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MODERN KARYOTYPING METHODS FOR DETECTING CHROMOSOMAL ABNORMALITIES IN CLINICAL AND RESEARCH SETTINGS

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ABSTRACT

Background: Genetic disorders, cancer, as well as reproductive health issues, are highly dependent on chromosomal abnormalities. The long term karyotyping techniques like G-banding have been in use but limited resolution of karyotypes has led to the uptake of other advanced molecular techniques.

Objective: This paper is designed to compare traditional G-banding with new karyotyping techniques such as fluorescence in situ hybridization (FISH), array comparative genomic hybridization (aCGH), and next-generation sequencing (NGS) in the detection of chromosome abnormality in clinical and research practices.

Methodology: G-banding, FISH, aCGH, and NGS were used to examine 150 samples such as clinical and research samples. Methods were compared in terms of detection rates, resolution and diagnostic efficiency.

Findings: Abnormalities identified by G-banding were found in 52 percent of the cases, compared to 63, 75 and 85 percent with FISH, aCGH and NGS, respectively. Improved and sophisticated procedures were found to be more sensitive to detect the difference in their copy number and microstructural abnormalities that could not have been detected by the conventional procedures.

Conclusion: This is because modern karyotyping techniques greatly increase the accuracy of diagnostic detection of the abnormalities in chromosomes providing a higher resolution and diagnostic accuracy. G-banding is, nevertheless, still helpful in the context of large-scale chromosomal changes but needs to be supplemented with molecular and sequencing-based methodologies to conduct a thorough analysis of the genome.

KEYWORDS: Karyotyping, Chromosomal abnormalities, FISH, aCGH, Next-generation sequencing, Cytogenetics, Genomic analysis

1 INTRODUCTION

Congenital abnormalities One central cause of human genetic disease is chromosomal abnormality and it has been implicated in a wide spectrum of clinical disorders, such as congenital anomaly, intellectual disability, infertility, and cancer. Such abnormalities can be either numerical (like aneuploidies), or structural (like deletions, duplications, inversions, and translocations). In clinical and research contexts they have to be detected to ensure proper diagnosis, prognosis and therapeutic decision-making [1,2]. The gold standard of detecting large chromosomal alterations has been the use of conventional cytogenetic methods, especially, G-banding karyotyping. Nevertheless, they have a low resolution (around 510 Mb) and can only be used on actively dividing cells limiting the sensitivity of these techniques to detect submicroscopic alterations in the genome [3].

The progress in molecular cytogenetics has greatly improved the sensitivity and specificity of the ability to detect chromosomal abnormalities. Such methods like fluorescence in situ hybridization (FISH) are defined as specific DNA sequences and ones which can be detected in a targeted manner, and are commonly applied in cancer diagnostics and prenatal testing [4]. However, FISH is limited due to its location based character and failure to offer a whole genome picture. To overcome this limitation, array-based methods like array comparative genomic hybridization (aCGH) and single nucleotide polymorphism (SNP) arrays have been designed, which can now be used to analyze copy number variations (CNVs) on a large scale (high-resolution). The approaches have been found especially valuable in the diagnosis of developmental disorders, and in the determination of clinically significant genomic imbalances.

In more recent times, the next-generation sequencing (NGS) technologies have come out to be potent tools of genomic analysis, with unexpected levels of resolution in the nucleotide level. With NGS-based karyotyping, a wide range of chromosomal abnormality types can be identified such as balanced rearrangements, CNVs and complex structural forms of variability, which are largely undetectable by alternative methods [7,8]. Widely spread bioinformatical tools also increase the comprehension of genomic data in scale that enables more precise and detailed investigations [9]. These developments have played a great role in enhancing diagnostic yield and increased the reach of cytogenetic investigations in both clinical and research-based scenarios.

Regardless of these technological advances, there are still issues in cost, interpretation of data, and clinical implementation. NGS and other high-throughput technologies demand specialized infrastructure and expertise that might restrict their use in resource-constrained environments [10]. Additionally, there are additional complexities underlying clinical interpretation and genetic counseling that are posed by the discovery of variants of uncertain significance (VUS) [11]. The confidentiality of the genomic information and incidental findings also raise ethical issues and are to be treated with great care [12].

