also represents evolutionary relationships and suggests primitive or advanced features of an organism (22).

MATERIALS AND METHOD

This study included articles from various search engines for data collection namely Pubmed, Google scholar, Cochran's, MeSH core, bioRxiv, MedxRiv, articles to till date using the keywords.

Inclusion criteria:

Articles related to karyotyping, chromosomal abnormalities, different techniques were included in the study.

Exclusion criteria:

Articles not related to karyotyping were excluded.

METHODOLOGY

Chromosome preparation:

Sources of chromosomes are from tissues with uncleaved cells that undergo division. Peripheral genius blood mostly lymphocytes (23). Skin (fibroblast), bone marrow is used to collect cells. For fetal chromosome patterns, amniotic fluid cellar chorionic villi are collected. 5-10ml heparinized venous blood is the most commonly used source, in which heparin prevents coagulation and interferes later with the separation of lymphocytes (24).

Culture:

Blood cells are grown in a suitable culture medium which contains phytohemagglutinin, acting as a mitogen. It stimulates the T-lymphocytes to divide. Agglutinate red blood cells are a commonly used culture medium. 5ml of water medium contains 1ml fetal bovine serum, 0.2ml phytohemagglutinin. The cultures are incubated at 37C for 48.72 hours (25).

The arrest of division:

Mitosis is interrupted at metaphase with spindle inhibitors such as colchicine 0.01%. The chromosome number, size, shape at metaphase are of species-specific. In non-dividing cells, the chromosomes are not visible even with aid of histologic stains for DNA in electron microscopy. All cytogenetic works are done at the metaphase stage. The culture is again incubated for 45 minutes. The contents are then transferred to a tube and centrifuged for 800 rpm 5 minutes. The supernatants are discarded and mixed.

Suspension in hypotonic solution:

Prewarmed hypotonic saline is added to the culture which causes the RBCs to lyse and stimulates osmotic swelling of lymphocytes which results in the spreading of the chromosomes. The culture is incubated at 37^0C for 5 minutes and then centrifuged at 800 rpm speed for 5 minutes. The supernatant is removed.

Fixation:

A freshly preparedfixative consists of 3 parts of methanol and 1 part of glacial acetic acid. This is added as two changes of fixative of an interval of 45 minutes.

Slide preparation:

The cells are resuspended in fresh fixative slides prepared by placing a drop of cell suspension on a previously cooled cleaned slide and then dried, followed by staining (26). The chromosomes are arranged in a standard format called ideogram or karyogram

RECENT ADVANCES

There are some methods of technology to detect chromosome aberrations even in tiny parts of the chromosome. The different methods are banding technique, Fluorescent in situ hybridization (FISH), comparative genomic hybridization (CGH), and spectral karyotyping which detects a deletion, translocation, duplication of chromosome for diagnosis (27).

Chromosome staining banding technique:

Numerous methods are available for identifying chromosomes and for preparing karyotypes for diagnostic purposes. Banding patterns are the barcodes for cytogeneticists to easily identify chromosomes, and to detect subtle deletion, inversions, insertions, translocation fragile sites, and other complex rearrangements and also refine breakpoints. The ability to analyze chromosomes depends on the length of chromosomes, level of fixed, spread, and staining of the chromosome (28) (Figure 2). Large numbers of cells are examined for clinical purposes by automatic screening light microscopy with computer control and analysis which facilitate identification of chromosomal abnormalities.

