

Research Article: Embryology

Histologic and morphometric study of human placenta in gestational diabetes mellitus

Soma Saha¹, Sharmistha Biswas^{1*}, Deepraj Mitra¹, Anjan Adhikari², Chandranil Saha³¹ N.R.S. Medical College, ² Department of Pharmacology of R. G. Kar Medical College and ³ Tollygunge Metro Railway Hospital; Kolkata, West Bengal, India

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Summary

Aims: The aim was to study morphometry, site of umbilical cord insertion and histological changes in placentae of women with gestational diabetes mellitus and compare the results with those of normal pregnancies and observe the perinatal outcome.

Methods: It was an observational, correlational study of 130 placenta specimens collected from labour room and operation theatre of Department of Gynaecology & Obstetrics, Institute of Post Graduate Medical Education and Research, Kolkata. The subjects were mothers who attended antenatal clinic of the hospital regularly and delivered their babies in the same hospital. Cases were selected randomly, and divided in two groups: group A consisted of mothers having normal, uncomplicated pregnancy, group B consisted of mothers whose pregnancies were complicated by gestational diabetes mellitus. Morphometry, site of umbilical cord insertion and histological changes in placentae of all women were recorded. Perinatal outcome of the cases were also registered. The statistical methods used were chi-square test and Mann-Whitney U test.

Results: It was observed that the placentae of diabetic mothers were significantly bigger in size, weight, volume, area, thickness, diameter and circumference than those of normal mothers. Also, in diabetic mothers, there was significant increase in villous oedema, fibrin deposition, calcification and congestion of blood vessels. These placental changes were significantly correlated with birth weights of babies. Out of 70 mothers in Group B, 65 had live births, 5 had still-born babies.

Conclusions: Placentae of women with GDM showed several changes that may be associated with impaired functioning, leading to bad perinatal outcome.

Key words

Gestational diabetes, placenta, perinatal outcome, morphometry, histology.

Introduction

Placenta is the mirror of maternal and foetal status. It is a membranous vascular organ that develops in female mammals and mediates materno-foetal exchange. In humans it is developed from two sources - foetal and maternal.

Pregnancy is a diabetogenic state by virtue of various physiological changes which cause insulin resistance. In normal pregnancy, glucose tolerance decreases by

* Corresponding author. E-mail: drsharmisthabiswas@rediffmail.com.

third trimester, though plasma levels of insulin increase. Gestational diabetes mellitus (GDM) is defined as any degree of glucose intolerance with onset or first recognition during pregnancy (Metzgar et al, 1998).

In gestational diabetes usually there is delivery of large babies (Radaelli et al., 2003; Segregur et al., 2009) and large placentae (Lao et al., 1997; Taricco et al., 2003). The mothers also have high risk of hypertensive disorder, abortion, still birth, pre-term labour, puerperal sepsis etc. Infants born to mothers with glucose intolerance are at an increased risk of morbidity and mortality related to respiratory distress, growth abnormalities (large for gestational age, small for gestational age), hypoglycaemia and congenital malformations (Kuhl et al., 1985; Potter, 2011). Thomson (1969) observed that the placenta of diabetic women tends to be heavier and paler due to villous oedema. According to Gauster et al (2012), the placenta of poorly controlled diabetic women is enlarged, thick, and plethoric.

Fox (1969) and Priscilla White (1971) had shown that placentae from class A (GDM) diabetic mothers might be expected to weigh about 600 – 900 grams. Histological abnormalities such as the presence of nucleated fetal red blood cells, fibrinoid necrosis, villous immaturity and chorangiomas were observed more often in the diabetic placentae compared with control placentae (Evers et al., 2003).

Thorough and minute examination of placenta after birth can reveal placental changes that occur due to GDM. The aims and objectives of the present study was to study morphometry, site of umbilical cord insertion and histological changes in placentae of women with gestational diabetes mellitus (GDM), compare the results with those of normal pregnancies, and observe the perinatal outcome of pregnancies with GDM.

