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Histological Changes in Placental Syncytiotrophoblasts of Poorly Controlled Gestational Diabetic Patients

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Abstract. It seems reasonable to expect that biochemical changes occurring in the pregnant woman with diabetes should be reflected in the placenta structure. However, it has not been possible to correlate placental morphology with glycemic control in a comparison between those with long life diabetes and poorly controlled gestational diabetes. In the present study we have histologically studied the syncytiotrophoblast of human placentae from overt diabetic and poorly controlled gestational diabetic patients. Using specific staining techniques and direct light microscopy we qualitatively studied these placentae and compared them with the normal placentae. We found fibrin thrombi, villous oedema, hyperplasia and thickening of basement membrane in the placentae of poorly controlled gestational diabetic mothers. Direct microscopy revealed that these various changes in syncytiotrophoblast structure were marked in the poorly controlled gestational placenta compared with overt diabetics, and could have been due to the presence of histochemical compounds e.g. general carbohydrates and lipids. These studies may indicate that poor control of diabetes during the gestation as indicated by high level HbAlc may result in the accummulation of carbohydrate compounds and fat droplets in the placental basement membrane, leading to structural changes in the placental cells.

Key words: Histology, Syncytialtrophoblast, Placenta, Gestational Diabetes.

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GESTATIONAL diabetes mellitus (GDM) is indicated by abnormal glucose tolerance with onset or first recognition during pregnancy, but which was normal before and will usually be normal after pregnancy [1]. It is associated with increased risk of maternal complications and an adverse outcome of the pregnancy, including miscarriage, stillbirth, macrosomia, and congenital anomalies [2]. The definition applies whether insulin is used for treatment or the condition persists after pregnancy but does not exclude the possibility that the glucose intolerance may have antedated the pregnancy.

In general, previous studies have suggested that abnormal placental structure results in diminished placental function and relates to perinatal loss and morbidity [3, 4]. Diabetes in pregnancy is associated with risk to the fetus in terms of malformation, morbidity and mortality.

The morphological changes in placentae of Type 1 IDDM have been investigated [5] based on their histological features, and the most frequently observed lesion was that of relative placental immaturity despite near-optimal blood glucose control. Furthermore morphological studies on syncytiotrophoblast microvillous membrane of placenta in these types of patients [6] showed a significant increase in the surface density of the microvilli and in the microvillous surface enlargement features as compared to those of the controls. Moreover placentae from GDM class A have been studied [7] and the histological findings confirmed that this is a thickening of the capillary villous vessels. Quantitatively the percentage of peripheral villous surface area covered with microvilli has been determined in the placenta [6] and found to

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be 89%. Furthermore the oedema observed may be correlated to the swelling of hyaluronic acid molecules [8].

The pathogenesis of these abnormalities is still far from being fully understood but there is a widespread impression that their extent and degree are related not only to the severity and duration of the maternal diabetes mellitus but also to the degree of control of the diabetic condition during pregnancy.

The present study characterized the histological changes in the microvillous membrane of the placental trophoblast from metabolically normal mothers and poorly controlled gestational diabetic (GDM) women who had abnormal metabolic control i.e. pregnant women who did not strictly follow the management of diabetes mellitus during pregnancy. Moreover women with overtly diabetic pregnancy were included in the study.

Subjects, Materials and Methods

Control subjects

The control group comprised 6 women randomly chosen from the outpatient obstetric care unit of the Nasyrria Obstetrics and Gynecology Clinics, Riyadh, Saudi Arabia. These women met the following criteria: normal oral glucose tolerance test (OGTT) with 75 g glucose [9], no endocrine abnormalities, no family history of diabetes and no hypertension at the end of pregnancy.

Gestational diabetic women

Six pregnant subjects were included in this study. GDM was diagnosed according to WHO criteria where one hour OGTT value exceeding 8.9 mMol/L was considered to indicate gestational diabetes. Diagnosis for GDM was performed at the beginning of the second trimester where it was noted for the first time during this pregnancy. None of the pregnant women studied with positive OGTT followed the treatment as recommended elsewhere [10] when they attended the antenatal clinic in the late third trimester on the initial presentation. These patients who were consistently not following the strict management of GDM were called poorly controlled GDM patients. Three patients from this group were obese (BMI>27) and among these three patients two were hypertensive.

