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Placental lesions associated with oligohydramnios in fetal growth restricted (FGR) pregnancies

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ABSTRACT

Introduction: Aim of the study was to investigate the association between placental pathology and oligohydramnios in pregnancies complicated by fetal growth restriction (FGR).
Methods: Placentas from 221 consecutive FGR pregnancies and 63 healthy controls were studied. Pathological lesions were described according to consensus nomenclature and standardized criteria; both elementary lesions and constellations of lesions (patterns) were considered. Statistics included analysis of linear trends and multinomial logistic regression.
Results: Amniotic fluid index (AFI) was normal in 56 (25.3%) FGR pregnancies, whereas mild, moderate and severe oligohydramnios were diagnosed in 32 (14.5%), 44 (19.9%) and 89 (40.3%) subjects, respectively. In FGR pregnancies, after adjustment for potential confounders, membrane meconium staining (chi-square = 28.6, $p < 0.001$), chronic villous hypoxia pattern (chi-square = 18.8, $p < 0.001$) and fetal thrombotic vasculopathy pattern (chi-square = 9.2, $p = 0.002$) were positively and linearly correlated to AFI decrease. Odds ratios of meconium and chronic villous hypoxia were 9.2 (95% CI = 2.6–32.9) and 4.2 (95% CI = 1.3–13.6) in FGR pregnancies with normal AFI and 25.2 (95% CI = 6.9–91.8) and 9.7 (95% CI = 3–31.5) in those with severe oligohydramnios ($p = 0.005$ and $p = 0.023$ compared to normal AFI, respectively).
Discussion: In FGR pregnancies, reduction of amniotic fluid volume is directly correlated to histological features of placental under-perfusion, meconium staining of membranes and fetal vascular damage. These findings support the clinical notion that in FGR pregnancies oligohydramnios is a risk factor of fetal hypoxia and possibly of increased adverse neonatal outcomes.

1. Introduction

Amniotic fluid volume is regulated by trans-placental passage of water from mother to fetus, and by mechanisms of liquid production, mainly fetal urine and lung fluid, and absorption, such as fetal swallowing [1]. Since the volume of daily fetal urine and lung fluid output exceeds the amount of liquid swallowed by the fetus, an additional route of absorption has been hypothesized, the so-called intramembranous pathway which refers to the passage of free water directly from the amnion cavity into fetal vessels [2]. In pregnancies complicated by fetal growth restriction (FGR), amniotic fluid volume progressively decreases with worsening of fetal conditions [3] and is associated with an increased risk of caesarean section, Apgar score <7 at 5 min, and possibly increased perinatal mortality [5,6]. In this setting, oligohydramnios is considered a result of the redistribution of fetal blood flow towards brain and heart and away from lungs, digestive tract, kidney and torso caused by increased placental resistance [4].

Although sonographic serial evaluation of amniotic fluid volume has a definite prognostic value in the antenatal evaluation of fetal well-being in FGR pregnancies, there are very few data on the relationship between oligohydramnios and placental histology [7–9]. The purpose of this study was to evaluate placental histopathological features in FGR pregnancies complicated by a reduction of amniotic fluid volume. Placental findings of FGR pregnancies

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were also compared with a control group of at-term healthy pregnancies.

2. Materials and methods

2.1. Patients

The study included 221consecutive pregnancies complicated by FGR with intact fetal membranes, diagnosed and followed-up at the high-risk pregnancy clinic of the Obstetrics and Gynecology Department of the IRCCS Policlinico San Matteo Foundation in the period 2008–2012. Eligibility criteria were: a) singleton pregnancy; b) absence of fetal malformations, known chromosomal anomalies and congenital infections; c) enrolment for prenatal care during the first trimester of pregnancy; d) pregnancy complicated by FGR eligible for conservative treatment based on antenatal surveillance data. Placentas from 63 consecutive, uncomplicated singleton pregnancies at term with normal fetal growth served as controls.

Demographic data were collected at enrolment; clinical data were collected during subsequent antenatal visits and at discharge. The study was approved by the local Institutional Review Board and all patients gave their informed, written consent.

FGR was diagnosed when abdominal fetal circumference at ultrasonographic (US) examination fell below the 10th percentile of reference curves [10], confirmed on at least two consecutive measurements taken two weeks apart after the standard US obtained at 18–22 weeks of pregnancy. A conservative management plan for FGR was adopted according to a predefined protocol that included antenatal visits, US surveillance and cardio-tocographic monitoring. US evaluation included weekly assessment of amniotic fluid volume and fortnightly fetal biometry. Doppler studies of fetal circulation included weekly or biweekly measurement of blood flow velocity waveforms of the umbilical artery, middle cerebral artery and ductus venosus depending on the initial severity of blood flow abnormalities [11]. Spontaneous rupture of fetal membranes was excluded on the basis of history, clinical and US examination and evaluation of vaginal pH and fibronectin. Amniotic fluid volume was expressed using the amniotic fluid index (AFI) and measured sonographically as the sum of the deepest vertical pool in each of the four maternal abdominal quadrants [12]. Measured AFI values immediately before birth were compared with the expected values of our local reference curve. AFI standard deviation scores (SDS) were used to evaluate the severity of amniotic fluid volume reduction. AFI results were categorized as normal (AFI SDS > -1.64), or mild ($-2 <$ AFI SDS ≤ -1.64), moderate ($-3 <$ AFI SDS ≤ -2) or severe (AFI SDS ≤ -3) oligohydramnios.

