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Histological and histochemical changes in placenta of diabetic pregnant females and its comparision with normal placenta

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ABSTRACT

Objective: To investigate the histological and histochemical changes in placenta of diabetic pregnant females and compare them with normal placenta. **Methods:** The histological and histochemical features of 60 placenta, 30 obtained from normal pregnant females and 30 from diabetic pregnant females, were studied. These placenta were obtained from Department of Obstetrics and Gynaecology, GSVM Medical College Kanpur and ERA's Lucknow Medical College and Hospital Lucknow. **Results:** On histological examination, the diabetic placenta showed increased syncytial knots, fibrinoid necrosis, trophoblastic basement membrane thickening, villous stromal fibrosis, villous oedema, crowding of villi, thickening of vessel wall and fibrin deposition. On histochemical study it was found that the PAS reactivity was stronger in diabetic placenta as compared to normal. Sudan Black reactivity was higher among diabetic placenta in comparison to normal placenta. **Conclusions:** It is concluded that distinct histological and histochemical changes could be seen in placenta of diabetic pregnant females.

1. Introduction

Placenta is the most important and vital organ of intrauterine life. It is fountainhead of human existence. The placenta is formed from elements of membrane which surround the developing fetus and the uterine endometrium and provide the means for physiological exchange between the fetal and maternal circulation and it shows various histological and histochemical changes in case of diabetic mother. Histologically a term placenta shows large number of villi and syncytial knots. In these knots, syntiotrophoblast nuclei are aggregated together in clusters leaving zones of thin cytoplasm devoid of nuclei in between. The diffusion barrier between maternal and fetal circulation comprises of five layers-trophoblast, trophoblastic basement membrane, core of supporting tissue, capillary endothelial basement membrane and endothelium. Yang in his study found histological changes like villous immaturity, proliferation of small fetal vessels, increased syntitiotrophoblast knots in diabetic placenta[1]. Okail found histological changes in syncytiotrophoblast structure were more marked in poorly controlled gestational diabetes placenta[2]. Evers *et al*. in their study on diabetic placenta found various histological abnormalities like fibrinoid necrosis, villous immaturity in the placenta[3].

The present work aims to increase our knowledge about the histological and histochemical changes in diabetic placenta because very few studies have been done on histochemical changes in placenta of a diabetic mother and further research in this field will go a long way in finding out a solution in evaluating the destructive changes in diabetic placenta in the initial stages of pregnancy.

2. Materials and methods

The study was carried out in Department of Anatomy in GSVM Medical College Kanpur, India and ERA's Lucknow Medical College Lucknow, India. A total of 60 placenta were collected immediately after delivery for the study. Out of these 60 placenta, 30 were from normal pregnant females and 30 from diabetic pregnant females. The placenta were washed with normal saline and then put to gross examination. Four, 2 cm pieces of the placental tissue were taken that included the fetal surface and intact maternal surface (within 2 cm of placental margin). The tissue was

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processed and fixed for routine haematoxylin and eosin stain, for special stains like PAS staining for glycogen and Sudan Black B staining for lipids. The slides were then examined under light microscope.

3. Results

Histological analysis of the diabetic placenta in current study revealed increased syncitial knots (Figure 2) in 80% cases as compared with normal placenta (Figure 1). The areas of fibrinoid necrosis also showed the same pattern. The trophoblastic basement membrane thickening was seen in all diabetic placenta. Villous stromal fibrosis was significantly increased and villous oedema (Figure 2) was also observed in all diabetic placenta. Further crowding of villi was prominent and areas of glycogen deposition and lipid deposition (Figure 4&6) were found to be significantly increased in diabetic placenta as compared with normal placenta (Figure 3&5).