Overtly diabetic women

Six diabetic pregnant women were studied. Diabetes was classified according to White [11]. These subjects were treated with insulin if the fasting and/or post prandial blood glucose was greater than 5.8 mMol/L and greater than 6.7 mMol/L respectively. Metabolic control was carried out in the hospital initially for instituting therapy and continued on an outpatient basis [12].

Informed consent from all patients and control subjects were obtained before they were allowed to participate in this study.

Methods

1) Glycemic control: The OGTT was performed between the 20th and the 28th week of gestation after overnight fasting. Loading was performed in the morning with 75 g glucose dissolved in 300–400 m*l* water. Blood samples were collected in EDTA tubes and separated serum was frozen at − 20°C for glucose analysis in a glucose analyzer (Beckman Instruments Inc., Brea, California, USA). Glycosylated haemoglobin (HbAlc) was measured in the whole blood by a microcolumn (Helena Laboratories, Beaumont, Texas, USA).

2) Placenta preparations: We were able to collect 17 placentae, 6 normal human placentae (NHP), 6 placentae from poorly controlled gestational diabetic patients (GDHP) and only 5 long standing overt diabetic human placentae (DHP) because one was lost when the patient left the city before the delivery. Samples of placentae were obtained after a full term vaginal delivery. Placentae with attached umbilical cords and membranes were received in the delivery room within 10 minutes of delivery, refrigerated and then sent to the histology and histochemistry laboratories. Within 1 to 6 h after receiving the placentae, umbilical cords were removed and the membranes were trimmed from the attachments on the edge of the placentae with 2 cm of its insertion. Excess blood clots were removed.

Histological preparations

1) Paraffin sections: The placenta were histologi-

cally processed as previously described by Teasedale [13]. Briefly, small pieces of the chorionic frondosum layer were taken from the placenta of each subject, then fixed for 2 weeks in cold (4°C) 10% buffered neutral formalin, Gendre's fixative and Elftman's fixative. After fixation, the tissues were thoroughly washed in running water before processing. The tissues were dehydrated in gradual concentrations of 70%, 80%, 90%, and absolute ethanol for 2 h in each solution at room temperature. Specimens were then cleared by passing them through two sets of chloroform for three hours each. Paraffin wax with a melting point of 65°C was used for impregnating and mounting the specimens. 5 to 7 μm thick sections were cut with an American optical microtome.

Sections were stained by histological staining techniques with haematoxylin-eosin, Mallory trichrome and Mallory-Azan after which histological examinations were performed.

Data analysis

Significance was 95% or more. All observations were carried out under a light microscope, and significant differences observed.

Results

General characteristics of the patients are shown in Table 1. Twelve patients were enrolled in this study, 6 of them were poorly controlled gestational diabetics and the rest were well controlled long standing overt diabetics. The age range of the patients was between 25 and 35 years and there was no significant age difference between gestational and overtly diabetics. An additional 6 pregnant normal controls were enrolled in this study and they were in the same age range when this study was conducted.

As shown in Table 1, the comparative fasting plasma glucose level mean ± SD was significantly higher in overt diabetes mellitus (6.4 ± 1.5 mM/L) than in gestational diabetics (5.9 ± 2.3 mM/L) in the second trimester. When following these patients up during the entire pregnancy, fasting plasma glucose levels were significantly higher in the third trimester among poorly controlled gestational diabetic mothers ($P<0.001$) than in frankly diabetic mothers. In the overt diabetic group, per cent HbAlc at the time of diagnosis was much higher than in the poorly controlled GDM patients, whereas in the third trimester the poorly controlled gestational diabetics had a higher value (9.6 ± 1.1%) than the overtly diabetic patients ($P<0.001$).

Histological findings

The histological findings in the normal placentae group were typical of their gestational age. The placenta from poorly controlled gestational diabet-

Table 1. General characteristics data for 6 poorly controlled gestational diabetic women, 6 overt diabetic pregnant women, and 6 normal pregnant controls*

	Gestational diabetics	Overt diavetics	Control subjects
Age(years)	26.4 ± 3.8	28.4 ± 4.2	25.6 ± 6.7
BMI**	26.7 ± 5.2	24.4 ± 3.6	23.5 ± 4.6
Second Trimester (Diagnosis time)			
Fasting plasma glucose (mM/L)	5.9 ±23.4[a]	6.4 ± 1.5[a]	4.6 ± 0.8
2–h plasma glucose (mM/L)	11.3 ± 1.8	9.3 ± 2.1	5.5 ± 1.1
HbAlc (%)	6.9 ± 1.2[b]	8.4 ± 1.6[b]	6.7 ± 1.0
Late third Trimester			
Fasting plasma glucose (mM/L)	9.7 ± 1.6[c]	7.8 ± 2.2[c]	4.2 ± 1.2
HbAlc (%)	9.6 ± 0.1[d]	9.1 ± 2.2[d]	5.6 ± 1.4