Women were admitted for close surveillance in the case of worsening maternal or fetal condition according to the judgment of the obstetric team (e.g. absent or reversed umbilical artery blood flow, severe preeclampsia). Betamethasone (12 mg, two doses, 24 h apart) was administered to the mother if preterm birth at less than 37 weeks gestation was anticipated.

After delivery, all placentas were immediately fixed in buffered formalin. After gross examination, standard samples for routine diagnosis were obtained: three samples in one block from the umbilical cord, a membrane roll and three blocks from the chorionic disk, two from the central part of the disk and one at the periphery, in areas devoid of macroscopic lesions. No attempt was made to select specific areas. Additional samples were taken to document specific or equivocal lesions. Late infarctions, hematomas, intervillous thrombi or other lesions that could be diagnosed at gross examination were not sampled for microscopy; their location, number and size were recorded and quantified, if required, by visual inspection of the placental cut surface. Overall, at least five blocks were obtained from each placenta (mean 6, range 5–14). Samples were routinely processed for histology and sections were stained using H&E. Stains for collagen (Masson trichrome) and iron (Perls' Prussian blue) were performed in selected cases to highlight small clusters of avascular villi or stem arteries obliteration and in the detection of membrane siderophages in chronic abruption, respectively.

For the purpose of the study, all slides were blindly reviewed using a predefined set of variables by two pathologists experienced in placental pathology. Pathologists were blinded to FGR and AFI subgroups, only gestational age was known at the time of revision. Histological lesions were defined according to the nomenclature and terminology proposed by the Society for Paediatric Pathology, Perinatal Section in 2004 [7–9,13].

Individual pathological variables considered in the analysis were: maternal inflammatory response (stage and grade); fetal inflammatory response (stage and grade) [13]; acute and chronic villitis; fetal vascular thrombi in the chorionic plate or proximal portion of villous tree; avascular villi with or without fetal vascular thrombi, any ($\geq$2 foci of $\geq$3 avascular villi) or severe ($\geq$2 foci/average 15 or more affected villi/slide) [8]; early and late infarction (site and percent volume); intervillous thrombi, (percent volume); retroplacental hemorrhage (percent volume) [7]; chronic abruption; free meconium or meconium-laden macrophages in chorionic

Table 1
Socio-demographic and obstetrical variables in 221 FGR pregnancies and 63 controls by amniotic fluid index (AFI) values.

Variables	Controls normal AFI Median (IQR) $N = 63$	FGR normal AFI Median (IQR) $N = 56$	FGR mild AFI reduction Median (IQR) $N = 32$	FGR moderate AFI reduction Median (IQR) $N = 44$	FGR severe AFI reduction Median (IQR) $N = 89$	*P* value
Maternal age (years)	31.5 (28–36.7)	31.5 (27–35)	32.5 (25–36)	32 (28–36)	32 (30.2–37.7)	0.81
Body mass index (kg/m^2)	21 (20.4–22)	22.4 (20.2–25.3)	22.7 (20–25.5)	22.6 (20.7–24.4)	23.6 (21.8–25.6)	<0.001
Weight gain (g)	10 (9–11)	10 (7–14)	10 (7.5–14)	10 (9–14)	10 (8–13)	0.28
Amniotic fluid index (mm)	117.5 (100–130)	110 (100–115)	90 (82.5–95)	70 (65–75)	40 (35–50)	<0.001
	N (%)	*N* (%)	*N* (%)	*N* (%)	*N* (%)	
Schooling years						
8	18 (28.6)	25 (44.6)	10 (31.2)	13 (29.5)	34 (38.2)	0.6
13	21 (33.3)	16 (28.6)	9 (28.1)	18 (40.9)	26 (29.2)	
>13	24 (38.1)	15 (26.8)	13 (40.6)	13 (29.5)	29 (32.6)	
Smoking						
No	63 (100)	39	27	33	72	<0.001
Yes	0	13 (23.2)	5 (15.6)	7 (15.9)	9 (10.1)	
Quit in pregnancy	0	4 (7.1)	0	4 (9.1)	8 (9)	
Parity						
0	29 (46)	34 (60.7)	28 (87.5)	29 (65.9)	64 (71.9)	0.01
1	24 (38.1)	15 (26.8)	3 (9.4)	12 (27.3)	17 (19.1)	
>1	10 (1.6)	7	1 (3.1)	3 (6.8)	8	
Previous spontaneous abortions						
No	52 (82.5)	44 (78.6)	24 (75)	36 (81.8)	69 (77.5)	0.01
1	11 (17.4)	9 (16)	8 (25)	3 (6.8)	19 (21.3)	
>1	0	3 (3.6)	0	5	1 (1.1)	
Caesarean section	20 (31.7)	44 (78.6)	26 (81.3)	33 (75)	80 (89.9)	<0.001
Preeclampsia	0	20 (35.7)	13 (40.6)	18 (40.9)	31 (34.8)	<0.001
Umbilical artery Doppler flow velocities						
Normal	63 (100)	35 (62.5)	21 (65.6)	19 (43.2)	33 (37)	<0.001
Increased	0	12 (21.4)	7 (21.8)	17 (38.6)	27 (30.3)	
Absent	0	8 (14.3)	2 (6.2)	7 (15.9)	16 (18)	
Reversed	0	0	2 (6.2)	1 (2.3)	13 (14.6)	

