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The importance of Spectral Karyotyping (SKY)

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Abstract
Spectral karyotyping (SKY) is one of the most useful cytogenetic techniques for detecting the whole genome in all types of chromosomal abnormalities in any individual. This is because all types of chromosomal abnormalities affect many genes, leading to cancers and many types of syndromes such as Edward's and Down's syndromes, which result in mental disabilities, malformations, and/or improperly developed body parts in affected individuals. This review will explain how Spectral karyotyping works and how it is performed. In addition, the classification of chromosomal abnormalities is explained.

Keywords: Spectral karyotyping, Edward syndrome, Down syndrome, chromosomal abnormality

1. Introduction

The cell of each human should have 23 homologous pairs of chromosomes, of which 22 are autosomes and 1 is a sex chromosome (XX or XY). Exceptions are erythrocytes, platelets and gametes/gametes, which have only 23 chromosomes [1]. Two meters of deoxyribonucleic acid (DNA) are organized into 46 chromosomes with a total of 20,000 to 25,000 genes that provide the genetic information necessary for growth and development. Chromosomal abnormalities usually occur when a defect occurs during meiosis or mitosis in the embryo or after the child is born. Many genetic disorders and birth defects are due to chromosomal abnormalities because there is a complete loss or increase in genetic material. This usually occurs in 0.6% of live births and results in malformations, developmental disorders, and/or disabilities. In addition, chromosomal abnormalities can lead to a 25% chance of stillbirth and miscarriage and a 50-60% chance of first trimester miscarriage [2]. Two methods are used to determine whether chromosomal abnormalities are present: fluorescent in situ hybridization (FISH) and spectral karyotyping [3] Spectral karyotyping (SKY) has been used since 1996 [4] to detect and register all chromosomes simultaneously, which corresponds to the meaning of the word 'karyotype" referring to the complete set of chromosomes [5]. Each homologous chromosome pair is recorded and detected with a different fluorescent color [6]. More than 500 research papers have been written based on SKY because this technique can easily detect chromosomal abnormalities [4]. Thus, cellular functions, banding patterns, chromosome length, evolutionary relationships, and changes in chromosome structure and/or number can be detected [7]. This has the great advantage of detecting many diseases and birth defects, such as Down syndrome and polycystic kidney disease, so that appropriate treatments can be initiated [8]. Moreover, this method integrates clinical and medical genetics, as the Spectral karyotype is a source of diagnostic information [9]. However, it can detect cryptic alterations, chromosomal rearrangements, and translocations, but not intra-chromosomal rearrangements [6]. SKY is important because it can detect rare diseases such as mental retardation, which affects fewer than 5 in 10,000 people [10], and also provides genetic counseling for infertility and disease management [11].

2. The history and development of cytogenetics and SKY

In 1882, the famous scientist Walter Flemming was the first to generate a human chromosome with 22-28 chromosomes from corneal epithelial cells [12]. In 1888, the term 'karyotyping' was introduced by the famous anatomist Heinrich Wilhelm Gottfried Waldeyer-Hartz [13]. In 1923, Theophilus Painter claimed that there were 48 chromosomes when he reconstructed images of cell nuclei obtained from testicular tissue that had been placed in kerosene and stained with an iron-hematoxylin solution. He also explained how sex could be determined by the presence or absence of a Y chromosome. However, in 1956, Joe Hin Tjio and Albert Levan determined that there were 46 chromosomes instead of 48. They were able to do this because instead of using tissue sections, they used crushed tissue in which the cells were frozen with colchicine during metaphase. Hypotonic solutions were used to visualize all chromosomes and their report was confirmed by different methods [12][15]. This solution is excellent for obtaining chromosomes and was discovered separately four years earlier by three famous scientists Makino, Hsu and Hughes. Colchicine is effective in isolating chromosomes at the metaphase stage and allows chromosomes to remain at the metaphase stage because it destroys spindle fibers and prevents chromosomes from entering anaphase [13]

Several chromosome staining methods have been developed that provide more details about chromosome structure. One method is called G-banding because Giesma is used to stain heterochromatic regions and especially cytosine-rich chromosome regions where the centromeres become visible. The second method is known as Q-banding, in which a fluorochromic dye called quinacrine stains the adenine- and thymine-rich regions [14] [15]. There are other types, including C-banding (allows visualization of centromeres) and R-banding (binds to G-bands). R-banding allows visualization of the nucleolar organization regions (NOR) of acrocentric chromosomes (i.e., chromosome 13, 14, 15, 21, and 22) [16], where ribosomal ribonucleic acid (RNA) genes are located [13] [15]. These banding techniques led to the discovery of numerous syndromes attributable to chromosomal abnormalities. The first person to discover a chromosomal abnormality was Marthe Gauther in 1958 [17]. A year later, Jerome Lejeune reported that Down syndrome was caused by the presence of an extra chromosome [12].

