Evaluation of Morphological (Macroscopic and Microscopic) Parameters of Placentas in Pregnancies Complicated by Preeclampsia, Diabetes and Preeclampsia and Diabetes

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Abstract

This study was done to compare the morphometric parameters of placentas in well controlled patients with preeclampsia, diabetes, and preeclampsia-diabetes with that of normal uncomplicated placentas.

Patients &Methods: A total of Twenty four placentas were freshly collected. Six placentas for control group and eighteen placentas for complicated group (preeclamptic- diabetic and preeclamptic--diabetic subgroups). The placentas were grossly examined (shape, number of cotyledons, weight, and thickness). After suitable fixation, tissue processing and sectioning, the sections were stained by hematoxylin and eosin to study the general morphology and morphometry of the following parameters: number of terminal villi, number of syncytial knots, number of apoptotic cells and number of fetal capillaries.

Result: The weight of the baby in D- subgroup was significantly increased when compared with control group and with P and P-D subgroups . Microscopical morphometry regarding number of terminal villi, apoptotic cells, syncytial knots and fetal capillaries were increased in complicated subgroups. The thickness of basement membrane was 0.8µm in control group and it was increased in all complicated subgroups. The highest value was reported by D- subgroup (2 µm).

Keywords: *Parameters, pregnancies, Preeclampsia, Diabetes*

Introduction

The placenta acts as a natural barrier between the maternal and fetal blood circulations and fulfills a wide range of endocrine and transport functions. The location between the two bloodstreams makes the placenta not only a crucial regulator of fetal nutrition, gas exchange, and maternal immune tolerance, but makes this feto-maternal organ also a target for maternal and/or fetal metabolic alterations associated with pregnancy pathologies. One of these pregnancy pathologies is gestational diabetes mellitus (GDM)[(1)]. DM is a common clinical condition that affects approximately 1 to 15% of pregnant women[(2)]. GDM is a metabolic disease defined as progressively impaired glucose intolerance with the onset or first recognition during pregnancy[(3)] . Fetuses with intrauterine exposure to hyperglycemia more often present with macrosomia, birth trauma, neonatal hypoglycemia, and respiratory distress syndrome [(4).]

The second pregnancy pathology that affects the placenta is preeclampsia which is a hypertensive disorder of pregnancy defined by the American College of Obstetrics and Gynecology (ACOG) as “new onset of hypertension on two separate occasions with proteinurea arising de nova after the 20th weeks of pregnancy in a previously normotensive non-proteinuric woman[(5)]. Pre-eclampsia is a human pregnancy-specific disorder with an incidence of 2-8% worldwide [(6)]**.** The incidence being 3 to 7 % in nulliparous and 1 to 3 % in multiparous. It is directly associated with 10 to 15 % of maternal deaths[(7)] . The diagnostic criteria for preeclampsia is systolic blood pressure of >140mm Hg or diastolic blood pressure of > 90mm Hg measured at rest on two different occasions at least six hours apart accompanied by proteinuria of > 0.3 g in a 24-hour urine specimen [(8)].

The placenta is a mirror which reflects the intrauterine status of the fetus. An adequate knowledge of the morphometric analysis of the placenta proves to be useful in the early assessment of placental sufficiency and also the state of fetal wellbeing. Hence, this study was done to compare the morphometric parameters of placentas in well controlled patients with preeclampsia, diabetes, and preeclampsia-diabetes with that of normal uncomplicated placentas.

Materials & Methods

A total number of 40 freshly delivered placentas were collected from the government hospital for women and children in Diyala. The placentas were collected soon after their expulsion, from normal deliveries. They were divided into two major groups; control group (10 women) and complicated group(30 wopmen) . The latter was divided into three subgroups (10 women each), pregnant women with preeclampsia(P); pregnant women with diabetes(D) and pregnant women complicated by both preeclampsia and diabetes(P-D). Clinical criteria for selection of patients were shown in table 1. The collected placentas were washed under running tap water and the membranes were thoroughly examined and trimmed. The umbilical cord was cut, leaving a length of 5cms from its placental site of insertion.

The specimens were then transported in formalin (10%) filled plastic containers to the Department of Anatomy.

