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The Yield of Chromosomal Microarray in Pregnancies Complicated with Fetal Growth Restriction Can Be Predicted According to Clinical Parameters

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Keywords
Chromosomal microarray · Fetal growth restriction · Gestational age · Birth weight percentile · Intrauterine growth restriction

Abstract
Introduction: We evaluated the yield of chromosomal microarray analysis in pregnancies complicated with fetal growth restriction (FGR) according to specific clinical parameters. ***Methods:*** The study was based on national records from the Israeli Ministry of Health. Chromosomal microarray analyses of amniocenteses performed nationwide for the indication of FGR, from January 2016 to March 2018, were included. The CMA yield was compared to 2 cohorts that reported the background risk. ***Results:*** Of 174 tests performed for the indication of FGR, there were 11 cases with a pathogenic/likely pathogenic result (6.3%). The yield of CMA was significantly higher in cases with major structural findings (29.4 vs. 3.4%, $p = 0.001$), compared to isolated FGR but not for minor structural findings (6.1 vs. 3.4%, $p = 0.5$). The rate of chromosomal aberrations was significantly higher for all cases with FGR, when compared to the background risk of a cohort of normal pregnancies (odds ratio [OR] 4.7, 95% CI 2.5–9 and OR 6.09, 95% CI 3.2–11.4) but not for iso-

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lated cases or cases diagnosed after 24 weeks of pregnancy. ***Conclusions:*** Chromosomal microarray analysis should be performed for all pregnancies complicated with FGR diagnosed before 24 weeks and for cases with major structural anomalies.

Introduction

Fetal growth restriction (FGR) is a condition in which the fetus fails to achieve its full growth potential in utero. Fetuses with FGR are at risk for perinatal morbidity and mortality [1–3] in the prenatal and postnatal periods. The common definition is estimated fetal weight <10th percentile or abdominal circumference <5th percentile for gestational age [4, 5]. However, a stricter cutoff of 2 standard deviations (SDs) below the mean is usually applied for the purpose of genetic workup. FGR has several possible etiologies, including placental factors, infection, maternal chronic conditions, and genetic causes [4]. Genetic causes are found in up to 20% of FGR cases [5–7]. These include chromosomal abnormalities, submicroscopic chromosomal syndromes, monogenic syndromes, and conditions related to epigenetic mechanisms [5, 7].

The American College of Obstetrics and Gynecology recommends genetic testing for cases of FGR detected in the midtrimester and for cases in which a structural anomaly is detected [8]. Previous studies reported the yield of chromosomal microarray analysis (CMA) in the presence of FGR to be in the range of 4.8–18.8%, with a significantly higher yield whenever additional fetal structural abnormalities are observed [9–12]. The reported incremental yield of CMA over karyotype in FGR cases is 4.8–10% [10, 12, 13]. The yield for CMA in pregnancies with no sonographic abnormalities has been reported to be in the range of 0.84–1.7% [14–16]. Data regarding the yield of CMA in FGR pregnancies according to specific clinical characteristics such as gestational age at which FGR was diagnosed, its severity, and other clinical parameters are sparse. The aim of this study was to assess the yield of CMA in pregnancies complicated with FGR in various clinical scenarios.

Material and Methods

We searched the electronic database of the Israeli Ministry of Health (MOH) for CMA tests performed in FGR pregnancies, from January 2016 to March 2018. All cases included in the study were tested following formal genetic counseling. The formal criteria for testing according to the Medical Genetics Association are fetal growth sonographic estimation of 2 SDs below the mean (below the 3rd percentile); however, some cases that were in the low-normal range (but below the 10th percentile) were also tested. Testing was financed by the MOH after approval by a clinical geneticist.

Clinical data were obtained from the Israeli national database and from patients' medical records. Detailed clinical information including maternal age, maternal chronic illness, familial background of genetic conditions, obstetrical history of recurrent spontaneous abortions, elevated nuchal translucency, biochemical screening results, gestational age at diagnosis, lowest weight percentile recorded during the pregnancy, and the presence of additional sonographic findings were retrieved. Additional findings were grouped into the following: major structural anomalies defined as conditions that create significant medical problems for the patient or that require specific surgical or medical management; minor anomalies that are findings that are not a normal variation but do not cause increased morbidity and soft signs. Pathogenic (P)/likely pathogenic (LP) copy number of variants (CNVs) classification was based on the American College of Medical Genetics and Genomics (ACMG) and the Clinical Genome Resource (ClinGen) 2020 guidelines [17, 18].