surface or placental membranes; massive perivillous fibrin deposition including maternal floor infarction (thickness, volume); increased syncytial knots (≤ or >30% of villi); villous agglutination; intervillous fibrin deposition (percentage of villous surface); distal villous hypoplasia; fibrinoid necrosis or atherosis of basal and capsular decidual arteries; capsular spiral artery hypertrophy; persistence of muscularized arteries in the basal plate; trophoblastic giant cells and immature intermediate trophoblastic in the basal decidua.

For macroscopic lesions, percentages of placental volume involved were calculated based on the number of lesions and their volume derived from the measurements of main and minor diameters.

Placental volumes were derived from measurements of major and minor diameters and depth.

Gross data were verified and integrated by microscopy when the nature of lesions was not clear by macroscopy (e.g. hemorrhagic infarcts vs. intervillous thrombi; peripheral atrophy vs. infarction).

For microscopic lesions, quantification was made by visual inspection of slides by trained pathologists.

For the purpose of statistical analysis, pathological lesions were assigned to one of five categories representing constellations of elementary lesions (patterns of damage): a) *infection/inflammation* including chorioamnionitis, any grade or stage, and/or acute or chronic villitis; b) *fetal thrombotic vasculopathy*, including avascular villi with or without obliterative fetal vasculopathy of stem villi and/or adherent or occlusive thrombi in chorionic or umbilical vessels; c) *ischemic injury*, defined by the presence of early or late infarctions involving > 10% of placental volume (>5% for central infarctions) and/or massive perivillous fibrin deposition (any grade) and/or intervillous fibrin deposition (>20% of intervillous space); 4) *superficial implantation* including atherosis and/or muscularized arteries in the basal plate and/or immature intermediate trophoblast plus trophoblast giant cells; 5) *chronic villous hypoxia* including villous infarction as defined above and/or hypo- (<2 capillary lumen) or hyper- (>6 capillary lumen) vascularization of terminal villi and/or syncytial knots (>30% of villi) and/or villous agglutination with excessive extra villous trophoblast.

Continuous variables were compared by Kruskal–Wallis one-way analysis of variance with Bonferroni correction for multiple pairwise comparisons and were reported as medians and interquartile ranges (IQR). Chi-square analysis was used to compare categorical variables. Partitioning of chi-square statistics with Bonferroni correction was used to compare categories in multi-way contingency tables. Linear trends in proportions were computed by chi-square for trends. The Spearman rank correlation coefficient was used to test for trends in continuous data. To evaluate the association between placental histological variables and severity of amniotic fluid volume reduction, multinomial logistic regression was used. In logistic equations, severity of amniotic fluid reduction was modeled as a five-level outcome variable (controls, normal, mild, moderate and severe oligohydramnios). Explanatory variables included those placental variables that were significantly different among the categories of FGR pregnancies as demonstrated by partitioning of chi-square. Gestational age (continuous), placental/neonatal weight ratio (continuous) and maternal smoking (categorical: no, yes, quit) were added as confounding variables. Significant differences between regression coefficients obtained from multinomial logistic equations were compared using the Wald test [14]. Logistic regression was also used to test for linear trends adjusting for confounding variables.

3. Results

During the period of the study 33 FGR pregnancies with fetal malformations, known fetal chromosomal anomalies or congenital infections were delivered at our Institution and were excluded from the analysis. The main characteristics of cases and controls included in the study are reported in Table 1. Cases were divided into four categories based on the severity of amniotic fluid volume reduction (AFI values). Severe oligohydramnios (AFI < −3 SDS) included eight women without measurable amniotic fluid (i.e. anhydramnios). A single deepest vertical pocket of amniotic fluid <2 cm was found in 94.4% (84/89) of subjects with severe oligohydramnios, in 13.6% (6/44) of those with moderate oligohydramnios and in none of the remaining subjects. Maternal pre-pregnancy BMI was higher among FGR cases compared to

Table 2
Neonatal indices at birth and perinatal complications in 221 FGR pregnancies and 63 controls by amniotic fluid index (AFI) values.

