ENHANCING PRENATAL DIAGNOSTIC YIELD OF CHROMOSOMAL ABNORMALITIES BY COMBINED IMPLEMENTATION OF ARRAY-CGH AND TRADITIONAL KARYOTYPING AT HANOI OBSTETRICS AND GYNECOLOGY HOSPITAL

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ABSTRACT

Background: While karyotyping remains the gold standard for prenatal diagnosis of chromosomal abnormalities, it is limited to detecting alterations larger than 5 Mb (over 5 million base pairs). In contrast, array CGH (Microarray-based Comparative Genomic Hybridization) provides a comprehensive analysis of all 24 chromosomes, enabling the detection of chromosomal imbalances, including aneuploidy, losses, and duplications. Additionally, array CGH can identify chromosomal abnormalities even in the absence of specific diagnostic indications. ***Aim:*** This study aims to assess the prevalence of chromosomal abnormalities using the array CGH technique in comparison with karyotyping at Hanoi Obstetrics and Gynecology Hospital. ***Methods:*** A total of 399 pregnant women with a gestational age of 17 to 28 weeks underwent amniocentesis at Hanoi Obstetrics and Gynecology Hospital 2020 and 2022. Amniotic fluid samples were simultaneously analyzed using both array CGH and karyotyping techniques. ***Results:*** The karyotyping method identified chromosomal abnormalities in 63 out of 399 cases (15.79%), while array CGH detected abnormalities in 98 out of 399 cases (24.56%). Both techniques identified 49 cases of aneuploidy. For larger deletions and duplications, array CGH detected 14 cases compared to 8 identified by karyotyping. In contrast, array CGH identified 16 cases of small deletions and duplications, whereas karyotyping identified only 1 case. ***Conclusion:*** Array CGH is a highly accurate diagnostic tool that effectively detects structural chromosomal abnormalities, particularly small deletions and duplications, which may be missed by karyotyping techniques. This underscores the importance of integrating array CGH into prenatal diagnostic protocols for enhanced detection of chromosomal abnormalities.

Keywords: *array CGH, prenatal diagnosis, aneuploidy.*

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I. INTRODUCTION

Chromosomal abnormalities remain a major concern among congenital disorders, drawing substantial attention in the field of obstetrics and gynecology both globally and in Vietnam. This is due to their severe clinical manifestations, including multiple anatomical anomalies, intellectual disabilities, and the absence of specific treatments. Worldwide, prenatal screening programs have been extensively developed to enable early detection of chromosomal abnormalities, facilitating tailored genetic counseling for each case. Additionally, array CGH (microarray-based comparative genomic hybridization) offers significantly higher resolution in detecting chromosomal deletions and duplications compared to traditional karyotyping.[1]

Amniotic fluid cell culture karyotyping remains the gold standard for the prenatal diagnosis of chromosomal abnormalities. However, this method has limitations, including its inability to detect

microdeletions and the lengthy turnaround time for results (up to three weeks). In recent years, advances in cyto-molecular techniques have considerably enhanced the early detection of chromosomal abnormalities, particularly those involving chromosomes 13, 18, 21, X, and Y, with results obtainable within 24 to 48 hours. Testing techniques such as fluorescence in situ hybridization (FISH), quantitative fluorescence PCR (QF-PCR), and multiplex ligation-dependent probe amplification (MLPA) have contributed to these improvements.[2]

The emergence of array CGH marks a significant advancement, not only for its ability to detect aneuploidies but also for identifying chromosomal abnormalities without prior diagnostic guidance, offering a distinct advantage over traditional methods. This study aims to improving in diagnostic yield of chromosomal abnormalities using combined testing using array CGH technology and tradional karyotyping at Hanoi Obstetrics and Gynecology Hospital.

II. SUBJECTS AND METHOD

Subjects

Pregnant women who underwent amniocentesis for the prenatal diagnosis of genetic abnormalities at the Center for Prenatal - Neonatal Screening and Diagnosis, Hanoi Obstetrics and Gynecology Hospital, between 2020 and 2022.

Inclusion criteria:

Amniocentesis was indicated for high-risk pregnancies with suspected genetic abnormalities, allowing for simultaneous karyotype and Array CGH testing under the following conditions

-Increased risk of chromosomal abnormalities based on maternal serum screening results (Combined test, Triple test, NIPT)

-Fetal ultrasound revealing morphological abnormalities, including soft markers (e.g., shortened nasal bone, increased nuchal translucency) and structural defects in organ systems (e.g., cardiovascular, neurological)

-History of a previous child with confirmed chromosomal abnormalities

-Parental history of chromosomal abnormalities in the mother and/or father Complete and thorough medical documentation for the amniocentesis procedure.