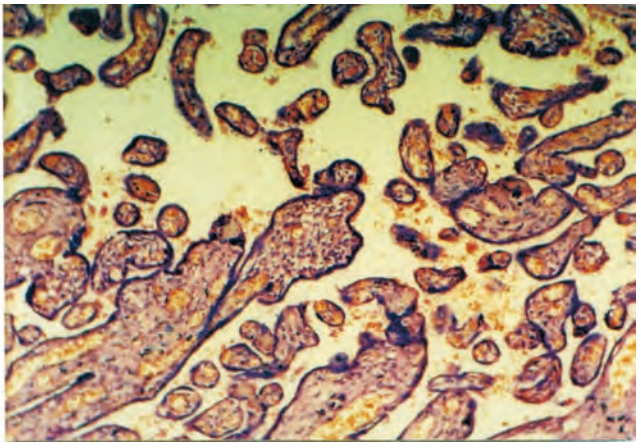

Figure 1. Photomicrograph showing normal placenta.
H&E stain (100×).

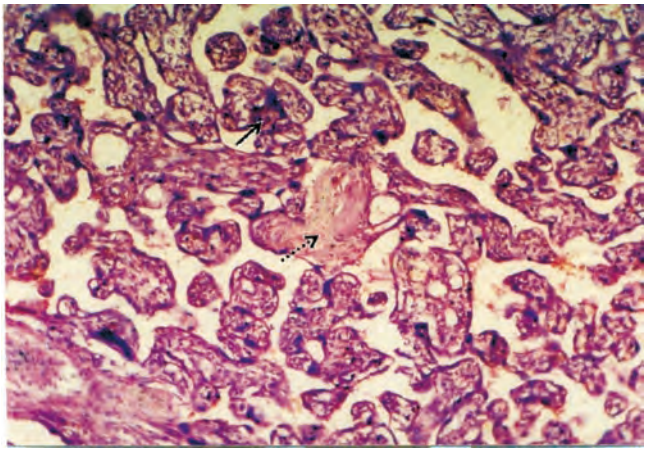

Figure 2. Photomicrograph showing increased syntitial knots (–>) and villous oedema (···>) in diabetic placenta.
H&E stain (100×).

Out of 30 cases of diabetic placenta, 24 cases showed increased syncytitial knots and areas of fibrinoid necrosis. In 18 cases villous stromal fibrosis was increased. The trophoblastic basement membrane thickening, villous oedema, crowding of villi and areas of fibrin deposition were found more in diabetic placenta (Table 1).

Histochemical analysis showed PAS reactivity for glycogen was much stronger in diabetic placenta (Table 2). 20% cases showed glycogen in traces and 80% cases moderate to severe reactivity. The Sudan Black reactivity gradings were mild in 20%, moderate in 20% and strong in 60% among the diabetic placenta (Table 3).

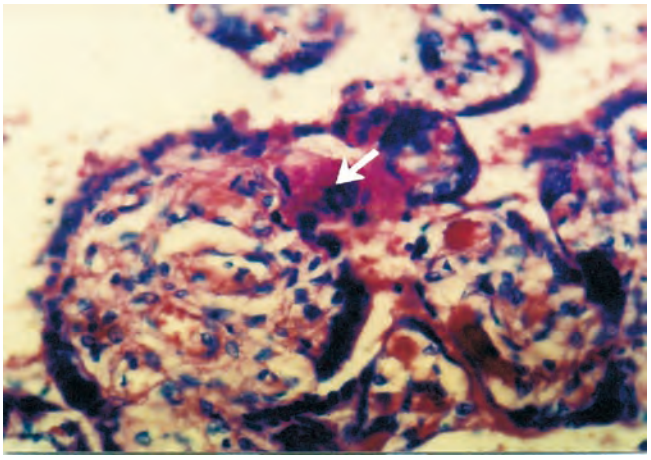

Figure 3. Photomicrograph showing areas of glycogen deposition in normal placenta.
PAS stain (400×).

