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HISTOMORPHOLOGICAL CHANGES IN MONOCHORIONIC MEMBRANE OF PLACENTAE IN DIABETIC MOTHERS AND ITS CORRELATION WITH FETAL OUTCOME

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ABSTRACT:
BACKGROUND: Currently the prevalence of diabetes mellitus (DM) among women of childbearing age is increasing with abnormal maternal glucose tolerance in 3- 10% of pregnancies. The placenta and placental membranes or amniochorionic membrane (ACM) are affected by DM during pregnancy produces variety of abnormalities which may results in disturbances in fetal growth and development.
OBJECTIVES: The aim of this study is to observe gross and microscopic changes in placenta and amniochorionic membrane in mothers with GDM and their correlation with fetal outcome.
MATERIALS AND METHOD: This Cross sectional study was conducted on 49 full term pregnant women with diagnosis GDM. After brief history the placenta along with its membrane was collected immediately after delivery. ACM were separated and collected after gross morphological examination, tissue is prepared for microscopic examination. Slides were observed under microscope and changes were noted. The data was entered and analyzed on SPSS-25
RESULTS: The placenta and placental membranes revealed significant changes in diabetes. The site of placental rupture in GDM placentae was precise; the placental insertion was most frequently circumvallate 38.8% with thick basement membrane. Macrosomia is significantly associated with abnormal ACM.
DISCUSSION: In GDM weight, thickness and diameter of placenta were increased. On microscopy fibrinoid necrosis, increased syncytial knots, trophoblastic basement membrane thickening, villous stromal fibrosis and villous edema was observed with strong correlation with fetal macrosomia.

KEY WORDS: Fetus; Histological; Macrosomia; Placenta; Placental membrane

INTRODUCTION:

The global incidence of GDM is round 14%. In Pakistan its prevalence ranges from 4.2-26% subjected to multiple factors such as geographical locale, sampling technique, awareness among study population and healthcare facilities. The fetomaternal complications of GDM occur both in pre natal and post natal period. GDM affects adversely on placenta, which is unique endocrine structure developing from both mother and fetus for supporting fetal development. In GDM several changes in the placental morphology occur, in response to variable levels of glucose during the critical periods of placental development [1, 2, 3].

Significant changes in placental morphology are change in placental size, increase in numbers of the villi, glycogen and fibrinoid deposition. Other modifications include villous immaturity, villous edema, fibrinoid necrosis, syncytial knots. Alteration in placental functions disturbs the nutritional supplies resulting in many fetal anomalies such as macrosomia, congenital malformations and intrauterine growth retardation. A good glycemic control will result in reducing diabetes induced fetomaternal complications [4].

ACM is an essential component of the placenta forms internal lining of the uterine cavity developing from extra-embryonic mesoderm. GDM can lead to vascular changes in the placenta, such as thickening of the basement membrane, endothelial cell damage, and fibrosis. These changes may result in impaired blood flow to the fetus, leading to intrauterine growth restriction (IUGR) or fetal macrosomia, depending on the severity of the vascular compromise [5, 6].

Alteration in ACM in placentae from mothers with GDM is very limited. Very scare data is available on changes in ACM in GDM. The role of ACM in contribution of pregnancy related comorbidities such as GDM is often neglected. In GDM often patients presents with infections, oligohydramnios and polyhydramnios which impacts function of ACM adversely [7].

Therefore this study is designed to understand the correlation between these histomorphological changes and fetal outcomes.

OBJECTIVES:

1) To observe the gross and microscopic changes in placental amniochorionic membrane in placentae from GDM mother.
2) To observe the correlation of changes in amniochorionic membrane with fetal outcome in GDM.

MATERIALS AND METHOD:

This Cross sectional study was conducted at Department of Anatomy with collaboration of Department of Gynecology and Obstetrics (OBG) and Department of Pathology, Liaquat University of Medical and Health Sciences (LUMHS), Jamshoro/Hyderabad after ethical approval from Research Ethical Committee. In this study 49 full term pregnant women with diagnosis of GDM admitted in the labor room of OBG, LUH, Hyderabad /Jamshoro were included. In all cases written informed consent from patient or attendant of the patients regarding study were obtained. The age of parturients is between 20–40 years and was from low socioeconomic group with parity ranged from primi gravida to gravida 6 [8].