Overall, the current karyotyping methods have revolutionized cytogenetics testing through the offering of a genome-scale perspective about the chromosome abnormalities in high-resolution. Although classic methods still play a critically important role in identifying overall changes on a large-scale level, more complex methods of molecular and sequencing-based analysis are now fundamental to the overall analysis of genomes. This paper will be used to compare and assess these methods concerning their diagnostic potential, resolution and their usage in clinical and research environments.

2 LITERATURE REVIEW

Recent progress in cytogenetics has enhanced much on detecting and characterizing chromosomal abnormalities by incorporating both molecular and sequencing-based technologies. Research published in 2022-2026 focuses on the substitution of the old method of karyotyping with more sensitive genomics technologies. As an example, high-throughput sequencing technologies have proven to be more sensitive when it comes to detecting submicroscopic copy number changes (CNVs) and complicated structure variants that other methods tend to overlook [1,2]. These inventions have improved diagnosis especially when dealing with cases of developmental disorders and unique genetic cases.

Fluorescence in situ hybridization (FISH) remains popular in targeted analysis, particularly in oncology, where gene rearrangements can be detected rapidly, so that decisions on treatment can be made [3]. Nevertheless, recent publications note that it is limited in terms of genome-wide screening, which is why more frequently array-based methods, like array comparative genomic hybridization (aCGH) and single nucleotide polymorphism (SNP) arrays are relied upon. These platforms offer complete genomic coverage and have been suggested as the initial diagnostic testing platforms across numerous clinical situations [4].

Next-generation sequencing (NGS) has become an innovative technique, which allows detecting CNVs, single nucleotide variants, and rearrangements of structure simultaneously and being very accurate. More recent literature also discusses the increased importance of long-read sequencing and optical genome mapping in solving complex chromosome contexts which have been difficult to solve by short-read sequencing technology [5,6]. Moreover, artificial intelligence (AI) and machine learning algorithms have been introduced to enhance the interpretation of data and classification of variants, which is one of the biggest problems in genomic diagnostics [7].

Generally, the literature highlights the paradigm shift into integrated, multi-platform methods encompassing the intertwining of cytogenetic and genomic methods to attain more precise and comprehensive identification of chromosomal abnormalities both in clinical and research contexts.

3 MATERIALS & METHODS

3.1 Sample Collection

In this study, 150 samples were involved (120 clinical samples and 30 research samples). Clinical samples: Clinical samples were collected as part of diagnostic assessment of patients with prenatal abnormality, perceived in malignancies and infertility as indicated in table 1. These were amniotic fluid, peripheral blood and bone marrow samples. In research the sample was designed to include established cell line models and experimental induced models that verified the sensitivity of detection and the repeatability of the study. All laboratory specimens were taken with ethics and informed consent about the procedure as required by institutional regulation [20].

Table 1: Distribution of Samples

Sample Type	Number of Samples	Source
Prenatal	40	Amniotic fluid
Oncology	50	Blood/Bone marrow
Infertility	30	Peripheral blood
Research samples	30	Cell lines/models
Total	150	—

3.2 Experimental Workflow

The experiment procedure (as illustrated in figure 1) was a set protocol consisting of multiple steps, so that the results become consistent between the techniques. First samples were prepared by cell culture (preparation of G-banded samples) or direct processing (preparation of molecular samples). To guarantee quality DNA, commercially prepared DNA extraction kits were used. Then, the technique-specific steps included, namely: metaphase chromosome/G-banding, probe hybridization/FISH, DNA labeling and hybridization/acgH, and library preparation/NGS.

