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Evaluation and Report of Array-CGH Utility in prenatal and postnatal diagnosis and abortion products referred to a Diagnostic Laboratory in Tehran

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ABSTRACT

Objective: Array comparative genomic hybridization (Array-CGH) has been used in diagnostic laboratories for the evaluation of individuals with intellectual disability/developmental delay, autism spectrum disorders, multiple congenital anomalies/dysmorphic features, prenatal diagnosis, and products of conception. Clinically available whole-genome aCGH can detect unbalanced chromosomal rearrangements/abnormalities with coverage of about one probe per 6 kb to one probe per 70 kb.

Material & Methods: We report the aCGH results of 142 patients referred to Sarem Cytogenetic laboratory, Sarem Women's Hospital for cytogenetic analysis between 2017 and 2020. They comprised 60 prenatal cases using amniotic fluid, 52 cases of products of conception, and 30 peripheral blood samples for post-natal cases. Chromosome analysis and aCGH were done for most of the referred samples.

Results: Four out of fifty-two aborted fetuses had pathogenic aCGH results including; two male fetuses with gain of whole chromosome 21 (compatible with trisomy 21), one male fetus with a gain of whole chromosome 9 (compatible with trisomy 9), and one female fetus with a pathogenic gain of 78.2 Mb on 13q13.3q34 and loss of 612 Kb on 20p13p13 which overlap with 175 and 7 OMIM genes, respectively. The later aborted fetus's karyotype result is 46,XX,der(20)t(13;20)(q13;p13) which is originated from the father. Also, five out of sixty prenatal amnion fluid's analysis demonstrated pathogenic chromosomal abnormalities. Ten out of thirty postnatal peripheral blood samples showed abnormal chromosomal aCGH results.

Conclusion: The results of this report emphasize the importance of the combination of classic karyotyping with aCGH in better management of the patients.

Keywords: Array Comparative Genomic Hybridization; Abortion Products; Prenatal; Postnatal; Chromosome Abnormalities.

Introduction

Diagnosis of the genetic disease is highly dependent on genetic techniques, in such a way that cytogenetics and molecular techniques are two fundamental arms of genetic-based diagnosis. Among these, classical cytogenetics (karyotyping) is the oldest and most reliable one, in regards to representing a whole picture of entire chromosomes and the number of chromosomes. Due to the contribution of molecular techniques, other cytogenetic techniques are superior to classical cytogenetics in terms of resolution power, such as Fluorescence in situ hybridization (FISH) and Array-based techniques. Compared to FISH, Array-based techniques are high-throughput. There are different Array-based techniques, based on the designed probes, and among these, Array Comparative Genomic Hybridization (Array-CGH) is considered routinely for diagnostic purposes [1-3].

Array-CGH, as a molecular-cytogenetic technique, has many applications for genetic testing of patients with unexplained intellectual disability/developmental delay (ID/DD), autism spectrum disorders (ASDs), and/or multiple congenital anomalies/dysmorphic features (MCA/DF), prenatal diagnosis, and products of conception [4]. Clinically available whole-genome Array-CGH can detect unbalanced chromosomal rearrangements/abnormalities (deletions and/or duplications) with coverage of about one probe per 6 kb to one probe per 70 kb, however, the traditional Giemsa-stained metaphase chromosome karyotyping identifies balanced and unbalanced chromosomal abnormalities with more than ~4 Mb length [2]. Validating the clinical utility and application of Array-CGH results in a timely manner would be totally informative and beneficial in future diagnostic decisions and clinical management [5-8]. Hence, in the present study we aim to evaluate and report the detection yield of Array-CGH genetic testing in the diagnosis of unexplained intellectual disability/developmental delay, autism spectrum disorders and/or multiple congenital anomalies/dysmorphic of Iranian patients, prenatal diagnosis, and products of conception referred to the Sarem Cytogenetic laboratory from 2017 to 2020. Here, we studied the Array-CGH results of 142 patients comprising prenatal, postnatal, and products of the conception samples and found that the detection yield of Array-CGH is highest in postnatal cases.