Variables	Controls normal AFI Median (IQR) $N = 63$	FGR normal AFI Median (IQR) $N = 56$	FGR mild AFI reduction Median (IQR) $N = 32$	FGR moderate AFI reduction Median (IQR) $N = 44$	FGR severe AFI reduction Median (IQR) $N = 89$	*P* value
Gestational age at birth (weeks)	39.1 (38.6–40.1)	35.5 (32.3–38.2)	35.7 (33.8–33.7)	34.2 (32–37)	33.1 (30.3–35.5)	<0.001
Neonatal weight (g)	3397.5 (3162.5–3597.5)	1909.5 (1140–2385)	1795 (1478.5–2277.5)	1645 (1090–2130)	1391.5 (918–1819)	<0.001
Birth weight z-scores	0.18 (−0.32–0.77)	−1.8 (−2.1; −1.5)	−1.7 (−2; −1.5)	−1.9 (−2.2; −1.6)	−1.7 (−2.1; −1.3)	<0.001
Umbilical cord pH	7.30 (7.25–7.34)	7.31 (7.27–7.35)	7.31 (7.29–7.36)	7.30 (7.28–7.33)	7.30 (7.26–7.38)	0.8
Umbilical cord base excess	−2 (−3.7; −0.7)	−3.4 (−1.35; 0.45)	−1.15 (−2.6; 0,3)	−2.1 (−3.7; −0.5)	−2 (−3.9; −0.5)	0.6
Fetal/neonatal outcome						
Livebirth	63(100)	55(98.2)	32(100)	42(95.4)	82(92.1)	0.28
Neonatal death	0	1(1.8)	0	1(2.3)	5(5.6)	
Fetal death	0	0	0	1(2.3)	2(2.3)	
Administration of surfactant	0	9 (16)	4 (12.5)	4 (9.1)	18 (20.2)	0.004
nCPAP[a]						
No	63 (100)	38 (67.8)	20 (62.5)	32 (72.7)	54 (60.7)	<0.001
≤24 h	0	9 (16)	10 (31.2)	10 (22.7)	24 (27)	
>24 h	0	9 (16)	2 (6.2)	2 (4.5)	11 (12.3)	
Mechanical ventilation						
No	63 (100)	46 (82.1)	27 (84.4)	39 (88.6)	65 (73)	<0.001
1 < 24 h	0	9 (16)	5 (15.6)	5 (11.4)	24 (27)	
2 > 24 h	0	1 (1.8)	0	0	0	
Respiratory distress						
No	63 (100)	36 (64.3)	20 (62.5)	24 (54.5)	41 (46.1)	<0.001
Mild to moderate	0	6 (10.7)	6 (18.8)	14 (31.8)	27 (30.3)	
Severe	0	14 (25)	6 (18.8)	6 (13.6)	21 (23.6)	
Bronchodysplasia	0	5 (8.9)	1 (3.1)	2 (4.5)	7 (7.9)	0.05
Sepsis	0	2 (3.6)	2 (6.2)	0	5 (5.6)	0.06
Cystic periventricular leukomalacia	0	0	1 (3.1)	1 (2.3)	0	0.25
Persistent brain hyperechogenicity	0	16 (28.6)	11 (34.4)	11 (25)	19 (21.3)	<0.001
Cerebral hemorrhage	0	4 (7.1)	1 (3.1)	2 (4.5)	5 (5.6)	0.14
Necrotising enterocolitis	0	2 (3.6)	2 (6.2)	2 (4.5)	8 (9)	0.06
Retinopathy of prematurity	0	1 (1.8)	1 (3.1)	1 (2.3)	7 (7.9)	0.06
Hypoglicemia	0	22 (39.3)	7 (21.9)	28 (63.6)	39 (43.8)	<0.001

[a] Nasal continuous positive airway pressure.

controls but within normal limits. FGR cases were more likely to be nulliparous, smokers and had higher rates of absent/reversed umbilical artery Doppler flow velocities. Umbilical artery reversed Doppler velocities were more frequent in pregnancies with severe amniotic fluid reduction in comparison to normal controls (chi-square = 14.8, $p = 0.001$) and FGR pregnancies with normal AFI (chi-square = 13.5, $p = 0.002$).

Gestational age at birth, birthweight and SDS of birthweight were significantly lower among cases than controls (Table 2). These variables were also correlated with the severity of amniotic fluid reduction. Median gestational age at diagnosis of FGR was 32.6 weeks (IQR = 28.4–36.1) for subjects with normal AFI, and 31.6 (IQR = 29.4–34.9), 32 (IQR = 30–35.2) and 31.2 (IQR = 27.9–34.2) ($p = 0.22$) in those with mild, moderate and severe amniotic fluid reduction, respectively. Birthweight was lower for infants with severe amniotic fluid reduction compared to normal AFI (post-hoc p value = 0.021). Among FGR pregnancies, the amniotic fluid reduction correlated inversely with gestational age (Spearman rho = 0.316, $p < 0.001$) and birthweight (Spearman rho = 0.3, $p < 0.001$) (Fig. 1) but not with severity of growth failure as expressed by birthweight SDS (Spearman rho = −0.02, $p = 0.8$). The median time from diagnosis of FGR to delivery was 15 days (IQR = 3–31) in pregnancies with normal amniotic fluid, 20.5 days (IQR = 15–36), 15 days (IQR = 2–31), and 9 days (IQR = 2–20, $p = 0.005$ compared to normal AFI) in pregnancies complicated by mild, moderate and severe amniotic fluid reduction, respectively. AFI SDS correlated directly with time to delivery (Spearman rho = 0.189, $p = 0.005$) confirming that in clinical management of FGR pregnancies, amniotic fluid volume was used as a marker of fetal well-being.

