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Identification of chromosomal aberrations in the diagnosis of certain genetic disorders

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Abstract **–** Cytogenetics is the analysis of blood or bone marrow cells that focuses on chromosomal rearrangements. Human body cells, exclusive of reproductive cells, have 23 pairs of chromosomes. So, any deviation from this is considered abnormal.
In our report, we testified by karyotyping bone marrow samples, taken from seven patients diagnosed with different hematological diseases, that the development of leukemia and other blood cancer involves changes in a cell's genetic material. Metaphase cells underwent G- Banding to identify specific chromosomal rearrangements and translocations. In hematological diseases, particularly in acute leukemias, abnormal and normal karyotypes may be present in the same sample. Structural and numerical chromosomal aberrations were examined in all karyotypes performed. Certain recurrent alterations act as hallmarks of a disease, which facilitates the identification of a specific disease by a cytogeneticist. Complex karyotypes characterized by the presence of three or more abnormalities, are generally associated with poor prognosis. The combination of conventional and molecular cytogenetics would be very useful in the diagnosis and, therefore, in the prescription of specific therapy for the treatment of diseases.

Keywords – Cytogenetics, Chromosomal aberrations, Hematopathology, Karyotyping, Diagnosis, Bone marrow, Targeted-therapy.

I. INTRODUCTION

The diploid human genome is composed of twenty to twenty-five thousand genes; However, haploid set is estimated to be 3.2 x 109 base pairs. Genes consisting of Deoxyribonucleic Acid (DNA) base pairs are located on chromosomes. Every nucleus includes twenty-three pairs of chromosomes that are always received from paternal and maternal lineages. DNA molecule, together with proteins called histones form a structure called chromatin. This means that chromatin is lower order of DNA organization whereas chromosomes are higher order of DNA organization (Watson, 2014).

DNA sequences or genes, composed of serially connected nucleotides are the functional heredity units. Every gene comprises of the particular set of instructions for a particular function or protein-coding, and any change in these instructions may have serious consequences.

We call changes in the structure of a gene: a mutation. A gene mutation refers to random alterations in DNA, it may occur in somatic and reproductive cells, most often, during replication and division, as well as exposure to mutagens or a viral infection. In this case, cells can make a defective protein or do not make a protein that the body needs. Whereas, changes to the structure or number of chromosomes are called chromosomal abnormalities. They can be a missing, extra, or irregular portion of chromosomal DNA.

Chromosome abnormalities may be detected or confirmed by comparing an individual's karyotype, or complete set of chromosomes, to a species-typical karyotype via genetic testing.

These chromosomal defects can be inherited from parents or be “de novo”. Others, can be acquired. In all cases chromosome studies are required to detect anomalies, as they often provoke malignancies because of the formation of hybrid genes and fusion proteins, deregulation of genes and overexpression of proteins, or loss of tumor suppressor genes. According to the Atlas of Genetics and Cytogenetics in Oncology and Haemathology, certain consistent chromosomal abnormalities can transform normal cells into leukemic cells such as the translocation of a gene, leading to its inappropriate expression.

After culturing cells, chromosome analysis is achievable using different tools, and this approach involves the pairing of homologous chromosomes, counting them and detecting malformations.

For this reason, the term cytogenetics appeared. The development of human cytogenetics has gained momentum during the past 20 years (Kerndrup and Kjeldsen, 2003). This field combines the study of chromosomes, their bands, all the karyotype of a specimen, in addition of cell division as well. It reveals the nature of the chromosomal defect which is an important trait that helps distinguish normal and cancer-causing cells.

In this work, we will present some recurrent forms of chromosomal alterations and the means of detecting them (Chapter 1), then we will deepen, by making an overview of some blood cancers where these alterations are mainly identifiable (Chapter 2). Later, in the experimental part, we explained the procedures followed to make karyotypes as well as detecting anomalies, and finally, we added our investigations and future prospects.

This work was designed to search for and detect numerical and structural anomalies in the genomes of distinct individuals, diagnosed with different diseases, with the aim of diagnosing or confirming an existent disorder, and/or estimating disease progression to other illnesses.

