Original Article

Change in Normal Morphology of Placenta and Its Possible Effects on Fetal Outcome in Diabetic Mothers as Compared to Non-Diabetic Mothers

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ABSTRACT

OBJECTIVES: To observe and compare gross and histological changes of the placenta of non-diabetic and diabetic mothers. And observe possible changes in the fetal weight of diabetic and non diabetic mothers.

STUDY DESIGN: Case control study.

PLACE AND DURATION: This study was conducted from June 2009 to July 2010 at the department of Anatomy of Liaquat University of Medical & Health Sciences Jamshoro. The placentae were collected from department of Gynecology & Obstetrics of Liaquat University Hospital.

MATERIAL AND METHODS: Eighty freshly delivered placentae were collected forty placentae from diabetic's mothers & forty placentae from parturient belonged to uncomplicated pregnancy (control group). Ages of all parturient were between 17 to 32 years. Fetal outcome and data was recorded. Placentae were measured on a weighing machine graduated in grams and diameter was measured with the help of a measuring tape in centimeters. Approximately five mm piece of from diabetic placenta was taken and processed for histological examination. Fetal weight was also recorded.

RESULTS: Morphological examination of placentae showed larger, heavier and more cotyledenous placentae group as compared to controls. Similarly microscopic examination revealed dilated blood vessels, necrotic and degenerative foci in placentae of diabetics as compared to controls.

CONCLUSION: Diabetes Mellitus produces profound gross as well as histological changes in placentae which might result in large for date babies because of fetal compromises. A good glycemic control might be a better option for reducing diabetes induced abnormalities.

KEY WORDS: Placenta, diabetes, histological changes.

INTRODUCTION

The placenta, a complex organ of short life-span which is responsible for the transfer of nutrients and waste products between the fetal and maternal circulations. The metabolic and endocrine activities of placenta are not clearly understood till date. The placenta must integrate signals from the fetus and the mother in an effort to match fetal demand with maternal nutrient supply [1, 2]. By doing so it actually plays a crucial role in fetal growth and well-being. Maternal diseases, which result in abnormal growth and development of placenta during early and mid-pregnancy are directly associated with decreased fetal growth in late pregnancy[3]. Diabetes Mellitus (DM) in pregnant women may be categorized into clinical diabetes or pregestational diabetes (women previously diagnosed with type 1 or type 2 diabetes) and gestational diabetes (GDM), which is stated as any degree of glucose intolerance with commencement or first recognition during pregnancy [4,5]. GDM represents nearly 90% of all pregnancies complicated by DM [6], and it affects nearly 2-5% of all pregnancies [7]. DM during pregnancy produces variety of placental abnormalities such as significant thickening of basal membranes of trophoblast, separation of basal membranes in basal capillaries[8], distension and proliferation of endothelial cells, disarrangements of perivascular space and decrease of vascular surface of terminal villi. The nature and extent of these changes depend on a number of factors, particularly the quality of glycemic control, achieved during the critical periods in placental development [1]. These pathological changes in the placentae of diabetic mothers are in turn important risk factors contributing to fetal anoxia and fetal compromise in pregnancy [9]. Furthermore abnormal maternal glycemic levels may alter the placental morphometric characteristics related to maternal-fetal exchanges [10]. Alterations in placental function due to uncontrolled diabetes result in disturbances in growth and development, macrosomia, congenital malformations and intrauterine growth retardation [10, 11]. This study is aimed to detect possible gross and micro-

scopic changes in the structure of placentae of diabetic mothers (pregestational and gestational both) which contribute to pregnancy outcome and compare those changes with placentae of mothers without diabetes mellitus or any other medical disease. This study in future will help us that good glycemic control can reduce the diabetic related changes in placenta and fetus.

MATERIALS AND METHODS

Eighty placentae of full term pregnancy were collected from labour room/ operation theatre of gynaecology and obstetrics department, LUH Hyderabad/Jamshoro Sindh, Pakistan. Out of these eighty placentae forty were from mothers with no known history of pre-existing diabetes mellitus or other medical disorders (control) and forty were collected from mothers with a history of uncontrolled diabetes mellitus. Subjects included in this study were aged between 20–40 years and were from low socioeconomic group. There were no racial, cultural or environmental differences among the subjects. The parity ranged from primi gravida to gravida 6. All placentae obtained were either by vaginal route or by caesarean section. A written consent and a proforma regarding personal biodata and medications, diet etc were signed by the subjects. The collected placentae were preserved in 10% formalin; the amnion and chorion were trimmed from the placenta in all cases. The umbilical cord was cut 5 cm away from its site of insertion.

