Purnima Tiwari*, Madhavi M. Gupta and Shyama Lata Jain

Placental findings in singleton stillbirths: a case-control study from a tertiary-care center in India

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Abstract

Objectives: Placental examination in a case of stillbirth can provide insight into causative/associated factors with fetal demise. The aim of this study was to compare placental and umbilical cord pathologies in singleton stillbirth and livebirth placentas, and to find prevalence of various associated maternal and fetal clinical factors.

Methods: This case-control study was conducted at a tertiary-care center in India over a period of 20 months. About 250 women who delivered stillborn fetus ≥28 weeks' gestation and 250 maternal-age-matched controls were recruited. Sociodemographic and clinical details were noted and placental gross and microscopic examination was done. Placental findings were compared between stillbirth and livebirth (overall), preterm stillbirth and preterm livebirth as well as term stillbirth and term livebirth in six categories – placenta gross, cord gross, membranes gross, maternal vascular malperfusion, fetal vascular malperfusion and inflammatory response. Prevalence of 11 maternal and fetal factors were studied in all categories of placental findings in both livebirth and stillbirth.

Results: Placental findings in all six categories were significantly associated with stillbirths ($p<0.05$). The placental findings associated with stillbirth with highest odds included placental hypoplasia (OR 9.77, 95% CI 5.46–17.46), necrotizing chorioamnionitis (OR 9.30, 95% CI 1.17–73.96) and avascular villi (OR 8.45, 95% CI 3.53–20.25). More than half of the women with stillbirths had medical disorders (n=130, 52.0%) and the most prevalent was hypertensive disorder (n=45, 18.0%).

Conclusions: Changes in placenta are associated with development of stillbirth. Therefore, antenatal investigations to identify placental dysfunction should be investigated to determine whether these reduce stillbirth. Also, placental examination in a case of stillbirth can detect/diagnose many maternal/fetal conditions and thereby can help in preventing future stillbirths.

Keywords: high-risk pregnancy; hypertension in pregnancy; placenta; preterm birth; stillbirth.

Introduction

The placenta plays a critical role in fetal development and in *in utero* health of fetus. Its detailed examination in any adverse perinatal event is of paramount importance and is justified by the facts that it lies at the maternal and fetal interface; serves as an essential organ to provide nutrition and blood supply to fetus; is affected by varieties of pathogenic or vascular insult affecting mother or fetus and any placental pathology can jeopardize fate of fetus *in utero* or of infant in later life. A systematic review of studies of placental pathology and stillbirth reported that placental pathologies can be responsible for fetal death in up to 65% of cases [1].

In previous studies, gross and microscopic pathologies of placenta and umbilical cord have been linked to stillbirths [2–18]. Smaller/larger placenta, long/short cord, thick/thin cord, circumvallate or circummarginate insertion of membranes, fetal vascular malperfusions (FVM), maternal vascular malperfusions (MVM) as well as various fetal and maternal inflammatory responses in placental disc have been linked to stillbirths. These findings have also been linked to individual clinical entity/disease in mothers (diabetes, hypertensive disorder of pregnancy, thrombophilia, premature rupture of membranes) or in fetus (fetal anomalies and fetal growth restriction) separately or in combination. However, majority of these studies provide insight into single/few placental findings, lack study of clinical factors (in each category of placental

***Corresponding author: Dr. Purnima Tiwari**, Department of Obstetrics & Gynaecology, All India Institute of Medical Sciences Nagpur, Nagpur, Maharashtra 441108, India,
E-mail: purnima_gupta30@yahoo.co.in
Madhavi M. Gupta, Department of Obstetrics & Gynaecology, Maulana Azad Medical College and associated Lok Nayak Hospital, New Delhi, India
Shyama Lata Jain, Department of Pathology, Maulana Azad Medical College, New Delhi, India

abnormality group) in same cohort of patients, have used non-standardized protocol for placental examination or lack control group to compare findings. Moreover, only few studies have compared findings in term and preterm stillbirth/livebirth placentas [2, 12, 18]. Furthermore, placentas from apparently healthy infants also have reports of abnormal histopathological lesions (particularly chorioamnionitis) [19]. Hence, we conceptualized this case-control study about placental findings in singleton stillbirths with the following objectives: (1) to compare placental and umbilical cord pathologies in singleton stillbirth placentas and singleton livebirth placentas; (2) gestation-specific analysis of pathologies in preterm stillbirth placentas vs. preterm livebirth placentas; and in term stillbirth placentas vs. term livebirth placentas; (3) prevalence of various maternal and fetal clinical factors in mothers with findings in their placentas. We believe that since there is dearth of literature on this topic from the country which shares the maximum burden of stillbirth in the world [20], therefore this study will help in identifying high risk mothers for stillbirths in current or subsequent pregnancies and will foster data in the field of stillbirth.