II. Materials and Method

Reagents:

- Preservative-free sodium heparin (green top tube)
- EDTA
- Growth media (such as MarrowMAX™ Bone Marrow Medium)
- COLCEMID
- Chemical fixative (CARNOYS)
- Hypotonic saline solution (0.9% sodium chloride)
- Trypsin
- Gurr buffer tablets
- Gurr stain Giemsa
- Immersion oil

Equipment:

- Bone marrow biopsy/aspiration needle
- Syringe
- Sterile gloves
- Blood collection tubes
- Sterile blood culture bottle
- Incubator
- Centrifuge
- Microscope slides and cover slips
- Pipette
- Test tubes
- Coplin glass staining jars
- Forceps
- 50°C warming oven
- Paper towels
- Light microscope and a computer (ViewSonic Monitor) with CytoVision ®/Genasis software
- Scissors
- Tape

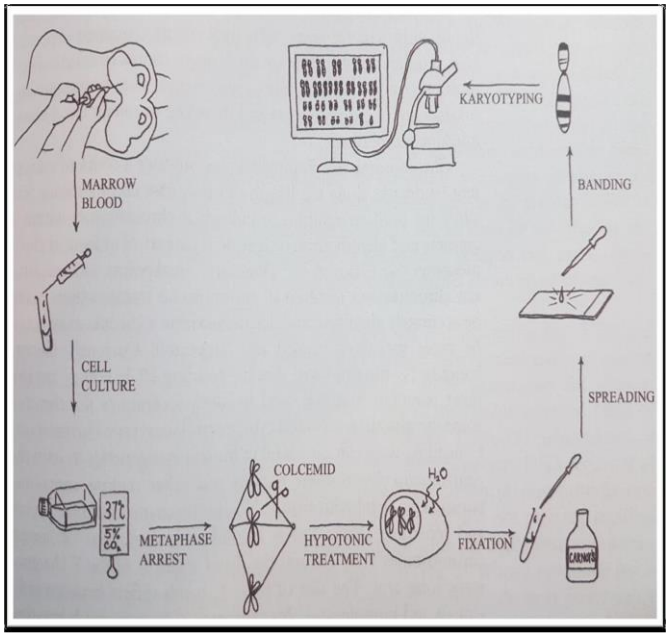

Fig. 1: Protocol for the preparation of a karyotype from a leukemic patient (Wan, 2014)

III. DISCUSSION

Cytogenetic analysis, the study of chromosomes by G bands, is used for karyotype analysis of abnormal cells, then it can be combined with FISH analysis to detect any genetic change.

Since we could determine the presence of malignant clones, this approach allowed us to agree on the fact that cytogenetic analyses can provide valuable and extremely suitable information of the analyzed genomes. In this way, the physician who collected samples from 7 patients and sent them to be cytogenetically analyzed can affirm the presence or the absence of the malignancy.

First, the selection of our samples for cytogenetic testing had a specific purpose which is to detect CAs in hematological diseases. Usually, bone marrow is the specimen of choice for leukemias and lymphomas (whether by aspiration of the fluid or biopsy of the cells). Because the length of in vitro culture depends on cell type, so, we used bone marrow samples as they are unstimulated and they contain spontaneously proliferating cells. They are harvested at 24 hours and if volume is sufficient, a 48-hour culture is also initiated followed by G-banding technique that produces specific alternating bands along each chromosome. [10]

Next, cell analyses of healthy people often show that they have normal karyotype with the correct number and structure of the complete set of chromosomes, 22 pairs (autosomes) and a pair of allosomes (sex chromosomes: X and Y), or they may be carriers of very small genetic changes. However, some karyotypes of unhealthy people may mimic the normal karyotype in some of their cells, and that was the case with the first patient that had at first place a normal karyotype with 46, XX, but in which, there was a masked hyperdiploid. In the same time, recent studies have demonstrated that a doubled clone can sometimes be mistaken as a typical hyperdiploid cell.

Based on both our findings and the reports available in the same context, we approve that in hematological disorders, particularly in acute leukemias, abnormal and normal karyotypes may be present in the same sample. That is why, we thought of the necessity of applying and testing samples with more sophisticated molecular cytogenetic techniques which can lead to getting the right interpretation of results, thus, right diagnosis of disorders.

Moreover, Attention is highly recommended in this type of analysis. Any small change in the procedures such as specimen transport to the cytogenetics laboratory in the inappropriate medium, microbial overgrowth, and technical errors involving cell harvest, slide preparation, or staining could result in assay failure. From our work, we assume that few additional minutes of COLCEMID treatment (that inhibits mitosis at metaphase by inhibiting spindle formation) can alter the obtained karyogram, not in the chromosomes' number or structure, but, in their size. They will appear shorter and thicker than they actually are, this was observed in the karyogram of the same patient (N°1). As a consequence, the crucial factor for the G banding is the wise use of time.

Additionally, cytogenetic analysis can sometimes overcome a misdiagnosis. Meaning that, an abnormality could be overlooked or incorrectly interpreted. For this reason, while inconclusive results are determined, checking out with molecular cytogenetics' tools is recommended to help or verify chromosome rearrangements. Nevertheless, and most importantly, the morphologic interpretation and correlation of results on all cases must be carried out by a qualified cytogeneticist and revised by a board-certified scientist (he may be the laboratory director).