SAMPLE COLLECTION:

After brief medical, obstetrical and drug history, demographic data regarding maternal age, weight, Hb A1c level, gestation age, parity was recorded on predesigned data sheet for analysis. All placentae were either by vaginal/caesarean delivery was included. The placenta along with its membrane was collected immediately after delivery from labor room or operation theatre at LUH. Fetal weight, APGAR score and outcome were noted. Amniochorionic membranes were separated and collected after gross morphological examination of placenta and amniochorionic membranes [9, 10].

The amniochorionic membranes were separated from the placenta disc with sharp scissor from the site of rupture. The ‘jelly roll’ method was applied to obtain a maximum amount of membranes. A “membrane roll” was made by taking a strip approximately 10 cm wide, and with forceps grasps the

portion representing the rupture site. Roll the membranes with the rupture site in the center and with the amnion inward. This roll can be briefly fixed and then cross-sections taken (preferably two) and submitted for microscopic examination. Tissue sample was collected in plastic container labeled with patient's identification code number, containing 10% buffered formalin for preserving the specimens [11].

For histological examination the placental membrane sections were fixed in 10% formalin for 3-4 days and then processed for routine tissue processing and sectioning. The APEC coated slides were examined under microscope at different magnification with pathologist. Microscopic changes were noted and images were captured with attached camera [12].

DATA ANALYSIS:

The data was entered and analyzed on SPSS-25. Mean, standard deviation was calculated for continuous variable and frequencies and percentages were calculated for categorical variables such as morphological variations to observe the effects on outcomes via Chi-square test and p-value of <0.05 was considered as statistically significant.

RESULTS:

In this study total 49 placentae with membranes from parturients diagnosed with gestational diabetes mellitus were studied. Mean age of study population was 37.65 years, parity was 1.32 while Mean ±SD of gestational age at the time of delivery was 38.71±0.73weeks and mean of FBS was 271.36.12±101.13 mg/dl and BMI was 29.95± 5.12 depicted in table 1 and gross placental parameters were examined and results were depicted in table 2

Table 1 Demographic characteristic of study population

	Minimum	**Maximum**	**Mean**	**Std. deviation**
Maternal age	34	43	37.65	3.50
Parity	1.00	2.00	1.32	0.47
Gestational age	38.00	40.00	38.7143	0.73598
FBS mg/dl	100.00	500.00	271.3673	101.13331
BMI kg/m^2	20.00	40.00	29.9592	5.12

Table 2 Placental Parameters

Placental Parameter	**Mean**	**Std. deviation**
Weight	593.1	9.6
Diameter	16.938	0.250
Thickness	3.0145	0.0735
No. of cotyledons	20.24	2.51

The site of placental rupture in GDM placentae was precise in 04 cases while remaining 45 shows imprecise rupture (table 3). The placental insertion was most frequently circumvallate 38.8%, while it was normally inserted in 26.5 % placenta. When considering the appearance of placental membranes, it was observed that 51% membranes were meconium stained, 24.5% were thick, and 22.4% were opaque and only 2% were thin (table 4)

Table 3 Site of rupture in placenta of GDM

	Frequency	Percent
Precise	4	8.2
Imprecise	45	91.8
Total	49	100.0

Table 4 Characteristics of placental membranes

		Frequency	Percent

Insertion	Marginal	17	34.7
	Circumvallate	19	38.8
	Normal	13	26.5
	Total	49	100.0
Appearance	Thin	1	2.0
	Thick	12	24.5
	Opaque	11	22.4
	Meconium stained	25	51.0
	Total	49	100.0

When fetoplacental membranes were observed for any abnormalities, it was revealed that 16.3% had retro-membrane hemorrhage and in 12.2% edema was seen. In majority of the membranes, there were placental infarcts, i.e., 71.4% in figure 1.