Material and methods

Placentae

Total 130 placentae were collected from labour room and operation theatre of Department of Obstetrics and Gynaecology, Institute of Post Graduate Medical Education and Research, Kolkata, India, during the period from June 2006 to May 2007. They were divided in two groups: group A consisted of placentae from mothers having normal, uncomplicated pregnancies; group B consisted of placentae from mothers whose pregnancies were complicated by GDM. Gestational ages were established from Last Menstrual Periods, and sometimes from ultrasonography reports. The mothers' obstetric and medical histories were taken from clinical data sheets.

In our study, mothers having a fasting plasma glucose level >126 mg/dl, or an occasional plasma glucose >200 mg/dl were included as having GDM. In the absence of this degree of hyperglycemia, in women with average or high-risk (marked obesity, personal history of GDM, glycosuria, or a strong family history of diabetes), initial screening was performed between 24 and 28 weeks of pregnancy by measuring the plasma glucose concentration 1 h after a 50 g oral glucose load (glucose challenge test), followed by a diagnostic oral glucose tolerance test on the women exceeding the glucose threshold value (>140 mg/dl) at glucose challenge test.

The study was performed after obtaining clearance from Institutional Ethical Committee. Before the placentae were collected, informed consent was obtained from the

mothers. The waste was disposed as per waste disposal norms. Morphological, morphometric, histological examinations were carried out on the freshly delivered placentae.

Morphometry

The following parameters were recorded:

Weight: by an electronic weighing scale.

Volume: estimated by the water displacement method.

Thickness: a thin long graduated needle was inserted in to the placenta, at the centre, at the margin and midway between the centre and margin. The average of the three readings was taken as the thickness of the placenta.

Diameter: measured twice with a measuring tape up to the nearest 0.5 cm. The mean of maximum (d_1) and minimum (d_2) diameters was taken as the diameter (d) of the placenta.

Area: The placental area was computed from:

$$A = (\pi/4) \times d_1 \times d_2 \text{ (Pryse- Davis et al., 1973).}$$

Circumference: the circumference P was estimated from $P = \pi \times d$

Cord insertion site: the minimum distance of the site of cord insertion to the margin of the placenta was measured and denoted as ' x '. Assuming the placenta to be a perfect circle the mean radius ' r ' was obtained from the formula $r = d/2$ and then the centrality (C) was computed from $100 \times (x/r)$. The placentae were divided into 4 groups:

Central cord insertion	($C = 76 - 100$);
Moderately eccentric cord insertion	($C = 51 - 75$);
Highly eccentric cord insertion	($C = 26 - 50$);
Marginal cord insertion	($C = 0 - 25$).

Histology

For histological examination, one whole-thickness placental section was taken from the central and one from the peripheral part of each placenta, fixed in 10% formol-saline for 3-4 days and routinely processed for histology. 5 μ m thick sections were stained with haematoxylin and eosin and examined under a light microscope at different magnifications to analyse the basal plate, amnion, chorionic plate, chorionic villi and intervillous spaces.

Examination of the baby

The time and mode of delivery were noted in each case. Whether the baby was live born or still born was recorded, the cause of still birth was ascertained. The parameters recorded in the labour room were: birth weight, Apgar score, umbilical artery pH (all noted by a paediatrician) and baby's length, which was measured using two hard boards and a measuring tape; any congenital anomaly was registered. Follow up of the baby was done in each case for 7 days to keep track of any neonatal complications.

Statistics

Quantitative data were analysed by Mann-Whitney U test and Pearson's correlation coefficient ' r '. Significance levels less than 0.05 were recorded and assumed as

significant. Standard deviation is presented as estimate of variance. Comparisons between normal and diabetic mothers were done using Mann-Whitney U test. The degree of correlation between the birth weight and some relevant placental parameters viz. weight, volume, area, thickness of placenta and foeto-placental ratio in each group were analyzed using Pearson's correlation coefficient 'r'.

