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Pathological Changes in Placentas of Diabetic Mothers & Its Association with Fetal Outcome

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Abstract: Diabetes mellitus is one of the metabolic disorder, which can affect the pregnant mother as well as the newborn because of its effect on the placenta, the incidence of which is keep on increasing. By studying the placentas of the diabetic mothers and comparing it with the placentas of normal mothers, we can understand the changes that occurs in placenta because of diabetes, like increased weight and thickness of the placenta, increase glycogen deposition or reduced VEGF expression which leads to various newborn abnormalities like increase birth weight (macrosomic babies), low APGAR score, neonatal diabetes etc. Here we compared 40 placenta of diabetic mothers with 40 placenta of normal mother and found that diabetes in mothers during pregnancy can cause various changes in the placenta like increase weight and thickness of placenta, increased syncytial knot, villous edema, villous fibrosis, increase in basement membrane thickness and reduced expression of VEGF which can affect the fetal outcome and thus requires further studies at the molecular level for the pathogenesis of these findings which might help in preventing the fetal morbidity and mortality.

Keywords: Diabetes, Immunohistochemistry, Placenta,VEGF

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I Introduction

Placenta is a Latin word which literally means a flat plate or cake[1]. Placenta is a very complex organ which has a very short life-span of 9 months and serves as a channel between the foetus and the mother for the selective forward transport of gases, nutrients and reverse transport of metabolic waste products[2]. Placenta separates the fetal and maternal circulation via endothelium and syncytiotrophoblast respectively[3]. The development of placental villous vessels continues throughout the pregnancy and comprises of two stages, vasculogenesis and angiogenesis. The stage of vasculogenesis occurs mainly during the period of first and second trimester, in which the mesenchymal cells of the villous core differentiate into the cords of vascular cells and by the process of dehiscence, it forms the vascular lamina. The cells required for the elongation and widening of vessels and the perivascular cells – pericytes are also derived from the mesenchymal cells. In stem villi, arteries and veins are differentiated from the vessels. The surrounding supporting structures of the walls of the vessels like smooth muscle cells, myofibroblasts and fibroblasts are also recruited from the villous stroma. On the other hand, the stage of angiogenesis takes place during the third trimester. In this process, the already existing stem villous vessels sprouts and give rise to new capillaries, and thus by this way vascularizing the emerging mature intermediate and terminal villi.[4] Metabolic disease associated with pregnancy such as diabetes mellitus and hypertension can affect the components of placenta for e.g. connective tissue component in chorionic villi and the basement membrane lining the chorionic villi[5]. The Center for Disease Control and Prevention (CDC) has shown that the crude incidence of the cases diagnosed with diabetes mellitus has increased, from 3.3 per 1000 to 7.4 per 1000, i.e. 124%, from the year 1980 to 2005 and hence, diabetes mellitus is now considered to be one of the major health problem in our society. Various studies suggested that the increased prevalence of diabetes mellitus (DM) amongst the women of child bearing age is due to increase in sedentary lifestyles, changes in dietary habits and the virtual epidemic of childhood and adolescent obesity[6].

GDM or Gestational Diabetes Mellitus is defined as variable degree of intolerance to glucose with either onset or first recognition during pregnancy. Maternal glucose intolerance occurs in 3-10% of pregnancies[7].

Pregnancy complications like gestational diabetes are reflected grossly and microscopically in the placenta. Placental examination can yield information about the existence and effects of maternal, placental or fetal disease, the cause of stillbirth, and potential risks in future pregnancies.

The various pathological changes occurring in the placenta of diabetic mothers are considered to be the important risk factors contributing to fetal anoxia and fetal compromise in pregnancy[8].

II Materials & Methods

The study was a prospective Case control study which was conducted in Department Of Pathology, Stanley Medical College after collecting the specimen from RSRM Govt Stanley hospital. 80 full term placentas were collected in 10% Neutral buffered formalin immediately after delivery, either following normal delivery or following caesarean section, out of which 40 placentas were from diabetic mothers and 40 were from normal mothers. The clinical history and the weight of the baby were recorded from the hospital record/casesheets. The purpose of this study was only to study our group and to obtain independent information and not to verify the existent information in the literature.

After thoroughly washing the specimens in tap water, weight of the placenta is taken with the help of spring balance weighing scale measuring the weight accurate to grams and thickness of placenta is measured at 3 positions, at center, at the periphery and between center and periphery, with the help of a knife which is then measured with measuring tape and an average value is taken. 2 sections were taken from each placenta and processed with conventional histological technique followed by staining of one section with Hematoxylin & Eosin stain, one with Periodic Acid Schiff (PAS) stain to highlight the thickness of the basement membrane and one section was used for immunohistochemistry using monoclonal antibody against VEGF.