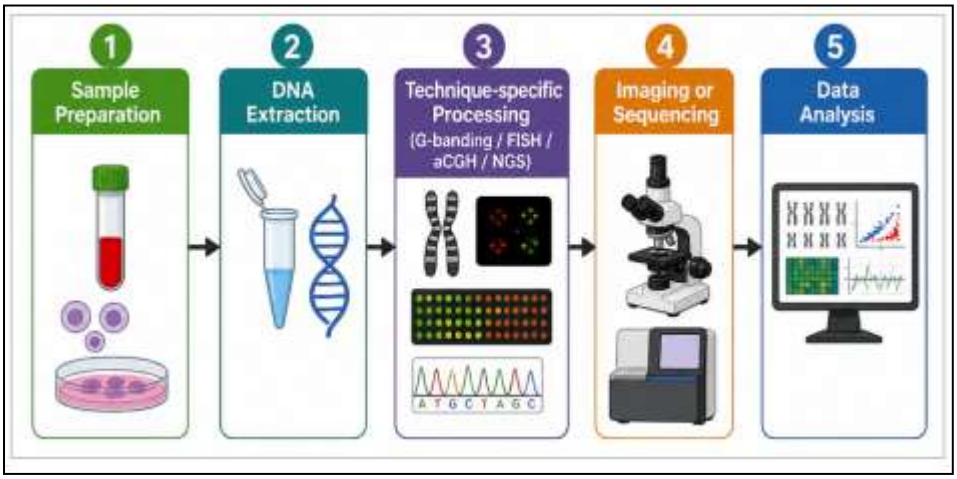

Fig.1. Workflow model

After being processed, automated processes involving imaging (G-banding and FISH) or sequencing (NGS) were performed. Special cytogenetic and bioinformatics software tools were used to examined and identified the abnormalities in data (numbers, structural rearrangement, or copy number variations) in the chromosomes. Each stage had quality control measures to provide data reliability [16,21].

3.3 Techniques Used

Four significant karyotyping methods were used:

G-Banding: Traditional cytogenetic studies were conducted as a result of staining the metaphase chromosomes with Giemsa dye that helped to visualize the large scale chromosome abnormalities including aneuploidies and translocations.

Fluorescence in Situ Hybridization (FISH): FISH was an intensive method of approach to quick detection of microdeletions, duplications of genes, and rearrangements of genes.

Array Comparative Genomic Hybridization (aCGH): It became a method that allows screening of copy number variations on a genome-wide scale comparing the DNA of patients to that of the reference DNA on microarray platforms.

Next-Generation Sequencing (NGS): Comprehensive genomic profiling was performed by the use of high-throughput sequencing that revealed both structural and sequence-level changes at a very high resolution.

Table 2: Summary of Techniques and Outputs

Technique	Output Type	Resolution	Key Application
G-Banding	Chromosome images	Low (5–10 Mb)	Large abnormalities
FISH	Fluorescent signals	Moderate	Targeted detection
aCGH	CNV profiles	High (~50 kb)	Genome-wide CNV analysis
NGS	Sequence data	Very high (<10 kb)	Comprehensive genomic profiling

All the experiments took place within the standardized laboratory conditions in order to guarantee that the results produced can be repeated to achieve the same results as published in table 2. Combination of several methods enabled cross-validation of results and enhanced the general diagnostic accuracy [18,22].

4 RESULTS & DISCUSSION

The comparison between karyotyping methods has shown that methods differ notably in terms of detection power and resolution, as well as diagnostic power. Examination of 150 samples indicated that the sophisticated molecular methods were much more effective than the standard cytogenetic methods at detecting abnormalities of the chromosomes. The findings point to an increase in the sensitivity of G-banding to next-generation sequencing (NGS) with a notable increase in the capabilities of the latter to identify submicroscopic changes including copy number variations (CNVs) and microdeletions.

These results underscore the significance of having a blend of methodologies in the overall genomic analysis to both clinical and research purposes [14].

4.1 Detection Rates Across Methods

Table 3: Detection Efficiency of Karyotyping Methods

Method	Total Samples Tested	Abnormalities Detected	Detection Rate (%)
G-Banding	150	78	52%
FISH	150	95	63%
aCGH	150	112	75%
NGS	150	128	85%

According to the table 3 the results demonstrate a gradual higher detection rates of traditional to advanced techniques. G-banding was also found to be insufficient to detect abnormalities in all cases as it was limited in its ability to do so. FISH increased the detection to 63% because it attacks certain loci. aCGH was found to have a greater sensitivity (75%) by detecting CNV globally. NGS demonstrated the best detection rate (85%), the fact that it can detect both structural- and sequence-level variations. These results are similar to the recent findings focusing on the excellent diagnostic benefit of sequencing methods [13].