Results

In group A, which included mothers having normal, uncomplicated pregnancy (60 cases), mean placental weight was 504.42 g (range 345-570 g), mean placental volume was 419.11 cm³ (range 300-520 cm³), mean placental area was 182.52 cm² (range 113.04-211.36 cm²), mean placental thickness was 1.67 cm (range 0.9-2.2 cm), mean placental diameter was 15.4 cm (range 12.0-17.8 cm), mean placental circumference was 48.35 cm (range 37.68-55.89 cm). The birth weight of babies ranged from 1.68 to 2.9 kg, mean 2.53 kg. The foeto-placental ratio, calculated dividing the baby birth-weight by placental weight, ranged from 4.7 to 5.7, mean 4.99 (Table 1).

In the 70 cases of group B (pregnancies with GDM), mean placental weight was 565.75 g (range 510-665 g). Mean placental volume was 487.65 cm³ (range 410-550 cm³). Mean placental area was 219.65 cm² (range 216.34-271.57 cm²). Mean placental thickness was 3.15 cm (range 2.7-3.5 cm). Mean placental diameter was 16.66 cm (range 14.6-18.6 cm). Mean placental circumference was 52.32 cm (range 45.8-58.4 cm). The birth weight of babies ranged from 3.19 to 4.2 kg, mean 3.9 kg. The foeto-placental ratio ranged from 5.04 to 6.2, mean 5.8 (Table 2).

In group A, cord insertion was central in 15 placentae, moderately eccentric in 21 placentae, highly eccentric in 3 placentae and marginal in 21 placentae. In group B, cord insertion was central in 10 placentae, moderately eccentric in 30 placentae, highly eccentric in 7 placentae and marginal in 23 placentae. Therefore, out of total 130 cases, umbilical cord insertion was central in 19%, moderately eccentric in 31.5%, highly eccentric in 7.7% and marginal in 33.8% (Table 3).

Histology

The histological features remarked were syncytial knots, cytotrophoblastic cell proliferation, fibrinoid necrosis, fibrosis of villous stroma, calcified and hyalinised spots within villi. Calcifications appeared as intracellular as well as extracellular basophilic deposits when stained with haematoxylin and eosin. Hyalinisation of villi appeared as an acellular, avascular, homogeneous area within villi. Also endothelial proliferation and fibromuscular sclerosis of stem arteries and the presence of Hofbauer cells were noted. These features were seen in both groups of placentae, but their frequency was higher in the diabetic group.

Delivery outcome

In group A, 60 mothers gave birth to 60 living babies. No still born or early neonatal death was noted. Of these, 55 were born at term and 5 before term. In group B (GDM) there were 70 deliveries with 65 live births and 5 still births. Out of 65 live

Table 1 – Placental morphometry, birth weight and foeto-placental ratio in group A (normal pregnancies, N = 60).

Parameter	Mean	Minimum	Maximum	Standard Deviation
Placental weight (g)	504.42	345	570	48.11
Placental volume (cm ³)	419.11	300	520	48.49
Placental area (cm ²)	182.52	113.04	211.36	28.66
Placental thickness (cm)	1.67	0.9	2.2	0.28
Placental diameter (cm)	15.4	12	17.8	1.49
Placental circumference (cm)	48.35	37.68	55.89	4.66
Birth weight of baby (kg)	2.53	1.68	2.9	0.24
Foeto-placental ratio	4.99	4.7	5.7	0.24

Table 2 – Placental morphometry, birth weight and foeto-placental ratio in group B (gestational diabetes mellitus, N = 70).

