Stereological study of human placental barrier in pregnant women suffering from gestational diabetes

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الخلاصـــــة

هذه الدراسة المستقبلية المجسمة للحاجز المشيمي أجريت في مستشفى الطفل والولادة وكلية الطب في البصرة وتضمنت أخذ عينات نسيجية من عشرين مشيمة للنساء الحوامل المصابات بداء السكر خلال الحمل وعشرة عينات نسيجية من مشيمة نساء حوامل غير مصابات بداء السكر خلال الحمل جميع العينات فحصت تحت المجهر الضوئي وعلى الشاشة الملحقة به لغرض قياس المسافات المشيمية للحاجز المشيمي وتمت مقارنة المسافات المشيمية للنساء الحوامل المصابات بداء السكر خلال الحمل مع المسافات المشمية للنساء الحوامل الطبيعية فوجد هناك زيادات في المعدلات لجميع المسافات المشمية للحاجز المشيمي في النســـاء الحوامل المصابات بداء السكر خلال الحمل ولكن هناك زيادة واضحة في معدل المسافة بين كريات دم الأم والأوعية الدموية في الزغابات المشيمية (M B P)

ومعدل المسافة بين كريات دم الجنين وبطانة الأوعية الدموية (F B P) وكذلــك

معدل المسافة الكلية بين كريات دم الأم وكريات دم الجنين داخل الزغابات (T DD) .فضلاعن التغيرات الشكلية والتركيبية وهي كما يلي:-

1 – أختلاف سمك الجدران المحيطة بالزغابات المشمية وتثخن الغشاء القاعدي وكانت أكثر وضوحا" في الزغابات المشيمية للنساء الحوامل المصابات بداء السكر.

2 - كثرة أحتشاء الزغابات المشيمية للنساء الحوامل المصابات بداء السكر.

3 - و جدان بعض الاوعية الدموية في الزغابات كانت متعددة الطبقات مع موت موضعي لتلك الزغابات .

4 - زيادة في الاوعية الدموية والخلايا المحيطة بالزغابات المشيمية للنساء الحوامل المصابات بداء السكر.

5 – تليف موضعي للزغابات المشيمية للنساء الحوامل المصابات بداء السكر.

Abstract

A prospective case control study conducted at Basrah maternity and child hospital and Basrah medical college. The study includes twenty samples of placental tissue from gestational diabetic pregnant women and ten samples of placental tissue from normal pregnant women (control group). Tissue sections of all samples were stained by Haematoxylin and eosinand examined under light microscope with screen for stereological investigation to the placental barrier stereological quantitation of placental barrier; comparison between different placental barrier parameters of the placenta in normal and gestational diabetes. Although there is an increase in all means of placental barrier parameters in placenta of gestational diabetes pregnant women than placenta of normal pregnant women but there is no statistical difference at ($P<0.05$).

There are only exception that (TDD,FBP and MBP are statistically differ at($P<o.05$) tables (1,2). In addition: Qualitative (morphological observations and structural) changes are obtained as following:-

Differences in thickness of the syncytiotrophoblast and basement membrane thickness in villi more marked in gestational diabetes.

Thrombosis of fetal arteries and infracted villi more than normal some regions have extensive infraction than other regions.

Villous vessels with multilayered and fibrinoid necrosis of villi more in gestational diabetes.

Capillary proliferation and cellularity were increased in gestational diabetes.

Fibrosis of villi in gestational diabetes.

Introduction

Placenta is a wonderful organ developed as a part of conceptus. Placenta is a fleshy structure that develops mostly from foetal chorionic tissue(arising from trophoblast) and maternal decidua basalis during pregnancy (1,2).

The placental chorionic villi recognized as the essential structural and basis for functional activity (3). Enumorus number of chorionic villi projecting in to the maternal blood in the inter villous spaces to facilitate increased transport the nutrient material and other elements between the mother and her foetus. The chorionic tissue separates the maternal blood from foetal blood vessels within the villi. It is histologically known as haemochorial placenta(4).

During the second and third trimesters, a varety of changes take place in the placenta that are physiological or pathological. Which are associated with intrauterine growth retardation .At term the villi consist largely of foetal capillaries with little or no stroma beyond that required for their support. Cytotrophoblast is not commonly seen with the light microscope, and the remaining syncytium is so thin that, in some areas referred to as epithelial plates represents specific different may be adapted for specialized types of exchange between the maternal and foetal vascular compartment which lead to increase diffusion capacity(5).

The substances classified in to four groups on the basis of the physiological significance of the material to be transferred:-

Substances essential for life such as oxygen and water that are rapidly transferred by simple diffusion.