This work was designed to investigate the CAs implicated in several hematologic/genetic disorders and the obtained experimental data were similar to a great number of research that have been performed to detect and locate recurrent balanced and unbalanced chromosomal changes leading to different hematological and genetic disorders:

Among the identified chromosomal alterations in DLBCL: gains (19p, 21, Y, etc.) and many translocations such as [t(7;15)(q22;q22)], these outcomes were similar to the findings reported in a scientific article about Chromosome abnormalities in DLBCL (Zhao et al., 2013).

Concerning CML, the presence of Ph chromosome is its main hallmark, where it is found in more than 90% of the diagnosed cases (Hagemeijer, 1987).

Thereafter, chromosomal abnormalities were detected in 4 patients diagnosed with MDS pathologies, including the deletions of chromosome 5q and 7q, trisomy 8, and complex

karyotypes. These marks matched the results provided in "Genetic abnormalities and pathophysiology of MDS" research, (Hosono, 2019)

Gathering all the results above, and because those chromosomal abnormalities can have many different effects that result in several malignancies, depending on the specific abnormality, it is important to call attention to the cytogenetic testing and its importance in confirming the findings of complete blood count (CBC) or other tests used to spot diseases like lymphomas and myelomas. In this way, the cytogeneticist ascertains the pathology diagnosed by a physician and indicate the chromosomal rearrangements involved in it. This will offer a clearer idea of the biology of the malignancy and its prognosis, which helps the doctor in finding the specific treatment and predicting the response to it.

Last but not least, in the genome sequencing era, molecular cytogenetics and microarrays are the best way of determining any hematological disorder, so that personalized therapy can be realized to treat those affected.

IV. CONCLUSION

Even though karyotype analyses can be more cost-effective when analyses must be performed on a large number of cells from a heterogeneous population, but they occupy an important position in genomic analyses and can be combined with higher resolution molecular methods that focus more on submicroscopic level changes in detecting certain disorders.

Recent studies reveal that complex karyotypes are associated with unfavorable prognosis, and thus are considered independent prognostic markers regardless of the disease type. In the same time, blood disorders had shown critical and recurrent chromosomal changes that became hallmarks of certain hematological disorders.

Risk Factors and causes of leukemias and lymphomas are not yet fully known, yet, exposure to radiation or certain chemicals, chemotherapy in the past, etc., can contribute to these types of cancer. Sometimes, even certain blood disorders such MDS can develop into other malignancies [11]. Although, smoking is an important contributing factor in many types of cancer, such as lung cancer and acute leukemia as it weakens the immune system. However, there is no proven link between alcohol consumption and an increased risk of any type of leukemia, including AML. Still, the presence of a risk factor does not mean the certainty of falling ill, but a greater probability than the absence of such a factor.

The highlighted hematological diseases are diseases that keep the body from making normal, healthy cells. Eventually, a person will start to lack RBCs that carry oxygen, platelets that prevent easy bleeding, and WBCs that protect the body from illness. The result can be deadly. In 2019, a total of 6,348 deaths from leukemia were registered in Italy. Deaths from leukemia seemed to be more common among males than among female [12].

Moreover, if we do not detect any cytogenetic alteration, we cannot conclude that there is an absence of a particular disease or that the prognosis is better than if a genetic abnormality was observed. For example, in ALL there are many different genetic rearrangements that are each associated with a different outcome.

Our investigations aimed to focus on the applied filed of Molecular and cellular biology. In our point of view, further technological advance should be made to overcome the limitations of diverse existing techniques. Consequently, gathering both known and newly incorporated techniques in the fields of bioinformatics, genomics, conventional and molecular cytogenetics would help to diagnose instantly the malignant disorders that resulted from genetic rearrangements.

Finally, since detecting CAs can facilitate the screening and diagnosis of the suspected disorder, so combination of conventional and molecular cytogenetics is really an informative way that enables physicians to confirm the diagnosis and assess the prognosis, thereby, choose the specific treatment and predict the response to it. By so, development into other malignancies could be restrained. Inevitably, a close relationship between the pathologist and the cytogeneticist is essential if maximum useful information is to be produced from the cytogenetics studies, especially of hematological diseases. Even though in many cases there is no cure for CAs. However genetic counseling and physical therapy may be recommended. Accordingly, targeted therapy can take place, and introducing personalized medicine would improve the survival rate.

From our perspective, a true understanding of all that is happening at the molecular level, the ongoing exploration of the fundamental epigenetic events

involved in genetic aberrations, and the pursuit of investigative clinical studies are helping to develop new strategies for the diagnosis, prevention and treatment of human diseases. Due to the fact that in recent times, they are already using epigenetically targeted therapies in clinical trials, which, at least for now, are showing promising results in hematological malignancies.

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