Materials and Methods

Patients

This retrospective study was performed at the Sarem Cytogenetic laboratory from 2017 to 2020. We totally included 142 cases referred to the Sarem Cytogenetic laboratory for cytogenetic analysis and Array-CGH analysis. One-hundred-forty-two collected cases contain 60 prenatal samples from amnion fluid, 52 products of abortion, and 30 peripheral blood cases. Clinical history was obtained from the patient's record according to the referral forms completed during genetic counseling. All procedures were in accordance with Sarem Women's Hospital's ethical standards (Ethical Number: SRI98102805F). This study was approved by the ethical committee of the Institutional Review Board (IRB) of Sarem Fertility & Infertility Research Center (SAFIR), Sarem Academy of Sciences.

Chromosome preparation and analysis

For prenatal cases, three cultures were set up for all samples using AminoMAX complete medium (Gibco, ref 11269-016). The peripheral blood and products of conception were also cultured and processed according to the standard protocols. The cultures were followed up and harvested when the growth of the cells was sufficient and chromosomal analysis was carried out by GTG- banding, if required, C-banding and NOR-staining were utilized. The chromosomal analysis was carried out and the results were reported from standard and international guidelines of the International System for Human Cytogenomic Nomenclature (ISCN). For each case, at least 20 metaphases were studied and in the case of abnormal findings, the number of metaphase analyses was increased to 50. MetaSystems software (MetaSystems, Germany) was used for karyotyping. The frequency of numerical and structural abnormalities is categorized and discussed with their referral indexes.

Array comparative genomic hybridization (Array-CGH)

Whole genome oligo Array-CGH was carried out using SurePrint G3 ISCA V2 8X60K whole genome oligo Array version 2 and was analyzed using Agilent Cytogenomic software v4. The Array consists of 60000 spots with overall median probe spacing of 60Kb and higher in close to 400 targeted disease regions. Standard genomic DNA labeling, hybridizations, hybridization, and normalization were done following standard protocols following [9]. For image analysis of slides, BlueFuse Multi Software, Version 3.1 (BlueGnome Ltd. Cambridge CB21 5XE UK) was utilized. Heteromorphic variants (Polymorphic Copy number variants (CNVs)) and the gene-free regions were removed from further studies.

Results

Totally, 142 cases were included in this study, and Array-CGH was carried out. Of these, 42.2% (60 cases)

were amnion fluid samples, 36.6% (52 cases) were products of abortion samples, and 21.1% (30 cases) were peripheral blood samples (Table 1).

Table 1: Frequency of abnormal Array-CGH results in Prenatal, Postnatal, and Products of Conception groups.

Number of patients / Type of referral	Total Cases (Male : Female)	Abnormal Array-CGH Findings (Male : Female)
Prenatal	60 (39:21)	5 (4:1)
Products of Conception	52 (26:26)	4 (3:1)
Postnatal	30 (14:16)	10 (5:5)
Total	142 (79:63)	19 (12:7)

Dividing based on gender, 44.4 % (63/142) are female and 55.6 % (79/142) are male. Among all participants, 19 cases (13.4%) had relatively pathogenic Array-CGH findings, including 12 (63.2%) males' and 7 (36.8%) females' karyotype (Table 2, 3, and 4).

In a more detailed view, five out of sixty (8.3%) amnion fluid analyses, four out of fifty-two (7.7%) aborted fetuses, and ten out of thirty (33%) postnatal peripheral blood sample analyses demonstrated pathogenic chromosomal abnormalities. Collectively, the imbalance of the long arms of 1 and 22 chromosomes is dominant in this study.

Among 22q abnormal cases, we found two DiGeorge syndrome, one 8-years-old affected boy and a one-year-old affected girl with 2.6 Mb, and 2.5 Mb deletions, respectively. Next, DiGeorge Syndrome was confirmed by MLPA (Fig.1A). Among 1q abnormal chromosomal cases, we diagnosed two cases with 1q21.2 Duplication syndrome, which had paternal origin (Fig.1B).

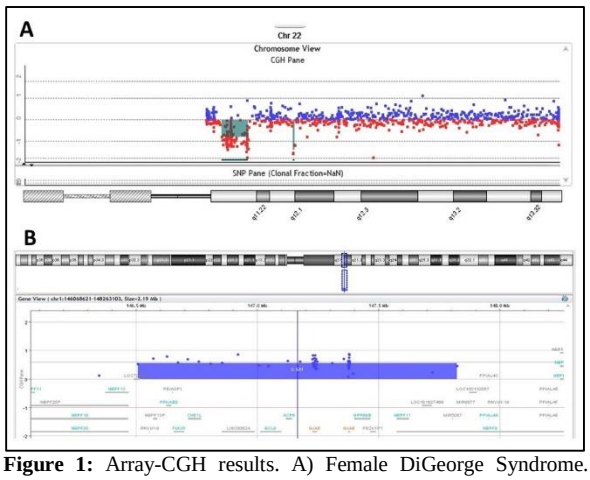

Figure 1: Array-CGH results. A) Female DiGeorge Syndrome. B) Male of 1q21.1 duplication.