Substances important in foetal nutrition (glucose, aminoacids, lipids and vitamins; transport of these may involve active processes.

Substances regulating metabolic activity including certain hormones is believed to diffuse slowly across the barrier.

Macromolecules of immunological importance (maternal antibodies) are taken by molecules pinocytosis of syncythiotrophoblast. Many substanses are present in a higher concentration in the foetal blood than in that of the mother e.g sodium, inorganic phosphate, calcium, iron and aminoacids. These substances would appear tobe transported against a concentration gradient i.e involve active transport and require metabolic energy (3,6). Pregnancy is a load causing alterations not just in the mother's pelvic organs but all over the body. Foetol physiology is different from that of an adult, but it interacts with mother's system, causing adaptation and change of function in her body (7).

Gestational diabetes is one the most common complications of pregnancy. It usually develops during the second half of pregnancy, when hormones interfere with the body's ability to use its insulin. Most women with gestational diabetes have no symptoms. Although some may experience extreme thirst, hunger or fatigue. Women at increased risk of gestational diabetes include those who are over age 30, are obese, have a family history of diabetes.

Effect of diabetes on the fetus are more serious than those on the mother, these include congenital abnormalities, hypoglycemia, hyperviscosity syndrome, macrosomia, hyaline membrane disease, hypocalcemia, apnea and bradycardia in addition to traumatic delivery(8,9)

The aim of this study is to determine the placental barrier thickness in gestational diabetes by using serological study which is can not detected by morphological observation of structural changes and compared with normal placenta.

Materials and Methods:

The study includes twenty gestational diabetic mothers to examine their placentas after delivery whether by

cesarean section or vaginal delivery, their age range(20-40 years) and their parity range(1-13).

Complete stereological study for different parameters of Placental barrier including Total diffusion distance (TDD)

Distance between maternal erythrocyte(ME) and foetal erythrocyte(FE) in foetal vessel(FV).

Maternal blood plasma (MBP)

Distance between maternal erythrocyteand outside of syncytiotrophoblast for the chorion.

Foetal blood plasma (FBP)

Distance between foetal erythrocyte inside foetal vessel of the chorion and endothelium of the foetal vessel.

Villous blood plasma (VBM)

Distance between endothelium of foetal blood vessel and syncytiotrophoblast.

Materno – foetal distance (ME-FV)

Distance between maternal erythrocyte and endothelium of foetal blood vessel.

All parameters of gestational diabetic are compared with ten cases of normal healthy pregnancy. They had approximately same age range and parity.

Preparation of tissues for microscopical examination: placental tissue samples were collected directly after delivery and placed in 10% formaldehyde as fixative solution . Dehydration was done by using ethanol. Then tissues were embedded in paraffin wax. The paraffin section were stained by haematoxylin and eosin(10) for examination by light microscope with screen (Reichert Austria Nr. 381116), and scientific plastic ruler.

Data were analyzed statistically using student "t" test of significance and "P" value. The test of significance was done between normal and gestational diabetes values of different placental parameters as in tables (1,2).

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Results

Stereological quantitation of placental barrier: The main results are describes in tables(1,2). These tables shows comparisons between different placental barrier parameters in placenta of gestational diabetes and normal pregnant women. However in the gestational diabetic placenta there is an increase in all mean of placental barrier parameters, (Total diffusion distance(TDD) ,Maternal blood plasma (MBP) ,Foetal blood plasma (FBP) ,Villous blood plasma(VBP) ,Materno-foetal distance(ME-FV) than the placenta of normal pregnant women but there are no statistical difference at (p < o.o5) tables(1,2).

There are only exception that (TDD,FBP and MBP are statistically differences at (P<o.05).

Table(1) show means of different placental barrier parameters in placenta for (20) gestational diabetes and normal (10) pregnant women

Table 1	Numbers	Mean (Micron)
TDDG	20	4.7334 ± 0.6389
TDDN	10	4.1192 ± 0.8475
FEG	20	0.2500 ± 0.000
FEN	10	0.2500 ± 0.000
FBPG	20	0.331 ± 0.2388
FBPN	10	0.1159 ± 0.1602
VBPG	20	3.6281 ± 0.5451
VBPN	10	3.3977 ± 0.7126
MBPG	20	0.2940 ± 0.2383
MBPN	10	0.1058 ± 0.2125
MEG	20	0.2500 ± 0.000
ME- FVG	20	3.7866 ± 0.6596
ME-FVN	10	3.5035 ± 0.8038

Table(2) show statistical analysis (t test for Equality of means) of different placental barrier parameters for gestational and normal placenta