Materials and methods

Design

This case-control study was conducted at a tertiary care and referral institute in central India over a duration of 20 months after approval from the Institutional Ethics Committee. Cases were women aged 18–40 years and delivered singleton stillborn baby at ≥28 weeks' gestation and controls (maternal age-matched) were women who had liveborn babies at ≥28 weeks' gestation [21]. Participants were recruited from antenatal and labor wards. All the consecutive stillbirths and every 30th liveborn placenta (after reviewing the institute's previous year record for stillborn: live–born ratio) were recruited. The mothers who had premature induced termination of pregnancy because of lethal congenital anomalies and where the complete placenta was not available for examination (morbidly adherent placenta) were excluded from the study. In total, 267 stillbirths occurred over 20 months, among them 13 placentas were either too macerated to examine or were incomplete and four patients did not give consent, hence were excluded from study. Informed consent was obtained from all individuals included in this study. Cases and controls were also followed up till six months postdelivery. We noted the maternal and fetal clinical details along with placental gross and microscopic findings in each of the two groups – stillbirth and livebirths, and compared the prevalence of each placental finding in the two groups. We further divided each group into preterm (<37 weeks') and term (≥37 weeks') because of the variable prevalence of some placental findings according to the gestation and compared the prevalence of different placental findings between preterm stillbirths and preterm livebirths, and between term stillbirths and term live births.

Clinical details

Demographic characteristics of women (age, education, body mass index [BMI]), their clinical details (history of previous stillbirth, medical disorder in current pregnancy such as diabetes/hypertensive disorders of pregnancy [HDP]/thrombophilia/heart disease/autoimmune disorders, history of decreased fetal movement); obstetric details (parity, booked/unbooked pregnancy, any labor complication such as prolonged labor, meconium stained liquor, premature rupture of membrane, abruption, fetal distress); spontaneous/induced labor, gestational age at delivery and fetal details (fetal growth restriction, congenital anomaly/aneuploidy, birth weight) were noted in predesigned proforma.

The BMI range 18.5–24.9 kg/m^2 was considered normal range [22]. The woman was considered booked when she had a minimum of four antenatal visits at our center. Among medical disorders, "diabetes" term included gestational diabetes, type one or type two diabetes mellitus; "hypertensive disorders" term included chronic hypertension, gestational hypertension, pre-eclampsia and eclampsia; "thrombophilia" included all types of hereditary/acquired thrombophilia but excluded autoimmune disorders which were included in autoimmune disorders in separate category; and, thyroid disorders included both hypothyroid and hyperthyroid conditions. Other disorders studied were liver disorders, renal disorders and active infections (latent and past infections were excluded). In some women, the diagnosis of particular disorder/disease was made in postnatal period. If a woman had two or more than two disorders, we included these entities separately. A standard algorithm was used for the estimation of gestational age at death for stillborns and gestational age at birth for liveborns [23]. Small for gestational age fetus was defined when stillborn/infant had birthweight <10th centile as per reference population [24]. Fetal anomalies were confirmed after birth by detailed external examination of baby and/or infantogram/autopsy (where parental consent was available).

Placental and cord examination

After delivery of the placenta, it was examined along with cord for its completeness, for any retroplacental hematoma, meconium staining or maceration. After that, it was washed thoroughly and examined for any gross anomaly (succenturiate lobe); cord was examined for coiling, cord length, velamentous insertion, presence of three umbilical vessels and true knot; and membranes were examined for circumvallate or circummarginate insertion. Placenta was weighed and its dimensions were measured after trimming cord and extraplacental membrane before fixating it in formalin solution. The entire placenta, cord and membranes were sent in a formalin container to the pathology lab where sampling of placenta and histologic examination of placenta was done according to standard protocol by a general pathologist [25]. As per this standard protocol, we analyzed the placental findings under six categories – three categories of gross findings (gross findings of placenta, of umbilical cord and of membranes) and three categories of microscopic findings (maternal vascular malperfusions, fetal vascular malperfusions and maternal/fetal inflammatory responses).

The placenta was classified as hypoplastic when its weight was below 10th centile for standard gestational age [26]. Coiling index was calculated by dividing the total number of coils by total length of umbilical cord in centimeters. We considered 0.20 ± 0.10 coil/cm as

normal coil index [27]. We also studied the prevalence of 11 clinical fetal and maternal factors (two fetal, nine maternal) associated with the six categories of placental findings: fetal – small for gestational age, gross congenital anomaly; maternal – diabetes, hypertensive disorders, thrombophilia, heart disease, autoimmune disease, prolonged labor, premature rupture of membranes, fetal distress and abruption.

Statistical analysis

Mean and standard deviation were used for analyzing continuous variables. Categorical variables were analyzed with percentages and frequencies. Continuous data were analyzed using t-test, whereas Fisher's exact test or Chi-square test were used, when appropriate, for the analysis of categorical data. Difference was considered significant, if p-value was <0.05. Statistical analysis was done on Stata version 13.1 (StataCorp, College Station, Texas 77845 USA). Considering 5% confidence interval and 80% power of a reference study, the sample size estimated was 178 cases and 178 controls, respectively [3].

