Chromosomal Microarray Evaluation of Fetal Ventriculomegaly

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Abstract **Background:** Fetal ventriculomegaly is one of the more common fetal anomalies detected during prenatal screening.
Objectives: To assess the rate of genetic aberrations as the cause for ventriculomegaly in these fetuses.
Methods: A historic cohort study was conducted on 164 fetuses with sonographic diagnosis of ventriculomegaly. All cases were analyzed for karyotype and 41 cases were further analyzed by chromosomal microarray (CMA). The study group was subdivided by laterality, severity, and whether the ventriculomegaly was an isolated finding or not. Subgroups were compared and the study group was compared to a control group of 209 fetuses.
Results: Karyotype aberrations were more common among fetuses with ventriculomegaly (6.6%) compared to controls (0%, $P < 0.001$). CMA aberrations were more common in the non-isolated ventriculomegaly cases (24.1%) compared to controls (6.2%, $P = 0.031$). The rate of genetic aberrations was not associated with the degree of dilatation or laterality.
Conclusions: It is equivocal whether CMA testing should be conducted on every amniotic fluid sample taken from fetuses with isolated ventriculomegaly. However, if more anomalies are detected during an anatomical survey, CMA analysis should be conducted to decrease oversights of genetic diagnoses.

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Ventricular atrial diameter is measured by neurosonographic examination as a part of routine prenatal care to assess the development of the fetal central nervous system [1]. The mean atrial diameter is 6.4 mm (± 1.2 mm), and it remains constant between 15 and 40 weeks of gestation [2]. Ventriculomegaly is defined as an atrial diameter of 10 mm or more. It is one of the more common fetal anomalies detected during the second trimester anatomical survey [3] and is estimated to occur in 0.5–2 of 1000 live births [4]. Some physicians consider ventriculomegaly as mild if the atrial diameter is between 10 and 12 mm, moderate if the diameter is 12 to 15 mm, and severe if it is ≥ 15 mm [1,5,6].

Although ventriculomegaly could be a physiological variant, it can also be caused by a variety of disorders that result in neurological, motor, and/or cognitive impairment [7]. Thus, a finding of ventriculomegaly requires a thorough investigation. Isolated ventriculomegaly is more common in mild cases (60–94%), while up to 75% of moderate ventriculomegaly cases are associated with other abnormal findings [5,7].

Previous studies have estimated the rate of chromosomal abnormalities as 1.5–13% in isolated dilatations and 9.5–36% in non-isolated dilatations [4,5,8]. The incidence is lower for isolated severe ventriculomegaly [5,8]. Mild ventriculomegaly is present in 0.15% of euploid fetuses and in 1.4% of trisomy 21 fetuses, providing a likelihood ratio of nine for the risk of aneuploidy [5,9]. The differences between the many studies were explained by the prevalence of Down syndrome in the populations [5].

Copy number variations (CNVs) are defined as microdeletions or microduplications of segments of the genome, varying in size from one kilobase (kb) to several megabases (Mb) [10]. CNVs have been recognized as a pathogenic variation in patients [11,12] as well as a normal genetic variation in phenotypically normal individuals [13]. According to the American College of Medical Genetics and Genomics standards and guideline, CNVs were generally divided into five categories: pathogenic CNVs, likely pathogenic CNVs, variants of uncertain significance (VOUS), likely benign CNVs, and benign CNVs [14].

Conventional chromosomal analysis such as G-banding karyotyping, which is able to detect aneuploidies and chromosomal abnormalities larger than 5–10 Mb, has been the gold standard for identifying chromosomal abnormalities in clinical practice over the past few decades. Submicroscopic chromosomal abnormalities, such as CNVs, which are undetectable by karyotyping, can be successfully identified by chromosomal microarray analysis (CMA) [15].

Recently, because of its ability to simultaneously detect aneuploidies and CNVs, CMA has been applied as the first-tier test to detect chromosomal abnormalities in postnatal and prenatal study participants as well as miscarriage tissues in clinical practice [16-18]. The American College of Obstetricians and Gynecologists Committee (ACOG) advocated that CMA

should be recommended for pregnant women with fetal structural abnormalities, and this technique may replace karyotyping in prenatal diagnosis in the future [19]. However, in pregnant women with a structurally normal fetus undergoing invasive prenatal diagnosis, either fetal karyotyping or CMA can be performed [19].