Exclusion criteria:

-Cases of amniotic fluid sampling for the diagnosis of mono-genetic disorders, including Thalassemia, Duchenne muscular dystrophy, etc and/or fetal infections.

-Cases where amniotic fluid testing was conducted without simultaneous karyotyping and Array CGH techniques

-Cases with incomplete medical records pertaining to the amniocentesis procedure, among others

Method

Study design: Retrospective cross-sectional descriptive study.

Sample size and method of sampling: Convenience Sampling: Collection of 399 Study Samples Meeting Research Criteria.

Principle of Array CGH

The array CGH technique allows for the comparison of DNA samples against a control sample to detect deletions or duplications through hybridization, where a single strand of one DNA molecule pairs with a complementary strand from another. This method assesses all 46 chromosomes simultaneously, facilitating the identification of chromosomal imbalances. In array CGH, thousands of short DNA segments, known as

probes, are arranged on a glass slide to form an array. The test sample is fragmented, and both test and control samples are labeled with distinct fluorescent dyes (Cy3 and Cy5). These mixed samples hybridize with fixed probes on the array. The number of probes used depends on the reagents' resolution; more probes lead to higher resolution. After hybridization, unbound segments are washed away, and a microarray scanner detects the fluorescent signals. The fluorescence intensity from the patient's sample is then compared to that of the control, allowing for the identification of variations in genetic material at specific loci.

III. RESULTS

Table 1. ***Characteristics of the Study Population***

	Group	Number (n=399)	Percentage (%)
Maternal age	< 18	2	0.5
	18 – 34	285	71.4
	≥ 35	112	28.1
	Mean (Min – max)	**29.1 ± 5.83 (17 - 46)**	
History of previous pregnancy with congenital defects,	No	390	97.7
	Yes	9	2.3
Maternal chromosomal abnormality	No	392	98.25
	Yes	7	1.75
Family history of congenital defects, chromosomal abnormality	No	376	94.2
	Yes	23	5.8

The average age was 29.1 ± 5.83 years, with a minimum age of 17 and a maximum age of 46. Most of the women were in the 2nd group of 18 to 34 years, accounting for 71.4%. 9 women had a previous pregnancy complicated by congenital anomalies or genetic abnormalities. In addition, seven women had maternal genetic abnormalities, and 23 cases had a family history of congenital defects.

Table 2. ***Indications for amniocentesis***

Indication for amniocentesis	n=339	Percentage %
Abnormal ultrasound (US) findings	305	76.44
High-risk maternal serum screening	14	3.51
High-risk cff DNA (NIPT) screening	60	15.03
History of previous pregnancy/ child with congenital defects/ chromosomal abnormality	8	2.01
Maternal/ Paternal chromosomal abnormality	3	0.75
Abnormal US findings + high-risk serum screening	2	0.5
Abnormal US findings + high-risk NIPT	5	1.25
Abnormal US findings + history	2	0.5
Total	**399**	**100**

Among the cohort of pregnant women referred for amniotic fluid testing, those with abnormal ultrasound findings comprised the largest proportion.

Table 3. ***Classification of abnormal ultrasound findings***

Abnormal US findings		n=321	Percentage %
Soft markers	Increased nuchal translucency (NT)	49	15.26
	Absent/ Hypoplastic nasal bone	40	12.46
	Chroid plexus cysts	11	3.43
	Intra cardiac echo focus	1	0.31
	Echogenic bowls	7	2.18
Cardiovascular		64	19.94
Nervous		31	9.66
Cranial - Facial – Cephalic – Cervical		29	9.03
Bone – Joints - Extremities		23	7.17
Digestive - Abdominal		18	5.61
Uro - genital		28	8.72
Pneu – pleural – diaphragmatic		15	4.67
Fetal growth restriction		2	0.62
Hydrops fetalis		2	0.62
Fetal tumors		1	0.31
Total		**321**	**100**

Increased nuchal translucency (NT) and absent/ hypoplastic nasal bone are the two soft markers most strongly associated with the indication for amniocentesis. Among cases of anatomical abnormalities detected on ultrasound, cardiovascular abnormalities have the highest prevalence, with 19.94%. A total of 37 cases presented with multiple morphological abnormalities on ultrasound.