Results

Maternal and fetal factors

The mean age of patients was similar in stillbirth and livebirth groups (29.5 and 30.0 years, respectively). The majority of women with stillbirth belonged to middle class of socioeconomic status (n=165, 66.0%), were literate (n=178, 71.2%), had normal BMI (n=152, 60.8%), were booked (n=133, 53.2%), were primipara (n=162, 64.8%) and had spontaneous onset of labor (n=162, 64.8%) (Table 1). More than half of the women with stillbirths had medical disorders (n=130, 52.0%) and the most prevalent were hypertensive disorders (n=45, 18.0%). The proportion of patients with labor complications was higher in the stillbirth group (30.4%) than livebirths (15.2%). Other complications in the two groups included scar dehiscence (7 vs. 3), ruptured uterus (1 vs. 0), cord prolapse (4 vs. 3), shoulder dystocia (3 vs. 2) and placenta previa (10 vs. 12). Total 135 stillborns were preterm while only 11 liveborn were preterm babies. The total number of antepartum stillbirths was 165 (66%) and intrapartum stillbirths was 85 (34%). Among the congenital anomalies in the stillbirth group, the most prevalent were neural tube defects (n=25) followed by non-immune hydrops (n=16), cardiac defects (n=11), anencephaly 4(n=), trisomy 21 (n=3), multicystic dysplastic kidney (n=1) and bilateral renal agenesis (n=1). In control group also, neural tube defects were the most prevalent congenital anomalies (n=5).

Table 1: Comparison of maternal and fetal factors in stillbirths and livebirths.

S. No.	Factors	Stillbirths (n= 250) (%)	Livebirths (n=250) (%)
1	Mean age, years	29.5 ± 1.0	30.0 ± 0.9
2.	Socioeconomic status		
	Upper	29 (11.6)	31(12.4)
	Middle	165 (66.0)	143 (57.2)
	Lower	56 (22.4)	76 (30.4)
3.	Education		
	Illiterate	72 (28.8)	56 (22.4)
	Literate	178 (71.2)	194 (77.6)
4.	Body mass index, kg/m^2		
	Underweight	40 (16.0)	34 (13.6)
	Normal	152 (60.8)	175 (70.0)
	Overweight	58 (23.2)	41 (16.4)
5.	History of previous stillbirth		
	Yes	34 (13.6)	12 (4.8)
	No	216 (86.4)	238 (95.2)
6.	Booking status		
	Unbooked	117 (46.8)	19 (7.6)
	Booked	133 (53.2)	231(92.4)
7.	History of medical disorder in current pregnancy	130 (52.0)	60 (24.0)
	Diabetes	38 (15.2)	9 (3.6)
	Hypertension	45 (18.0)	17 (6.8)
	Thrombophilia	8 (3.2)	1 (0.4)
	Autoimmune	11(4.4)	1 (0.4)
	Heart Disease	24 (9.6)	8 (3.2)
	Other	20 (8.0)	35 (14.0)
8.	History of decreased fetal movement		
	Yes	68 (27.2)	5 (2.0)
	No	182 (72.8)	245 (98.0)
9.	Parity		
	Primipara	162 (64.8)	79 (31.6)
	Multipara	88 (35.2)	171 (68.4)
10.	Labor onset		
	Spontaneous	162 (64.8)	196 (78.4)
	Induced	88 (35.2)	54 (21.6)
11.	Complications in labor	76 (30.4)	38 (15.2)
	Prolonged Labor	31 (12.4)	10 (4.0)
	Meconium stained liquor	15 (6.0)	8 (3.2)
	PROM	17 (6.8)	7 (2.8)
	Abruption	16 (6.4)	9 (3.6)
	Fetal distress	24 (9.6)	15 (6.0)
	Other	25 (10.0)	20 (8.0)
12.	Gestational age at delivery		
	<34 weeks	75 (30.0)	4 (1.6)
	34-37 weeks	60 (24.0)	7 (2.8)
	>37 weeks	115 (46.0)	239 (95.6)
13.	Gross congenital anomaly		
	Yes	62 (24.8)	11 (4.4)
	No	188 (75.2)	239 (95.6)
14.	Small for gestational age fetus		
	Yes	60 (24.0)	25 (10.0)
	No	190 (76.0)	225 (90.0)

Placental findings

Placental findings overall and on gestation-specific analysis

The placental findings associated with stillborn placentas with highest odds included placental hypoplasia (OR 9.77, 95% CI 5.46–17.46), necrotizing chorioamnionitis (OR 9.30, 95% CI 1.17–73.96) and avascular villi (OR 8.45, 95% CI 3.53–20.25). All the six broad categories of placental findings, i.e., placental gross (OR 7.83, 95% CI 4.86–12.60), cord gross (OR 4.84, 95% CI 3.11–7.55), membrane gross (OR 1.74, 95% CI 1.01–2.99), maternal vascular malformations (OR 4.14, 95% CI 2.82–6.09), fetal vascular malformations (OR 7.75, 95% CI 4.72–12.74) and inflammatory findings (OR 2.34, 95% CI 1.58–3.47), were more common (p value <0.05) in stillborn placentas than liveborn placentas (Table 2).