* Data expressed as the mean±SD. ** BMI(Body mass index) calculated as weight in Kg over height in m^2(Kg/m^2). a, b, c, d, all at $P<0.001$.

ics and overt diabetic patients exhibited different histological features from the normal. The poorly controlled gestational diabetic placentae (Fig. 1C) showed marked villous oedema of the chorion frondosum layer and marked fibrin thrombi in the syncytiotrophoblast. Marked hyperplasia of the cytotrophoblast was also observed with moderate thickening of the basement membrane of the syncytiotrophoblast. In comparison, overt diabetic placentae (Fig. 1B) showed slight villous oedema of the chorion frondosum and moderate hyperplasia in the cytotrophoblast but weak occurrence of fibrin thrombi was observed. The basement membrane of the syncytiotrophoblast was slightly thickened in gestational diabetic syncytiotrophoblast (Fig. 1C). Fig. 1A shows the histological staining of the normal placenta in which the villous capillaries were normal and the membrane of the syncytiotrophoblast was normal. Results of the general histological staining are shown in Table 2. The table shows significant differences among the three types of placentae.

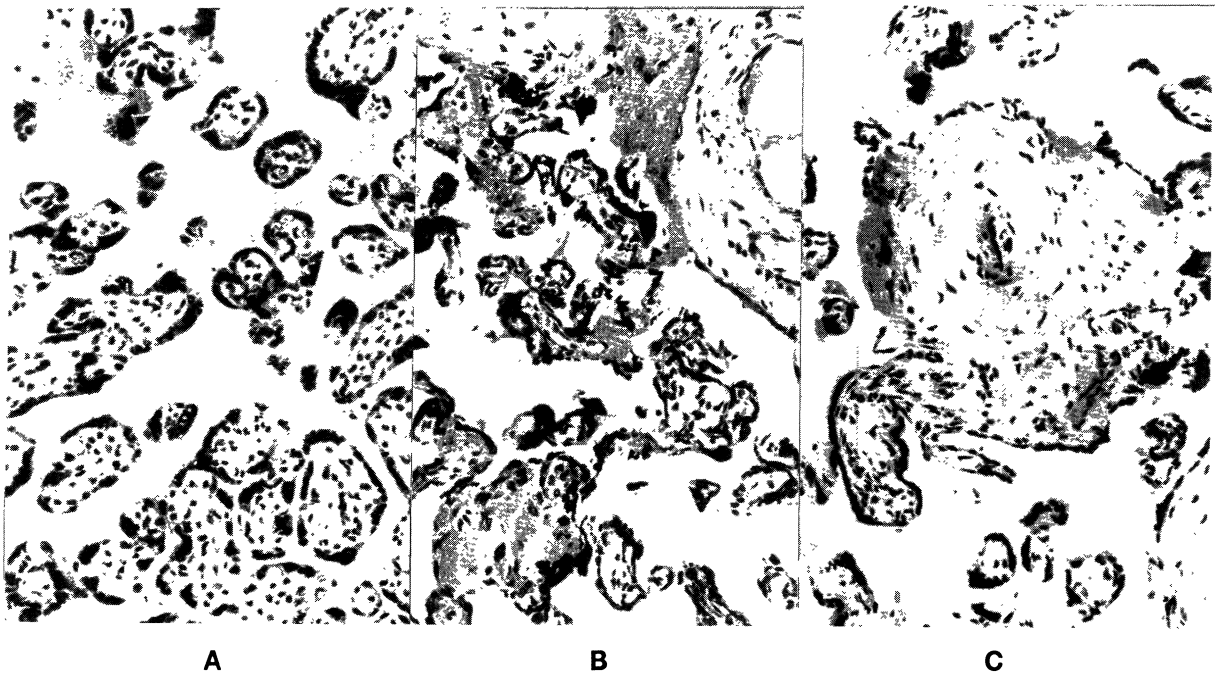

Fig. 1. A: A section through the chorion frondosum in the normal placentae. (Haematoxylin-eosin stain, Magnification ×240). B: A section through the chorion frondosum of placenta from overt diabetic placentae showing slight villous oedema, weak fibrin thrombi, moderate hyperplasia and slightly thickened of the basement membrane. (Haematoxylin-eosin stain, Magnification×240). C: A section through the chorion frondosum of placenta from poorly controlled gestational diabetic placentae showing marked villous oedema, marked fibrin thrombi, marked hyperplasia and moderate thickening of the basement membrane. (Haematoxylin-eosin, Magnification×240).

