Original Article

Gestational Diabetes

Placental Histology in Diet and Insulin Treated Gestational Diabetics

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ABSTRACT

Objective: To observe and compare placental histology for hypoxic changes in diet plus exercise alone versus diet plus exercise and insulin treatment in patients with Gestational Diabetes Mellitus (GDM).

Study Design: Comparative / analytic study.

Place and Duration of Study: The study was conducted in Lyari General Hospital and Mamji Hospital after approval from the Institutional Review Board (IRB) and Ethical Committee of Dow University of Health Sciences from Jan 2010- Jan 2011.

Materials and Methods: After written informed consent, **30** patients were diagnosed to have GDM with RBS between 126-129mg/dl were given diet control plus exercise therapy (Group A). **39** GDM patients with RBS greater than 130 mg/dl were kept on diet plus exercise and insulin (Group B). After delivery placentae were collected from 25 patients in each group. Histological slides of placentae were prepared and parameters of hypoxia such as villous immaturity, villous fibrinoid necrosis, syncytial knots, chorangiosis, calcification etc. were observed and compared between the two groups using light microscope. Results were evaluated by SPSS 16 using student t- test and chi square test.

Results: Statistically non-significant results were obtained for the hypoxic parameters. However numerically more calcification was found in Group A while villous immaturity, villous fibrinoid necrosis and syncytial knots were present more in Group B.

Conclusion: Histological examination of placentae showed presence of hypoxic features in both Group A and Group B patients with more propensities in diet plus exercise and insulin treated GDM group.

Key Words: Diabetes, Gestational, Placenta, Hypoxia, Diet Management, Insulin

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INTRODUCTION

Pregnancy is a condition when females are more prone to develop diabetes due to a strong diabetogenic effect of maternal and placental hormones. Diabetes which occurs during pregnancy is known as gestational diabetes mellitus with FBS equals to or more than 5.5 mmol/L (100mg/dl) and post prandial glucose levels greater than 7 mmol/L(126mg/dl)".[1]

Placenta supplies nutrition and oxygen to the baby and also provides detailed information regarding infant intrauterine encounters[2]. The proper maturity of placental vessels is important for normal fetal growth and survival[3] Glucose can cross placenta so excessive glucose is stored as glycogen in the body of the fetus under the influence of fetal insulin, resulting in macrosomic babies and large sized placentae with compromised function[4] The whole process produces many maternal and fetal complications including cesarean sections, eclampsia in mothers and stillbirths, intrauterine deaths, respiratory distress syndrome and hyperbilirubinemia in babies attributed to hypoxic changes.[5]

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Grossly placenta is a disc shaped highly vascular organ. Microscopically normal placenta comprises of multiple villi. They have grape like outgrowth of vessels with sinusoidal dilated capillaries to reduce the blood pressure in this area for adequate gaseous exchange[6] Maturity of these villi is necessary for proper exchange of gases and by term mostly placental structure comprises of mature villi. During the first trimester the syncytio-nuclei are regularly placed, but as the pregnancy advances these nuclei aggregate to form knots, known as "syncytial knots" due to ongoing apoptosis and necrosis of the tissue[7]. Syncytial knots significantly indicate utero-placental ischemia or fetal stress[8]. There are not more than 5 blood vessels within each villi. If their number exceeds more than 10 vessels then it is named as chorangiosis and is said to be associated with fetal congenital abnormalities and fetal hypoxia[9]. Fibrinoid necrosis may occur due to thrombus formation in maternal blood but peri-villous fibrin is the result of damaged trophoblastic tissue and is indicator

of fetal hypoxia, intrauterine growth retardation and fetal death[10]. Visible calcification can be seen, and histologically, these are structure less basophilic areas which are a sign of placental degeneration.[11]

Conservative management for GDM includes diet control with mild exercise that is 30 minutes of walk thrice weekly. If maternal glucose levels are not controlled with diet and exercise alone then pharmacological treatment is added to the management plan. The mainstay of treatment is insulin. It acts through tyrosine kinase receptor which is finally directed towards intra cytoplasmic proteins (insulin second messenger system). This in turn increases translocation of glucose receptors on the cell membrane (GLUT 4) and enhances the intracellular entry of glucose, increases glycogen synthesis, lipolysis and lipogenesis[12].