On gestation-specific analysis, gross placental/cord findings which were more prevalent in preterm stillbirth placentas included retroplacental hemorrhage, succenturiate lobe, abnormal coiling index, abnormal length of cord and meconium staining of membranes. In histology, retroplacental hemorrhage, absence of spiral artery

Table 2: Comparison of placental findings between stillbirths and livebirth.

Pathologic findings	SB[a] (n=250) (%)	LB[b] (n=250) (%)	OR (95% CI)	p-Value
1. Gross findings – placental disc	**119 (47.6)**	**26 (10.4)**	**7.83 (4.86–12.60)**	**<0.001**
i) Placental hypoplasia	96 (38.4)	15 (6.0)	9.77 (5.46–17.46)	<0.001
ii) Retroplacental Hemorrhage	13 (5.2)	7 (2.8)	1.90 (0.75–4.86)	0.178
iii) Succenturiate Lobe	10 (4.0)	4 (1.6)	2.57 (0.79–8.28)	0.116
2. Gross findings – umbilical cord	**106 (42.4)**	**33 (13.2)**	**4.84 (3.11–7.55)**	**<0.001**
i) Hypocoiled	18 (7.2)	7 (2.8)	2.69 (1.11–6.57)	0.029
ii) Hypercoiled	22 (8.8)	9 (3.6)	2.58 (1.17–5.73)	0.020
iii) Long/short cord	47 (18.8)	12(4.8)	4.59 (2.37–8.89)	<0.001
iv) Velamentous cord insertion	5 (2.0)	1 (0.4)	5.08 (0.59–43.81)	0.139
v) Two vessel cord	8 (3.2)	3 (1.2)	2.72 (0.71–10.38)	0.143
vi) True knot	4 (1.6)	1 (0.4)	4.05 (0.45–36.49)	0.213
3. Gross findings – membranes	**39 (15.6)**	**24 (9.6)**	**1.74 (1.01–2.99)**	**0.045**
i) Meconium staining	32 (12.8)	18 (7.2)	1.80 (1.03–3.47)	0.039
ii) Circumvallate insertion	4 (1.6)	5 (2.0)	0.80 (0.211–3.00)	0.737
iii) Circummarginate insertion	3 (1.2)	1(0.4)	3.02 (0.31–29.27)	0.340
4. Maternal vascular malperfusions	**139 (55.6)**	**58 (23.2)**	**4.14 (2.82–6.09)**	**<0.001**
i) Distal villous hypoplasia	19 (7.6)	8 (3.2)	2.49 (1.07–5.80)	0.035
ii) Accelerated villous maturation	5 (2.0)	1 (0.4)	5.08 (0.59–43.81)	0.139
iii) Infarct	21 (8.4)	6 (2.4)	3.73 (1.48–9.40)	0.005
iv) Retroplacental hemorrhage	26 (10.4)	7 (2.8)	4.30 (1.71–9.47)	0.001
v) Decidual arteriopathy				
(1) Arterial thrombosis	15 (6.0)	2 (0.8)	7.91 (1.79–34.99)	0.006
(2) Fibrinoid necrosis of vessels	38 (15.2)	11 (4.4)	3.89 (1.94–7.81)	<0.001
(3) Absence of spiral artery remodeling	15 (6.0)	23 (9.2)	0.63 (0.32–1.24)	0.180
5. Fetal vascular malperfusions	**110 (44.0)**	**23 (9.2)**	**7.75 (4.72–12.74)**	**<0.001**
i) Avascular villi	43 (17.2)	6 (2.4)	8.45 (3.53–20.25)	**<0.001**
ii) Thrombosis (arterial/venous)	37 (14.8)	12 (4.8)	3.45 (1.75–6.78)	**<0.001**
iii) Intramural fibrin deposition	45 (18.0)	25 (10.0)	1.98 (1.17–3.34)	**0.011**
6. Inflammatory response	**98 (37.2)**	**54 (21.6)**	**2.34 (1.58–3.47)**	**<0.001**
i) Maternal				
(1) Acute chorionitis	12 (4.8)	5 (2.0)	2.47 (0.86–7.12)	0.094
(2) Acute chorioamnionitis	57 (22.8)	27 (10.8)	2.44 (1.48–4.01)	<0.001
(3) Necrotizing chorioamnionitis	9 (3.6)	1 (0.4)	9.30 (1.17–73.96)	0.036
ii) Fetal				
(1) Chorionic vasculitis/cord phlebitis	12 (4.8)	19 (7.6)	0.61 (0.29–1.29)	0.198
(2) Umbilical arteritis	8 (3.2)	2 (0.8)	4.10 (0.86–19.50)	0.076

[a]Stillbirth; [b]livebirth.

remodeling, thrombosis of vessels, intramural fibrin deposition, acute chorioamnionitis and chorionic vasculitis/cord phlebitis were more prevalent in preterm stillbirth placentas than preterm livebirth placentas. Placental gross findings more common in term stillbirth than term livebirth included placental hypoplasia, retroplacental hemorrhage, abnormal coiling index, abnormal length of cord and meconium staining; among the histological findings, infarction of placental parenchyma, retroplacental hemorrhage, fibrinoid necrosis of vessels, avascular villi, thrombosis of vessels, intramural fibrin deposition and acute chorioamnionitis were more prevalent in term stillbirths (Table 3).