Two cases of chromosomal translocation were seen, one in prenatal cases and one in products of conception. Although the karyotype result of the former sample, which was a 20-week male fetus, is not much informative, the Array-CGH results were accurate and demonstrated the partial monosomy of 1q43q44 and partial trisomy 9p24.3p23 which has a paternal origin.

In the latter sample, a 20-week female aborted fetus because of abnormal sonography, both karyotype and Array-CGH results confirmed each other which is an unbalanced translocation (46,XX, der(20)t(13;20)(q13.3;p13)pat with paternal origin with an apparently balanced reciprocal translocation between the long arm of chromosome 13 and the short arm of chromosome 20 (46,XY,t(13;20) (q13;p13) (See Fig.2A,2B,2C). Interesting couple, which was first cousins and referred because of spontaneous abortion of their 20 weeks' fetus. Array-CGH results demonstrated that both parents had 1q21.1 duplication syndrome (OMIM number: 12475-Fig.1B).

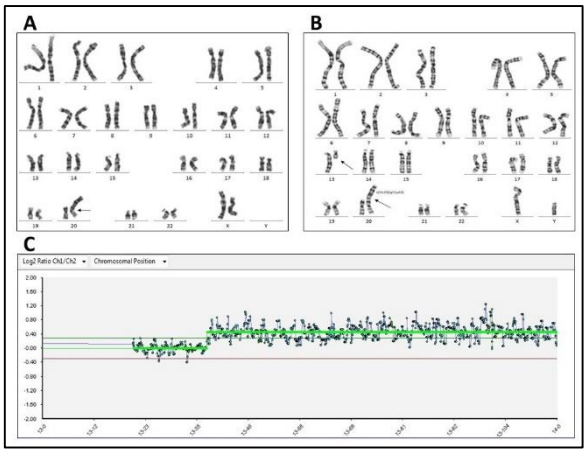

Figure 2: Cytogenetic results of an aborted fetus from consanguineous marriage and her father. A) Female fetus karyotype. B) Father's karyotype. C) Array-CGH result of Chromosome 13q13.3q34 duplication.

Discussion

For better diagnosis, prognosis, and clinical management of unexplained (ID/DD), autism spectrum disorders (ASDs), and/or multiple congenital anomalies/dysmorphic features (MCA/DF), identification of the genetic defects is essential and high-throughput techniques with best resolutions are needed [10]. However, sometimes, a combination of genetic techniques is required for better detection and confirmation. Thus, assessing the diagnostic power of current methods at time intervals –regionally- can be helpful in choosing the best diagnostic approach for the next complex cases.

Here, we studied the power of the Array-CGH technique in ID/DD, ASDs, and MCA/DF, prenatal diagnosis, and products of the conception of some Iranian patients and found that collectively; the detection yield of the Array-CGH technique is about 33.33 % for postnatal peripheral blood samples, 8.33% for prenatal samples and 7.69% for the product of abortion samples. In line with others' findings, the results of this report emphasize that a combination of traditionally karyotyping with Array-

CGH could contribute to the clinical geneticist/clinicians diagnosing the genetic basis of disease more accurately.

In this report, the total detection yield of Array-CGH for ID/DD, ASDs, and MCA/DF was 13.4 % which was in the range of detection yield of other studies (5.3-35%) [10,9]. The best detection yield was 33% for the postnatal samples and the lowest detection yield was 7.7 % for aborted fetus samples. However, utilizing karyotyping, FISH, MLPA, and Array-CGH in our previous studies, the detection yield was 12.5-19 % for Iranian patients with intellectual disabilities and 16 % for Iranian MCA patients with the diagnosis of one *de novo* DiGeorge Syndrome, similar to the findings of the present study [9,12].

Considering that the most microdeletions and or microduplication findings in this study were de novo cases with no clinically affected and/or abnormal chromosomal findings in the parents that are inconsistent with other findings [13,14]. Nevertheless, six out of 19 (31.6%) abnormal chromosomal cases had parental origin including five cases with a paternal origin and one maternal origin.