CMA findings were reviewed by 2 authors (R.S.H. and I.M.) and grouped into 4 categories:

1. Normal (including benign and variants of unknown significance – likely benign categories),
2. P/LP variants,
3. Microdeletion/duplication with low penetrance, and
4. Variants of unknown clinical significance (VUS).

The categorization was based on laboratory reports, as well as new information gained from the medical literature and from the authors' experience. For VUS, only cases with deletions of ≥1 Mb and duplications of ≥2 Mb were included. These variants are reported by the lab, according to the guidelines determined by Israeli Society of Medical Geneticists.The yield of CMA included both microscopic and submicroscopic aberrations. Microarray results were also categorized into "karyotype detectable" (i.e., CNVs of at least 10 MB) or not "karyotype detectable" in order to assess the incremental yield of CMA over karyotype.

Two cohorts were used to assess the background risk. A large local cohort of 5,541 cases with normal prenatal ultrasounds in a large, hospital-based clinical laboratory [16]. This cohort included cases tested for advanced maternal age, abnormal maternal first- or second-trimester serum screening and cases with no indication for testing. The prevalence of CNVs for this cohort was 1.4% (78 cases). A second cohort of 10,614 cases was extrapolated from a meta-analysis by Srebniak et al. [15]. We calculated the background risk by adding the risk for submicroscopic chromosomal abnormalities to the risk for chromosomal abnormalities [19]. This risk was 1:384, based on the average maternal age of our cohort. The prevalence of abnormal CNVs for this cohort was 1.1%.

The study was approved by the Institutional Review Board for Human Subjects (September 6, 2016, registration number – MOH2016). CMA testing was performed by 12 laboratories as described previously [20]. Genomic coordinates were evaluated in accordance with genome build GRCh37/hg19 in all laboratories. All analyses performed in the different laboratories met the standards and guidelines of the American College of Genetics

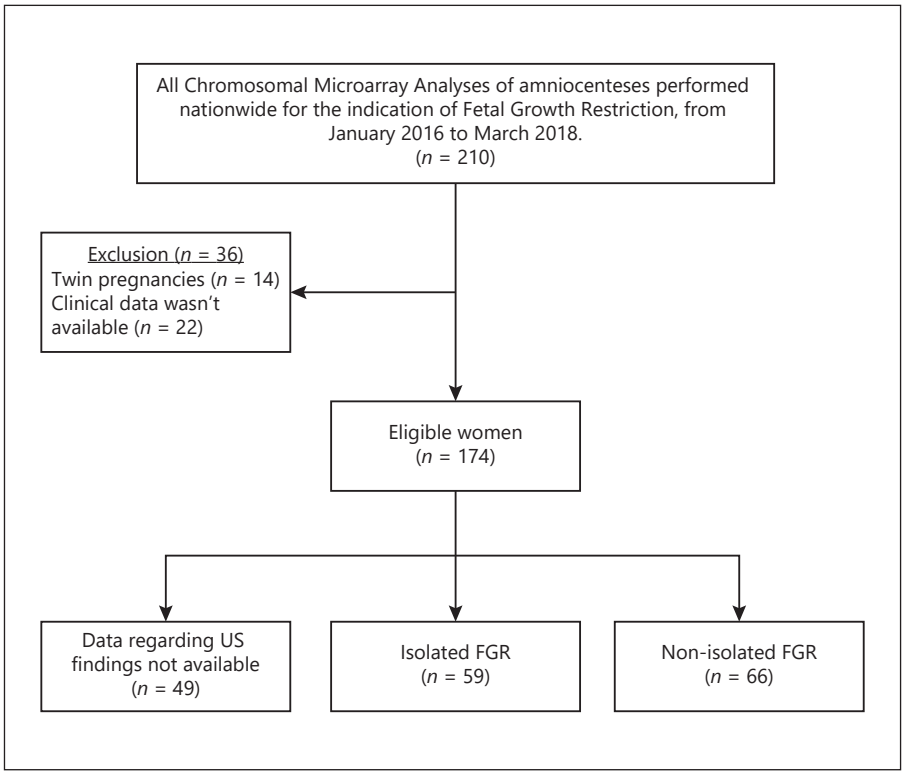

Fig. 1. Study sample formation. CMA, chromosomal microarray analysis; FGR, fetal growth restriction.