All placentae were weighed on weighing machine graduated in grams (gm), after rinsed with running tape water and dried with blotting paper. The diameter of placentae was measured with the help of measuring tape in centimeters. Also gross examination was carried out for any abnormalities or missing cotyledons. After gross examination all placentae were cut along maximum diameters into two parts. Those parts were further cut into three pieces. The cut portions of the placentae were randomly selected and preserved in 10% formalin solution for further processing. Jars were labeled with code numbers and Hospital record number.

Microscopic Examination

Fixed placental tissues went through a series of processes starting from dehydration and clearing to wax impregnation before being sectioned. Total processing time was 24 hours. The tissues were sectioned at 5µm by rotary microtome and slides were prepared using APES coated glass slides. Slides were placed on metallic hot plate at 60^{o}C for 30 minutes before being stained.

Haematoxylin and Eosin staining of tissue slides

Once all the slides were prepared, they were passed through xylene-1 for 10 minutes and xylene -2 for another 10 minutes. The slides were then placed in to 100%, 95%, 80%, and 70% alcohol for 5 minutes each in order to dehydrate the samples. Slides were placed into the Harris Haematoxylin solution for 5-10 minutes after rehydrated with water. Tissues were differentiated in acid alcohol to remove excess stain before bluing the nuclei in a saturated solution of lithium carbonate. The tissues were again washed in tap water then placed in to 1% eosin for approximately 1-2 minutes. Finally the tissues were dehydrated by passing through increasing concentrations of alcohol and cleared in xylene for 5 minutes. The slides were then allowed to dry before being mounted in DPX and covered with cover slips. The prepared slides were examined under light microscope with 10 HPF.

Statistical analysis

The data was analyzed and entered in statistical prism 5^{TM} software. Numerical data i.e. weight, size and cotyledons were presented as mean$\pm$SD and student's t-test was applied to compare the mean between two groups (case and control). All the data was calculated on 95% CI. A p value < 0.05 was considered significant.

RESULTS

A total of 80 subjects were included in the study, 40 were non-diabetic mothers (control) and 40 were diabetic mothers (case). The mean weight of placenta in control group was 499.0 gm and in diabetic mothers placenta it was 967.5 gm, the difference between them was -468.5. Second parameter was placental size which in control was 499.6 cm in control and in diabetic mothers, it was 975.0 cm and the difference between these two was -475.4 in mean values which are statically significant (P-value <0.05).

Gross examination of placentae showed that placentae from diabetic mothers (figure 1a) are larger in size as compared to control (figure 1b and table 1) group.

Statistical analysis of macroscopic examination of number of cotyledons in placentae from control mothers had a mean of 16.13 and in diabetic mothers it was 24.46 with the difference of -8.333 which is significantly different from non-diabetic control group as shown in table 1.

Gross Examination

Gross examination of placentae showed that placentae from diabetic mothers (figure 1a) are larger in size as compared to control (figure 1b) group.

Statistical analysis of macroscopic examination of number of cotyledons in placentae from different age

groups of diabetic mothers showed no significant difference, while the number of cotyledons in placentae of different age groups of diabetic mothers was significantly different from non-diabetic control group as shown in table 2.

The size of placentae from diabetic mothers is significantly large in comparison with non-diabetic control group as shown in Table 2.

The weight of placentae of diabetic and non-diabetic control group was significantly different (P-value <0.05).

Histological Examination

On microscopic examination of placentae of diabetic mothers showed multiple foci of degenerative changes (dilated blood vessels and necrosis) with a mean of 2.300 and in control 0.8000with difference in mean was 1.500 as shown in fig 2a and 2b respectively, which were significantly different from non-diabetic control group in table 1 clearly show a difference in both above mentioned groups.

On histological examination of diabetic placenta, shows sub-trophoblastic basement membrane thicker in placentae with a mean of 1.923 then compared with non diabetics with a mean of 0.5641 the difference in mean was 1.359 as shown in figure 2c and table 1. Statistical analyses revealed significant differences between diabetic and non-diabetic control placentae (P-value <0.05). On microscopic examination, vessel thrombosis seen with mean of 0.5641 in diabetic mothers mean was 2.513 and difference of mean was 1.949 and nucleated RBCs (fig 2d) seen in control mothers was 0.3077(mean) while in diabetic patients the mean was 0.8205 but still their no. is significantly higher with a mean of 0.5128 than those were in non diabetic control group.