All the slides were examined under light microscope by double blinding the observer. Hematoxylin & Eosin slides were examined for the features like Synctial knots, villous edema, villous fibrosis and fibrinoid necrosis. There is no specific/strict criterias given when to call an increase in Synctial knots, villous edema, villous fibrosis and fibrinoid necrosis, and hence each case was studied and percentage of findings seen in 10 HPF were taken as average number of finding in that case. The PAS slides were examined to assess the thickness of the basement membrane of the villi which was categorized into 5 categories, Hazy (±), trace (+), mild (++) , moderate (+++) and severe (++++). The immunohistochemistry slides were analyzed according to a subjective evaluation of intensity of reaction of VEGF into 4 categories negative (-), mild (+), moderate (++) and severe (+++) in the trophoblastic cells and in endothelial cells.

III Result

On gross examination, we found out that the placentas from diabetic mothers were heavier and thicker than those of normal placenta. The maximum weight of placenta in diabetic mother was 800 gms while in case of normal mother it was 750 grams and the difference between the mean weights of placenta in 2 groups were statistically significant (Table1). The maximum thickness of placenta in diabetic mother was 4.0cms , while in normal mother it was 2.5 cms with a statistically significant difference between the mean thicknesses of placenta in 2 groups (Table1). The difference of average weight of the newborn in diabetic mothers and the normal mothers were also statistically significant (Table1).

Histopathological examination of the sections from the placentas of diabetic mothers showed increased villous edema (Fig.1), villous fibrosis (Fig.2), syncytial knots (Fig.3) and fibrinoid necrosis (Fig.4) as compared to the placentas of normal mother which was found to be statistically significant with a value of $p < 0.05$ using Mann-Whitney U test (Table2).

There is an increase in glycogen deposition in placentas of diabetic mothers with basement membrane thickening as demonstrated by PAS staining, with 15% of diabetic cases showing trace staining (Fig.5), 50% showing mild staining (Fig.6), 30% showing moderate staining (Fig.7) and 5%cases showing strong staining (Fig.8), (Table3).

There is reduced expression of VEGF in the fetal endothelial cells and trophoblastic cells in placentas of diabetic mother as compared to the normal placentas (Table 4 & 5).

IV Figures

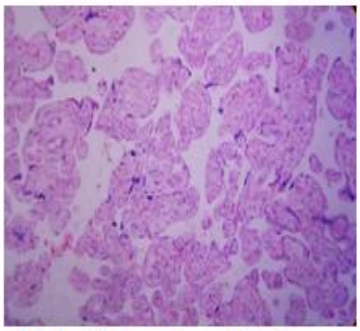

Fig.1 - Villous edema (10x)

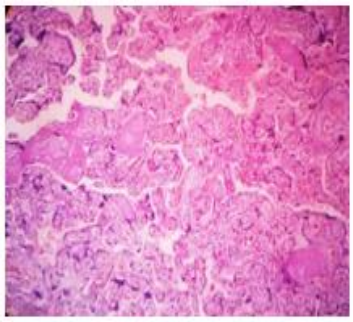

Fig.2 - Villous fibrosis (10x)

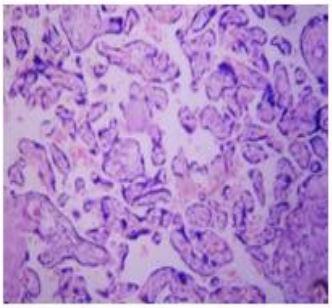

Fig.3 - Increased syncytial knot (10x)

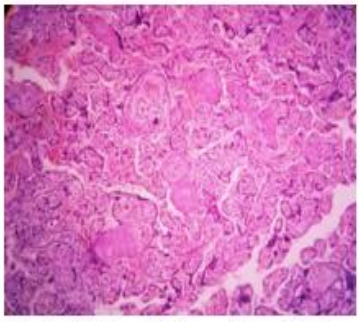

Fig.4 - Fibrinoid deposition (10x)

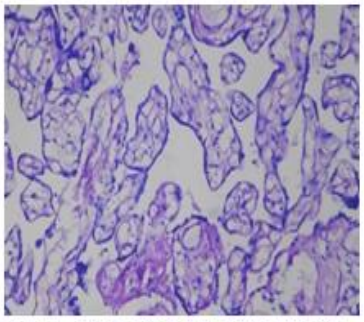

Fig.5 - Trace staining of basement membrane by PAS (40x)

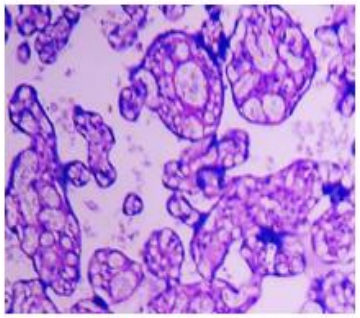

Fig.6 - Mild Staining of basement membrane by PAS (40x)