Table 1. Clinical characteristics

Characteristic	Mean ± SD/ *N* (%)
Maternal age	31.5±4.9
Gestational age at diagnosis	25.6±6.15
Gestational age at amniocentesis	28.2±6.7
Lowest weight percentile‡	3.9±2.5
EFW >5 percentile‡	19/92 (20.6)
EFW ≤5 percentile‡	73/92 (79.3)
EFW ≤3 percentile‡	54/92 (58.7)
EFW ≤1 percentile‡	10/92 (10.8)
Additional sonographic findings (non-isolated FGR)‡	66/125 (52.8)
Abnormal biochemical screen‡¶	15/93 (16.1)
Chronic maternal illness‡, §	13/127 (10.2)
Familial background of genetic conditions‡	28/120 (23)
IVF pregnancy‡	7/65 (10.77)
History of FGR‡	4/90 (4.44)
Clinical characteristics suggestive of placental insufficiency‡	29/112 (25.89)

SD, standard deviation; FGR, fetal growth restriction; IVF, in vitro fertilization. ‡ Among the cases with available data. ¶ Abnormal biochemical screen: first or second trimester screening test for Down syndrome. § Chronic maternal illness includes asthma, hypercouglation syndrome, thalassemia, epilepsy.

and Genomics for constitutional cytogenomic microarray analysis, including postnatal and prenatal applications [17, 18], adopted by the recommendations of the Israeli Medical Genetics Association [13].

Statistical Analysis

Fisher's Exact test or χ^2 were used to test the differences between CMA yield in relation to different parameters and compared to the background risk. $p < 0.05$ was considered statistically significant. Odds ratio (OR) is derived using the OR formula for a 2-dimensional table with a confidence interval of 95%. Python statistics library version 3.5.1 (scipy.stats) was used for statistical analysis.

Analysis was performed only for cases where data regarding the specific parameter analyzed were available. Cases with missing data regarding a specific parameter were not included in the analysis. In the analysis CMA results classified as abnormal (P/LP) (group 2) were compared to groups 1, 3, and 4 combined (normal CMA, low penetrance, and VUS).

Power Analysis

The number of cases needed to detect a 3-fold increase in the rate of abnormal CMA results from the prevalence of abnormal CMA results reported in the local background cohort of 5,541 cases (1.4%) was 203 and for a 4-fold increase the minimal sample size needed was 102 (power of 0.8). The number of cases needed to detect a 3-fold increase in the rate of abnormal CMA results from the prevalence of abnormal CMA results reported in the meta-analysis cohort of 10,614 cases (1.1%) was 260 and for a 4-fold increase the minimal sample size was 131 (power of 0.8).

Table 2. Summary of P/LP CMA and microdeletion/duplication with low penetrance findings