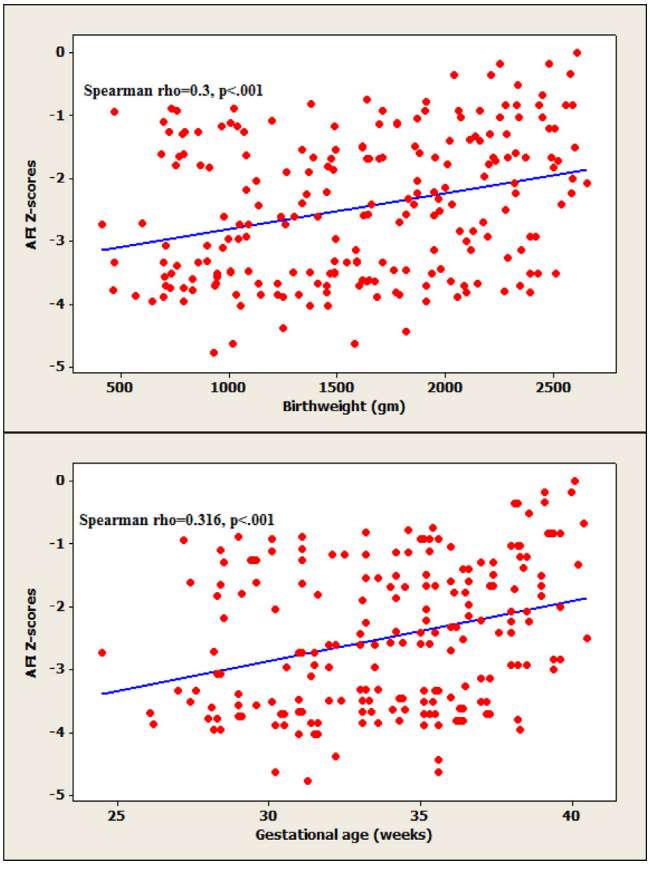

Fig. 1. Association between severity of amniotic fluid volume reduction as expressed by amniotic fluid index (AFI) *Z*-scores, gestational age and birthweight.

An umbilical artery pH < 7 and/or a base excess < −12 was recorded in five cases (5.6%) of severe oligohydramnios, in two cases of moderate oligohydramnios (4.5%) and in none of the remaining FGR or control pregnancies ($p = 0.04$). Compared to controls, FGR infants had higher rates of prematurity complications such as need for administration of surfactant, ventilation support, respiratory distress syndrome or pulmonary bronchodysplasia. Partitioning of chi-square statistics failed to demonstrate a significant difference in neonatal outcomes among AFI subgroups.

Table 3 reports the association of placental gross variables among groups. FGR pregnancies were associated with lower disk and cord diameters compared to controls, but post-hoc analysis did not show significant differences among categories of amniotic fluid reduction. In FGR pregnancies, AFI SDS correlated significantly with placental weight (Spearman rho = 0.18, $p = 0.007$) and maximum placental diameter (Spearman rho = 0.198, $p = 0.003$), but not with maximum placental thickness (Spearman rho = 0.04, $p = 0.54$), cord length (Spearman rho = 0.08, $p = 0.25$) or cord diameter (Spearman rho = 0.05, $p = 0.45$).

Individual histological lesions associated with AFI included late infarctions, meconium staining, villous agglutination, distal villous hypoplasia, intervillous fibrin deposition, increased number of syncytial knots, atherosis of spiral arteries and placental site giant cells. Among combined patterns of damage, ischemic injury, superficial implantation, chronic villous hypoxia and fetal thrombotic vasculopathy were more frequent among cases than controls (Table 4). No differences were seen for individual or combined inflammatory lesions.

Among FGR cases, a significant linear trend for increasing severity of amniotic fluid reduction was observed for meconium staining (chi-square for linear trend = 51.4, $p < 0.001$), chronic villous hypoxia and fetal thrombotic vasculopathy patterns (chi-square for linear trend = 49.5, $p < 0.001$ and 20.2, $p < 0.001$, respectively) (Fig. 2). In the partitioning of total chi-square and after correction for multiple comparisons, FGR placentas with severe amniotic fluid reduction had significantly increased rates of meconium staining ($p = 0.028$) and chronic villous hypoxia ($p = 0.034$) compared to FGR with normal amniotic fluid volume. After adjustment for the confounding effect of gestational age, placental/neonatal weight ratio, and cigarette smoking, meconium staining (chi-square = 28.6, $p < 0.001$), chronic villous hypoxia (chi-square = 18.8, $p < 0.001$) and fetal thrombotic vasculopathy (chi-square = 9.2, $p = 0.002$) were positively and linearly correlated to the severity of amniotic fluid reduction.