In all the collected placentas, the following parameters were studied: weight , shape, placental thickness and the number of cotyledons. From each placenta, tissue pieces measuring 0.25×0.25 cm were taken 2 cm from the attachment of umbilical cord[9]. After 24 hours fixation in 10% formalin (Fluka AG. Chemicals, Buchs),the fixed tissues were processed for routine paraffin- wax embedding. From each paraffin tissue block, 2 sets of sections were prepared. The 1st set was used for routine heamatoxylin - eosin stain; the 2nd set for the Periodic Acid Schiff's' reaction (PAS) [10]. In our study microscopic morphometry included the following parameters : number of apoptotic cells, number of terminal villi , number of syncytial knots ,number of fetal capillaries and thickness of trophoblastic basement membrane(microns) using 40X objective and 10X ocular at 6 random fields per sections.

The data collected were analyzed using the computer facility with the available software statistical packages of SPSS 17 (Statistical Packages for Social Science, version 17.0). Results were presented in simple measure of mean ± S.D.(standard deviation).The significance of difference among quantitative variables of groups was assessed using one –way analysis of variance (ANOVA) [11].

Results

In all groups , the placenta is disk like, and round to oval. The fetal surface appeared congested in P-subgroup, ranged from red-purple to shinny translucent in D- and D-P subgroups. Subchorionic fibrin/fibriniod deposits and infarcted area were significantly increased in all complicated subgroups (table 2).

· Macroscopic parameters of the placenta(Table 3 and 4):

The number of cotyledons were significantly decreased in all complicated subgroups. The weight of the placenta in control group was 510 gm. In P subgroup was significantly decreased (375.8gm) and in D- and D-P subgroups were non significantly increased and they were 550gm and 521.6gm respectively.

The weight of the baby in control group was (3191gm) . In D- subgroup was significantly increased (4300gm) when compared with control group and with P and P-D subgroups .

The thickness of placenta was 2.5 cm in control group while in all complicated subgroups, the thickness of placenta was 2.2cm

· Microscopic Examination of Placenta(Tables 5 & 6):

The number of villi in control group was 10.5 and it was significantly increased in P, D and P-D subgroups when compared with control group .

The number of apoptotic cells in control group was 11.4. and it was significantly increased in P and D subgroups and non-significantly increased in P-D when compared with control group.

The syncytial knots were 12.4 in control group and their number was significantly increased in P, D & P-D subgroups when compared with control group.

The thickness of basement membrane was 0.8µm in control group and it was increased in all complicated subgroups. The highest value was reported by D-subgroup (2 µm).

The number of fetal capillaries was 8.3 in control group .It was significantly decreased in P– subgroup when compared with control groups and with D-and P-D subgroups and was significantly increased in D- and P-D subgroups when compared with control and P-groups.

Discussion

Round or oval placentas are the predominant human placental form, but many other shapes exist (Bilobed placenta, Placenta membranacea, Succenturiate placenta, Fenestrated placenta, Ring (zonary) placenta [12]. Anomalies may develop from abnormal fetal genes expressed by the placenta, an abnormal maternal environment, or an abnormal fetal-maternal interaction [13].As all the subjects were apparently healthy and there was no evidence of maternal malnutrition. The hemoglobin level was about 10gm/ dl in all subjects included in this study. This may be the reason of normal shape. Only in severe malnutrition, abnormal shape has been reported by previous workers [14,15].

In our study, fibriniod deposits moderately increased in all complicated groups which mean that focal degeneration of syncytiotrophoblast increased in these groups. This finding is in agreement with older findings by Horman who considered that factors such as hypoxia and acidosis might lead to syncytial degeneration and eventually result in replacement of the syncytium by fibriniod[16].

The number of cotyledons, in complicated group, was significantly decreased. This may be explained by an altered distribution of fetal blood in complicated placenta resulting in different modes of arrangement of intracotyledonary vessels of complicated pregnancy[17].

The placental weight was significantly decreased in preeclampsia. This finding corroborate with the study of Fox [18]. The placental weight in D and P-D subgroups was non significantly increased as it had been found by Clarson et al. that when diabetes is well controlled during pregnancy, the placental weight does not deviate from that of normal organs [19].