Case	Diagnosis week	Percentile	Additional USA findings	CMA result (ISCN*) array GRCh37/hg19	Karyotype detectable
1	22.6		PLSVC, retrognathia	**Wolf-Hirschhorn** arr 4p16.3p15.33 (68,345–12,620,851) × 1	Yes
2	24	2.5	CPC prominent forehead, clenched hands	**Trisomy 18**	Yes
3	24.6		Right club hand, pulmonary stenosis, narrowing of aortic arch and widening of the ascending aorta. Small stomach and kidneys	arr chr4 p16.3del -p15.32 (1–17,588,863) × 1 arr chr7q36.3 (156,646,277–159,321,559) × 3 Unbalanced translocation	Yes
4	26	2.5	SUA, inlet VSD, thickening of the pulmonary valve, hand deformities	**Trisomy 18**	Yes
5	17	1	No	**Trisomy 21**	Yes
6	20		Echogenic bowel, CCP, SUA, dolichocephaly	**Wolf-Hirschhorn** arr 4p16.3p16.1 (68,345–9,569,582) × 1	No
7	17.7		Unilateral pyeloectasis	Arr 15q11.2 (22,770,421–23,625,785) × 1 arr 12p1 (23,995,360–24,414,292) × 1	No
8				arr 16p11.2 (28,824,490–29,051,191) × 1mat	No
9	22.7		VSD hypoplastic RV, clenched hands	**Trisomy 18**	Yes
10	22		No	arr 17q12 (34440088–36307773) × 3	No
11	14		CPC, SUA Intracardial echogenic focus	arr (1–22) × 3), (X) × 2, (Y) × 1 Triploid male	Yes
Microdeletion/duplication with low penetrance					
1	32.3	7	No	arr 15q11.2 (22,299,434–23,288,275) × 1	No
2	34.6	3	Oligohydramnios	arr 15q11.2 (22,299,434–23,288,275) × 1	No

P/LP, pathogenic/likely pathogenic; CMA, chromosomal microarray analysis; CPC, chorid plexus cyst; SUA, single umblical artery; VSD, ventricular septal defect; PLSVC, persistent left superior vena cava.

Results

Clinical Characteristics of the Study Cohort

A total of 210 CMA were performed as part of FGR workup during the study period. We excluded 14 cases of twin pregnancies (Fig. 1) and 22 cases for which we had no clinical data. Clinical data were available for 174 singleton pregnancies (Table 1).

The mean maternal age in the cohort was 31.5 ± 4.9 years. Mean gestational age at diagnosis was 25.6 ± 6.15 weeks. The mean lowest estimated fetal growth percentile was 3.9 ± 2.5. Our cohort included 59 cases of isolated FGR and 66 cases with additional findings. Data regarding specific ultrasound findings in the additional 49 cases were not available.

The Yield of CMA According to Different Clinical Characteristics

Out of the 174 cases of singleton pregnancies for which clinical data were retrieved, 159 had no abnormal CMA findings (91.4%), 7 had chromosomal anomalies detectable by karyotype (4.0%), and 4 had a submicroscopic P/LP CNV (2.3%). In 2 cases (1.2%) a microdeletion/duplication with low penetrance was detected, and in 2 cases (1.2%) variants of unknown clinical significance were detected (Table 2). The total yield of CMA testing was (6.3%) (11 CNVs of 174 tests).

There were 3 cases of trisomy 18, 1 case of trisomy 21, 1 case of triploidy, 3 cases of Wolf-Hirschhorn syndrome (2 cases with deletions and 1 case of an unbalanced translocation [deletion of chr4 p16.3–p15.32 and duplication of chr7q36.3]) and 3 cases of submicroscopic chromosomal anomalies. In the P/LP group, 7 cases were detectable by karyotype and the incremental yield of CMA over karyotype was 2.3%.

Table 3. The yield of CMA according to clinical parameters

Parameter	CMA			*p* value
	normal, *N* (%)	abnormal, *N* (%)	total	
Isolated FGR	57 (96.6)	2 (3.4)	59	0.07
Non-isolated FGR	58 (87.9)	8 (12.1)	66	
Normal fetal echo	100 (95.2)	5 (4.8)	105	**<0.005**
Abnormal fetal echo	1 (25)	3 (75)	4	
Diagnosed >24 GW	59 (96.7)	2 (3.3)	61	**0.047**
Diagnosed ≤24 GW	53 (86.9)	8 (13.1)	61	
Diagnosed >26 GW	51 (100)	0	51	**0.005**
Diagnosed ≤26 GW	61 (85.9)	10 (14.1)	71	
Lowest percentile ≤5%	69 (94.5)	4 (5.5)	73	0.3
Lowest percentile >5%	19 (100.0)	0	19	
Lowest percentile ≤3%	51 (94.4)	3 (5.6)	54	0.5
Lowest percentile >3%	37 (97.4)	1 (2.6)	38	
Lowest percentile ≤1%	9 (90.0)	1 (10.0)	10	0.35
Lowest percentile >1%	79 (96.3)	3 (3.7)	82	
Signs of placental insufficiency	27 (100.0)	0 (0.0)	27	0.26
No signs of placental insufficiency	63 (95.5)	3 (4.5)	66	
Maternal age ≥40 years	6 (85.7)	1 (14.3)	7	0.29
Maternal age <40 years	150 (94.9)	8 (5.1)	158	
Abnormal screening test[±]	14 (93.3)	1 (6.7%)	15	0.8
Normal screening test[±]	74 (94.9)	4 (5.1)	78	
Previous FGR	4 (100.0)	0 (0.0)	4	0.58
No previous FGR	80 (93.0)	6 (7.0)	86	
Hx of recurrent abortions	6 (85.7)	1 (14.3)	7	0.5
No Hx of recurrent abortions	84 (92.3)	7 (7.7)	91	
IVF pregnancy	7 (100.0)	0 (0.0)	7	0.4
No IVF	53 (91.4)	5 (8.6)	58	