Table 4. ***Detection Rates of Genetic Abnormalities by Two Methods Based on the Type of Abnormality***

Abnormalities	Detected by array CGH	Detected by karyotyping	p
Aneuploidy	49	49	>0.05
Deletion/ Duplication larger than 5Mb	14	8	>0.05
Deletion/ Duplication smaller than 5 Mb	29/56	1	0.012
Mosaism	5	4	>0.05
Marker chromosome	1	1	>0.05

Array CGH identified 124 abnormal cases, significantly outperforming karyotyping, which detected only 63 abnormal cases. In cases involving microdeletions or microduplications, array CGH demonstrated a markedly higher detection rate, identifying 29 clinically significant Copy Number Variants (CNVs) out of 56 detections, from 399 cases (7.27%), compared to karyotyping, which detected just 1 out of 399 cases (0.25%).

Table 5. ***Detection Rates of Genetic Abnormalities by Two Methods Based on Indication for Amniocentesis***

Indication	n	Detected by aCGH	Detected by karyotyping	Increased detection rate	OR
Abnormal ultrasound (US) findings	305	59/83	26	18.69	2.57
High-risk maternal serum screening	14	1	1	0	1
High-risk cff DNA (NIPT) screening	60	33	29	6.67	1.31
History of previous pregnancy/ child with congenital defects/ chromosomal abnormality	8	1	1	0	1

Indication	n	Detected by aCGH	Detected by karyotyping	Increased detection rate	OR
Maternal/ Paternal chromosomal abnormality	3	0	0	0	nd*
Abnormal US findings + high-risk serum screening	2	0	0	0	nd
Abnormal US findings + high-risk NIPT	5	5	5	0	nd
Abnormal US findings + history	2	1	1	0	nd
Total	**339**	**124**	**63**	*nd: not determined	

In the group undergoing amniocentesis due to ultrasound-detected abnormalities, array CGH identified 59 clinically significant abnormalities out of 83 detected, out of 305 cases (19.34%), reflecting a 9.3% higher detection rate compared to karyotyping, which detected 26 out of 305 cases (8.34%).

Table 6. ***Detection Rates of Genetic Abnormalities by Two Methods Based on Abnormal ultrasound findings***

Abnormal ultrasound findings	n	Detected by aCGH	Detected by karyotyping	Increased detection rate	OR
Soft markers (Increased NT, absent/ hypoplastic nasal bone, chroid plexus cysts, intracardiac echo focus, echogenic bowls)	149	32	19	8.72	1.87
Cardiovascular	64	24	11	19.56	2.78
Nervous	31	5	2	16	2.88
Cranial - Facial – Cephalic – Cervical	29	4	1	10.34	0.16
Bone – Joints - Extremities	23	9	5	17.4	2.31
Digestive - Abdominal	18	4	1	16.6	1.42
Uro - genital	28	4	2	7.14	2.16
Pneu – pleural – diaphragmatic	15	2	1	6	2.15
Fetal growth restriction	2	0	0	0	Nd
Hydrops fetalis	2	1	1	0	1
Fetal tumors	1	1	0	0	nd
Other	6	0	0	0	nd
Total	321	86	43		

Array CGH identified 124 abnormal cases significantly outperforming karyotyping in detecting chromosomal abnormalities in cases with abnormal ultrasound findings.

IV. DISCUSSION

The average maternal age in this study was 29.1 ± 5.83 years, with 71.4% of participants aged 18 to 34. The youngest mother was 17, and the oldest was 46, indicating that most participants were within the reproductive age range. Chromosomal aneuploidy is known to be associated with maternal age, with numerous studies showing an increased frequency, especially after age 35, due to errors in oogenesis leading to non-disjunction during meiosis II. In contrast, chromosomal microdeletion/microduplication syndromes, such as DiGeorge, Cri du Chat, Prader-Willi, and Angelman syndromes, are not influenced by maternal age, with no significant variation in incidence across age groups.