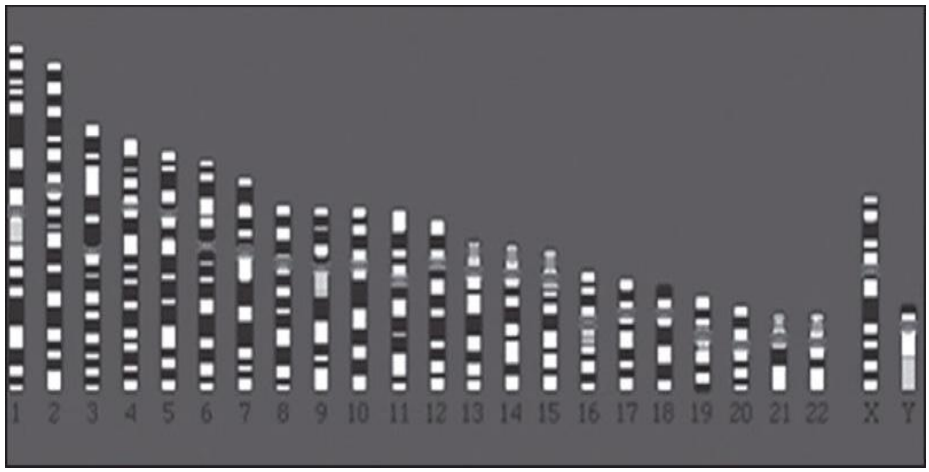

Figure 2: Photography of chromosomes by Banding Technique(29)

General techniques:

Some **human** chromosomes are distinguished under morphological grounds alone, for example, length of arms, the position of primary and secondary constriction. Autoradiography is used especially for S phase identification of chromosomes 4, 5, 13, 14, 15, 17, and 18.

Procedure:

The suitable tissue preparation with nuclear emulsion is done in a dark room and stared for several weeks in dark and then photographically developed and fixed. Discrete silver grains are seen over sites that emit radiation. Their position indicates sites of incorporation of radioisotope which is called autoradiograph or radio autograph (30).

Auto orcein method:

It is an original staining technique that permits the study of chromosomes morphology. It is prepared by adding 1-2g orcein to 45ml of horn acetic acid.

Technique:

Few drops of stain are added to the prepared slide on lower slip and gently firm pressure is applied with filter paper or glass rod. The excess of stain is removed by applying filter paper at the edge of the coverslip (31). Chromosomes are stained deep purple. It is replaced by banding techniques.

Banding Techniques:

The first banding technique was introduced by Caspersson in 1969. It includes G (Giemas) banding, Q (Quinacrine) banding, RC (reverse) banding, C (Centromeric heterochromatin) banding, T (Telomeric) banding and nucleolar organizing regions (NORs) high-resolution banding (32).

G (Giemsa) banding:

It is the most common method which produces permanent slides that are studied under standard light microscopy. It shows the same banding pattern as quinacrine. It has greater resolution so no need for the use of fluorescent microscopy used to pair and identify individual chromosomes (33). G banding is most consistently produced by pretreatment of chromosomes with trypsin before staining with Giemsa (34). G bands consist of 300 to 400 alternate dark and light bands that resemble characteristics of each chromosome and reflect chromosomal condensation (35). Other banding techniques that are specifically used are, R banding: It is the opposite of G or Q banding, C banding: It is used to localize the heterochromatic regions of chromosomes, T banding: Stains the telomeric end regions of the chromosomes (36).

Molecular cytogenetics:

Fluorescent in situ hybridization (FISH):

It is a chromosome-specific or chromosome breakpoint specific DNA probes that facilitate the confirmation of presumed chromosomal aberrations. It has high sensitivity and specificity ((37). Visualization of the chromosomal location and nuclear location of a specific DNA sequence (38). It permits the detection of specific nucleic acid sequences of morphologically preserved chromosomes. It is performed on either metaphase or interphase cells which involves denaturing genomic DNA by using heat and formamide(39).

Comparative genomic hybridization:

It identifies DNA copy number changes in tumor genomes in a single hybridization experiment (40). Metaphase chromosomes and tumor cell culture are not required. Based on quantitative two-color fluorescence in situ suppression hybridization. Genomic DNA from tumor specimens isolated. Reference and control DNA of individual normal karyotype labeled differently in standard nick tRNA, action for example green fluorescent for tumor genome and red fluorescence for normal genome (Figure 3). Labeled genomes are pooled and hybridized and human metaphase spreads. It prevents cross-hybridization. Chromosomal lost or chromosomal subregions detect tumor genomes result in red fluorescence. Many studies have shown the same technique (41).