Parameters	Mean	Minimum	Maximum	Standard Deviation
Placental weight (g)	565.75	510.00	665.00	41.04
Placental volume (cm ³)	487.65	410.00	550.00	47.12
Placental area (cm ²)	219.65	167.34	271.57	31.34
Placental thickness (cm)	3.15	2.70	3.50	0.40
Placental diameter (cm)	16.66	14.60	18.60	1.18
Placental circumference (cm)	52.32	45.84	58.40	3.70
Birth weight of baby (kg)	3.90	3.19	4.20	0.24
Foeto-placental ratio	5.80	5.04	6.20	0.33

Table 3 – Insertion site of umbilical cord.

Group	N	Central	Eccentric		Marginal
			Moderately	Highly	
Normal	60	15 (25.0%)	21 (35.0%)	3 (5.0%)	21 (35.0%)
Diabetic	70	10 (7.7%)	30 (42.8%)	7 (10.0%)	23 (32.9%)
Total	130	25 (19.0%)	41 (31.5%)	10 (7.7%)	44 (33.8%)

births, 57 babies were born at term and 8 at pre-term. Of the 5 still born, 4 babies were pre-term and 1 was at term. 3 babies from group B mothers died within the first week of birth, all were pre-term and suffered from respiratory distress syndrome, one of them also had severe congenital diaphragmatic hernia and meconium aspiration syndrome.

Discussion

The placental weight may become the single most important factor in determining foetal growth. In the present study, in Group A, minimum placental weight was noted to be 345 g and maximum 570 g, mean (\pm standard deviation) 504.42 ± 48.11 g. Our findings were similar to those of Hertig (1960) and Woodling et al. (1976) whose normal placental weight ranged from 450 to 550 g; our findings were slightly higher than those observed by Rath et al. (1994).

In our study, in group B, the least placental weight recorded was 510 g and the highest was 665 g, mean 565.75 ± 41.04 g. This was heavier than the mean placental weight found in normal pregnancies. According to Teasdale (1981) placental weights of diabetic mothers showed only a tendency to be heavier than the gestationally matched controls, though the difference was not statistically significant.

As represented in Table 4, the placental weight and all the other placental morphometric parameters were significantly lower in normal group than in diabetic group. Cord insertion on the contrary showed a similar distribution between the two groups of placentae. Woods and Malan (1978) studied 940 placentae and found no correlation between the birth weight and the site of cord insertion in normal term infants. In a study conducted by Pathak et al (2010) the cord insertion was found not to be significantly different between non-affected pregnancies and pregnancies affected by GDM, pre-eclampsia, pregnancy induced hypertension, and small for gestational age babies.

In Group A the minimum birth weight of baby was observed to be 1.68 kg and the maximum 2.9 kg, mean 2.53 ± 0.24 kg. This value is slightly lower than that observed by Younosazi and Haworth (1969) and Damania et al. (1989) but quite concordant with the values found by Rath et al. (1994), probably due to same geographic distribution of the study populations. In our study, diabetic mothers gave birth to larger babies, as expected. Foetal macrosomia (>90 th percentile for gestational age or >4000 g in the term infant) occurs in 15-45% of diabetic pregnancies (Potter, 2011).

We found that in group A the mean value of foeto-placental ratio was 4.99 ± 0.24 (range 4.7-5.7). This value is lower than that observed by Rath et al. (1994) but similar to the values recorded by Shah and Jagiwala (1985). In group B, mean foeto-placental ratio was 5.80 ± 0.33 (range 5.04 - 6.2).

Histologically, the placentae from pregnancy complicated with GDM showed increased incidence of syncytial knots, cytotrophoblastic cell proliferation, calcification of villi, fibrinoid necrosis, hyalinisation, basement membrane thickening and thickening of the wall of stem arteries. Also we have seen Hofbauer cells in these placentae. These findings corroborate those of Fox (1978) and Perrin Eugene (1984). Daskalakis et al. (2008) noted that the presence of degenerative lesions such as fibrinoid necrosis and vascular hyperplasia (chorangiomas) was apparent mainly in the diabetes group.