Table 2. General histological findings in the chorionic frondosum microvillous syncytial membrane of the three different human subject placentae*

Histological findings	Normal human placentae	Overt Diabetic human placenta	Poorly controlled gestational diabetic human placentae
Fibrin thrombi	None	Weak	Marked
Thickening of basement membrane	None	Slight	Moderate
Villous oedema	None	Slight	Marked
Hyperplasia	None	Moderate	Marked

* Observations were done with a light microscope.

Discussion

The data collected in the present study have shown that the placentae of poorly controlled gestational diabetic mothers and the long standing well controlled diabetic mothers differ markedly from the control mothers by having significantly different histological abnormalities. Firstly our findings showed marked hyperplasia in the insufficiently controlled gestational diabetic placentae, slight hyperplasia was observed in well controlled life diabetic placentae, and in normal placentas the hyperplasia was absent. Winick and Noble [14] working on the placentae content measurements found an increase in the number of cells of normal size in the placentae of gestational diabetes. Jones and Fox [5] observed hyperplasia in gestational diabetes and in diabetic placentae during the phase growth in the human placentae which terminates at around the third trimester of gestation [13, 14].

Secondly villus oedema was clearly observed in gestational diabetic placentae and slightly in well controlled diabetic placentae. Brudenell and Doddridge [15] reported that villus oedema is common in diabetic placenta. Also Shen-Schwarz [16] have observed the same features. In our study the thickening of the basement membrane of syncytiotrophoblast was clearly seen in poorly controlled gestational diabetic placentae compared with that in the well controlled diabetic placentae. Further studies reported that thickening of the basement membrane of syncytiotrophoblasts occurs in diabetic placentae [17, 18].

The villus oedema observed may be due to the accummulation of acidic mucosubstances, Nelson *et al.* [19] reported the presence of acidic components at higher density on the surface of membrane microvilli than on intermicrovillous surface membrane in normal pregnancies. Similarly it was reported that the thickening of the basement membrane in gestational diabetic and diabetic placentae is the result of mucopolysaccharide deposition seen by intense Alcian blue staining at pH 2.5. Moreover, mucopolysaccharide deposition was also observed in all the cases of villous oedema [20]. The oedema observed may be correlated to the swelling hyaluronic acid molecules, and an increase in mucopolysaccharide content, mainly hyaluronic acid, was found in overtly diabetic placentae [21].

The changes seen by us in placentas from women with poorly controlled gestational diabetes may be summarized as patchy syncytial necrosis, an increased number of syncytial secretory droplets, dilatation of the syncytial rough endoplasmic reticulum, increased syncytial lysosomal activity, cytotrophoblastic hyperplasia, an increased number of active cytotrophoblastic cells, degenerative changes in occasional cytotrophoblastic cells, focal thickening of the trophoblastic basement membrane, and narrowing of the lumen of already small fetal vessels by enlarged endothelial cells.

In our present study poorly controlled gestational diabetic patients, as indicated by glycemic control (glucose and HbAlc levels), showed a marked increase in the thickness of the basement membrane of the syncytiotrophoblast, this phenomenom being of minimal degree in the overt diabetic placenta and absent from the normal control placenta. It seems clear that the placental changes are independent of uteroplacental ischemia and are related solely to the presence of maternal diabetes mellitus. Therefore these qualitative and quantitative differences in placental findings between poorly controlled gestational and well controlled overtly diabetics support the hypothesis that the maternal disease may produce a variety of alterations in the metabolic microenvironment of the placenta. This being the case, it is logical to assume that the less severe and better controlled the patient's diabetic stage, the less striking would be the placental abnormalities. The elimination of hyperglycemia will not therefore itself prevent the development of placental abnormalities and this must be due to some as yet unknown constituent factor in the diabetic status which is only partially influenced by diet or insulin.

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