Fetal hypoxia, growth restriction, intrauterine death and still birth are common in diabetic pregnancies. Microscopic examination of diabetic placentae could provide an insight into these problems. This study was designed to observe and compare placental histology for presence of hypoxic changes in diet plus exercise alone versus diet, exercise and insulin treated GDM patients.

MATERIALS AND METHODS

The study was conducted in Lyari General Hospital and Mamji Hospital after approval from the Institutional Review Board (IRB) and Ethical Committee of Dow University of Health Sciences from Jan 2010- Jan 2011. With written informed consent patients were enrolled for the study. Screening was carried out in high risk females, attending antenatal clinic by random blood sugar checking in OPD with glucometer. Confirmation was done with Oral Glucose Challenge Test and Oral Glucose Tolerance Test, according to WHO criteria and finally **69** diabetic patients were enrolled in the study. Two groups of GDM patients were made on the basis of RBS. Females having RBS less than 130 mg/dl were given diet control therapy for a week and then RBS was rechecked. **30** patients with RBS between 126-129mg/dl were kept in Group A, with diet control therapy and mild exercise. They were counseled to take 2000-2500 kcal/day and diet charts were provided accordingly. They were further asked to do 90 minutes of walk in a week. **39** Patients with RBS greater than 130 mg/dl were treated with s/c insulin therapy (2/3 NPH + 1/3 regular insulin, 0.8IU in 2nd trimester and 0.9 IU in 3rd trimester) administered in two doses (before breakfast and dinner) along with diet control and exercise (Group B) (1). All these patients were followed in obstetric diabetic OPD up to 32 weeks and then weekly till term. On every visit RBS was checked by glucometer and the dose of the drug was adjusted if needed. Placentae were collected at the end of the study with 25 patients in each group. These were preserved soon after delivery in 10% formalin in containers of adequate sizes. These containers were transported to Dow Diagnostic Research Lab (DDRL) for microscopic evaluations. For microscopic evaluation, histological slides were prepared. In this procedure first blocks were set by taking out placental tissues from 12 o clock, 6 o clock and center of the placenta of adequate size and were fixed in the plastic cassettes. Then these cassettes of placental tissue were processed step wise starting from dehydration in an ascending concentration of alcohol for a few hours. After that tissue clearing was done using xylene. Embedding of the tissue was done using liquid paraffin. After cooling of the blocks, finally, cutting of 4 µm thick sections were performed by manual microtome. The tissue sections were preserved on histology glass slides and were allowed to dry. Staining was then done with hematoxyline, eosin, PAS and trichome stains**.** Hypoxic parameters as villous immaturity, villous fibroid necrosis, syncytial knots, chorangiosis and calcification were observed using light microscope and findings were documented on a predesigned data form. The results were evaluated by SPSS 16 using student t- test and chi square test accordingly.

RESULTS

Both the groups had age and weight matched GDM mothers. Significant differences were present in FBS and RBS at the time of enrollment (0.005 and 0.00 respectively) (Table1).

Table No.1: Maternal characteristics Comparison between group A and group B N=50

Numerical Variables	Group A n=25 mean± SD	Group B n=25 mean± SD	Significance
Maternal weight (Kg)	78.54± 6.93	77.90± 9.03	0.78
Maternal age (year)	30.08 ± 3.16	31.60 ± 4.27	0.15
FBS (gm/dl)	88.88±8.79	102.08±20.06	0.005*
RBS (gm/dl)	148.72±38.9	239.16±69.7	0.00*

Table No.2: Microscopic examination of placentae comparison between Group A and Group B. N=50

Categorical Variables	Group A n=25	Group B n=25	Significance
Villous immaturity:			
Present	10	14	0.25
Absent	15	11	
Villous fibrinoid necrosis:			
Present	19	22	0.26
absent	6	3	
Syncytial knots:			
Present	14	15	>0.99
absent	11	10	
Chorangiosis:			
Present	13	13	>0.99
Absent	12	12	
Calcification:			
Present	10	7	0.37
Absent	15	18	

Group A: Pregnant GDM on diet control treatment

Group B: Pregnant GDM on diet control and insulin treatment.