Data presented here include only cases where data regarding the specific parameter analyzed were available. CMA, chromosomal microarray analysis; FGR, fetal growth restriction; GW, gestational week; IVF, in vitro fertilization. [±] First or second trimester screening test for Down syndrome.

Isolated FGR

Among cases with available ultrasound clinical information and isolated FGR, 2 (3.4%) had P/LP CNV versus 8 (12.1%) in the non-isolated FGR group ($p = 0.07$, Table 3). Findings in the isolated FGR group included Down syndrome and 17q12 duplication ([34440088–36307773] × 3).

Non-Isolated FGR

Among the 66 cases with available ultrasound clinical information and non-isolated FGR, 49 had minor structural findings (such as soft signs and pericardial effusion) or other findings related to parameters out of the normal range (such as oligohydramnios/polyhydramnios, head circumference of −2 SDs). The other 17 cases had major structural anomalies, including hypospadias, persistent left superior vena cava, micrognathia/retrognathia, ventricular septum defect, cardiomegaly, aortic stenosis, club foot, and ventriculomegaly. The yield of CMA was significantly higher in cases with major structural findings, when compared to isolated FGR (29.4 vs. 3.4%, $p = 0.001$),

Table 4. P/LP CNV rates according to additional sonographic findings

Parameter	Cases, *n*	P/LP CNV	*p* value*
Isolated FGR	59	3.4%	
Non-isolated FGR	66	12.1%	0.07
FGR with minor structural findings	49	6.1%	0.5
FGR with major structural findings	17	29.4%	**0.001**

P/LP, Pathogenic/likely pathogenic; CNV, copy number of variant; FGR, fetal growth restriction. * Compared to isolated FGR.

Table 5. The yield of CMA for FGR cases compared to background risk

Parameter	Cases, *n*	Clinically significant CMA results,* *n* (%)	Compared to a background risk of 1.4% in a population of 5,541 cases with normal ultrasound [16] OR (95% CI) *p* value	Compared to a background risk of 1.1% in a population of 10,614 cases with normal ultrasound‡‡ [15] OR (95% CI) *p* value
All FGR cases	174	11 (6.32)	**4.7 (95% CI 2.5–9) p < 0.0001**	**6.05 (95% CI 3.2–11.4) p < 0.0001**
Isolated FGR	59	2 (3.39)	2.4 (95% CI 0.6–10.2) *p* = 0.2	3.14 (95% CI 0.76–13.05) *p* = 0.9
Non-isolated FGR	66	8 (12.12)	**9.6 (95% CI 4.4–20.9) p < 0.0001**	**12.4 (95% CI 5.8–26.5) p < 0.0001**
Diagnosis >24 weeks gestation	61	2 (3.28)	2.4 (95% CI 0.6–9.89) *p* = 0.22	3.04 (95% CI 0.73–12.6) *p* = 0.11
Diagnosis >22 weeks gestation	85	5 (5.88)	**4.38 (95% CI 1.72–11.1) p = 0.0007**	**5.6 (95% CI 2.2–14.1) p < 0.0001**
Lowest percentile >1%	82	3 (3.66)	2.66 (95% CI 0.82–8.6) *p* = 0.089	3**.4 (95% CI 1.06–10.9) p = 0.028**
Lowest percentile >3%	38	1 (2.63)	1.89 (95% CI 0.25–13.9) *p* = 0.52	2.42 (95% CI 0.3–17/8) *p* = 0.37