It's worth noting that although ID/DD, and ASD can be caused by various factors such as maternal hypoxia, the submicroscopic chromosomal abnormalities have a great impact on the etiology of ID/DD, ASDs, and MCA/DF [15,16]. Thus, Array-CGH is an ideal diagnostic test here. However, the interpretation of copy number variation is extremely challenging, and detecting pathogenicity mostly is influenced by deletions and the number of OMIM genes, specifically the number of dose-sensitive genes, and not the size of the region. Therefore, in regards to the new findings and based on the heterogeneous etiology of ID/DD, ASDs, and MCA/DF, the re-evaluation of uncertain significant findings might be essential in the future [5].

It should be noted that the Array-CGH technique has limitations to detect balanced chromosomal abnormalities (such as inversions), and is also unable to distinguish low-level mosaicism of less than 20% in samples (17). Moreover, other limitations of this study were the low sample size with heterogeneous patients in each group, the retrospective design, and limited clinical data. Increasing the sample size and collecting more clinical data in the next time interval reports will improve the assessment of the diagnostic yield and clinical utility of Array-CGH for unexplained ID/DD, ASDs, and MCA/DF patients.

Conclusion

In conclusion, for clinical management purposes, in line with others, this study suggested that Array-CGH could be the first-tier diagnostic approach for unexplained ID/DD, ASDs, and MCA/DF cases. However, it seems that sometimes it couldn't be a standalone detection method and other genetic techniques such as classical karyotyping are also required for confirmation or accurate diagnosis.

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Authors' contributions

F.B., F.V.R., F.M.: Participated in study design, data collection and evaluation, drafting and statistical analysis. F.B., I.B.: Were responsible for overall supervision. R.K., F.B., I.B.: were responsible for the laboratory work and the analysis of the results. K.B., S.G., M.K.: were responsible for patients' collection, clinical examination, and genetic counselling. F.V.R., F.M.: Drafted the manuscript, which was revised by F.B. All authors read and approved the final manuscript.

Conflict of Interest

Authors have not any sources of funding and potential conflicting interest.

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Table 2: Abnormal Array-CGH findings in prenatal group

Gender and age	Array Results	Size of Gain/Loss	Pathogenicity based on ACMG	Karyotype Results	Reasons for Referral & possible Follow ups
F,26w	Arr (GRCh37) 22q11.21 (18230460_21561514)x3 (Overlaps with 50 OMIM genes)	Gain of 3.3 Mb	Pathogenic	46,XX	Abnormal sonography Fetus:26 w Mother:30 y
M, 12w	Arr(GRCh37) 22q11.21 (21081260_21561514)x1 (Overlaps with 9 OMIM genes)	Loss of 480 Kb	Likely pathogenic compatible with microdeletion of A A22q11.21q11.21	46,XY	Abnormal sonography Fetus:12 w Mother:27 y
M, 18w	Arr (GRCh37) 13q12.12 (23566962_24910743)x3, (X,Y)x1 (Overlaps with 8 OMIM genes)	Gain of 1.3 Mb	Likely pathogenic. Compatible with microduplication of A13q12.1	46,XY	Abnormal sonography Normal male child was born Fetus: 18 w Mother: 31y
M, 20w	Arr (GRCh37) 1q4344 (242638796_249212668)x1 (Overlaps with 22 OMIM genes) Arr (GRCh37) 9p24.3p23 (211086_10218576)x3 (Overlaps with 34 OMIM genes)	Loss of 6.57 Mb Gain of 10 Mb	Pathogenic. Compatible with partial monosomy of 1q43q44 and partial trisomy A9p24.3p23	46,XY, ?1q44	Increased risk of chromosomal abnormality in sonography Fetus: 20 w Mother: 29y Fetus was Expired
M, 16w	Arr (GRCh37) A15q13.3 (31972646_32438A943)x3 (Overlaps with one OMIM gene, CHRNA7)	Gain of 466 Kb	Uncertain significance	46,XY	Former offspring and one of parents (father) had this abnormality. Fetus had been aborted Former oligo Array CGH study of offspring. Fetus: 16 weeks Mother: 33y