	T	Sig(2- tailed)	Mean difference
TDDG	2.225	0.034	0.6142
TDDN	2.022	0.062	0.6142
FBPG	2.588	0.015	0.2172
FBPN	2.951	0.007	0.2172
VPBG	0.985	0.333	0.2304
VPBN	0.900	0.383	0.2304
MBPG	2.110	0.044	0.1882
MBPN	2.194	0.040	1882
MEFVG	1.031	0.311	0.2831
MEFVN	0.963	0.350	0.2831

Qualitative (morphological observation for structural changes)

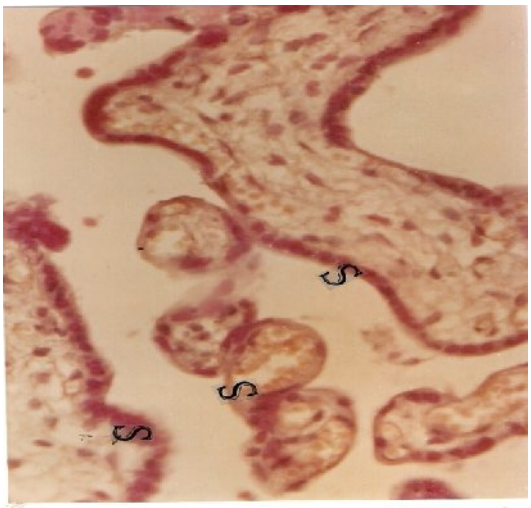
1-Differences in thickness of the syncytiotrophoblast(S)and basement membrane thickening in villi more marked in gestational diabetes(Fig1).

2-Thrombosis of fetal arteries and infracted villi more than normal some regions have extensive infraction(I) than other regions (Fig2).

3-Villous vessels with multilayered and fibrinoid necrosis(F) of villi more in gestational diabetes (Fig3).

4-Capillary proliferation (V) and cellularity were increased in gestational diabetes(Fig4).

5-Fibrosis of villi in gestational diabetes.



Fig(1) Section of placenta shows differences in thickness of the syncytiotrophoblast (s) X 742.5

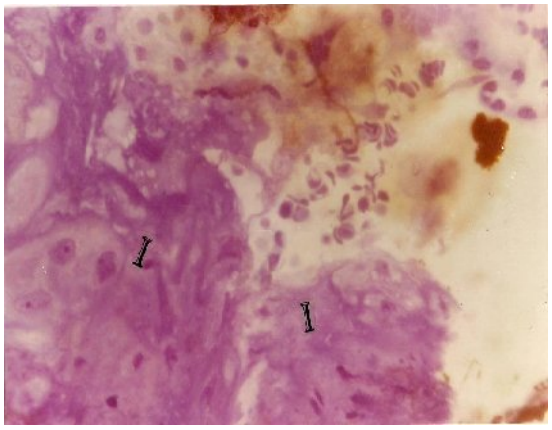


Fig (2) Thrombosis of the fetal arteries and infracted villi with region of extensive infarction (I) X 742.