This study reveals that the fetal weight was significantly decreased in P-subgroup. This finding corroborate with the studies of other workers[18,19,20] . Rath stated that in hypertension; arrangement of the intracotyledonous vasculature is altered resulting in low birth weight of babies[17]. The birth weight in P-D and D subgroups was increased and it was significant in the latter. The weight gain in diabetic's placentas may be attributed to macrosomia and compensatory hyperplasia. Macrosomia affects the fetus and fetal part of placenta i.e. chorionic plate. The macrosomia may be attributed to fetal hyperinsulinemia in response to hyperglycemia in fetuses of diabetic mothers[21].

Thickness of the placenta depends upon the length of the villi[22] . In present study, the thickness of placenta (length of stem villi) in all complicated subgroups was non significantly decreased when compared with control group.

Longitudinal growth of the capillaries within the mature intermediate villi exceeds that of the villi themselves so the capillaries coil and form loops that bulge from the villous surface, forming grape-like outgrowths known as terminal villi[23]. The development of this low impedance capillary network parallels the proportional rise in fetal cardiac output entering umbilical arteries to about 40% at term[24]. In our study, the number of terminal villi was significantly increased in all complicated subgroups and this means that the capillary growth exceeds the longitudinal villous growth in these groups which may occur secondary to an increase of fetal cardiac output entering umbilical arteries in these groups.

Apoptosis is initiated via the extrinsic or intrinsic pathway. Both pathways rely upon a cascade of protein interactions orchestrated by a family of 14 cysteine proteases, caspases, which are able to cleave structural proteins producing the morphological appearances typical of apoptosis. In addition, active caspases potentiate the apoptotic signal by activating a variety of pro-apoptotic proteins[25]. The extrinsic pathway is controlled by members of the tumour necrosis factor (TNF) death receptor family[26]. The intrinsic pathway is initiated by cellular stress; such as DNA damage, reactive oxygen species, the unfolded protein response, or removal of growth factor support(27). In our study, the significant increase in apoptotic cells in P- and D- subgroups may be attributed to an exaggeration of extrinsic and/or intrinsic pathways.

Syncytial knots are consistently present, increasing with increasing gestational age, and can be used to evaluate villous maturity. Increased syncytial knots are associated with conditions of uteroplacental malperfusion and are important in placental examination(28). Therefore, the significant increase of syncytial knots in P- and D- subgroups was due to fetal malperfusion of placental villi.

It is well known that molecular composition of the basement membrane changes during maturation of villous trophoblastic and endothelial basement membranes, these changes are particularly obvious in area of trophoblast proliferation and villous sprouting(29). . Due to increased incidence of apoptosis, large number of parenchymal cells (trophoblast, endothelial cells) has been observed to be eliminated and replaced by fibrous tissue(30). This fibrous tissue was synthesized by fibroblasts of villous stroma. Fibroblasts also take part in the synthesis of subtrophoblastic basement membrane (31). In this way, secondary to increase incidence of apoptosis in all complicated subgroups, large number of parenchymal cells (trophoblast, endothelial cells) had been replaced by fibrous tissue and this may play a role in increase thickness of basement membrane.

Placental angiogenesis can be subdivided regarding its mechanisms and the geometry of the resulting vascular bed(32,33,34) into branching angiogenesis (multiple sprouting of micro vessels produces a complex multiply branched capillary web)and non-branching angiogenesis(Vascular bed expands by elongation of existing capillary loops). In this way, the significant decrease in number of fetal capillaries in P- subgroup may be due to non-branched angiogenesis which may become the only mode of angiogenesis which is suppressed by binding of PlGF to VEGFR-1. The significant increase of fetal capillaries in D-and P-D subgroups has occurred as branched angiogenesis may become the only mode of angiogenesis which may be stimulated by binding of VEGF-A to its receptors.

Table 1 Shows clinical criteria for control and complicated groups.

Group	Blood pressure	Fetal heart rate	PaO2	Hb	GUE	FBS	APGAR
Control	125/78.3	135	95%	10.0	-VE	normal	8
P*	166.6/95	127.5	95%	10.43	Protien++	normal	8.16
D *	119.16/78.3	120	96%	10.38	66.6%suger -ve 43.4%+ve	141.8	8.3
P-D*	168.3/95.83	128.3	94.8%	10.83	Protein ++	151.5	8.16

*Preeclamptic patients were treated with aldomet, Diabetic patients were treated with insulin and preeclamptic –diabetic patients treated with aldomet and insulin.