FIGURE 1 A, 1 B, 1 C:
Gross picture of maternal surface of Placenta of control mother (A), Placenta of diabetic mother (B, C). Figure indicates increased number of cotyledons large size placenta of diabetic mothers as compared to control placenta

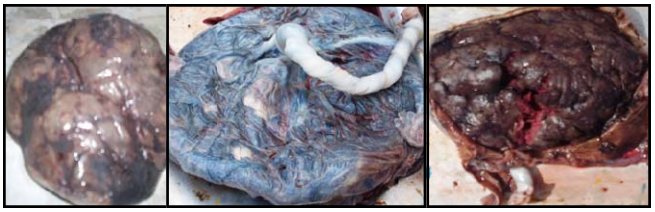

Figure 1 A Figure 1 B Figure 1 C

FIGURE 2(A, B, C, D and E):
Photomicrograph of 5 µm sections of placenta of diabetic mothers (diabetic mothers) stained with H& E under microscope (Magnification X 40). Figure 2 A showing normal and B show dilated blood vessels and necrosis (yellow arrows) respectively. Figure 2 C shows thickened basement membrane (yellow arrows); similarly 2 D indicates few nucleated RBCs (black arrows)

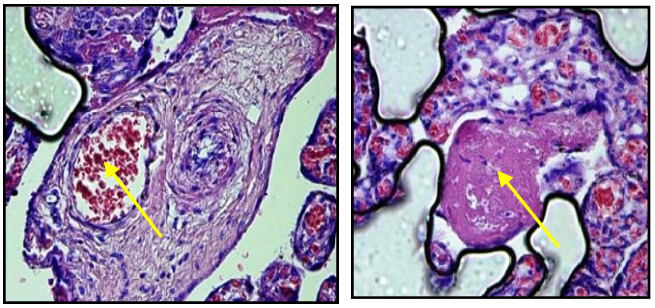

Figure 2 A Figure 2 B

TABLE I: SHOWING PLACENTAL PARAMETER ON GROSS AND MICROSCOPY AND FETAL WEIGHT

Placental parameters on gross	Non Diabetic mothers Mean±SD(control) n =40	Diabetic mothers Mean±SD(case) n =40	Difference b/w mean	P value
Weight of placenta (gm)	499.0±21.00	967.5±32.50	-468.5±38.69	<.0.0068
Size of placenta (cm)	499.6±11.91	975.0±20.21	-475.4±23.46	<0.0001*
No. of cotyledons	16.13±0.6884	24.46±0.02917	-8.333±0.7477	<0.0001*
Placental parameters on microscopy				
Degenerative changes in placenta	0.8000±0.1086	2.300±0.1144	1.500±0.1577	<0.0001*
Vessels thrombosis	0.5641±0.1207	2.513±0.1031	1.949±0.1587	<0.0001*
Subtrophoblast membrane thickness	0.5641±0.1207	1.923±0.1293	1.359±0.1769	<0.0001*
Presence of nucleated RBC	0.3077±0.09113	0.8205±0.1211	0.5128±0.1516	<0.0001*
Fetal weight (kg)	2.871 ± 0.05091	4.845 ± 0.07372	1.974 ±0.08959	<0.0001*

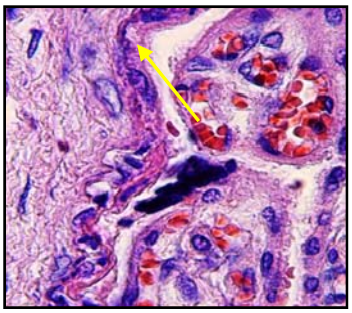

Figure 2 C

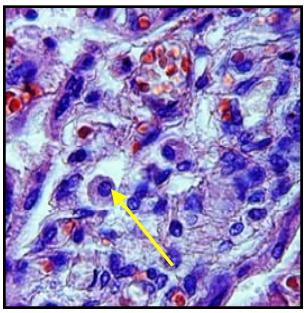

Figure 2 D

DISCUSSION

Diabetes mellitus is a common metabolic disorder, defined by chronic hyperglycemia. It is a major cause of morbidity and mortality from long term diseases of major organ systems such as cardiovascular, renal, central nervous system etc [12]. Apart from affecting major organ systems of body diabetes mellitus during pregnancy produces complications both in mother and offspring [13, 14].

It is believed that if metabolic control is good, perinatal mortality should not be higher than general population. But, macrosomia or large babies continuous to be a problem in higher than average proportions of such cases [15].

The placenta is highly specialized organ of pregnancy that supports normal growth and development of the developing fetus [2]. The fetus, placenta and mother form a triangle of dynamic equilibrium. Disturbances in any of these affect the others [16]. The main functional unit of placenta is chorionic villi. In the beginning fetal blood is separated from maternal blood by means of four layers of placental membrane[2].