Various placental findings and maternal/fetal clinical factors

Placental gross findings

The absolute number of women in both the groups (cases and controls) with concerned clinical factor was compared in each category of placental finding (Figure 1A–F). The most prevalent maternal factor among cases in gross placental category was hypertensive disorders (n=15) followed by abruption (n=12) and growth restricted fetus were more in number (n=45) than anomalous fetus (n=34) (Figure 1A). Out of total 96 stillbirth hypoplastic placentas,

Table 3: Gestation-specific analysis of placental findings.

Pathologic findings	PT SB[a] (n=135) (%)	PT LB[b] (n=11) (%)	p[1]-Value	T SB (n=115) (%)	T LB[d] (n=239) (%)	p[2]-Value
1) Gross findings – placental disc						
i) Placental hypoplasia	43 (31.9)	4 (36.4)	0.758	53 (46.1)	14 (1.7)	<0.001
ii) Retroplacental hemorrhage	7 (5.2)	4 (36.4)	0.002	6 (5.2)	3 (1.3)	0.041
iii) Succenturiate lobe	4 (3.0)	3 (27.3)	0.003	6 (5.2)	1 (0.4)	0.180
2) Gross findings – umbilical cord						
i) Hypocoiled	10 (7.4)	3 (27.3)	0.040	8 (7.0)	4 (1.7)	0.018
ii) Hypercoiled	12 (8.9)	4 (36.4)	0.01	10 (8.7)	5 (2.09)	0.008
iii) Long/short cord	13 (9.6)	8 (72.7)	<0.001	34 (29.6)	4 (1.7)	<0.001
iv) Velamentous cord insertion	4 (3.0)	1 (9.1)	0.309	1 (0.9)	0 (0.0)	0.264
v) Two vessel cord	5 (3.7)	2 (18.2)	0.050	3 (2.6)	1 (0.4)	0.110
vi) True knot	1 (0.7)	1 (9.1)	0.074	3 (2.6)	0 (0.0)	0.075
3) Gross findings – membranes						
i) Meconium staining	13 (9.6)	6 (54.5)	<0.001	19 (16.5)	12 (5.0)	<0.001
ii) Circumvallate insertion	1 (0.7)	1 (9.1)	0.074	3 (2.6)	4 (1.7)	0.056
iii) Circummarginate insertion	2 (1.5)	0 (0.0)	0.594	1 (0.9)	1 (0.4)	0.604
4) Maternal vascular malperfusions						
i) Distal villous hypoplasia	17 (12.6)	1 (9.1)	0.735	11 (9.6)	10 (4.2)	0.064
ii) Accelerated villous maturation	23 (17.0)	1 (9.1)	0.503	9 (7.8)	11 (4.6)	0.263
iii) Infarct	6 (4.4)	1 (9.1)	0.498	15 (13.0)	5 (2.1)	<0.001
iv) Retroplacental hemorrhage	13 (9.6)	5 (45.5)	0.002	13 (11.3)	2 (0.8)	<0.001
v) Decidual arteriopathy						
(1) Arterial thrombosis	7 (5.2)	1 (9.1)	0.589	8 (7.0)	1 (0.4)	0.007
(2) Fibrinoid necrosis of vessels	17 (12.6)	4 (36.4)	0.042	21 (18.3)	7 (2.9)	<0.001
(3) Absence of spiral artery remodeling	12 (8.9)	8 (72.7)	<0.001	3 (2.6)	15 (6.3)	0.135
5) Fetal vascular malperfusions						
i) Avascular villi	20 (1.5)	4 (36.4)	0.077	23 (20.0)	2 (0.8)	<0.001
ii) Thrombosis (arterial/venous)	14 (10.4)	4 (36.4)	0.020	21 (18.3)	8 (3.4)	<0.001
iii) Intramural fibrin deposition	15 (11.1)	6 (54.5)	<0.001	30 (26.1)	19 (8.0)	<0.001
6) Inflammatory response						
i) Maternal						
(1) Acute chorionitis	8 (5.9)	2 (18.2)	0.144	4 (3.5)	3 (1.3)	0.177
(2) Acute chorioamnionitis	37 (27.4)	7 (63.6)	0.019	20 (17.4)	12 (5.0)	<0.001
(3) Necrotizing chorioamnionitis	6 (4.4)	1 (9.1)	0.498	3 (2.6)	0 (0.0)	0.075
ii) Fetal						
(1) Chorionic vasculitis/cord phlebitis	7 (5.2)	9 (81.8)	<0.001	5 (4.4)	10 (4.2)	0.943
(2) Umbilical arteritis	4 (3.0)	1 (9.1)	0.309	4 (3.5)	1 (0.4)	0.056

[a]Preterm stillbirth; [b]preterm livebirth; [c]term stillbirth; [d]term livebirth.