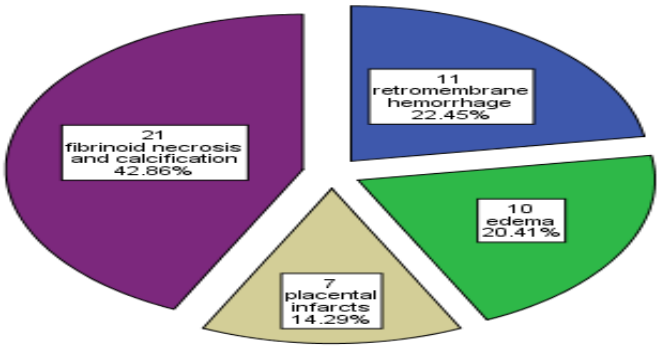


Figure 1 Pie chart; histological findings in amniochorionic membranes

Mean of body weight ad Apgar score of baby at the time of delivery was 3.78kg ±0.34 and 6.02±1.2 respectively. The Apgar score after 5 minutes was 7.65±1.33.Table 5

Table 5 Body weight and Apgar score of new born in GDM patients

	Minimum	Maximum	Mean	Std. Deviation
Weight of baby	2.30	4.00	3.7816	0.34014
Apgar score at time of birth	4	8	6.02	1.233
Apgar after 05minutes	5	9	7.65	1.332

The most common fetal outcome in patients of GDM was macrosomia, i.e., in (18)36.7% of births. The frequency (%) of hyperbilirubinemia and admission to NICU/RDS was 15(30.6%) and 15(30.6%) respectively. The spina bifida was present only in one pregnancy out of 49 shown in figure 2.

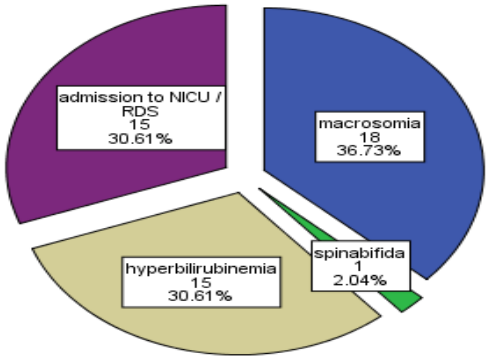
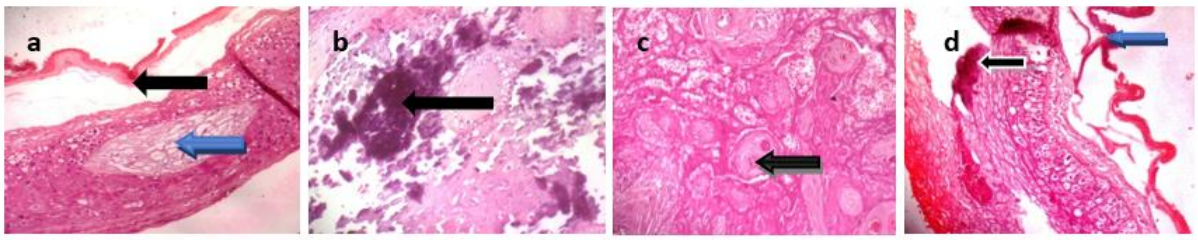


Figure 2: Pie chart showing fetal outcome in GDM mothers

Table 6: Association of histological findings with fetal outcome

Histological findings	Fetal outcome				Total
	Macrosomia	Spinabifida	Hyperbilirubinemia	Admission to NICU	
Retromembrane hemorrhage	6	1	1	3	11
	33.3%	100.0%	6.7%	20.0%	22.4%
Edema	1	0	6	3	10
	5.6%	0.0%	40.0%	20.0%	20.4%
Placental infarcts	1	0	1	5	7
	5.6%	0.0%	6.7%	33.3%	14.3%
Fibrinoid necrosis and calcification	10	0	7	4	21
	55.6%	0.0%	46.7%	26.7%	42.9%
Total	18	1	15	15	49
	100.0%	100.0%	100.0%	100.0%	100.0%