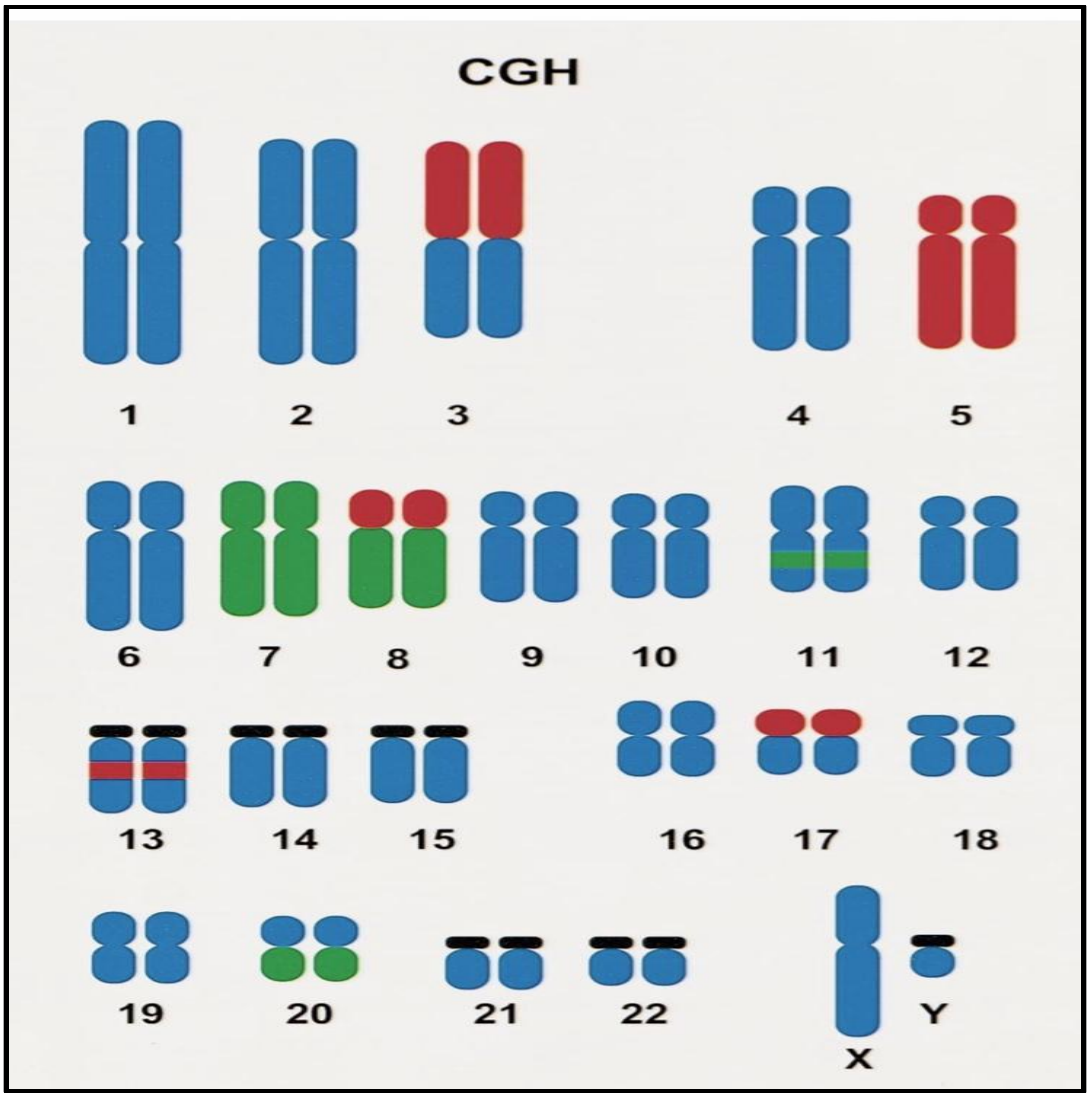

Figure 3: Photography of chromosomes by Comparative Genomic Hybridisation Technique(42).

Spectral karyotyping:

It also allows detecting tumor genome chromosomal aberrations in a single experiment (Figure 4). It utilizes the combination of epifluorescence microscopy, CCD imaging, Fourier spectroscopy. Recent studies show that spectral karyotyping was able to refine karyotype interpretation in the majority of the cases (41). It is also used to detect carcinoma (43)(44).