40 were associated with SGA fetus while out of 15 livebirth hypoplastic placentas, 10 had SGA baby.

Umbilical cord gross findings

Total 11 fetuses were SGA in stillbirth group: five had abnormal coiling index, three had both abnormal coiling index and short/long cord and one had long cord while hypertensive disorder were the most prevalent maternal factor in this category (23 women: 12 had abnormal coiling index, eight had short/long cord, two had two vessel cord and one had true knot) (Figure 1B).

Membrane gross findings

Meconium staining of placenta was significant finding in stillbirth group (overall, preterm, term) in this category. In stillbirth group, total 16 fetus had congenital anomalies: 10 had meconium staining, three had circumvallate insertion and three had circummarginate insertion of cord. The majority of women (13) had prolonged labor (10 had meconium staining) in this category (Figure 1C).

Maternal vascular malperfusions

Distal villous hypoplasia (OR 2.49, 95% CI 1.07–5.80), infarction of placental tissue (OR 3.73, 95% CI 1.48–9.40), retroplacental hemorrhage (OR 4.03, 95% CI 1.71–9.47), arterial thrombosis (OR 7.91, 95% CI 1.79–34.99), fibrinoid necrosis of vessel (OR 3.89, 95% CI 1.94–7.81) were significant findings in stillbirth group. The number of anomalous fetus (n=17: nine had distal villous hypoplasia) was higher than SGA fetus (n=12) in stillbirth group and hypertensive disorder was the most prevalent maternal factor (25: 15 had absence of remodeling) followed by heart disease (21: seven had arterial thrombosis and five had fibrinoid necrosis) (Figure 1D).

Fetal vascular malperfusions

Avascular villi (OR 8.45, 95% CI 3.53–20.25), thrombosis of vessels (OR 3.45, 95% CI 1.75–6.78), intramural fibrin deposition (OR 1.98, 95% CI 1.17–3.34) were significant placental findings in stillbirth group. In this group, total 21 fetus had congenital anomalies and 15 fetus had arterial thrombosis in placenta out of these 21 fetus. The majority of the mothers who had FVM in their placentas had hypertensive disorder in both stillbirth group and livebirth group (33 and 15) (Figure 1E).

Inflammatory response

Among all kind of inflammatory responses, acute chorioamnionitis (OR 2.44, 95% CI 1.48–4.01) and necrotizing chorioamnionitis (OR 9.30, 95% CI 1.17–73.96) were significant placental inflammatory responses in stillbirth group. The majority of women had hypertensive disorder in both stillbirth and livebirth groups (26 vs. 17) (Figure 1F). We had one interesting case where an unbooked patient

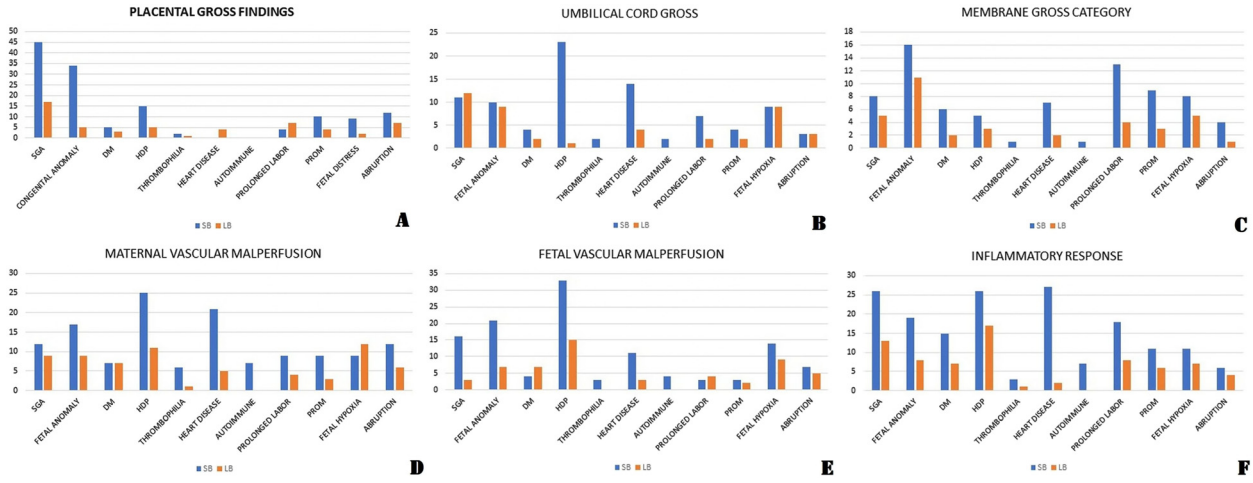

Figure 1: Prevalence of various maternal and clinical factors in different category of placental findings in stillbirth group and livebirth group. (A) Placental gross category. (B) Umbilical cord gross category. (C) Membranes gross category. (D) Maternal vascular malperfusion category. (E) Fetal vascular malperfusion category. (F) Maternal/fetal inflammatory response category.

