

Histopathological Study of Placenta in Gestational Diabetes in a Tertiary Care Hospital

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Abstract: Gestational Diabetes Mellitus (GDM) is described as glucose intolerance with first recognition during pregnancy, and disappears with delivery. Gestational diabetes complicates approximately 2-4% of pregnancies and it causes variable changes in placenta. The study is undertaken to identify the histo-morphological findings of all placenta and to correlate normal and abnormal placental findings in relation to gestational diabetes mellitus (GDM). Thirty placental specimens from gestational diabetes mothers and thirty placental specimens from non gestational diabetic mothers were taken up for the study. The specimens were received in the Department of Pathology, Sri Venkateshwaraa Medical College and Hospital for a period of 18 months was studied. The clinical data related to maternal and foetal details and the blood sugar levels of mother were recorded. Detailed microscopic examination of the variables such as fibrotic villi, villous edema, chorangiomas, syncytial knots, fibrinoid necrosis, syncytial knots, thickness of basement membrane. The data were analysed using SPSS 20.0 version. Microscopic examination showed increase in following features in GDM placentas Fibrosis of villi, Villous edema, Chorangiomas, Fibrinoid necrosis, Syncytial knots, Thickened basement membrane, villous immaturity. In all these variables p value was found to be statistically significant. Diabetes in pregnancy alters the placental morphology. Histopathological examination of placenta is indispensable and has to be carried out as a routine protocol.

Keywords: Placenta, Gestational diabetes, Histopathology.

INTRODUCTION

The placenta acts as a vital organ which is responsible for the transfer of nutrients and waste products between the fetal and maternal circulations and metabolic and endocrine activities of the placenta are not clearly understood [1]. Diabetes mellitus is a chronic metabolic disorder that causes hyperglycemia and it is a major health problem worldwide. According to the World Health Organization (WHO) [2], diabetes has emerged as an epidemic affecting 246 million people across the world [3]. India has the second largest number of people with diabetes in the world currently estimated at 63 million [4].

Research also suggests that, the prevalence of diabetes mellitus (DM) among pregnant women is increasing because of inactive life styles, poor diet and adolescent obesity [5]. Diabetes mellitus in pregnant women is categorized into clinical diabetes or pregestational diabetes and gestational diabetes (GDM).

Gestational diabetes mellitus (GDM) refers to any degree of glucose intolerance with onset on first recognition during pregnancy [6]. It affects 2 to 5% of all pregnancies [7, 8].

During pregnancy extra demand on the pancreas causes some women to develop gestational diabetes [6]. Gestational diabetes mellitus is associated with increased perinatal morbidity like macrosomia, neonatal hypoglycaemia, hyperbilirubinemia, shoulder dystocia and respiratory distress syndrome. In offspring's, it increases genetic risk of developing adolescent obesity and diabetes. In mothers, it is a strong risk of developing permanent diabetes in life [6].

Abnormal maternal glucose tolerance occurs in 3- 10% of pregnancies. , any type of diabetes mellitus during pregnancy produces variety of placental abnormalities [9]. These changes depend on a number of factors like glucose level during the critical periods

in placental development [10]. Changes in placental morphology due to uncontrolled diabetes result in development of macrosomia, congenital malformations and intrauterine growth retardation in fetus[11].

MATERIALS AND METHODS

Type of study

It is a descriptive study.

Duration of the Study

October 2015 to April 2017, a period for 18 months

Nature of study

It is a descriptive study on placental specimens which are received in the Pathology Department of Sri Venkateshwaraa Medical College, Hospital and Research Center, Puducherry. Ethical clearance obtained from institutional ethical committee.

Sample size

Control group – 30 and Diabetic group – 30. Thirty placental specimens from mothers who had been diagnosed with GDM and thirty placental specimens from mothers who had not diagnosed with GDM were taken for the study after obtaining Ethical Committee Clearance.

Inclusion Criteria

- All placentas of term pregnancies between weeks of 36 to 42 weeks of gestation from diagnosed GDM mothers.

Exclusion criteria

- Gestational diabetes in association with hypertension.
- Gestational diabetes in association with any toxemia of pregnancies.
- Any other known chronic illness or metabolic disorders in pregnant mothers.

METHODOLOGY

The clinical data related to maternal details, blood glucose levels (fasting, post prandial and HbA1c) and the mode of delivery were recorded. The placenta from both control and GDM group was collected immediately following expulsion, and washed in the running tap water to remove the blood clots.

The shape of the placenta was assessed by stretching it flat on the cutting board. The weight of the placenta was measured on a standard digital weighing apparatus. Placental diameter was measured using a thread and the thickness was measured at the center of placenta after giving serial cuts. The fetal surface was examined for colour, transparency, sub chorionic thrombus, insertion of umbilical cord and attachment of fetal membranes. The extra placental membranes were inspected for completeness, adherent blood clot, colour and transparency.