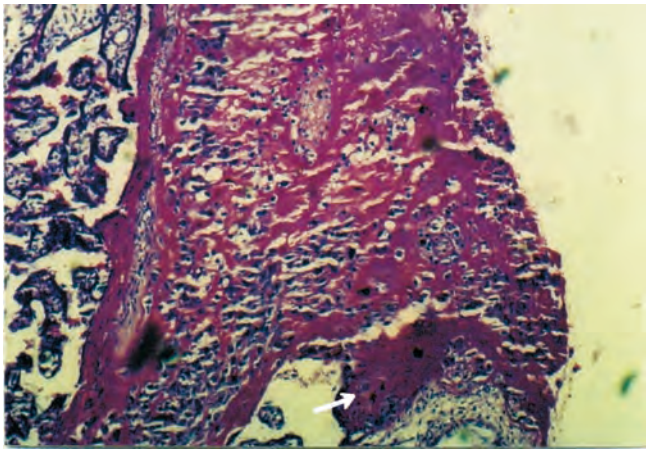

Figure 4. Photomicrograph showing areas of glycogen deposition in diabetic placenta.
PAS stain (100 ×)

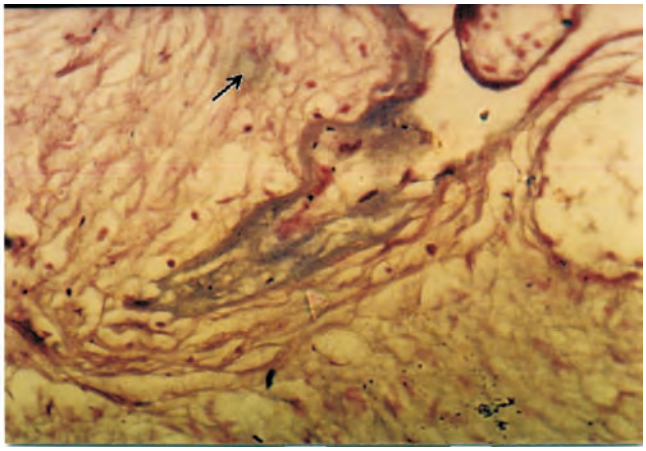

Figure 5. Photomicrograph showing areas of lipid deposition in normal placenta.
Sudan black stain (400×).

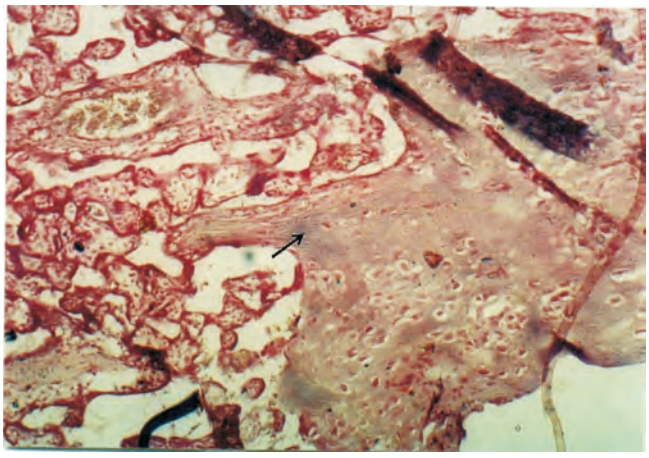

Figure 6. Photomicrograph showing areas of lipid deposition in diabetic placenta.
Sudan black stain (100×).

Table 1
Microscopic finding of diabetic placenta (n=30) [n (%)].

Features	Normal	Increased
Syncytial knots	6 (20)	24 (80)
Fibrinoid necrosis	6 (20)	24 (80)
Trophoblastic basement membrane thickening	–	30 (100)
Villous stromal fibrosis	12 (40)	18 (60)
Villous oedema	–	30 (100)
Crowding of villi	–	30 (100)
Fibrin deposition	–	30 (100)

Table 2
PAS reactivity [n (%)].

	Normal placenta	Diabetic placenta
Hazy + –	12 (40)	–
Traces +	12 (40)	6 (20)
Mild++	6 (20)	–
Moderate +++	–	12 (40)
Strong ++++	–	12 (40)

Table 3
Sudan black reactivity [n (%)].