During this period, British scientists discovered that Klinefelter and Turner syndromes were caused by abnormalities of sex chromosomes [17]. In 1960, the Philadelphia chromosome was discovered as the first abnormal chromosome causing a cancer known as chronic myeloid leukemia (CML) [18]. This provided evidence that Theodor Boveri had predicted in 1914 that chromosomal alterations could cause cancer [19]. This allowed researchers to interpret that there are two types of genes that cause cancer, namely tumor suppressor genes and oncogenes, and changes in the genome due to changes in copied chromosome number, mutations, and other factors [20]. At the same time, phytohemaglutinin (PHA) was used to enable rapid growth of lymphocytes as these cell types were obtained from peripheral blood [21]

In the 1960s and 2000s, more advanced techniques for the detection of chromosomal abnormalities were developed, such as fluorescence in situ hybridization (FISH), chromogenic in situ hybridization (CISH), comparative genomic hybridization (CGH), silver-enhanced in situ hybridization (SISH), and SKY [22][23][24]. SKY has been used since 1996 because it combines several techniques, such as cell cultivation and harvesting, slide preparation, staining, and microscopy [25].

3. How does SKY work and how is it done?

The steps of this technique include cell cultivation, harvesting, washing process and many other steps. This experiment is performed under aseptic conditions because the cells must not be contaminated. The first step is the preparation of the sample, i.e. the collection of white blood cells, red blood cells and fetal skin cells and their cultivation in vitro. This depends on what type of diagnostic is being performed and what area of the body is to be examined [26]. One of these cells is placed in cell culture medium, usually RPMI-1640, and a few drops of PHA are added. These samples are placed in a 5% CO_2 incubator at 37C for 72 hours (approximately 3 days) to allow the cells to grow and proliferate. The samples are removed from the incubator and a few drops of colchicine are added to stop the cells in metaphase [27].

Trypsin and EDTA are used to remove the cells and detach them from the vessel [28]. Hypotonic solutions such as Ohnuki solution or potassium chloride (KCl) are used to isolate the chromosomes as the nucleus lyses [29]. In addition, the sample should be centrifuged several times until a clean pellet is found.

The second step is to prepare the slide and place it under the microscope to capture the image and interpret the results. The slide is prepared and placed in a staining solution such as Giemsa or Giemsa-Trypsin-Giemsa (GTC) to visualize the chromosomes [30]. Also, different types of fluorescent dyes such as Texas red are used to mark the different types of chromosomes with different colors [31]. After preparation of the slide, the chromosomes are arranged in the correct order under the microscope, usually with a 10x, 45x, and 100x objective [27][32]. The chromosomes are arranged according to their length, the number of bands and the position of the centromere. The image can be captured by a camera, and the chromosomes are photographed. If a camera system is connected to a computer and a microscope, the results can be viewed without a microscope [33]. Using this image, scientists can observe and search for abnormal changes in the number, structure, and morphology of chromosomes. What is particularly useful about SKY is that it allows us to visualize the entire set of genomes in a three-dimensional (3D) view [34].

4. When SKY is required?

It is usually performed when a doctor expresses concern about a patient's symptoms, and blood is usually drawn from the stem cells. SKY is needed when people have developmental problems that may affect more than one organ (multiple malformations). This can lead to disproportionate growth and reduction in size of organs [35]. Not to mention that organs are also affected by cancer in the late stages. Therefore, SKY is useful to detect patients with cancers such as esophageal cancer in which chromosome 8 is affected [36].
It is also recommended to have first-degree relatives tested, as this disease can be hereditary. In this case, cells from peripheral blood or bone marrow are taken from them [37]

Amniocentesis is performed in mothers if they become pregnant after the age of 35, as there is a higher risk of chromosomal abnormality in their offspring [38][39]. Amniotic fluid is collected from the uterus during this procedure, and complications that occur, such as miscarriage, bleeding, and infection, are extremely rare [37]. In addition, SKY should be tested in couples in which the female partner has difficulty conceiving or may miscarry because the egg and sperm create an embryo with an unbalanced abnormality. This is usually due to a translocation, as a reciprocal or Robertsonian translocation can lead to infertility [39].

In addition, testing should be performed in women who enter premature menopause before the age of 40, as chromosomal abnormalities such as Turner syndrome are known factors that cause premature menopause. Of course, there are other risks that lead to premature menopause, such as smoking, autoimmune diseases, hereditary diseases, human immunodeficiency virus (HIV) infections, and many others [40].

5. Types of chromosomal abnormality

There are three ways to classify chromosomal abnormalities as presented by SKY. First, chromosomal anomaly is divided into numerical anomaly and structural anomaly [41]. Numerical anomalies are more common when there is an extra or missing chromosome (this anomaly is also called aneuploidy) [42] and occur when chromosomes fail to separate during meiosis I or II [43]. For example, in rare cases, people may have a monosomy, as in Cri-du-Chat and Turner syndromes, where there are 45 chromosomes instead of 46, while in more common cases, people may have a trisomy, as in Down syndrome, Edward syndrome, and Platau syndrome, where there are 47 chromosomes instead of 46 [44]. When a fetus has an extra chromosome copy, it is usually fatal and results in spontaneous miscarriage or stillbirth. Those born with trisomy have less severe outcomes than those with monosomy [43].