We attempted to find out the degree of correlation between the birth weight and few relevant placental parameters viz. weight, volume, area, thickness of placenta and foeto- placental ratio in each of the 2 groups of mothers by using Pearson's correlation coefficient 'r' (Table 5). In group A a strong correlation was found between the birth weight of baby and placental weight, volume and thickness, a fair correlation between birth weight and placental area and a poor correlation between birth

Table 4 – Comparison between normal (group A) and diabetic mothers (group B) using Mann-Whitney U test.

Parameters	Group A (N = 60)	Group B (N = 70)	P
Placental weight (g)	504.42 + 48.11	565.75 + 41.04	0.001
Placental volume (cm ³)	419.11 + 48.49	487.65 + 47.12	0.001
Placental area (cm ²)	182.52 + 28.66	219.65 + 31.34	0.004
Placental thickness (cm)	1.67 + 0.28	3.15 + 0.4	0.001
Placental diameter (cm)	15.40 + 1.49	16.66 + 1.18	0.025
Placental circumference(cm)	48.35 + 4.66	52.32 + 3.7	0.001
Baby weight at birth (kg)	2.53 + 0.24	3.90 + 0.24	0.001
Foeto-placental ratio	4.99 + 0.24	5.80 + 0.33	0.001

Table 5 – Pearson's correlation coefficient 'r' between birth weight and relevant placental parameters.

Groups	N		Placental parameters				Foeto-placental ratio
			Weight	Volume	Thickness	Area	
Normal	60	Baby weight at birth	0.88	0.84	0.82	0.63	0.26
Diabetic	70	Baby weight at birth	0.75	0.67	0.52	0.58	0.44

Interpretation of correlation coefficient: 'r' > 0.7 = strong correlation; 0.7 ≥ 'r' > 0.5 = good correlation; 0.5 ≥ 'r' > 0.3 = fair correlation; 0.3 ≥ 'r' = poor correlation.

weight and foeto-placental ratio. In group B, there was a strong correlation between birth weight and placental weight, a good correlation between birth weight and placental volume, thickness and area, and a fair correlation between birth weight and foeto-placental ratio. From this correlation study we found that the birth weight of baby was strongly associated to placental weight in each case, i.e. if there was an increase in placental weight the birth weight of baby was also increased and vice versa. Asgharnia et al (2008) also commented that there were statistically significant relationships between placental weight and birth weight in their experience.

For perinatal outcome our findings were in accordance with Potter (2011) who observed that infants born to mothers with glucose intolerance were at an increased risk of morbidity and mortality related to respiratory distress, growth abnormalities (large for gestational age, small for gestational age), congenital malformations, hypoglycaemia etc. According to Evers et al (2003) unexplained intra-uterine foetal death is still a problem in diabetic pregnancies. They observed that in these pregnancies impaired placental function, in terms of abnormal placental weight or abnormal placental histology, or both, may account for this phenomenon.

From this study we conclude that infants born to mothers with GDM are at increased risk of morbidity and mortality and that the placentae of women with gestational diabetes mellitus show several abnormalities that can be associated with impaired functioning, leading to bad perinatal outcome.