*statistically significant difference

Student's t test applied

In placental histology villous immaturity, villous fibrinoid necrosis, syncytial knots, chorangiosis, and calcification were found to be non-significant statistically in both groups (Table 2). Though numerically, calcification were seen more in diet plus exercise alone (Group A) placentae whereas villous immaturity, villous fibrinoid necrosis and syncytial knots were present more in diet plus exercise and insulin treated (Group B) placentae.

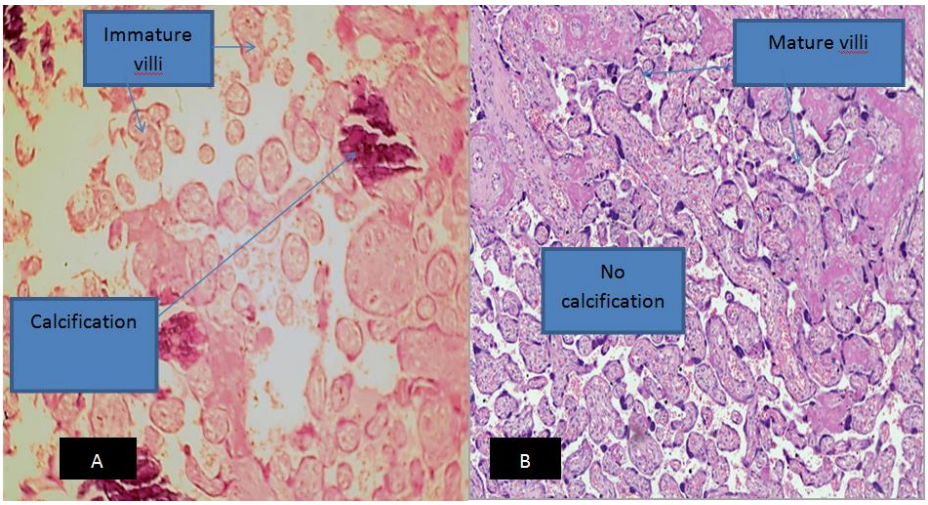

Figure No.1: Histological slides of GDM placenta treated with diet and exercise showing immature villi and calcification (A) whereas mature villi and no calcification is seen in placenta of diet, exercise and insulin treated