A structural abnormality is due to the rearrangement of one or more chromosomes and is caused by chromosomes being replaced unevenly or two chromosome breaks not being repaired properly. The structure of chromosomes is altered in various ways, such as deletions, inversions, translocations, duplications, and ring chromosomes [45]. This can occur through a secondary form of malignant transformation leading to cancer [46]. In addition, structural abnormalities can be divided into two types, balanced and unbalanced structural

abnormalities. A balanced structural abnormality is caused by a rearrangement of genetic material, such as inversion or translocation, without a complete gain or loss of genetic material. This results in the generation of germ cells in which not all chromosomes are duplicated. An unbalanced structural abnormality is caused by the loss or gain of genetic material [47], as a chromosome segment may be removed or duplicated, or another chromosome segment is inserted [48]. Examples of unbalanced chromosomal abnormalities include Wolf-Hirschhorn syndrome, WAGR syndrome, and Cri-du-chat syndrome, which usually occur in 1 in 50000 people. Other common syndromes include Prader-Willi/Angelman syndrome, which usually occurs in 1 in 15 000 people, and DiGeorge syndrome, which usually occurs in 1 in 4 000 people. It is important to point out that even a ridiculously small amount of unbalanced structural abnormalities can unfortunately affect many genes, leading to serious consequences [47].

Another way to classify chromosomal abnormalities is by the time period in which the problem occurs. The first type is called a constitutional chromosomal abnormality and affects almost all cells of the embryo as it develops in the mother's uterus. Examples include Edwards's syndrome (trisomy 18), Patau syndrome (trisomy 13), Klinefelter and triple X syndromes (when an extra X chromosome is present), Turner syndrome, and Down syndrome (trisomy 21). It is important to note that Klinefelter syndrome affects males, while triple syndrome affects females [49]. Acquired chromosomal abnormalities affect the person throughout life and are usually limited to a specific type of tissue [50]. For example, a person may suffer from CML, which can occur between the ages of 40 and 60, but rarely in young people. This disease occurs when part of the DNA from chromosome 22 is inserted into chromosome 9, and this chromosomal change is known as the Philadelphia chromosome, as mentioned earlier. This type of chromosome is not hereditary because the chromosomal changes occur in somatic cells, and it is one of the signs that lead to cancer. The bone marrow begins to produce more tyrosine kinase, which causes the stem cells to grow into many white blood cells called granulocytes. These unhealthy cells begin to pile up, and eventually there is less room for healthy erythrocytes, white blood cells and platelets. This can lead to infections, bleeding and anemia [51].
A third way is to classify chromosomal abnormalities based on whether or not all cells examined have a chromosomal abnormality. One type is called homogeneous abnormality, which means that all cells have abnormal chromosome pairs, e.g., all bone marrow cells examined have abnormal chromosomes because proliferation of normal cells is inhibited. Another type is called mosaic abnormality and means that some cells have chromosomal abnormality while the other cells are normal or carry some other type of abnormality. For example, in a patient who has acute lymphoblastic leukemia (ALL) with normal cells and several types of cancer cells [41].
The risk of a child suffering for aneuploidy includes mother's and father's age, unhealthy lifestyle of parents such as smoking, improper prenatal scanning, consanguinity, and the reproductive performance [52].
It is noted that a mother over the age of 35 has a 9.1% higher chance for their newborn to be affected by Down syndrome and other types of syndromes. The reason is that the mother age as their eggs starts to have more abnormal number of chromosomes age. It is found that by the age of 40, there will be around 60% eggs that have abnormal number of chromosomes. Furthermore, another reason is that there is a lower amount of cohesion and securin found in eggs as the mother ages. The lower amount of these proteins causes the chromosomes to divide unevenly, and this research was done on mice [53].

Unfortunately, there is no specific cure for these diseases or cancers caused by chromosomal abnormalities, but it is possible to manage symptoms, as genetic counseling and physical and occupational therapy are recommended. Those with Turner syndrome, for example, may undergo surgery to correct heart defects. Prenatal diagnostics help pregnant women terminate the pregnancy if it is legal and acceptable [54].

6. Conclusion

Spectral karyotyping is an overall useful advanced technique that can detect all types of chromosomal abnormalities as it can detect cancer and diseases. This allows medical professionals to create treatment plans and find out if the treatment plan is successful. Spectral karyotyping did not come about overnight; it took decades to develop this technique and discover more details about chromosomes. Chromosomal abnormalities usually occur when cells do not divide properly and can occur both during embryonic development and throughout a person's life. Even though an estimated 1 in 150 people have a chromosomal abnormality, it can have serious consequences such as cancer and developmental delays. It is important to know that maternal age over 35 years and environmental factors that cause cancer are the main risk factors for a cancer or syndrome.

There is no cure for diseases and/or cancers due to chromosomal abnormalities, but it is possible to live with them and in some cases it is possible to terminate the pregnancy.

Declaration of competing interest

The authors declare that they have no known financial or non-financial competing interests in any material discussed in this paper.

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