The umbilical cord length was measured and it was examined for the number of vessels, true knots, false knots and insertion (central, eccentric or velamentous). The membranes were trimmed and a strip was taken as “Swiss roll”. The cord was cut at about 4cms from its insertion. The cut end of the cord was examined for the number of blood vessels. Portions of the cord from the proximal and distal regions were taken for paraffin embedding. The maternal surface of the placenta was inspected for completeness, adherent blood clots, and number of cotyledons, calcification and infarction. The whole placenta was left for fixation in 10% formal saline for 24-48 hours. After fixation the placenta was cut into vertical strips (bread loaf manner) of 0.5 cms thickness each and the macroscopic lesions were examined. The villous parenchyma was examined for calcification, infarction and intervillous thrombosis. The tissue bits for microscopic examination were taken from, cut end of umbilical cord, at placental junction, strip of membrane roll, margins of placenta, central area of placenta, any fibrotic area, any calcified area and any infarcted area. The tissue bits were then processed through series of dehydration steps and embedded in wax blocks. Tissue sections of 5 µm thicknesses were cut from paraffin embedded blocks and stained by conventional Hematoxylin and Eosin (H & E) stain.

Evaluation of Histological Features

Fibrosis of villi

Stromal collagen gradually increases throughout pregnancy and at term the stroma usually shows a delicate network of fibrous tissue [12]. Fibrosis in >3% of villi was recorded in H&E stained slides in both control and diabetic placenta. Results are expressed as a percentage.

Villous edema

Villous edema in >3% of villi was recorded in H&E stained slides in both control and diabetic placenta. Results are expressed as a percentage.

Chorangiosis

Chorangiosis is 10 fields with 10 villi with 10 capillaries per villus [23]. Chorangiosis was recorded in H&E stained slides in both control and diabetic placenta. Results are expressed as a percentage.

Villous immaturity

Villous immaturity in >3% of villi was recorded in H&E stained slides in both control and diabetic placenta. Results are expressed as a percentage.

Fibrinoid Necrosis

Fibrinoid necrosis is a homogeneous eosinophilic deposit in placenta and considered as a hallmark of an immunological reaction within the villi [12]. The number of placentas showing fibrinoid

necrosis in more than 3% of villi was noted and the total of such villi was then expressed in percentage.

Thickened basement membrane

Aberrations of cytotrophoblastic proliferation and function are presumed to cause basement membrane thickening [12]. Thickened basement membrane of both trophoblast in H&E slides in both control and diabetic placenta. Results are expressed as a percentage.

Syncytial Knots

They have been considered as accumulation of apoptotic nuclei which is a degenerative phenomenon in response to trophoblastic ischemia or hypoxia [12].

Syncytial knots were recorded in both control and diabetic group when present in more than 30% of villi and the results are expressed as a percentage.

STATISTICAL ANALYSIS

Descriptive analysis using Chi-Square Test and T test.

Statistical Software

The statistical software SPSS 20.0 version was used for the analysis of the data and Microsoft excel have been used to generate graphs, tables etc.

RESULTS

Table-1: Show the frequency, percentage and p-value of fibrosis of villi control group and diabetic group

| FIBROSIS OF VILLI | Control Group (N=30) | | Diabetic Group (N=30) | | X ² value | p-value |
|-------------------------------|----------------------|--------------|-----------------------|--------------|----------------------|---------|
| | Frequency | Percentage % | Frequency | Percentage % | | |
| Present in more than 3% villi | 16 | 53.3% | 24 | 80% | 4.800 | 0.028* |
| Present in less than 3% villi | 14 | 46.7% | 6 | 20% | | |

*significant at 0.05 level
 **significant at 0.01 level
 NS – Not significant

Table-2: Show the frequency, percentage and p-value of villous edema control group and diabetic group

| VILLOUS EDEMA | Control Group (N=30) | | Diabetic Group (N=30) | | X ² value | p-value |
|-------------------------------|----------------------|--------------|-----------------------|--------------|----------------------|---------|
| | Frequency | Percentage % | Frequency | Percentage % | | |
| Present in more than 3% villi | 11 | 36.7% | 24 | 80% | 11.58 | 0.001** |
| Present in less than 3% villi | 19 | 63.3% | 6 | 20% | | |

*significant at 0.05 level
 **significant at 0.01 level
 NS – Not significant

Table-3: Show the frequency, percentage and p-value of chorangiomas control group and diabetic group

| CHORANGIOSIS | Control Group (N=30) | | Diabetic Group (N=30) | | X ² value | p-value |
|--------------|----------------------|--------------|-----------------------|--------------|----------------------|---------|
| | Frequency | Percentage % | Frequency | Percentage % | | |
| Present | 15 | 50% | 23 | 76.6% | 4.593 | 0.032* |
| Absent | 15 | 50% | 7 | 23.4% | | |