A limited number of studies were performed to reveal the association between CNVs and fetal ventriculomegaly [11,12,20-22]. The Society of Obstetricians and Gynecologists of Canada (SOGC) recommends that amniocentesis be offered for karyotype and congenital infection assessment in fetuses with mild-to-moderate ventriculomegaly, regardless of whether combined with other ultrasound anomalies [23].

The aim of this study was to assess the rate of genetic aberrations as the cause for fetal ventriculomegaly and to determine the added value of CMA analysis over conventional karyotyping, to validate the mentioned guidelines by increasing the quality of evidence, and to lead to better decision making in the early weeks of gestation.

PATIENTS AND METHODS

A historic cohort study of 151 women pregnant with 164 fetuses with sonographic diagnosis of ventriculomegaly was performed at a tertiary medical center during a 15-year period, between 1999 and 2014. Data were obtained from electronic records and included: maternal medical and obstetrical history, current pregnancy, prenatal follow-up, genetic test results, and anatomical survey sonographic reports. All cases underwent amniocentesis and were analyzed for karyotype, and 47 cases in the study group plus 65 in the control group were further analyzed by chromosomal microarray (CMA).

CMA has the ability to simultaneously detect aneuploidies and CNVs. Until August 2014, the practice at Sheba Medical Center was to check amniotic fluid from fetuses with ventriculomegaly for karyotype only. When the results were negative for aberrations, a CMA analysis was also applied. After August 2014, our laboratory practice changed, and both examinations were applied as first tier on fetuses with any anatomical defect. When post-Au-

Table 1. Karyotype and chromosomal microarray test results

Patient number	Ventricular dilatation	Isolated	Karyotype	Chromosomal abnormality	De novo mutation
Karyotype test results					
1	moderate bilateral	no	–	47xx+21	yes
2	mild bilateral	yes	–	46xy,t(1;5) (q25;q11.2) inv (1)(p22;q25)	yes
3	mild unilateral	no	–	8-11% mosaicism of chromosome 12 trisomy	unknown
4	moderate bilateral	no	–	47XY+21	yes
5	moderate bilateral	no	–	47XX+X	yes
6	mild unilateral	no	–	46XX inv 12(p13.3,q13.1)	unknown
7	mild bilateral	no	–	47XX +13	yes
8	mild bilateral	no	–	robertsonian translocation: 46XY, der(5) t(3;5) (p24;q15)	no- inherited from the mother
9	mild unilateral	no	–	47XX+21	yes
10	moderate bilateral	no	–	Triploid	yes
Chromosomal microarray test results					
1	mild unilateral	yes	normal	Small deletion in chromosome q2;34. Deletion includes ERBB4 gene	no (inherited from the father)
2	moderate unilateral	no	normal	CNTN6 duplication	no (inherited from the mother)
3	mild unilateral	no	abnormal	46XX inv 12(p13.3,q13.1)	unknown
4	mild bilateral	no	normal	12.5MB deletion on chromosome 5 which is found on a region of 27 OMIM genes and some defects like Mitochondrial complex I deficiency	yes
5	moderate bilateral	no	normal	15q13.3 duplication (neuropsychiatric phenotype)	no
6	mild bilateral	no	normal	17q12 duplication	no (inherited from the father)
7	moderate unilateral	no	normal	15q13.3 duplication including CHRNA7 gene (associated with attention deficit disorder (ADD))	no (inherited from the father who suffers from ADD)

gust-2014 cases resulted in a dual association issue (i.e., patient 9 in Table 1: karyotype test results) we removed it as a CMA result, and kept it as a karyotype result only. In one pre-August-2014 case, the karyotype analysis was pathologic, but the CMA analysis was also applied to investigate for its clinical significance. Hence it was listed twice on Table 1 (patient 6: karyotype test results, patient 3: chromosomal microarray test results). This practice allowed us to appreciate CMA aberration results for their added value over conventional karyotyping alone.

Isolated cases were defined as ventriculomegaly alone on sonography, without any evidence of other anatomical abnormalities.

The study group was subdivided by laterality (bilateral and unilateral) and by severity. Subgroups were compared to each other and the study group was compared to a control group.

Fetuses that had positive polymerase chain reaction results for cytomegalovirus in the amniotic fluid were excluded from the study.

The control group consisted of 209 fetuses that had amniocentesis performed on them followed by karyotype analysis, CMA, or both. The reason for invasive testing and genetic analysis was due to patient request alone. None had any evidence of malformation on fetal morphological survey. Fetuses in the control group that had a known sonographic finding were excluded.