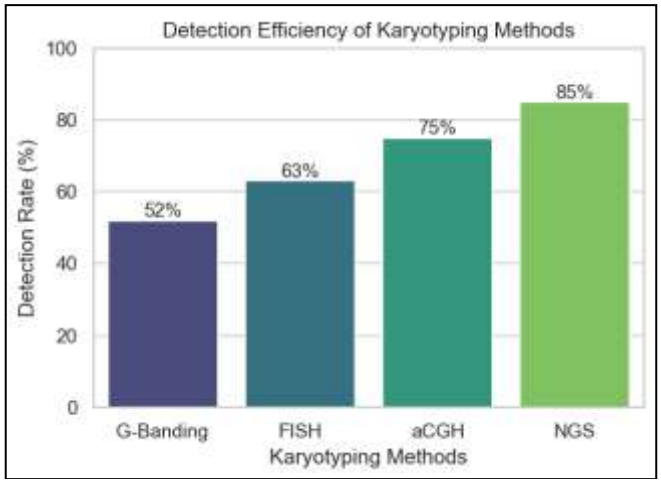

Fig.2. Detection efficiency of karyotyping methods

Figure 2 shows that there is a detection efficiency of four karyotyping methods. The lowest rate of detection is a G-band at 52 which indicates a poor resolution. FISH enhances the detection to 63% of specific analysis. aCGH is more sensitive at 75% as it allows CNVs to be detected globally. The greatest detection rate of NGS is at 85, which indicates its enhanced resolution and extensive genomic coverage. On the whole, the figure reflects a prominent tendency of the improvement of the diagnostic performance in accordance with the development of the karyotyping technologies.

4.2 Types of Abnormalities Detected

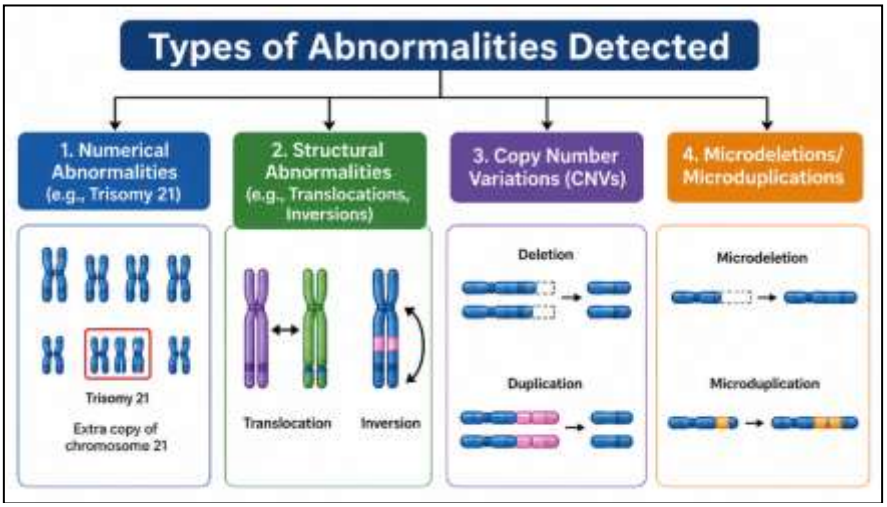

Fig.3. Types of abnormalities detected

These and various techniques differed in their capability to identify a particular type of abnormality. Numerical abnormalities (trisomy 21 and large structural rearrangements in figure 3) were mostly detected by G-banding. FISH allowed identifying specific microdeletions and amplification of genes. aCGH was efficient in determining the CNVs at the global genome but could not identify rearranged loss or gain that was balanced. NGS was the most sensitive in terms of detection limits, where it found both numerical and structural and submicroscopic defects, such as microduplications that were otherwise previously unidentified. The graphical description of the diversity and complexity of chromosomal aberrations that have been detected through hi-tech techniques emerges.