In multinomial logistic regression, the odds ratios of meconium staining and chronic villous hypoxia were significantly higher in each category of FGR when compared to controls, and were higher among pregnancies complicated by severe amniotic reduction as compared to those with normal amniotic fluid (Table 5).

4. Discussion

The results of this study showed that in FGR pregnancies amniotic fluid reduction was significantly and directly correlated with the severity of placental lesions. Both in univariate and multivariate analyses, histological patterns of chronic villous hypoxia and acute fetal distress (as indirectly shown by meconium staining of membranes) were more common among FGR pregnancies complicated by severe amniotic fluid reduction (less than 3 SDS from gestational age-adjusted reference values) than in those with normal amniotic fluid volume. In addition, univariate and multivariate tests for linear trends suggested that histological features of fetal thrombotic damage including avascular villi, obliterative fetal

Table 3
Gross placental morphometric variables in 221 FGR pregnancies and 63 controls by amniotic fluid index (AFI) values.

Variables	Controls normal AFI Median (IQR) $N = 63$	FGR normal AFI Median (IQR) $N = 56$	FGR mild AFI reduction Median (IQR) $N = 32$	FGR moderate AFI reduction Median (IQR) $N = 44$	FGR severe AFI reduction Median (IQR) $N = 89$	*P* value
Placental weight[a] (g)	489 (412.5–544)	305 (245–339.5)	300 (265–400)	295 (240–334.5)	271.5 (220–321)	<0.001
Placental max diameter (cm)	17.1 (16–19)	14.2 (13–16.75)	15.1 (14–16)	14.5 (14–17)	14.3 (12–15)	<0.001
Placental min diameter (cm)	15.1 (14–16)	12.3 (11–13)	12.8 (12–13)	13.1 (10.5–14)	11.9 (10–13)	<0.001
Placental max thickness (cm)	3 (2.5–3)	2.8 (2.5–3)	2.7 (2–3)	2.5 (2–3)	2.5 (2–3)	0.06
Placental min thickness (cm)	1.5 (1–2)	1.7 (1.5–2)	1.6 (1.4–2)	1.5 (1–2)	1.5 (1.2–2)	0.81
Cord length[a] (cm)	32.1 (24–38.5)	25.4 (18.5–37)	27.2 (18–31)	27.4 (19.5–33)	27.9 (19–32)	0.05
Cord diameter (cm)	1.2 (1–1.5)	1.1 (0.85–1.2)	1 (0.9–1.2)	1.3 (0.8–1)	1.1 (0.9–1.2)	<0.001
Placental/neonatal weight ratio	0.142 (0.124–0.163)	0.164 (0.138–0.221)	0.185 (0.161–0.215)	0.170 (0.150–0.198)	0.195 (0.160–0.234)	0.55

[a] Measurements taken after fixation.

vasculopathy and/or thrombosis of large vessels were linearly related with the severity of amniotic fluid reduction.

Data on placental histology of oligohydramnios in FGR pregnancies are scant, retrospective, and have been mainly analyzed without properly accounting for confounders [9]. Since most of the histological features associated with FGR and amniotic fluid reduction are gestational age-dependent, a multivariable approach adjusting for potential confounding variables seems desirable. The

Table 4
Placental histological injuries, elementary lesions and patterns, in 221 FGR pregnancies and 63 controls by amniotic fluid index (AFI) values.