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References

- Aherne W., Dunhill M.S. (1966) Quantitative anatomy of the placenta. *J. Pathol. Endocrinol.* 91: 123-139.
- Asgharnia M., Esmailpour N., Poorghorban M., Atrkar-Roshan Z. (2008) Placental weight and its association with maternal and neonatal characteristics. *Acta Med. Iran.* 46: 467-472.
- Damania K.R., Salvi V.S., Ratnaparkhi S.K., Daftary S.N. (1989) The placenta in hypertensive disorders of pregnancy. *J. Obstet. Gynaecol. India* 39: 28-31.
- Daskalakis G., Marinopoulos S., Krielesi V. (2008) Placental pathology in women with gestational diabetes. *Acta Obstet. Gynecol. Scand.* 87: 403-407.
- Evers I.M., Nikkels P.G., Sikkema J.M., Visser G.H. (2003) Placental pathology in women with type 1 diabetes and in a control group with normal and large-for-gestational-age infants. *Placenta* 24: 819-825.
- Fox H. (1978) *Pathology of the Placenta.* W.B. Saunders, London.
- Gauster M., Desoye G., Tötsch M., Hiden U. (2012) The placenta and gestational diabetes mellitus. *Curr. Diab. Rep.* 12: 16-23.
- Hertig, A.T. (1960) Pathological aspects. In: Villee C.A. (Ed.) *Placenta and Fetal Membranes.* Williams & Wilkins, New York . Pp 109-124.
- Kuhl C., Hornnes P.J., Andersen O. (1985) Etiology and pathophysiology of gestational diabetes mellitus. *Diabetes* 34 Suppl 2: 66-70.
- Lao T.T., Lee C.P., Wong W.M. (1997) Placental weight to birth weight ratio is increased in mild gestational glucose intolerance. *Placenta* 18: 227-230.
- Metzger B.E., Coustan D.R., Eds. (1998) *Proceedings of the Fourth International Workshop-Conference on Gestational Diabetes Mellitus.* *Diabetes Care* 21 (Suppl. 2): B1-B167.
- Pathak S., Hook E., Hackett E., Murdoch E., Sebire N.J., Jessop M, Lees C. (2010) Cord coiling, umbilical cord insertion and placental shape in an unselected cohort delivering at term: relationship with common obstetric outcomes. *Placenta* 31: 963-968.
- Perrin Eugene V.D.K. (1984) *Pathology of Placenta.* Churchill Livingstone, New York. P. 77.
- Potter C.F. (2011) *Infant of diabetic mother.* Medscape Reference. On line at: <http://emedicine.medscape.com/article/974230-overview>; accessed June 25, 2013.
- White P. (1949) Pregnancy complicating diabetes. *Am. J. Med.* 7: 609-616.
- Pryse-Davies J., Beazley J.M., Leach G. (1973) A study of placental size and chorioamnionitis in a consecutive series of hospital deliveries. *Br. J. Obstet. Gynaecol.* 80: 246-251.
- Radaelli T., Varastehpour A., Catalano P. (2003) Gestational diabetes induces placental genes for chronic stress and inflammatory pathways. *Diabetes* 52: 2951-2958.

- Rath G., Garg K., Anand C., Kawle M. (1994) Vascular pattern of human placenta in complicated pregnancy: A corrosive cast study. *Ann. N.A.M.S. India* 30: 17-22.
- Segregur J., Bukovic D., Milinovic D. (2009) Fetal macrosomia in pregnant women with gestational diabetes. *Coll. Antropol.* 33: 1121-1127.
- Shah R.K., Jagiwala K.S., Vyas P.K. (1985) Placental morphology and fetal growth in normal and abnormal pregnancies. *J. Obstet. Gynecol. India* 35: 1089-1094.
- Taricco E., Radaelli T., Nobile de Santis M.S. (2003) Foetal and placental weights in relation to maternal characteristics in gestational diabetes. *Placenta* 24: 343-347.
- Teasdale F. (1981) Histomorphometry of the placenta of the diabetic women: class A diabetes mellitus. *Placenta* 2: 241-251.
- Thomson. Placenta in relation to birth weight. *J. Obstet. Gynaecol. Br. Commonw.* 76: 865-872.
- Woodling B.A., Kroener J.M., Puffer H.W., Furukawa S.B., Anderson G.Jr., Ochoa R.G., Warner N.E. (1976) Gross examination of the placenta. *Clin. Obstet. Gynecol.* 19: 21-44.
- Woods D., Malan A.F. (1978) The site of umbilical cord insertion and birth weight. *Br. J. Obstet. Gynaecol.* 85: 332-333.
- Younoszai M.K., Haworth J.C. (1969) Placental dimensions and relations in pre-term, term and growth retarded infant. *Am. J. Obstet. Gynaecol.* 103: 265-271.