*significant at 0.05 level
 **significant at 0.01 level
 NS – Not significant

Table-4: Show the frequency, percentage and p-value of villous immaturity in control group and diabetic group

| VILLOUS IMMATURITY | Control Group (N=30) | | Diabetic Group (N=30) | | X ² value | p-value |
|--------------------|----------------------|--------------|-----------------------|--------------|----------------------|---------|
| | Frequency | Percentage % | Frequency | Percentage % | | |
| Present | 15 | 50% | 25 | 83.3% | 7.500 | 0.006** |
| Absent | 15 | 50% | 5 | 16.7% | | |

*significant at 0.05 level
 **significant at 0.01 level
 NS – Not significant

Table-5: Show the frequency, percentage and p-value of fibrinoid necrosis control group and diabetic group

| FIBRINOID NECROSIS | Control Group (N=30) | Diabetic Group (N=30) | X ² value | p-value |
|--------------------|----------------------|-----------------------|----------------------|---------|
|--------------------|----------------------|-----------------------|----------------------|---------|

| | Frequency | Percentage % | Frequency | Percentage % | 4.355 | 0.036* |
|--------------------------------|-----------|--------------|-----------|--------------|-------|--------|
| Present in more than 3% villi. | 19 | 63.3% | 26 | 86.6% | | |
| Present in less than 3% villi. | 11 | 36.7% | 4 | 13.4% | | |

*significant at 0.05 level
 **significant at 0.01 level
 NS – Not significant

Table-6: Show the frequency, percentage and p-value of thickened basement membrane control group and diabetic group

| THICKENED BASEMENT MEMBRANE | Control Group (N=30) | | Diabetic Group (N=30) | | X ² value | p-value |
|-----------------------------|----------------------|--------------|-----------------------|--------------|----------------------|---------|
| | Frequency | Percentage % | Frequency | Percentage % | | |
| Present | 14 | 46.7% | 24 | 80% | 7.177 | 0.001** |
| Absent | 16 | 53.3% | 6 | 20% | | |

*significant at 0.05 level
 **significant at 0.01 level
 NS – Not significant

Table-7: Show the frequency, percentage and p-value of syncytial knots control group and diabetic group

| SYNCYTIAL KNOTS | Control Group (N=30) | | Diabetic Group (N=30) | | X ² value | p-value |
|---------------------------------|----------------------|--------------|-----------------------|--------------|----------------------|---------|
| | Frequency | Percentage % | Frequency | Percentage % | | |
| Present in more than 30% villi. | 18 | 60% | 29 | 97% | 11.88 | 0.001** |
| Present in less than 30% villi. | 12 | 40% | 1 | 3% | | |

*significant at 0.05 level
 **significant at 0.01 level
 NS – Not significant

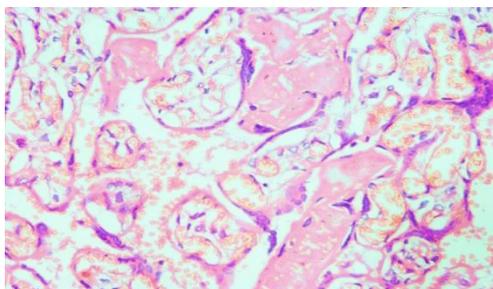


Fig-1: Photomicrograph showing fibrinoid necrosis (H&E, 40x)

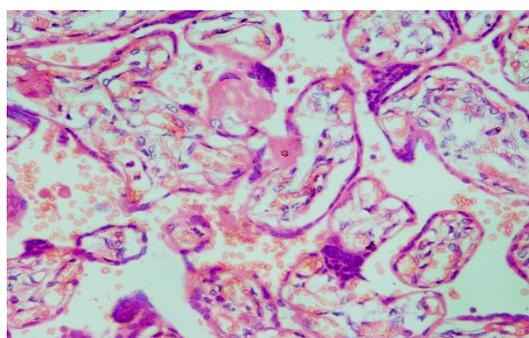


Fig-2: Photomicrograph showing syncytial knots. (H&E, 40x)

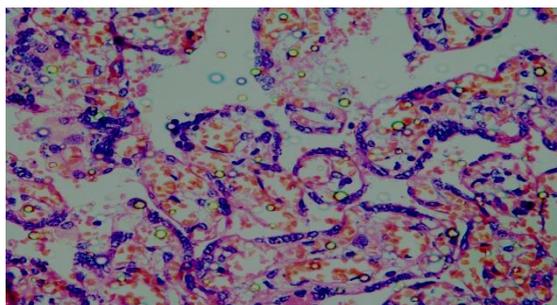


Fig-3: Photomicrograph showing chorangiogenesis (H&E, 40x)

DISCUSSION

Diabetes mellitus is a common metabolic disorder, characterized by chronic hyperglycemia. It is a major cause of morbidity and mortality from long term diseases of major organ systems [13]. Apart from affecting major organ systems, diabetes mellitus during pregnancy produces complications both in mother and offspring [14].