Table 2 shows deposition of fibrin\ fibrinoid in control and complicated groups.

Group	Fibrin\Fibriniod (numbers of patches)
Control	<10
P	>20
D	>20
P-D	>20

Sub chorionic fibrin/Fibrinoid per/fetal surface area

Table (3): shows Mean ± standard deviation of macroscopical parameters

Parameter	Control	Complicated		
		P	D	P-D
Number of cotyledons	38 4.5	27.5 5.1	31.6 2.3	30.6 5.24
Weight of Placenta/gm.	510 52.1	375.8 24.1	550.0 50.5	521.6 51.5
Thickness of placenta/cm	2.5 0.3	2.2 0.3	2.2 0.3	2.2 0.3
Weight of Baby/gm.	3191.6 162.5	3158.3 638.2	4300.0 316.2	3558.3 454.3

Table (4) shows Multiple comparison(ANOVA)of macroscopic parameters.

		Control	P	D	P-D
Weight of placenta	Control		0.09*	0.00*	0.004*
	P	0.009*		0.246	0.738
	D	0.000*	0.246		0.406
	P-D	0.004*	0.738	0.406	
Weight of the baby	Control		0.863	0.000*	0.063
	P	0.863		0.000*	0.043*
	D	0.000*	0.000*		0.000*
	P-D	0.063	0.043*	0.000*	
Number of cotyledons	Control		0.000*	0.024*	0.010*
	P	0.000*		0.130	0.247
	D	0.024*	0.130		0.712
	P-D	0.010*	0.247	0.712	
Thickness of placenta	Control		0.332	0.363	0.363
	P	0.332		0.951	0.951
	D	0.363	0.951		1.000
	P-D	0.363	0.951	1.000	

Table (5) shows Mean ± Standard deviation of microscopic parameters

***The mean difference is significant at the 0.05 level.**

Parameter	Control	Complicated		
		P	D	P-D
Number of villi	10.5 1.15	15.4 1.4	14.6 0.7	15.2 3.7
Number Apoptotic cells	11.4 0.7	14.4 1.5	14.7 0.6	12.4 1.1
Number of Syncytial Knots	12.4 0.8	17.6 1.0	15.0 1.0	13.5 1.0
Thickness of basement membrane/ µm	0.8 8.5E-02	1.9 6.7E-02	2.0 0.1	1.1 6.0E-02
Number of fetal Capillaries	8.3 0.7	6.1 0.3	15.5 0.9	15.5 1.21

Table(6) shows Multiple comparison(ANOVA)of microscopic parameters.

		Control	P	D	P-D
Number of villi	Control		0.000*	0.000*	0.017*
	P	0.360		0.000*	0.000*
	D	0.046*	0.000*		0.002*
	P-D	0.016*	0.000*	0.002*	
Number of apoptotic cells	Control		0.000*	0.769	0.073
	P	0.551		0.000*	0.000*
	D	0.029*	0.000*		0.131
	P-D	0.015*	0.000*	0.131	
Number of syncytial knots	Control		0.000*	0.000*	0.035*
	P	0.442		0.000*	0.000*
	D	0.342	0.000*		0.005*
	P-D	0.406	0.000*	0.005*	
Thickness of basement membrane	Control		0.894	0.879	0.974
	P	0.051		0.984	0.920
	D	0.982	0.984		0.905
	P-D	0.978	0.920	0.905	
Number of fetal capillary	Control		0.000*	0.000*	0.000*
	P	0.011*		0.000*	0.000*
	D	0.001*	0.000*		0.994
	P-D	0.000*	0.000*	0.994	

*The mean difference is significant at the 0.05 level.

Conclusions

An adequate knowledge of the morphometric analysis of the placenta with its clinical relevance proves to be useful in the early assessment of placental sufficiency and also the state of the fetal wellbeing. In mothers who have had no previous antenatal checkup, a thorough examination of the placenta helps in the early diagnosis of the fetal complications, soon after parturition and thus helps in the early treatment of the baby by neonatologists.

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Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the College Of Medicine and all experiments were carried out in accordance with approved guidelines.

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