Table 3: Abnormal Array-CGH findings in postnatal group

Gender and age	Array Results	Size of Gain/Loss	Pathogenicity based on ACMG	Karyotype Results	Reasons for Referral & possible Follow ups
M, 33y	Arr(GRCh37) Yq12 (59031421_59335913)x2 (Overlaps with 3 OMIM genes)	Gain of 304.5 Kb on Yq12q12	Uncertain significance	46,XY,add(15) (p13)	Recurrent abortions, 32y
M, 8y	Arr(GRCh37) 22q11.21q11.21 (18894835_21505417)x1 (Overlaps with 44 OMIM genes)	Loss of 2.6 Mb	Pathogenic DiGeorge syndrome	Not Done	Heart defect, 8 y. MLPA confirmed
M, 14y	Arr(GRCh37) 15q13.3(32260104_32426869)x3 (Overlaps with 1 OMIM genes, *CHRNA7*) Arr(GRCh37) 16q24.3(89807374_89864425)x1 (Overlaps with 1 OMIM genes, *FANCA*) Arr(GRCh37) Yp11.2(6839085_7430343)x2 (Overlaps with 3 OMIM genes, *PRKY, TBL1Y, DFNY2*)	Gain of 167 Kb Loss of 57 K. Gain of 591 Kb	Uncertain significance	"	Intellectual Disability
F, 1d	Arr(GRCh37) 22q11.21q11.21 (18894835_21440514)x1 (contains 43 OMIM genes)	Loss of 2.5 Mb	Pathogenic DiGeorge syndrome	"	Dysmorphic features MLPA confirmed. Fetus with left kidney agenesis, polydactyly, cleft palate, ASD
F, 2d	Arr(GRCh37) 14q13.3q21.1 (37553056_42776040)x1 (Overlaps with 6 OMIM genes)	Loss of 5.22 Mb	Pathogenic Compatible with monosomy of 14q13.3q21.1	"	Ambiguous genitalia, ichthyosis and ear deformity
F, 33y	Arr(GRCh37) 1q43 (240716738_243218573)x3 (contains 9 OMIM genes) Arr(GRCh37) 15q11.2 (22765628_23217514)x3 (Overlaps with 4 OMIM genes)	Gain of 2.5 Mb on 1q43q43. (Maternal origin.) Gain of 452 Kb on 15q11.2q11.2	Uncertain significance Microduplication on 1q43q43	"	Oligo-Array-CGH mother: Gain on 1q43q43
M, 34y	Arr(GRCh37) 1q21.1q21.2 (146507518_147824207)x3 (contains 18 OMIM genes)	Gain of 1.32 Mb (Parental origin.)	Pathogenic 1q21.1 Duplication syndrome (≠612475)	"	First cousin marriage
F,32y	Arr(GRCh37) 1q21.1q21.2 (146507518_147824207)x3 (Overlaps with 18 OMIM genes)	Gain of 1.32 Mb on 1q21.1q21.2. (Parental origin.)	Pathogenic 1q21.1 Duplication syndrome (≠612475)	"	Mother of a fetus with imbalance on 1q21.1q21.2 and multiple congenital anomalies detected in sonography
M, 6y	Arr(GRCh37) 9p24.2p24.3 (2049845_3497979)x3 (Overlaps with 5 OMIM genes)	Gain of 1.4 Mb	Likely pathogenic Compatible with microduplication of 9p24.2p24..	"	Intellectual Disability
F, 43	Arr(GRCh37) Xp22.31p22.31 (6628264_7491648)x3 (Overlaps with 2 OMIM genes)	Gain of 863.4 kb	Uncertain significance	"	Intellectual Disability

Table 4: Abnormal Array-CGH findings in products of conception group

Gender and age	Array Results	Size of Gain/Loss	Pathogenicity based on ACMG	Karyotype Results	Reasons for Referral & possible Follow ups
M, 21w	Arr (GRCh37) 21q11.2q22.3(15485037_48090288)x3	Gain of whole chromosome 21	Pathogenic Trisomy 21	47,XY, +21	Products of Conception
Trisomy 21	47,XY, +21	Products of Conception	Pathogenic Trisomy 21	47,XY, +21	"
M, 16w	Arr(GRCh37) 21q11.2q22.3(15485008_48090317)x3	Gain of whole chromosome 21	Pathogenic Trisomy 9	47,XY, +9	"
Trisomy 21	47,XY, +21	"	Pathogenic	46,XX,der(20)t(13;20)(q13;p13)pat	"