	Normal placenta	Diabetic placenta
Hazy+ –	–	–
Traces+	9 (30)	–
Mild++	15 (50)	6 (20)
Moderate+++	6 (20)	6 (20)
Strong++++	–	18 (60)

4. Discussion

Diabetes represents a spectrum of metabolic disorders in which blood sugar levels are abnormally high. Diamant *et al* state that there is an increase in phospholipid and triglyceride content in placentas from GDM and type1 diabetic pregnancies[4]. Sala *et al* state that there is regional variation in the frequency of fibrinoid degeneration in human term placenta[5]. They suggest that hypoxia or relative stasis at increased sugar levels could stimulate the fibrinoid degeneration of villi. Jones and Desoye state that there is an increased glycogen deposit around placental vessels in maternal diabetes[6]. Mayhew and Jairam state that essentially normal microscopical morphology is preserved in placenta of diabetic subjects with good glycaemic control[7]. Clapp state that altering the source of maternal dietary carbohydrate may prove to be valuable tool in management of pregnancies at risk for anomalous feto-placental growth and for the prevention of obesity of insulin resistance in non-pregnant state[8]. Evers *et al* report that histological abnormalities such as presence of nucleated fetal RBC′s, fibrinoid necrosis, villous immaturity is more often seen in diabetic placenta[3]. Elchalal *et al* report that lipid deposition and formation of lipid droplets in term of trophoblastis are stimulated by insulin[9]. Alonso *et al* conclude that during gestational diabetes, placenta suffers structural and functional alterations which are indicative of the protective role of placenta[10]. Jauniaux and Burton state that significant increase in fetal and placental weight, placental volume, volume of intervillous spaces and the trophoblast is found in the placenta of diabetic pregnant females[11]. Jansson *et al* suggest that altered placental function may be a mechanism contributing to fetal overgrowth in diabetic pregnancies[12]. Gernot Desoye, Sylvie Hauguel-de Mouzon report that glycogen increments in diabetes are found around villous vessels and capillaries and also fatty acids accumulate in fetal part of placenta of diabetic mothers[13]. Lang *et al* state that the human fetal placental endothelial cells have a mature arterial and venous phenotype with adipogenic and osteogenic differentiation potential[14]. Riza *et al* report presence of villous immaturity, chorangiosis and ischaemia are significantly increased in placentas of women with diabetes[15]. Hiden *et al* suggest that disregulation of growth factors and their receptors may be involved in placental and fetal changes like enhanced growth and hypervascularisation observed in diabetes[16]. Pathmaperuma *et al* conclude that hyperglycemia causes intracellular glycogen accumulation and reduces lipid droplet accumulation and no other effects on trophoblast metabolism or function[17]. Ansari *et al* find that there is significant increase in percentage of placental villi showing syncytial knot formation, cytotrophoblastic proliferation, basement membrane thickening and hypovascularity in pre-eclamptic patients[18]. Verma *et al* state that control of hyperglycemia only partially prevents the development of placental abnormalities which must be due to some other constituent factor of diabetic state[19-21].

In the present study in addition to increased syntitial knots, fibrinoid necrosis and fibrin deposition, villous oedema, crowding of villi and trophoblastic basement membrane were present in most of the diabetic placenta. These histological changes of placenta are chiefly due to metabolic disturbances which leads to accumulation of carbohydrate and fat in the placenta. This carbohydrate accumulation is identified histochemically using specific

stain for glycogen (PAS stain). This study revealed higher PAS reactivity in most of the diabetic placenta. The fat accumulation is identified histochemically using Sudan Black stain and its reactivity was stronger in all the diabetic placenta studied.

Diabetes is fast emerging as a global epidemic, India is going to become the global capital of diabetes by year 2015. Pregnancies complicated by diabetes and the subsequent perinatal complications are an area of concern. Developing countries like India, with new found affluence, are breeding grounds for life style diseases. With small family norms and fewer pregnancies, fetal well being is of prime importance.

In this study, distinct histological and histochemical changes were seen in placenta of diabetic pregnant females. Excessive fibrosis, intervillous fibrin deposition and various other degenerative changes are detrimental to the outcome of pregnancy. Developing markers, histological and histochemical, for fetal wellbeing in diabetic mothers, would be great tools for addressing this grave disease with potential negative outcomes in pregnancy. Further research to study these changes *in vivo* would be worthwhile.

Conflict of interest statement

We declare that we have no conflict of interest.

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