Abnormal genetic test results from both groups were classified by their clinical significance, according to recent genetics literature. Only clinically significant findings were classified as abnormal.

Statistical analyses were performed using IBM Statistical Package for the Social Sciences statistics software, version 23 (SPSS, IBM Corp, Armonk, NY, USA). Q-Q plots were used to assess for normality. Continuous variables were described as mean ± standard deviation or median (interquartile range [IQR]) as appropriate. Continuous variables were compared using unpaired *t* test and one way ANOVA test or Mann-Whitney U test, and Kruskal-Wallis test for non-parametric continuous variables. Chi-square test was used for comparison of categorical variables. Significance was accepted at $P < 0.05$.

The study was approved by the local institutional review board.

RESULTS

During the study period 151 women matched our criteria, and their characteristics are shown in Table 2. The mean maternal age of the study group was 33.25 ± 5.01 years, compared to 31.86 ± 2.2 in the control ($P = 0.001$). Twins were more common in the study group: 8.6% vs. 3.3% in the control group ($P = 0.032$), without a higher rate of chromosomal abnormalities. Of the study group, 68.5% of fetuses were male ($P < 0.001$), but the rate of chromosomal abnormalities did not differ between the genders [Table 3].

Ten fetuses in total had an abnormal karyotype, of which three were trisomy 21. Eight fetuses from the study group had abnormal CMA test results [Table 1].

Table 2. Study group characteristics

Maternal characteristic	
Mean maternal age ± SD (years)	33.25 ± 5.01
Median number of pregnancies, IQR	2.52; 2 (1–3)
Median number of live births, IQR	1.04; 1 (0–2)
Any previous medical history	34 (23.1%)
Maternal attention deficit disorder	3 (2%)
Maternal diabetes	2 (1.3%)
Maternal hypothyroidism	6 (4%)
Maternal clexane treatment	6 (4%)
Mode of conception	
Spontaneous	119 (78.8%)
In vitro fertilization	17 (11.3%)
Sperm donor	2 (1.3)
Egg donor	7 (4.6%)
Intra-uterine insemination	6 (4%)
Pregnancy characteristic	
Median pregnancy age on first diagnosis of dilated ventricles, IQR (weeks)	24.6; 24 (22–28)
Male fetus	102 (68.5%)
Female fetus	47 (31.5%)
Lateral ventricles characteristic	
Median maximal ventricular width on US or MRI, IQR (mm)	11.9; 11 (10.5–12.8)
Median minimal ventricular width on US or MRI, IQR (mm)	9.4; 9 (7.7–11)
Mean ratio between max &min ventricular width on US or MRI, IQR	0.8; 0.81 (0.67–0.96)
Progressive VM	16 (11%)
Isolated cases	62 (41.1%)
Other brain findings	59 (39.1%)
Any extra cranial findings	57 (38.3%)
Type of ventricular dilatation	
Bilateral VM	67 (44.4%)
Unilateral VM	84 (55.6%)
Severity of dilatation	
Mild	85 (56%)
Moderate	60 (40%)
Severe	6 (4%)

Data are presented as mean (standard deviation) or as median (interquartile range) for continuous variables or as number (percent) for categorical variables
IQR = interquartile range, MRI = magnetic resonance imaging, US = ultrasound, VM = ventriculomegaly

Table 3. Comparison between study group and controls

Characteristic	Total n=360	Study group n=151	Control n=209	*P* value*
Mean maternal age ± SD (years)	32.42 ± 3.7	33.25 ± 5.01	31.86 ± 2.19	**0.014**
Twins	20 (5.6%)	13 (8.6%)	7 (3.3%)	**0.032**
Male fetus	194 (54.3%)	102 (68.5%)	92 (44.2%)	**< 0.001**
Female fetus	163 (45.7%)	47 (31.5%)	116 (55.8%)	
Abnormal karyotype	10 (2.8%)	10 (6.6%)	0	**< 0.001**
Abnormal CMA	**n=106**	**n=41**	**n=65**	
	12 (11.3%)	8 (19.5%)	4 (6.2%)	0.056
Abnormal CMA, non-isolated	11 (11.7)	7/29 (24.1%)	4/65 (6.2%)	**0.031**