	Controls normal AFI ($n = 63$) *N* (%)	FGR normal AFI ($n = 56$) *N* (%)	FGR mild AFI reduction ($n = 32$) *N* (%)	FGR moderate AFI reduction ($n = 44$) *N* (%)	FGR severe AFI reduction ($n = 89$) *N* (%)	
Elementary lesions						
Cord vascular lesions	0	3 (5.3)	2 (6.2)	2 (4.5)	5 (5.6)	0.28
Acute villitis	0	2 (3.6)	0	0	1 (1.1)	0.28
Chronic villitis	13 (20.6)	10 (17.9)	5 (15.6)	9 (20.5)	16 (18)	0.97
Corionamnionitis						
No	53 (84.1)	53 (94.6)	30 (93.7)	40 (90.9)	84 (94.4)	0.15
Mild	10 (15.9)	3 (5.3)	2 (6.2)	3 (6.8)	5 (5.6)	
Severe	0	0	0	1 (2.3)	0	
Retroplacental haematoma	7 (11.1)	3 (5.3)	4 (12.5)	5 (11.4)	9 (10.1)	0.74
Early infarction	2 (3.2)	5 (8.9)	5 (15.6)	6 (13.6)	10 (11.2)	0.25
Late infarction	5 (7.9)	30 (53.6)	15 (46.9)	15 (34.1)	34 (38.2)	<0.001
Chronic abruption	0	0	0	0	3 (3.4)	0.16
Meconium staining	5 (7.9)	23 (41.1)	15 (46.9)	23 (52.3)	59 (66.3)[a]	<0.001
Syncytial knots						
No increase	54 (85.7)	8 (14.3)	3 (9.4)	6 (13.6)	6 (6.7)	<0.001
≤30%	9 (14.3)	14 (25)	12 (37.5)	17 (38.6)	29 (32.6)	
>30%	0	34 (60.7)	17 (53.1)	21 (47.7)	54 (60.7)	<0.001
Any	9 (14.3)	48 (85.7)	29 (90.6)	38 (86.4)	83 (93.3)	
Villous agglutination						
None	45 (71.4)	45 (80.3)	28 (87.5)	35 (79.5)	63 (70.8)	<0.001
Mild	18 (28.5)	2 (3.6)	1 (3.1)	5 (11.4)	7 (7.9)	
Severe	0	9 (16)	3 (9.4)	4 (9.1)	19 (21.3)	
Intervillous fibrin deposition						
None (0–5%)	62 (98.4)	15 (26.8)	13 (40.6)	17 (38.6)	23 (25.8)	<0.001
Mild (6–20%)	1 (1.6)	32 (57.1)	17 (53.1)	24 (54.5)	56 (62.9)	
Severe (>20%)	0	9 (16)	2 (6.2)	3 (6.8)	10 (11.2)	<0.001
Any	1 (1.6)	41 (73.2)	19 (59.4)	27 (61.4)	66 (70.8)	
Distal villous hypoplasia	1 (1.6)	11 (19.6)	5 (15.6)	13 (29.5)	21 (23.6)	<0.001
Mural hypertrophy of spiral arteries	15 (23.8)	24 (42.8)	10 (31.2)	17 (38.6)	38 (42.7)	0.12
Atherosis of spiral arteries	1 (1.6)	13 (23.2)	3 (9.4)	11 (25)	19 (21.3)	0.002
Muscularised arteries at implantation site	11 (17.5)	19 (33.9)	8 (25)	16 (36.4)	29 (32.6)	0.15
Giant trophoblastic cells at implantation site	2 (3.2)	35 (62.5)	17 (53.1)	26 (59.1)	51 (57.3)	<0.001
Immature intermediate trophoblast at implantation site	1 (1.6)	35 (62.5)	17 (53.1)	29 (65.9)	52 (58.4)	<0.001
Patterns of lesions						
Infection/inflammation	9 (14.3)	5 (8.9)	2 (6.2)	4 (9.1)	5 (5.6)	0.44
Superficial implantation	14 (22.2)	37 (66.1)	19 (59.4)	28 (63.6)	56 (62.9)	<0.001
Ischemic injury	7 (11.1)	27 (48.2)	14 (43.7)	19 (43.2)	36 (40.4)	<0.001
Chronic villous hypoxia	6 (9.5)	26 (46.4)	19 (59.4)	25 (56.8)	63 (70.8)[a]	<0.001
Fetal thrombotic vasculopathy	0	8 (14.3)	6 (18.8)	10 (22.7)	25 (28.1)	<0.001

[a] $p < 0.05$ compared to FGR pregnancies with normal amniotic fluid volume.

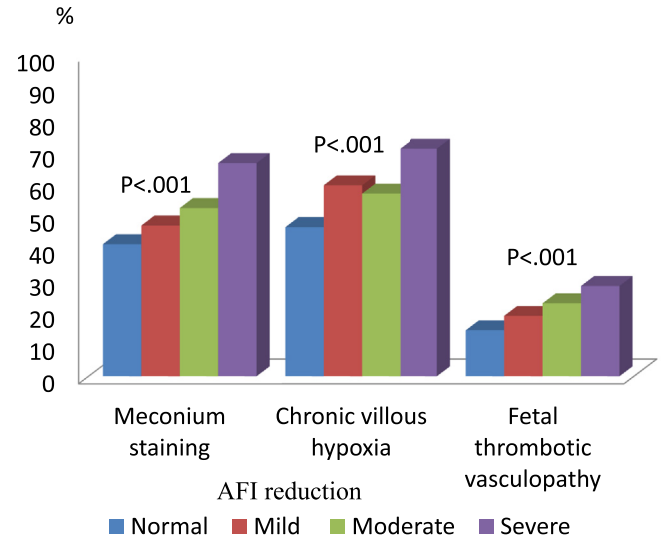

Fig. 2. Prevalence of meconium staining of membranes, chronic villous hypoxia, and fetal thrombotic vasculopathy according to the severity of amniotic fluid (AFI) volume reduction. (p values by chi-square for trend).

main strengths of our study are in the design of the investigation (i.e. pathologists blinded to the clinical characteristics of FGR, homogeneous group of consecutive FGR pregnancies followed-up in a single high-risk clinic, exclusion of fetal causes of oligohydramnios), the sample size allowing multivariate analysis of data, and the inclusion of a control group of healthy pregnancies. Potential limitations of the study include the referral origin of most FGR cases and their early diagnosis suggesting that mainly severe cases of fetal growth failure were enrolled.