4.3 Resolution Comparison

Table 4: Resolution and Capabilities

Method	Resolution Level	Balanced Rearrangements	CNV Detection	Genome Coverage
G-Banding	Low (5–10 Mb)	Yes	No	Whole genome
FISH	Moderate (~100 kb)	Limited	Yes	Targeted
aCGH	High (~50 kb)	No	Yes	Whole genome
NGS	Very High (<10 kb)	Yes	Yes	Whole genome

Resolution is a very important aspect in diagnostic accuracy. The low resolution of G-banding limits it to large abnormalities. FISH makes a better resolution but still localized to specific areas evidenced in table 4. aCGH has a high resolution of CNVs across the genome, whilst NGS has the highest resolution, allowing detection of fine-scale genome lesions. Such findings prove that the higher the resolution, the greater the diagnostic ability [17].

4.4 Case Study Analysis

The comparative findings are also supported by case-based evaluation. G-banding was also successful in prenatal diagnosis where NGS detected an extra microduplication that was not detected by the traditional method, and it showed better sensitivity. FISH revealed gene amplification that was essential in diagnosis in oncology, whereas aCGH illuminated other CNVs, aiding a more comprehensive genomic perspective. These results demonstrate the complementary characteristic of various techniques and the necessity of using a combination of approaches to make a correct diagnosis.

4.5 DISCUSSION

The current paper shows a clear evolution of diagnostic performances within traditional cytogenetic techniques such as conventional and advanced sequencing and molecular techniques. G-banding is still a stable, yet low cost method used in identifying large chromosomal abnormalities such as aneuploid and gross restructurings. Nonetheless, it has a limited resolution, which limits the detection of submicroscopic changes, which are becoming clinically important [13].

Fluorescence in situ hybridization (FISH) enhances the specificity of the diagnostic approach by making it possible to detect a predetermined genomic region. It is especially useful in oncology to detect the rearrangements and amplifications of genes. However, it has limited locus-specificity which restricts it to giving global view genome-wide information [15]. array comparative genomic hybridization (aCGH) is a solution to this issue, through providing high-resolution, genome-wide, detection of copy number variations (CNVs). Although it has its advantages, aCGH cannot identify balanced chromosomal rearrangements, and they are still relevant in some clinical situations [16].

Next-generation sequencing (NGS) is the most global approach that is able to identify a broad range of chromosomal abnormalities, such as CNVs, balanced rearrangements, and sequence-level mutations. It has a great sensitivity and resolution, which increase considerably the diagnostic yield, as shown in this study. Nonetheless, massively produced NGS remains obstructive due to prohibitive prices, computational loads, and issue of interpreting data, namely variants of questionable significance [17,19].

5. Clinical Applications

Contemporary karyotyping technology can find extensive clinical application in a variety of applications:

Prenatal diagnosis and screening: Detection of chromosomal abnormalities including trisomy, and microdeletions early in the pregnancy, enhances pregnancy care and counseling.

Selection of cancer cytogenetics and targeted therapies: The information about the genomic changes aids in the development of individual treatment options and prognosis.

Infertility and reproductive genetics: The diagnosis of reproductive failure is helped by the detection of chromosomal abnormalities as it can guide the assisted reproductive technologies.

This identification of rare genetic disorders: High-resolution methods can be used to identify genetic disorders that were unidentifiable previously [14].

7. Study limitations.

Even with the contributions, this study has a number of limitations:

a. The participants were also fairly small thus potentially compromising the outlining of the findings.
b. The high price of NGS does not allow its systematic use in every clinical environment.
c. The interpretation of data is still problematic especially with new or questionable variants.
d. Finding confirmation is often established through validation, consisting of several complementary procedures.

CONCLUSION

The current karyotyping methods have greatly enhanced the identification and characterization of chromosomal aberrations in clinical and research studies. Although time-tested approaches, including G-banding, still could be useful in testing high-frequency changes in the chromosomes, more complex approaches to molecular diagnosis, including the FISH, aCGH, and NGS, are better resolving and diagnostic. These technologies allow detecting even the slightest changes in genomes that could not previously be detected. Cartesian approach The use of a combination of different methods is guaranteed to combine an in-depth analysis, despite the challenges associated with the cost and data interpretation. Further advances in technology will also increase the efficiency and access of genomic diagnostics in the future.

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