Indications for amniocentesis to diagnose chromosomal abnormalities in 399 patients

are detailed in Table 2. In this study, the primary rationale for amniotic fluid sampling for array testing was the presence of morphological abnormalities detected on ultrasound, which accounted for 78.69% of cases. This included 76.44% with isolated ultrasound abnormalities and 2.25% with ultrasound abnormalities associated with other factors. With the widespread popularity of NIPT, amniocentesis due to high-risk NIPT results without abnormal ultrasound findings (60/399) accounts for 15.04%, a considerable rate.

According to guidelines from the American College of Medical Genetics and Genomics (ACMG), array CGH is recommended as the first-line option for prenatal diagnosis in the following circumstances:

- Fetuses with morphological abnormalities identified on ultrasound.
- A history of offspring with chromosomal abnormalities.
- Parents who are carriers of balanced chromosomal rearrangements, such as translocations or inversions.

Table 4 highlights the advantages of array CGH over karyotyping in the detection of deletions and duplications. Specifically, array testing identified an additional 14.08% of chromosomal abnormalities, resulting in a detection rate 30 times higher than that of karyotyping in detection micro structural chromosomal abnormality. Karyotyping, conducted at Hanoi Obstetrics and Gynecology Hospital using G-banding techniques, exhibited a resolution range of 400 to 550 bands on the haploid chromosome set, with each band averaging approximately 5 Mb in size. As a result, this method is restricted to detecting abnormalities larger than 5 Mb.

Theoretically, karyotyping can identify aneuploidies and deletions/duplications of 5 Mb or larger, while smaller abnormalities may remain undetected. However, this 5 Mb banding resolution threshold is relative. The interpretation of karyotype results is influenced by several factors, including the staining technique and the expertise of the geneticist in analysis and interpretation.

The effectiveness of array CGH in detecting pathogenic copy number variations (CNVs) is closely related to the indication for amniotic fluid testing. In cases where amniotic fluid analysis is warranted due to high-risk factors—such as a high-risk Combined test, Triple test, or NIPT screening, or a history of pregnancies with congenital anomalies—array CGH did not reveal additional abnormalities compared to conventional cell culture methods. Furthermore, studies involving larger sample sizes have demonstrated a very low detection rate of pathogenic CNVs in this population. A comprehensive review evaluating the efficacy of array CGH across 10 large studies involving 10,614 fetuses indicated that array CGH detected an additional 0.89% of clinically significant CNVs.[3]

In the group indicated for amniocentesis due to abnormal ultrasound findings, array CGH identified 59 out of 305 cases (19.34%), reflecting a 9.3% increase over karyotyping, which detected 26 out of 305 cases (9.5%). Subgroup analysis showing considerable detection rate of chromosomal abnormality by array CGH compared to karyotyping, especially in those with cardiovascular anormaly, with the most prominent cause of DiGeorge syndrome caused by chromosome 22 deletion. Similarly, a study involving 1,033 fetuses with ultrasound abnormalities conducted by

Srebniak et al. reported pathogenic CNVs in 5.5% of cases.[4] A larger investigation revealed pathogenic CNVs in 6.6% of 2,462 cases with ultrasound abnormalities.[5]

Consequently, array CGH should be regarded as the first-line test for detecting chromosomal abnormalities in prenatal diagnoses involving ultrasound-detected morphological anomalies. This recommendation is endorsed by prominent global organizations, including the American College of Medical Genetics and Genomics (ACMG), the Society of Obstetricians and Gynaecologists of Canada (SOGC), and the Genetics Society of Canada (GSC)

V. LIMITATIONS:

We acknowledge several limitations in our study. First, the sample size was small and non-representative, as not all cases received simultaneous testing with array CGH and karyotyping due to the high associated medical costs. While array CGH provides high-resolution detection of chromosomal abnormalities, it often uncovers numerous copy number variations (CNVs) of uncertain significance, many of which are benign or likely benign and considered chromosomal polymorphisms. This complicates the identification of genetic causes for congenital defects and may increase medical costs for parents seeking further testing. Given the advancements in prenatal diagnosis and genetic testing, we aim to expand the scope of this study in the future.

VI. CONCLUSION

Array CGH is one of the most effective techniques for comprehensive chromosomal analysis, enabling precise identification of chromosomal abnormalities and associated genes. This advancement enhances evaluation, monitoring, and treatment strategies. However, array CGH has limitations compared to karyotyping, including its inability to detect polyploidy, balanced translocations, and abnormalities with low mosaicism. Therefore, it is essential to use array CGH alongside conventional karyotyping to ensure accurate diagnosis of genetic disorders in fetuses.

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