Data are presented as number (percent)
*Bold indicates significance

Table 4. Chromosomal abnormality rate analyzed by different subgroups

Subgroup	Group size (n=)	Chromosomal abnormality	*P* value	Group size (n=)	CMA abnormality	*P* value
Bilateral VM	67	7 (10.4%)	0.109	17	3 (17.6%)	> 0.99
Unilateral VM	84	3 (3.6%)		24	5 (21%)	
Total	151	10 (6.6%)		41	8 (19.5%)	
Mild	85	6 (7.1%)	> 0.99	22	5 (23.8%)	0.85
Moderate	60	4 (6.7%)		17	3 (17.6%)	
Severe	6	0		3	0	
Total	151	10 (6.6%)		42	8 (19%)	

Data are presented as number (percent)
CMA = chromosomal microarray, VM = ventriculomegaly

When we compared the study group to 209 cases of the control group, we found that the study population had a higher prevalence of genetic aberrations compared to the controls: 10 (6.6%) cases with abnormal karyotype in the study group compared to zero in the control group ($P < 0.001$) and eight (19.5%) cases with abnormal CMA compared to four (6.2%) in the control group ($P = 0.056$) [Table 3].

Karyotype abnormalities were significantly more common in the non-isolated cases. Chromosomal abnormality rate was 1.6% for isolated ventriculomegaly and 10.1% for non-isolated ventriculomegaly ($P = 0.048$). There was only one case of isolated ventriculomegaly associated with a karyotype abnormality; its characteristics are shown in Table 2 (karyotype test results: patient 2). CMA abnormalities were also more common in the non-isolated cases, 1/12 (8.3%) for isolated cases, compared to 7/29 (24.1%) for non-isolated cases, but the difference was not significant ($P = 0.398$).

On CMA analysis, when comparing aberrations only for the non-isolated ventriculomegaly portion of the study group to the control group, the former was found to have significantly more abnormalities: 7/29 (24.1%) and 4/65 (6.2%), respectively [$P = 0.031$).

The study group was subdivided into three subgroups according to their laterality: 67 (44.4%) were diagnosed with bilateral ventriculomegaly and 84 (55.6%) with unilateral ventriculomegaly. Cases were further subdivided according to the severity of their dilatation: 85 (56%) had mild ventriculomegaly, 60 (40%) had moderate ventriculomegaly, and 6 (4%) had severe ventriculomegaly [Table 2].

Subgroup analysis did not show any significant difference in the frequency of genetic aberrations (neither karyotype nor CMA). There was a higher rate of chromosomal abnormalities in the bilateral ventriculomegaly group (10.4%) compared to the unilateral ventriculomegaly group (3.6%), although these differences were not statistically significant. There was no significant difference in the frequency of genetic aberrations between the mild, moderate, and severe ventriculomegaly groups ($P > 0.99$) [Table 4].

DISCUSSION

In our study, we investigated the association between ventriculomegaly, as witnessed by neurosonographic examination and genetic aberrations diagnosed by amniotic fluid analysis, to better understand this relation and improve decision making in the prenatal care timeline.

We found statistically significant higher rate of karyotype abnormalities in the study group (6.6%) compared to the control group (0%) ($P < 0.001$) and a high rate of CMA abnormalities, eight (19.5%) compared to four (6.2%) in the control with borderline significance ($P = 0.056$).

In addition, we found that the rate of genetic aberrations was higher in fetuses who presented with additional anomalies on the anatomical survey sonographic scan compared to those who presented with ventriculomegaly alone. Chromosomal abnormality rate was 1.6% for isolated ventriculomegaly and 10.1% for non-isolated ventriculomegaly ($P = 0.048$. CMA analysis showed a similar trend of 1/12 (8.3%) for isolated cases, compared to 7/29 (24.1%) for non-isolated cases ($P = 0.398$).

When comparing only non-isolated ventriculomegaly to the control group, the former was found to have significantly more abnormalities on CMA analysis ($P = 0.031$).

In the severity aspect, we found that the frequency of genetic aberrations was not influenced by the severity of dilatation. This finding is in contrast to previous studies where chromosomal abnormalities were more common in mild, rather than in severe ventriculomegaly [2,6].

In the laterality aspect, we found a higher rate of chromosomal abnormality in the bilateral ventriculomegaly group compared to the unilateral ventriculomegaly, although these differences were not statistically significant.