came at study center at 32 weeks period of gestation in labor with intra-uterine fetal death. She delivered stillborn fetus and her placenta was sent for examination. Gross examination was normal but placental histopathology revealed caseous granulomas with Langhans giant cells. The patient was asymptomatic at the time of presentation. On follow-up, she was diagnosed as a case of miliary tuberculosis and was started on anti-tubercular drugs as per protocol.

Discussion

The placenta plays a very crucial role in the development of fetus. Hence, placental evaluation holds paramount importance, particularly in adverse perinatal outcomes. We evaluated and compared placental and umbilical cord pathologies in singleton stillbirth placentas with singleton livebirth placentas, and did a gestation-specific analysis of placental pathologies in stillbirths and livebirths. We found that placental findings in all the six categories were more common in stillbirths than livebirths. Also, the placental findings associated with stillborn placentas with highest odds included placental hypoplasia, necrotizing chorioamnionitis and avascular villi.

While comparing placental weight, we found that smaller placentas had significant association with stillbirths especially with term stillbirths. Similar to our finding Hutcheon et al. found association between lower placental weight and risk of stillbirth [4]. Furthermore, SGA and hypertensive disorders were the most prevalent finding in this category, implying that hypertensive disorders may affect placental development and its weight which in turn can affect fetal growth [5]. Various reasons have been hypothesized for this association. McDonald et al. hypothesized that low level of pro-angiogenic factor leads to smaller placenta, which in turn is responsible for inadequate nutrient support to fetus and adverse perinatal outcome [6]. The role of high level of anti-angiogenic proteins and low proangiogenic proteins have been also seen in SGA fetus and pre-eclampsia in literature [28]. Placentas with succenturiate lobe were significantly associated with stillbirth after 37 weeks of gestation in our study as has been previously reported also [7]. Machin et al. and Tantbirojn et al. concluded that abnormal coiling index is associated with risk of stillbirth [8, 9]. We also found that umbilical cord coiling abnormalities significantly were more prevalent in the both the preterm and term stillbirth group. We also found that the higher numbers of babies were SGA in cord gross category. Sharma et al. conducted a cross-sectional study in 408 antenatal women and found that abnormal coiling index calculated at antenatal scan at 18–20 weeks of gestation was associated with preterm birth and low birth weight [29]. The clinical implication of above findings is that placental and cord (coiling index) assessment during antenatal scans is very important and can detect mothers at high risk of SGA babies, hypertensive disorders and fetal demise.

Meconium staining of placenta may indicate *in utero* fetal hypoxia. Fetal anomalies and prolonged labor both have been established as a risk factor for fetal hypoxia and hence may lead to passage of meconium that in turn may lead to fetal death [10]. Therefore, anytime during pregnancy or labor, presence of meconium in liquor or prolonged labor should alarm the obstetrician and such mothers should be kept under intense monitoring to save fetus. We could not find any significance of circumvallate and circummarginate insertion with studied outcome. However, contrary to our findings, Suzuki S found that incidence of intrauterine fetal death and other complications (premature delivery, oligohydramnios, etc.) was significantly higher in patients with circumvallate placentas than that in controls [11].

The normal and adequate blood flow to maternal vessels is essential to provide oxygen and nutrients to fetus and therefore, any maternal vascular pathology can affect growth of fetus and further outcome of pregnancy. Uterine vasculature aberrations from the normal remodeling process are central to the pathologic process in development of MVM. This leads to either abnormal high velocity and turbulent flow through un-remodeled spiral arterioles or reduced blood flow secondary to reduced vascular capacity. Among the livebirths, MVMs were the most frequent microscopic pathologies observed in our study (23%). Similarly, Pathak et al. found that chronic placental underperfusion was found in a substantial number of normal singleton pregnancies (7%) [19]. Man et al. found that MVMs were the most frequent placental abnormalities associated with stillbirth [18]. Similarly, MVMs were found in 56% of stillbirths in our study. Pre-eclampsia has strong association with MVM histopathological findings. Autoimmune disorders such as antiphospholipid antibody syndrome and systemic lupus erythematosus have also been found associated with MVM [30]. Similar to above findings; in our study, 55.55% of total hypertensive mothers and 63.63 % of total mothers with autoimmune disorders in stillbirth group had MVM in their placenta. The recurrent rate of such lesions can be upto 25% in next pregnancy [5]. Similar to our findings, MVM have been associated with fetal death in previous studies also [12–14, 30]. In literature, the association between fetal anomalies