Previous studies indicated that in FGR pregnancies, oligohydramnios is associated with fetal hypoxia, abnormal fetal Doppler and biophysical testing, and fetal distress in labor [3,5,6]. Placental lesions such as hyper-vascularization of terminal villi, distal villous hypoplasia and syncytial knotting were also described in early and late FGR as expression of chronic fetal hypoxia [15]. Our findings showed a direct linear correlation between histological features of chronic villous hypoxia and decreased amniotic fluid volume that is consistent with the central role of maternal underperfusion in FGR. They also support the notion that decreasing amniotic fluid volume is a surrogate marker of progressive worsening of maternal–fetal exchanges in FGR pregnancies. Accordingly, we observed that amniotic fluid reduction was associated with earlier fetal growth failure, a shorter time from diagnosis to delivery and progressive worsening of umbilical artery Doppler findings.

The presence of meconium staining of membranes, is considered a nonspecific feature of acute or subacute fetal hypoxia [15]. Although histological detection of meconium in term pregnancies correlates poorly with fetal hypoxia, in preterm pregnancies meconium-stained amniotic fluid has been associated with fetal acidemia, grade III–IV intraventricular hemorrhage and adverse neurological sequelae [16,17]. The histological detection of meconium among subjects with severe oligohydramnios might be favored by its higher concentration or by changes in the composition of the amniotic fluid [15].

We also found a significant linear trend, in both univariate and multivariate analysis, between decreasing amniotic fluid volume and fetal thrombotic vasculopathy as expressed by the presence of avascular villi and/or adherent thrombi in umbilical or fetal vasculature. These lesions have been associated with FGR and with increased risks of fetal brain damage [8]. The association between oligohydramnios and fetal thrombotic vasculopathy in FGR has been related to circulatory stasis associated with increased placental vascular impedance, vascular injury associated with the toxic effect of meconium, and the risk of cord compression [18]. Severe fetal vascular damage might also modify trans-placental water gradients and contribute to the net loss of amniotic fluid volume as it has been modeled for vasoconstriction [19].

At variance with other histological variables, lesions of maternal decidual vessels and/or a profile of superficial implantation did not correlate with the severity of amniotic fluid reduction. This was unexpected as decidual arteriopathy is strongly associated with both preeclampsia and 'idiopathic' FGR and correlates with abnormal uterine and umbilical artery Doppler findings [20]. To explain this finding, it should be considered that oligohydramnios is neither causally related to the etiology of FGR nor is an obligate complication of FGR being observed in only 20–30% of cases with acute deterioration of arterial or venous Doppler parameters [3].

The progressive reduction of amniotic fluid volume associated with FGR is thought to be attributable to the redistribution of blood flow favoring fetal heart and brain over lungs, digestive tract and kidney [15]. However, there is evidence that decrease of amniotic fluid volume in FGR pregnancies is not a purely fetal circulatory response to hypoxia [1,2]. In fact, the human placenta can promote water passage from mother to fetus by either hydrostatic or osmotic mechanisms probably regulated by water channel proteins [1,2]. In vitro and animal studies [21,22] suggest that hypoxia and preeclampsia can influence amniotic fluid volume by interfering both with trans-placental water passage and trans-membranous water reabsorption. Venoconstriction caused by intervillous space/villus flow mismatching, as occur in placental underperfusion, can alter the transvillous pressure and affect the transplacental passage of liquids [19,23]. Recent studies have shown that preeclampsia and FGR are associated with increased placental vascular resistance that can be visualized in tissue section by loss of immunostaining for cystathionine γ-lyase, an enzyme involved in the production of hydrogen sulfide, a major placental vasodilator [24,25]. These

Table 5
Multinomial logistic regression of independent pathological variables correlated with the presence of oligohydramnios and its severity.

Pathological variables	Controls normal AFI	FGR normal AFI	FGR mild AFI reduction	FGR moderate AFI reduction	FGR severe AFI reduction
		OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
Chronic villous hypoxia	Reference	4.2 (1.3–13.6)	7.1 (2–25)	6.3 (1.9–21)	9.7[a] (3–31.5)
Meconium staining of membranes	Reference	9.2 (2.6–32.9)	11.6 (3–45.1)	14.4 (3.9–53.3)	25.2[b] (6.9–91.8)

AFI = Amniotic fluid index.
OR (95% CI) = Odds ratio and 95% confidence intervals as obtained by multinormal logistic regression equations including a five-level outcome variable (controls, FGR/no reduction of amniotic fluid, FGR/mild reduction, FGR/moderate reduction, FGR/severe reduction). Explanatory variables included gestational age, placental/neonatal weight ratio, maternal smoking during pregnancy (yes, no, quitted) and either a hypoxic pattern of placental injury (yes, no) or meconium (yes, no).
[a] $p = 0.023$ compared to FGR pregnancies with normal amniotic fluid volume.
[b] $p = 0.005$ compared to FGR pregnancies with normal amniotic fluid volume.

findings offer new opportunities for testing this hypothesis in pathological samples.

In conclusion, we showed that in FGR pregnancies progressive amniotic fluid volume reduction is associated with histological findings of chronic villous hypoxia, meconium staining and fetal thrombotic vasculopathy. These findings support the clinical notion that in FGR pregnancies oligohydramnios is a marker of increased risk of fetal hypoxia and possibly of increased adverse neonatal outcomes.

Conflict of interest statement

No conflict of interest to be declared.

Acknowledgments

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