Most common histological finding in amniochorionic membranes of GDM patients was fibrinoid necrosis and calcification, i.e., 21(49.2%). The retro membrane hemorrhage was found in 11(22.4%) amniochorionic membrane histological sections and edema in 10(20.4%), while placental infarcts were present in 07 (14.3%) amniochorionic membranous tissues. Macrosomia is significantly associated with amniochorionic membranous histological findings, i.e., fibrinoid necrosis and retro membrane hemorrhage table 6 with Chi square value= 17.90 and *p*- value=0.03 with df-6



Photomicrograph (a) showing thick basal plate (Black) and edematous villi (blue) arrow,(b) calcification with black arrow, (c) thrombosed vessel with black arrow, (d) showing Fibrinoid necrosis (black) and thick basal plate (blue) with arrow (H&EX10)

DISCUSSION:

GDM is the carbohydrate intolerance of inconsistent severity with onset or first identification in pregnancy. The gross morphological as well as histological changes occur in the fetoplacental membranes of GDM mothers which may increase the risk of worse fetal outcome [13].

In present study placental weight is increased, similarly, found by Kuck et al. that placental weight in GDM mothers was 694.8±152.1 gm as compared to normal gestation i.e., 610.2±116.6gm. Another study conducted by Khaskheli in Pakistan also reports the same [9, 14].

Other placental parameters observed in this study include diameter and thickness of placenta and number of placental cotyledons, all was found significantly increased than normal. All these changes are in line with studies conducted by Gusteine, Verma and Memon [7, 8, 10].

While considering the appearance of placental membranes in GDM mothers, it was observed that 51% membranes were meconium stained, 24.5% were thick, 22.4% were opaque and only 2% were thin. Significant thickness in placental membrane was noted in this study similar is reported by Togrul in his study conducted in Turkey on GDM placentae [15].

In present study, the most common fetal outcome in patients of GDM was macrosomia, in (18)36.7% of births. The body weight of neonate is also found increased up to 3.78kg ±0.34 with apgar score of 6.02±1.2 (in 1 minute and 7.65±1.33 after 5 minutes) in this study. These finding are supported by the

Vieira MC, et al., that also found that fetal macrosomia and increased body weight of neonates has been found frequently with GDM and maternal obesity [16].
Next to macrosomia, hyperbilirubinemia and admission to NICU/RDS has been observed frequently with only one baby with spina bifida. Boskabadi H et al has found that maternal risk factor for neonatal hyperbilirubinemia is gestational diabetes mellitus. Zanardo V, and the coworkers also found that total serum bilirubin was significantly raised in neonated born to GDM mothers [17, 18].
Most common histological finding in amniochorionic membranes of GDM patients was fibrinoid necrosis and calcification, i.e., 21(49.2%). The retro membrane hemorrhage was found in 11(22.4%) amniochorionic membrane histological sections and edema in 10(20.4%), while placental infarcts were present in 07 (14.3%) amniochorionic membranous tissues. Macrosomia is significantly associated with amniochorionic membranous histological findings, i.e., fibrinoid necrosis and retro membrane hemorrhage. Few studies were conducted on amniochorionic membranes in GDM mothers. One of the research studies has supported the findings of present study by revealing that in GDM patients, there is polyhydramnios that predisposes to inflammatory changes and alter the function of amniochorionic membranes. They have found increased levels of IL-4 in amnion of GDM patients with increased permeability [19].
El Sawy NA, et al. also found fibrin deposition and edematous changes in placenta of GDM mothers, when compared to the placenta of non GDM mothers with normal pregnancy [20].
Similarly, Istrate-Ofiţeru has also found fibrinoisd necrosis and placentalinfarcts in GDM mothers. In GDM mother hyperglycemic status might lead to rapid fetal growth and macrosomia. Placental studies in GDM mothers found that there was fibrinoid necrosis, increased syncytial knots, trophoblastic basement membrane thickening, villous stromal fibrosis, villous edema, crowd of villi, thickening of vessel wall and fibrin deposition [21, 22].

CONCLUSION

This study concludes strong relationship between the placenta, placental membranes and diabetes mellitus. Clinically, the adverse effects of diabetes on the outcome of pregnancy are well established but we have seen their gross morphological and microscopical impacts on placental membranes for the first time in our setup.

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