(especially congenital heart defect) and risk of pre-eclampsia in mothers have been described and placental vascular malperfusions have been found as a common finding in both type of placentas implying the role of common etiologic factors in fetal anomalies and hypertensive disorders that in turn may lead to stillbirth [31]. If MVM are diagnosed in a case of stillborn placentas, further complete maternal check-up to look for hidden/latent hypertensive disorders or autoimmune disorders are warranted and more intensive monitoring in next pregnancy is of paramount importance. Various maternal conditions (thrombophilic disorders [protein C deficiency, protein S deficiency, factor V leiden mutation], preeclampsia and anti-phospholipid antibody syndrome) and various fetal conditions (fetal growth restriction, fetal anomalies, fetal infection and fetal death) have been associated with changes of FVM [15]. We also found that FVM were significantly more common in stillbirth placentas and 37.5% women with thrombophilia had FVM changes in placenta. More number of stillbirths had congenital anomalies in this category implying that screening for hypertensive disorders and thrombophilia is needed for such mothers who had placenta with FVM. However, we agree that it is difficult to differentiate that whether these FVM changes are before or after demise of fetus *in utero* and this can be a major limitation in interpretating data in this specific category.

Placenta is formed by three major units: placental disc, chorioamniotic membranes and umbilical cord. Acute inflammatory lesions can involve either one or two or all of these three units. Acute chorioamnionitis is defined as histologic evidence of neutrophilic infiltration in these membranes but this response may be due to infective or to non-infective etiology. In our study, all cases of fetal inflammatory responses were associated with acute chorioamnionitis. Inflammatory disorders especially chorioamnionitis have been associated with stillbirths [15, 32]. Lahra et al. concluded that absence of fetal inflammatory response is associated with antepartum unexplained stillbirths [16]. We also found that more livebirth placentas had fetal inflammatory response than stillbirth placentas; however, this difference was not significantly associated in our study. Recent literature suggests that non-infective inflammation of chorioamniotic membranes can also be caused by endogenous mediators known as "damage-associated molecular patterns (DAMPs)" or alarmins which should be explored more in future [17]. Similar to our finding, Williams et al. studied total 2,579 infants and concluded that histologic chorioamnionitis was associated with markers of fetal growth restriction in both preterm and term infants [33].

The strength of our study was that it was conducted in a tertiary-care referral institute with patients coming from various parts of the country and therefore has a heterogenous patient population. Secondly, studying the placental lesions associated with stillbirths has more significance, when it is done in those parts of the world which contribute the largest proportion of the caseload, as was done in this study. Also, we included livebirths as controls further increasing the strength of the study. This study is more informative as patients' clinical findings were also studied with their placental findings. However, this study had some limitations that should be considered while interpreting the results. First, the pathologists were not blinded to the outcome – stillbirth or livebirth. Secondly, on gestation-specific analysis, the sample size in the subgroups was relatively small. Therefore, more such kind of studies at larger scale are needed in evaluating the role and association of placental findings in stillbirths. However, as very few of this kind of studies are available from the developing world, our results will foster future research in this direction and will provide baseline data to the Obstetricians and the policy-makers alike. Also, there may be significant overlap between macroscopic findings and microscopic findings of placenta in our study, however, our categorization of the placental findings in the two broad groups (macroscopic and microscopic) was not mutually exclusive. The six categories of placental lesions were chosen to comprehensively characterize the placental findings including both macroscopic and microscopic findings. Moreover, some selection bias might be present in the study as majority of participants who had a stillbirth were of middle-class and were literate, contrary to the findings in literature. However, those contradictory findings might also reflect the population in the study area.

Our study identified that both gross (gross lesions of placenta, umbilical cord and membranes) and placental microscopic lesions (maternal vascular malperfusions, fetal vascular malperfusions and inflammatory response) were associated with stillbirths. Hypertensive disorders, fetal anomalies and growth restriction were the most prevalent clinical factors in stillbirth fetus with placental pathology. Many of such placental lesions (weight, succenturiate lobe, coiling index) can be diagnosed antenatally and these pregnancies should be monitored carefully to prevent intrauterine fetal death. Many placental lesions when identified postnatally (MVM, FVM) warrant evaluation of mothers to rule out hypertensive disorders and/or autoimmune disorders. Furthermore, identifying placental pathology in stillbirth placenta may allay anxiety of mother/parents to some extent where no direct fetal/maternal cause can be identified. To conclude, placental

examination in a case of stillbirth can detect/diagnose many maternal/fetal conditions and thereby can help in preventing future stillbirths in high-risk mothers.

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Ethical approval: Research involving human subjects complied with all relevant national regulations, institutional policies and is in accordance with the tenets of the Helsinki Declaration (as revised in 2013), and has been approved by the authors' Institutional Review Board (Institutional Ethics Committee, Maulana Azad Medical College, New Delhi, India) or equivalent committee. (F.11/IEC/MAMC/10/No. 199, dated 20th November 2013).

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