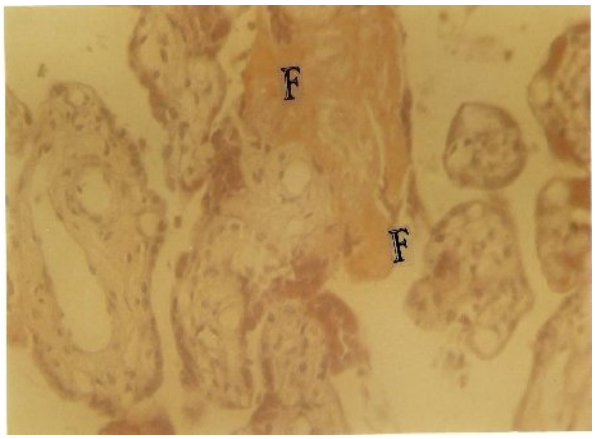


Fig (3) Villous vessels with multilayer and fibrinoid necrosis of villi .X742.5

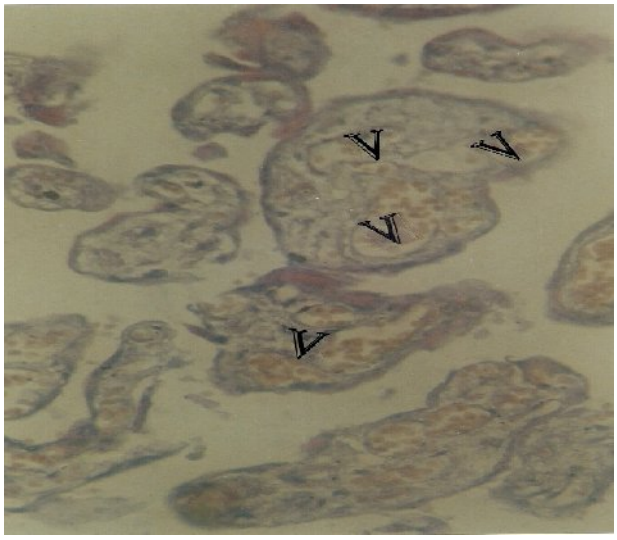


Fig (4) Capillary proliferation (V) and cellularity were increased X742.5

Discussion

The placenta is the central organ of fetomaternal exchange Which metabolically provides for the growth of the fetus and its membranes as a whole. The main structural components of the human placenta develop and continue to grow and differente until birth (11). Gestational diabetes is one of the most common complications of pregnancy during second half of pregnancy which may effect the placenta,fetus and mother .Despite many researches are engaged in study the placental barrier few works were concentrated on the use of quantitative measurement of the placental barrier .These works deal only with study of normal values of human placenta.The present work explains to certain extent the above observations on the probable effect of gestational diabetes in pregnant women on the placental barrier and morphological observation for structural changes. There are significant differences in the structure and thickness of the barrier at different stages of pregnancy and even different areas of each villus at agiven time, The thickness of the membrane is affected by the extent of distension of the villous capillaries. The actual exchange are between maternal blood in the intervillous space and foetal blood in the capillaries and sinusoid in the stroma of the chorionic villi. Factors concerned in the exchange will be the rate of circulation of blood in the intervillous space and foteal vessels, the thickness of the membrane and also the extent of the surface presented by the villi to the maternal blood .(12,13). In this study the normal (TDD Total diffusion distance (4.1192 micron) .So there is no difference compared with normal total diffusion distance normal range (2-6micron) of pirax (2) and normal range thickness placental barrier(1.2-4.9 micron) of Boyd (13). Although there is an increase in all means of placental barrier parameters in placenta of gestational diabetic pregnant women shows in tables (1,2) but there is no statistical difffernces at ($p < o.o5$) .

There are only exception that (TDD,FBP and MBP are statistically differences at $P< o.05$ (tables 1 , 2). Other structural change like Differences in thickness of the syncytiotrophoblast and basement membrane thickening in villi more marked in gestational diabetes. But these changes also seen in premature onset of labour, RH incompartibility ,hypertension , prolonged pregnancy, In pre-eclampsia superimposed essentional hypertension (14,15,16) and in placenta

of smoking mothers villous capillaries were smaller in diameter and situated nearer to the center of the villi (17). Decrease in the number of vasculosyncytial membranes and thickening of basement membrane in the placenta of smoking mothers have been described in alight microscopic study (17,18).Other workers found like Vander etal., the basal lamina thickening in the placenta of smoking mothers.It is suggested that thickening of the villous trophoblastic basement membrane is usually a response to the placental ischaemia .Asmussen found an increase in the collagen content of the villous stroma in smokers placenta(15,19). But in some cases the thickening is due to an immunological reaction like placenta from diabetic women or from cases of Rhesus incompartibility show thickening of basement membrane as a result of an immunological reaction .These are only exceptions to the general conclusion that thickening of this membrane is a response to placental ischaemia(20). Other finding in this work thrombosis of fetal arteries ,infracted villi and villous vessels with

multilayered and fibrinoid necrosis of villi in addition to capillary proliferation and increase in cellularity stroma.Which is more than normal placenta also found in the other complications in pregnancy. Martinek found relatively small diameter and multilayering of the capillary basement membrane occasionally observed in small proportion of villi in normal mature placenta(21).

Robertson also suggested that the lesion acute atherosis is apathgnonomic feature of preeclampsia(22,23).De wolf reported vascular lesions ofacute atherosis in five patient with foetal growth retardation with out hypertension(24) .We could find no arteriopathy specific for preeclampsia (25). Histological changes observed in placenta of hypertensive women were similar to those previously described in placenta of smoking mothers (17) ,Placenta of women with small gestation age infant for unknown aetiology (18)and placenta of women with intrauterine death(26).

Conclusions and recommendations

In present study by using stereological measurement can detect placental barrier thickening that are probably lead to placental function impairment which may effects the mother and foetal growth during intrauterine life.Future studies must be directed to study ultrastructural of placental barrier by electron microscope after control risk factar of gestational diabetes and hypertension before and during pregnancy.

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