FGR, fetal growth restriction; CMA, chromosomal microarray analysis; OR, odds ratio. * Pathoigenic and likely pathogenic. ‡‡ The background risk was calculated as the risk for submicroscopic chromosomal abnormalities plus the risk for chromosomal abnormalities [17] (1:384) based on the average maternal age of the cohort.

but not significantly higher for cases with minor structural findings (6.1 vs. 3.4%, $p = 0.5$; Table 4).

The yield of CMA was significantly higher for cases with abnormal echocardiogram, (75 vs. 4.8%, $p < 0.005$; Table 3). The number of additional sonographic findings was correlated with the rate of abnormal CMA results: of 66 cases with additional abnormal sonographic findings, there were 13 cases with >2 additional findings of which 5 had abnormal CMA results and 53 cases with <2 additional findings of which there were 3 cases with abnormal CMA results ($p = 0.001$).

Estimated Weight Percentile

Data regarding the lowest estimated weight percentile were available for 92 cases. P/LP CMA was found in 4, all had an estimated fetal weight lower than the 5th percentile. No correlations between the estimated weight percentiles and CMA yield were found in our cohort (3.7 vs. 10%, $p = 0.35$; 5.6 vs. 2.6%, $p = 0.5$; and 5.5% vs. 0, $p = 0.3$ for 1st percentile, 3rd percentile, and the 5th percentile respectively; Table 3).

Gestational Age at Diagnosis

Early detection of FGR was correlated to a significantly higher yield of pathogenic/likely pathogenic results. The yield of CMA for cases diagnosed at ≤24 weeks of gestation compared to cases diagnosed after 24 weeks was significantly higher (13.1 vs. 3.3%, $p = 0.047$). For FGR cases diagnosed after 26 weeks there were no P/LP results (Table 3). Other parameters such as maternal age, maternal chronic illness, familial background of genetic conditions, obstetrical history of recurrent abortions, elevated nuchal translucency, elevated Down syndrome risk according to biochemical screening, and prenatal workup were not significantly associated with an increased likelihood of pathogenic CNVs (Table 3).

The Yield of CMA Compared to the Background Risk (Table 5)

We assessed the yield of CMA in different clinical scenarios of FGR pregnancies compared to 2 cohorts that represented the background risk: a cohort of 5,541 uncomplicated pregnancies for which the yield of CMA was 1.4% [16] and a cohort of 10, 614 cases extrapolated from a meta-analysis by Srebniak et al. [15] for which the yield of CMA was 1.1%.

The rate of chromosomal abnormalities was significantly higher for all FGR cases as compared to the background population for both cohorts; OR for the cohort of 5,541 cases was 4.7 (95% CI 2.5–9), and OR for the cohort of 10,614 cases was 6.05 (95% CI 3.2–11.4) ($p < 0.0001$). While the yield for non-isolated cases (8/66, 12.12%) was

significantly higher than the background risk, ORs for the 2 cohorts were 9.6 (95% CI 4.4–20.9) and 12.4 (95% CI 5.8–26.5) respectively ($p < 0.0001$); for isolated FGR cases (3.4%) it was not significantly higher ($p = 0.9$). The yield was higher than the background risk for cases diagnosed with FGR after 22 weeks of gestation compared to both cohorts. OR for the 2 cohorts were 4.38 (95% CI 1.72–11.1, $p = 0.0007$) and 5.6 (95% CI 2.2–14.1, $p < 0.0001$), respectively, but not for cases diagnosed after 24 weeks ($p = 0.11$). For cases in which the lowest estimated birthweight percentile was below 1%, the yield was higher than the background risk.

Discussion

In our cohort of 174 FGR cases of singleton pregnancies the yield of both microscopic and submicroscopic aberrations detected by CMA was 6.3%. The rate of P/LP results for cases diagnosed at ≤24 weeks of gestation was significantly higher compared to cases diagnosed after 24 weeks. The yield of CMA in non-isolated FGR cases and in cases diagnosed before 24 weeks was significantly higher as compared to the background risk reported in uncomplicated pregnancies.