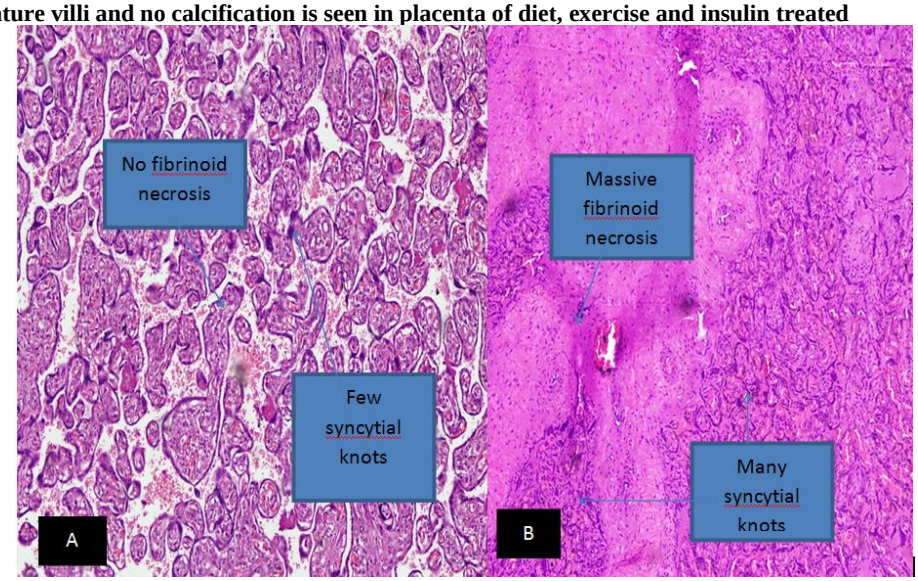

Figure No.2:Histological slide of GDM placenta treated with diet, exercise showing no fibrinoidnecrosis and few syncytial knots (A) whereas placenta treated with diet, exercise plus insulin showing syncytial knots & villous fibrinoid necrosis (B)

DISCUSSION

Placenta is an important organ for fetal survival and wellbeing. It has both maternal and fetal interactions, so has the influence in the environment of both.[13] Gestational diabetes is a carbohydrate and sugar intolerant state which occurs in 2-5% of all pregnancies. It needs proper management as it can be harmful to both mother and fetus[14].

Normal placental histology is important for its proper functioning. Any alteration in the placental histological structure such as immature villi, fibrinoid necrosis, chorangiosis, and excessive syncytial knots and calcification shows disruption of placental structure leading to defective supply of oxygen and nutrients to the fetus.

In our study more immature placental villi with abnormal structural and functional alteration were seen in insulin treated group on placental microscopy. Verma indicated that increase risk to fetal existence in comparison to diet control group. It has been pointed out that placentae of insulin treated patients had more

immature villous development than diet control placentae[15]. These immature villi are not capable enough to oxygenate the fetal blood thus are strongly associated with fetal hypoxia, growth restriction and still birth[16,17]. Maly stated that immature villi can be a causative factor behind fetal demise and growth retardation in GDM patients.[18]

According to our study results, Villous fibrinoid necrosis was found to be more in insulin treated gestational diabetics. It suggests more compromised state in placental circulation in comparison to diet control group. This is caused by excessive placental growth due to anabolic effects of fetal insulin which is probably exaggerated further by administration of exogenous insulin Morphologically placentae loses its structure and functional capabilities and this might be responsible for hypoxia and adverse fetal outcomes encountered in diabetic patients.[19]

Syncytial knots are clumped nuclei of dying placental cells protruding into inter-villous space These are considered abnormal if microscopically present within more than 30% of the villi. Extensive number of knots indicates utero-placental ischemia or fetal stress[20]. In our study more syncytial knots were seen in insulin treated placentae which indicates presence of more utero-placental ischemia or fetal stress in this group.

Rudge stated that placentae of patient kept on diet and nutritional therapy had more chorangiosis than insulin treated placentae[21] but we found different results in both groups, equal number of patients had chorangiosis. The reason of difference in results of both studies is probably the difference in sample size of both the studies. Following the patients throughout the pregnancy in the diabetic antenatal clinic, and then long individual study period of 37-38 weeks for a single sample also accounted for a relatively smaller sample size in our study. Hypoxia is an important stimulator of multiple transcription factors which play an important role in angiogenesis[22]. In diabetic pregnancies there is elevation of fetal fibroblast growth factor-2 levels which causes placental angiogenesis and hyper-capillarization[23].

Visible calcification can be seen on the maternal surface as multiple whitish areas very small and hard to touch. Histologically, these are structure-less basophilic areas (figure 1). It is the sign of placental degeneration but may also occur due to some underlying maternal pathology. In our study more placentae on diet plus exercise alone group showed calcification. Many factors like parity, increase intake of dietary calcium etc. are said to be involved in the process of increased placental calcification.[24-28]

Thus hypoxic parameters were present in the GDM placentae managed conservatively or with pharmacological treatment. However the magnitude of features were more in diet, exercise plus insulin treated GDM patients.

CONCLUSION

Hypoxic features were observed upon histological examination of placentae in both groups, one with diet plus exercise alone and other treated with diet plus exercise and insulin. However they were more common in the insulin treated GDM group.

Conflict of Interest: This study has no conflict of interest to declare by any author.

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