This study indicates that all placentae from pregnancies complicated by diabetes mellitus, regardless of age, parity and onset of diabetes were larger in size, more in weight and had more cotyledons as compare to non-diabetic control groups. These findings are comparable to the findings of a study carried out by Ashfaque and his fellows, which showed significant increase in the weight and size of placentae of diabetic mothers [17]. In another study gross examination of placenta revealed that weight of placenta from diabetic mothers was more as compared to controls, similarly histological alterations were also obvious in the form villous immaturity and dysmaturity in diabetic placenta as compared to controls [18].

The pathophysiology behind weight gain includes compensatory hyperplasia due to fetal macrosomia, which in turn results from reactionary hyperglycemia in fetuses of diabetic mothers. Another factor involving villous hyperplasia could be due to low oxygen tension in chorionic villous blood. Reduced blood flow through intervillous spaces is because of vascular compromises in diabetes mellitus [2]. A significant increase in fetal and placental weights, placental volume, volumes of the intervillous space and the trophoblast was found in the diabetic group compared to the controls in a study conducted by Jauniauxa, and Burtonb in 2006 [19]. They also found a significant increase in the volume of the intermediate and terminal villi, the surface area of the villi and of the fetal capillaries, and the thickness of the villous membrane was found in the macrosomic subgroup compared to the controls. Furthermore Kucuk M, Doymaz F. also mentioned that there was a direct proportion of gestational diabetes with placental and fetal weight which was not observed with only dose of 100mg of oral glucose [20].

These studies support our findings of increase in surface area and weight of placenta from mothers suffering from diabetes mellitus during pregnancy. However there are evidences of no placental abnormalities in diabetic mothers or only minimal deviations are found from control groups [21]. This scarcity of uniformity may reveal a combination of small series, grouping of different classes of maternal diabetes, differences in glycemic control between individual patients, recent improvements in antenatal care, and differing methodology.

These findings are in line with one previous study conducted to compare gross and histological pathologies of placentae from mothers complicated by gestational diabetes with controls. The study showed same histological abnormalities such as nucleated fetal RBCs, fibrinoid necrosis, as are observed in our study [22]. Similar findings were also observed in another study conducted by Madazli and fellows [23]. Although no gross abnormalities were observed in placentae of diabetic mothers by Verma and his fellows, however histological findings such as fibronoid necrosis, villous edema, villous fibrosis were exhibited which further support our findings. Histological pathologies such as the presence of nucleated fetal red blood cells, fibrinoid necrosis, villous immaturity and chorangiosis were observed more often in the diabetic placentae compared with the control placentae [24].

In second part of study fetal outcome was observed. Examination of fetuses showed that almost all diabetic mothers gave birth to large for date babies as compare to non-diabetic control group. Macrosomia or large babies affect the fetal part of placenta. It is a contributing factor for increase in the thickness, diameter and villi of the placenta. Fetal hyperinsulinism due to diabetes mellitus is associated with macrosomia and a higher rate of birth injuries and caesarean sections, neonatal hypoglycemia, respiratory distress and due to fetal programming the development of the sequelae of the metabolic syndrome in childhood or adolescence [25].

Unexplained intra-uterine fetal death is also a problem in diabetic pregnancies despite metabolic control, particularly in those with LGA-infant [26].

Apart from large for date babies, the offspring of women with diabetes also have an increased occurrance of congenital malformations as compared with the general population. There is growing evidence that both humans and animal studies, signifying an association between these malformations and poor glycemic control in the periconceptual period. Diabetes mellitus is one of the frequent metabolic pregnancy complications associated with an increased risk of maternal and neonatal morbidities. Indeed uncontrolled diabetes during pregnancy is linked with severe medical conditions in the fetus. If it remains uncontrolled during the period of organogenesis, it leads to severe congenital malformations of major organ systems including the central nervous system and cardiovascular system [25, 26].

Late pregnancy complications of uncontrolled diabetes effect in neonatal morbidity and mortality. A number of epidemiological studies show that despite good glycemic control, the number of congenital malformations in diabetic mothers is still higher than in non-diabetics [27].

These responses may attribute to fetal hyperglycemia due to hyperinsulinemia. Fetal hyperglycemia disturbs the osmotic environment with resultant cell and tissue injury [28]. High levels of glucose and ketone bodies in diabetes involve many factors in development of complications. One of the key factors is oxidative stress, which is proved in many experimental studies. The effects of free radicals are normally controlled by administration of wide range of antioxidants [29, 30].

As this study is conducted on low socioeconomic, uneducated and rural population of Pakistan and majority of women from these areas are unaware about the dietary plans during pregnancy and also are unaware regarding achievements of good glycaemic control and taking of medications or diets full of antioxidants and other nutrients. It is therefore advisable and must be a necessary act to create mass awareness in these backward areas of countries like Pakistan in order to save the life of mother and offspring.

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