Several studies reported the yield of CMA for FGR cases to be in the range of 4.8–18.8% [9–12, 21]. The cohorts in these studies involved various inclusion parameters and clinical characteristics.

The rate of pathogenic CMA in non-isolated FGR cases was found to be higher than in the isolated FGR cases, in most cohorts. The difference between the yield of CMA in the non-isolated (12.7%) compared to the isolated groups (3.4%) in our study is that it approached statistical significance ($p = 0.07$) and would have probably been significant with a larger cohort. The reported rates of 12% (3/27) [9] and 10.2% (5/49) [10] for non-isolated cases in previous studies were similar to our detection rate of 12.7% (8/63).

In order to better explore the yield of CMA in the presence of structural findings, we subgrouped these cases into minor and major structural anomalies. The yield of CMA was significantly higher in the major structural findings group, when compared to isolated FGR, but this was not true when comparing pathogenic CMA in the minor structural anomalies group to isolated FGR. While in our cohort the rate of pathogenic CMA was higher in the major structural findings group, 29.4% (5/17), compared to the minor findings group, 6.1% (3/49), Borrell et al. [10] reported differently. In their cohort, when differentiating between nonstructural abnormalities and major structural malformations the yield of CMA was similar: 10% (3/30) and 10.5% (2/19), respectively [10]. This difference may be due to a difference in the inclusion criteria. Borrell et al. [10] included only cases with normal rapid chromosomal analysis results for 5 chromosomes while in the current study CMA was the primary genetic test performed. In our cohort, 7 cases with pathologic CMA were karyotype detectable (4 detectable by rapid chromosomal analysis), of which 5 had major structural findings, 1 had a minor anomaly, and 1 was a case of isolated FGR.

As reported in previous studies [17, 22], the yield of CMA for FGR cases combined with prenatally detected congenital heart malformations was significantly higher.

We found no correlation between the severity of IUGR and the yield of CMA. In this study, most cases were below the 3rd percentile, according to the formal criteria for testing, and the vast majority of cases were below the 5th percentile. However, our study did include some cases in the low-normal range between the 3ed and 10th percentile. There are a number of reasons for performing invasive testing and CMA analysis in these cases including the limited time available for genetic workup in an ongoing pregnancy, the known margin of error for fetal biometric measurements, and the possibility that growth restriction can be progressive and parameters below the normal range may be detected in a follow-up scan later in the pregnancy. The inclusion of these cases allowed us to assess the yield of CMA in these borderline cases. We had 19 cases with fetal parameters between the 5th percentile and 10th percentile of which no abnormal CMA findings were detected and 38 cases above the 3rd percentile with 1 abnormal CMA result (2.6%). As far as we know, no previous study has assessed the yield of CMA according to the estimated fetal weight percentile. A larger cohort might help clarify the impact of the estimated fetal weight percentile on the risk for a P/LP CMA result.

Regarding microscopic chromosomal anomalies, there is an assumption that cases detected early in pregnancy are more likely to have a chromosomal abnormality as compared to cases detected later in the pregnancy [23]. In our cohort, all cases of pathologic CMA were diagnosed with FGR before 26 weeks of gestation; hence, the yield of CMA was strongly correlated with gestational age at diagnosis. Of the cases detected before 24 weeks, 42.8% (3/7) were submicroscopic anomalies compared to 33.33% (1/3) for cases detected after 24 weeks.

Peng et al. [24] explored chromosomal anomalies (microscopic and submicroscopic) in isolated FGR cases and

found higher rate pathologic findings in cases detected before 32 weeks of gestation compared to cases detected later in the pregnancy (12.7 vs. 1.8%, $p = 0.042$) [24]. An et al. [11] recently reported a similar trend in a cohort of isolated FGR cases detected at 19–23.8 weeks as compared to cases diagnosed at 24–32.8 weeks (9.6 vs. 9.3%, $p = 1.00$, respectively) [11]. This suggests that for cases where FGR is diagnosed earlier in the pregnancy, the motivation for invasive testing and CMA analysis should be higher.