In the present study total of 60 placentas were studied which included 30 from mothers with uncomplicated pregnancies, considered as control group and 30 from mothers with gestational diabetes mellitus considered as diabetic group. The microscopic lesions were quantified and compared between the two groups.

FIBROSIS OF VILLI

In term placenta less than 3 percent of villi show fibrosis and if more than 3 percent of villi in a placenta show fibrosis it is considered as abnormal. It is as a morphological hallmark of reduced villous perfusion [16]. In the present study, 53.3% were having fibrotic villi in more than 3% villi in control group whereas 80% were having fibrotic villi in more than 3% villi in diabetic placenta group. (Table 1) Fox H (1965), found that fibrotic villi is significantly high in diabetic placenta when compared to normal placenta [17].

VILLOUS EDEMA

In the present study it was seen that 11 (36.7%) placentas were having villous edema in more than 3% villi and whereas in the diabetic placenta group 24 (80%) placentas were having villous edema in more than 3% villi. Wasserman L, Shlesinger H *et al.* [18], noticed type of changes with focal distribution on the distal villi in most studied cases. It was concluded that, the appearance of the true villous edema in placentas of diabetic mothers is probably the result of the appearance of abnormal deposits of mucopolysaccharides in villous stroma (Table 2).

CHORANGIOSIS

Altshuler defined chorangiogenesis as at least 10 fields with 10 villi with 10 capillaries per villus [21]. In the present study it was observed that control group was

having 50% of chorangiogenesis whereas increased chorangiogenesis (76.7%) were seen in diabetic placenta group. Altshuler G, studied 19 cases with diabetic placenta, they found that increased number of villous capillaries (Chorangiogenesis) was a sign of chronic hypoxic changes [23] (Table 7).

VILLOUS IMMATURITY

In the present study it was observed that control group were having 50% of villous immaturity whereas increased villous immaturity (83.3%) was seen in diabetic placenta group. Lavinia Gheorman, I. E. Pleşea and V. Gheorman [20]. Found that villous immaturity showed significant increase in diabetic placenta group when compared with normal placenta group (Table 4).

FIBRINOID NECROSIS

Fibrinoid necrosis is seen as a nodular mass of homogeneous acidophilic material in the villi. Placenta in which fibrinoid necrosis involving up to three percent of placental villi is considered as normal and placenta in which the percentage of villi showing fibrinoid necrosis of more than three percent is considered as abnormal [24,25]. In the present study it was observed that control group was 63.4% of fibrinoid necrosis in more than 3% villi whereas increased fibrinoid necrosis was observed in diabetic placenta group in more than 3% villi (86.7%). Pankaj Saini, Jai Prakash Pankaj and Gyan Chand Agarwa [27]. found that fibrinoid necrosis was significantly higher in the diabetic group (6.70 ± 2.14) as compared to a mean value 2.25 ± 1.66 in the controls. (Table 5)

THICKENED BASEMENT MEMBRANE

In the present study it was observed that control group were having 47% of thickened basement membrane of whereas increased in thickened basement membrane (80%) was seen in diabetic placenta (Table 6). Tewari V, Tewari A and Bhardwaj N, found that thickness of basement membrane shows a significant increase in diabetic placenta group when compared with normal placenta group [15].

SYNCYTIAL KNOTS

Syncytial knots are composed of aggregates of small, closely packed densely staining nuclei protruding from the villous surface into the intervillous space. Knots in more than 30% of the villi are considered excessive [27]. In the present study it was observed that control group were having 60% villi had syncytial knots whereas increased syncytial knots (97%) was seen in diabetic placenta group. Tewari V, Tewari A and Bhardwaj N [15], found that out of 30 cases of diabetic placenta, 24 cases showed increased syncytial knots. (Table 7)

CONCLUSION

Placenta is a mirror which reflects the intrauterine status of the foetus. It is a vital organ for maintaining and promoting normal foetal development. Gestational diabetes mellitus (GDM) refers to any degree of glucose intolerance with onset or first recognition during pregnancy. Our present study examined the histopathological findings in placenta of gestational diabetes group compared to non-diabetic pregnancies group. Microscopic examination showed increase in following features in GDM placentas Fibrosis of villi, Villous edema, Chorangiomas, Fibrinous necrosis, Syncytial knots, Thickened basement membrane, villous immaturity. We arrived a conclusion that gestational diabetes shows profound changes on placenta. This may cause injury to both mother and foetus. Hence careful monitoring of blood glucose levels during gestation is of profound importance. Thus we conclude that histopathological examination of placenta is indispensable and has to be carried out as a routine protocol.

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