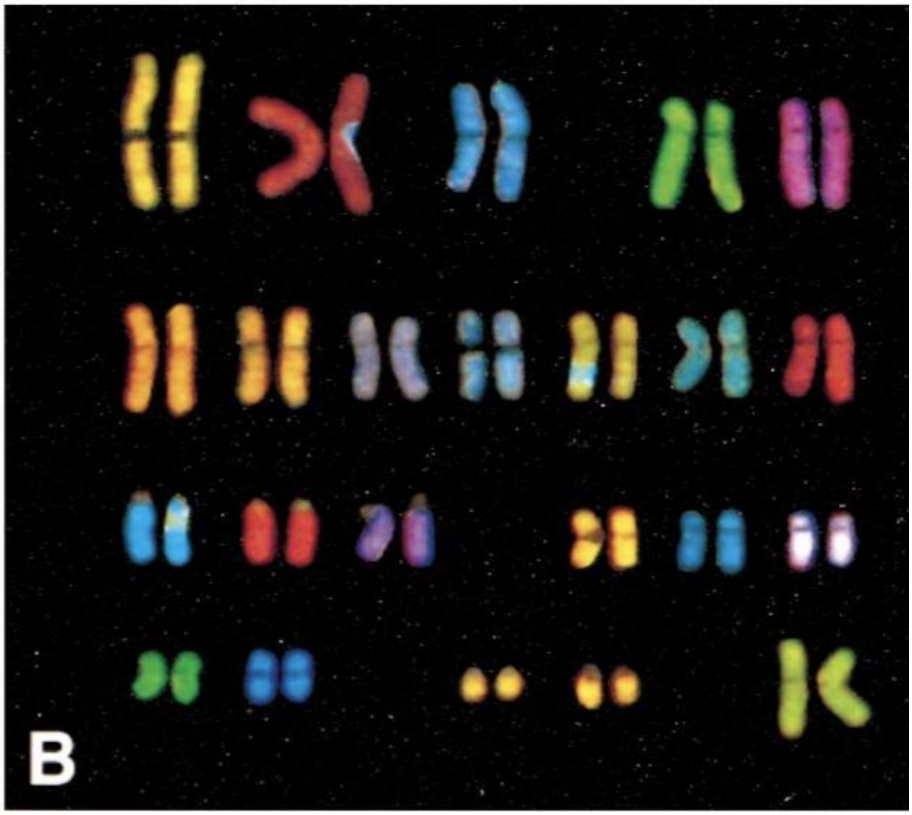

Figure 4: Photography of chromosomes by Spectral Karyotyping(45).

Dual-beam cytometry:
The metaphase chromosomes are suspended in a fluorescent stain solution through a laser beam rate of 1000 chromosomes per second.

Electrophoretic karyotyping:
It consists of 24 electrodes of gel electrophoresis. DNA transfer and hybridization using hybridization probes (46).

ADVANTAGES

It is used to analyze individual chromosomes (47). Neonatal discoveries of congenital abnormalities and prenatal diagnosis (48). Structural abnormalities of rearrangements of chromosome breakage, translocation, deletion, inversion, ring chromosomes, isochromosomes, mosaicism, and chimerism. Diagnose mental retardation (49) and neuropsychiatric disorder (50). Fluorescent in situ hybridization used to visualize chromosomal aberrations in non-dividing cells. Diagnose miscarriages (51). Spectral karyotyping identifies incentivize subtle chromosomal aberrations such as translocation of telomeric chromatin which cannot be detected by fluorescent in situ hybridization (FISH) and comparative genomic hybridization (CGH).

DISADVANTAGES

Despite the advantages, there are also some disadvantages. In chromosome banding technique assessment of karyotype changes in only classical cytogenetic analysis alone (52). The detection of the mitotic index is low. Quality of metaphase chromosome is of inferior quality high-resolution analyses cannot be performed. Fluorescent in situ hybridization (FISH) locus-specific or chromosome-specific probes (53) are used for this purpose. In comparative genomic hybridization, the main disadvantages analyzed were loss of spatial resolution and loss of heterozygosity (54).

CONCLUSION

In this review, we discussed the basics of Karyotyping, banding techniques, their types, and a few recent advances. Karyotyping and its techniques are the greater important diagnostic tool for chromosomal anomalies. The basic model of the karyotyping technique is the key to all the newer generation molecular and chromosomal investigations. It is also used to discover many preventive and treatment options for congenital anomalies and chromosomal disorders and also used to know about past evolutionary events.

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