When counseling couples with a diagnosis of FGR, one of the major issues is to aid them in their decision regarding invasive genetic testing. These cases are usually diagnosed in the mid-second trimester or third trimester; hence, this is relevant for couples that had not opted for invasive testing earlier in the pregnancy. In some cases, noninvasive prenatal screening (NIPS) had already been performed. In this study, out of 11 P/LP abnormal CMA results, there were 4 detectable by standard NIPS and an additional 3 cases could have been detected by NIPS platforms that includes specific submicroscopic aberrations. However, the sensitivity for NIPS is not 100% especially for submicroscopic aberrations.

We found that the yield of CMA in all FGR cases was significantly higher compared to the background population of both a local cohort [16] and a cohort derived from a meta-analysis [15]. The OR for all FGR cases was 4.7 (95% CI 2.5–9) and for non-isolated FGR it was 10.2 (95% CI 4.7–22.1).

The rate of isolated FGR cases and of cases diagnosed after 24 weeks was not significantly higher than that of the control population background. However, if we look at the yield of isolated cases (3.39% compared to the background risk of 1.4%) we can assume that a significant difference could have been reached if the cohort were larger. Therefore, this information should be conveyed to couples and factored into their decisions.

The spectrum of abnormal CMA results included both chromosomal microscopic and submicroscopic anomalies. The incremental yield of CMA over karyotyping was 2.3%. Interestingly, there were 3 cases of Wolf-Hirschhorn syndrome (MIM #194190) one of which was an unbalanced translocation, a common mechanism for this disorder [25]. This multisystem disorder consists of characteristic facies, delayed growth and development, hypotonia, hearing loss, intellectual disability, and seizures, as well as congenital heart defects, urinary tract malformations, and/or structural brain abnormalities. All 3 cases diagnosed in this cohort had additional findings; however, in 1 case these were only minor findings and in all 3 a specific diagnosis could not have been determined based on the combination of sonographic findings.

The current analysis has several important limitations. The study cohort does not include all suspected FGR cases. It consists of only cases where genetic testing was opted, indicating a potential bias for inclusion of cases that are more suggestive of a genetic abnormality. Hence, the true rate of abnormal CMA results may be lower than the calculated rate in our study. The retrospective nature of data acquisition did not include complete clinical data for every case; hence, the cohorts used for analysis of the different clinical aspects were limited to cases where information was available to us. However, we undertook great efforts to access the clinical files and retrieve the maximum information available for most cases. We also did not have access to postnatal data, which could be imported in counseling couples with FGR pregnancies. However, our aim was to assess the yield of CMA based on clinical parameters that are available during pregnancy when the decision regarding invasive testing is made. Another limitation was that CMA was performed by several laboratories using different platforms. Finally, our cohort size may have been underpowered for some of the subanalysis performed for specific stratification groups. Nevertheless, this study presents one of the largest cohorts of CMA results in pregnancies with FGR and focused on analyzing the results according to specific clinical characteristics.

Conclusion

The yield for CMA in cases of FGR pregnancies is significantly higher than the background risk for non-isolated cases and for cases diagnosed before 24 weeks. These findings should be conveyed to couples who opt for invasive genetic testing as part of FGR workup.

Statement of Ethics

The study was approved by the Ministry of Health Institutional Review Board (Helsinki Committee) for Human Subjects (September 6, 2016, registration number – MOH2016). The study is retrospective, there was no interaction with the study participants. Hence, the institutional review board granted us an exemption from obtaining informed consent.

Conflict of Interest Statement

The authors report no conflict of interest.

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Author Contributions

K.T.G. – project development, data collection and analysis, and manuscript writing. A.S. – project development, data collection, and manuscript editing. I.M. – data analysis and manuscript editing. S.B.S. – data collection and manuscript editing. L.S.D. – data collection and manuscript editing. H.D. – data collection and manuscript editing. R.M.C. – data collection and manuscript editing. L.G. – data collection and manuscript editing. M.F.Z. – data collection and manuscript editing. R.S.H. – project development, data collection and analysis, and manuscript writing.

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