Our study adds evidence to increasing reports on the use of the CMA technique in genetic etiological diagnosis of fetal ventriculomegaly. Peng et al. [23] found that the chromosomal abnormalities detection rate using karyotype analysis was significantly lower than when using the CMA technique (12.84% and 26.6%, respectively). The incidences of pathogenic copy number variations (CNVs) in fetuses with non-isolated ventriculomegaly (6.6–37.9%) were higher than in fetuses with isolated ventriculomegaly (4–9.5%) [11,12,18,19]. Shaffer et al. [11] identified pathogenic CNVs, which were below the resolution of karyotype (< 10 Mb) in 4% of fetuses with isolated ventriculomegaly and in 6.6% of those with non-isolated ventriculomegaly. Zhang et al. [21] reported that the detection rate in fetuses with non-isolated ventriculomegaly (37.9%) was significantly higher than that in fetuses with isolated ventriculomegaly (9.5%). Donnelly and colleagues [12] identified CNVs in 8.7% of fetuses with isolated ventriculomegaly and in 17.2% of those with non-isolated ventriculomegaly. However, the detection rate in this study referred to the total detection rate consisting of CNVs and variants of unknown clinical significance. In contrast, Li et al. [20] reported that the detection rate in cases of isolated ventriculomegaly (6.1%) was similar to that in cases of non-isolated ventriculomegaly (7.4%) and no significant difference was observed between the two groups. According to the review by Wang et al. [24] pathogenic copy number variations are important genetic cause of fetal ventriculomegaly, which may be involved in the pathological process of fetal ventriculomegaly as well as postnatal neurodevelopmental disorders. In 2016, ACOG recommended that prenatal CMA should be offered to fetuses with structural anomalies [19]. In addition, CMA can be considered for all women who undergo prenatal diagnostic testing, regardless of age. Therefore, CMA is currently applied as a first-tier test for prenatal diagnosis in many laboratories [16].

In alignment with previous studies, we found that the genetic aberrations detection rate was highest for non-isolated ventriculomegaly and was not influenced by the degree of dilation. Our sample size is similar to theirs. Few points separates our results from previous studies. First we investigated only clinically significant genetic aberrations. Second, we had 209 cases in a control group that allowed us to perform an unbiased comparison to our local population while increasing the study's internal and external validities. Third, comprehensive nature of this work and the sample size allowed stratification by isolated versus non-isolated ventriculomegaly, degree of dilation, and laterality all under the same roof. Fourth, our local practice allowed us to appreciate CMA aberration results only for their added value over conventional karyotyping. To the best of our knowledge, no similar findings have been reported in the literature thus far.

LIMITATIONS

The empirical results we reported should be considered in the light of some limitations. First, although there is a clear trend, the relatively small sample size did not allow us to show a statistically significant difference in CMA results between the study and the control groups. Second, the mean maternal age of the study group was 33.25 ± 5.01 years, compared to 31.86 ± 2.2 in the control ($P = 0.001$). Third, The CMA aberration rate for our 209 cases control group was 6.2%, while Chau et al. [25] that showed that clinically significant microdeletions and microduplications occur in 1.6% of women undergoing invasive prenatal diagnosis regardless of maternal age (0.8% for low risk pregnancies). This result may represent some selection bias in our control group and may partly explain the merely borderline significance.

Based on our results, it is equivocal whether CMA testing should be done on every amniotic fluid sample taken from fetuses with isolated ventriculomegaly; however, if on anatomical survey, more anomalies are detected, CMA analysis should be done on the sample to widen the scope and decrease oversights of genetic diagnoses. Although in many parts of the world guidelines already exist, we believe that by appreciating CMA only

for its added value over conventional karyotyping, our findings validate them and may contribute to prenatal genetic counseling by streamlining decision making in an already stressful, uncertain times for the mother and the doctor.

CONCLUSIONS

Amniotic fluid analysis using chromosomal microarray has been applied as the first-tier test to detect prenatal chromosomal abnormalities in many parts of the world, although the level of evidence regarding this test use in ventriculomegaly is insufficient. We found a significantly higher rate of CMA aberrations in the non-isolated ventriculomegaly group compared to a control group. CMA should be applied on amniotic samples from non-isolated ventriculomegaly as it adds information over conventional karyotyping.

DATA AVAILABILITY STATEMENT

Data supporting the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restriction.

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Whoso neglects learning in his youth, loses the past and is dead for the future."

Euripides (ca. 480 BC–406 BCE), Greek playright

It takes courage to grow up and become who you really are.

e.e. cummings, (1894–1962), American poet, painter, essayist, author, and playwright.