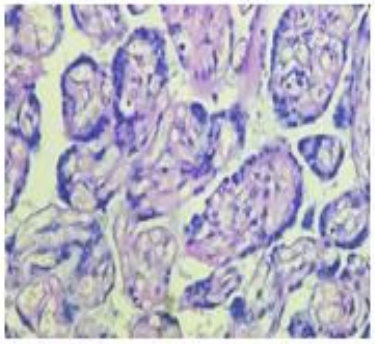

Fig.7 - Moderate staining of basement membrane by PAS (40x)

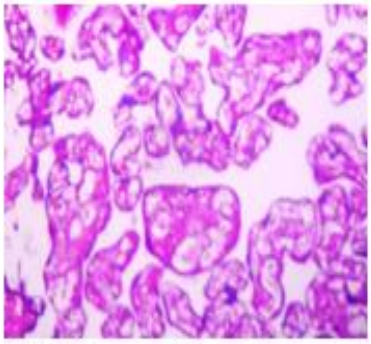

Fig.8 - Strong staining of basement membrane by PAS (40x)

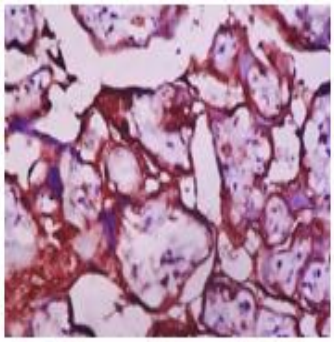

Fig.9 - Strong staining of VEGF in trophoblastic cells (40x)

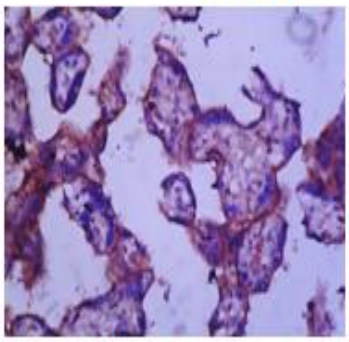

Fig.10 – Moderate staining of VEGF in trophoblastic cells (40x)

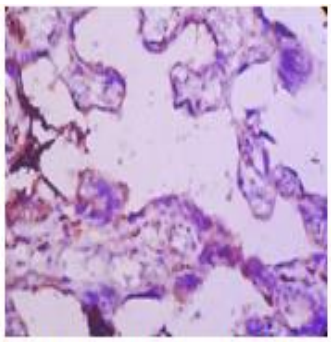

Fig.11 - Weak staining of VEGF in trophoblastic cells (40x)

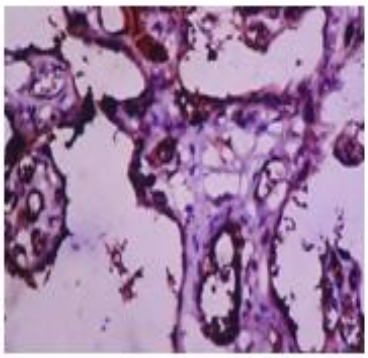

Fig.12 - Strong expression of VEGF in endothelial cells (40x)

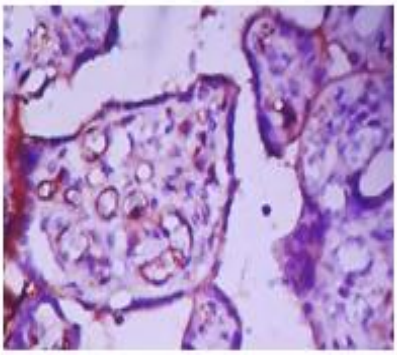

Fig.13 – Moderate expression of VEGF in endothelial cells (40x)

V. Tables

Table 1 - Mean and p value for the placental weight, central thickness and weight of the baby in normal and diabetic mothers

Parameters	**Normal Mean±SD**	**Diabetic Mean±SD**	**P value**
Placental weight	469.63±88.39	563.75±96.78	.000
Central thickness	1.79±0.37	2.51±0.57	.000
Baby weight	2.82±0.36	3.14±0.35	.000

Table 2 – Mann Whitney U test for comparison of histopathological changes in placenta of diabetic and normal mother

Parameters	**Normal Mean of rank**	**Diabetic Mean of rank**	**P value**
Villous edema	25.44	55.56	0.000
Villous fibrosis	28.58	52.43	0.000
Synctial knots	24.15	56.85	0.000
Fibrinoid necrosis	22.53	58.48	0.000

Table 3 – PAS staining in placentas of diabetic mother and diabetic mother

			GROUP		Total
			DIABETES	NORMAL	
PAS	HAZY	Count	0	17	17
		% within GROUP	.0%	42.5%	21.3%
	TRACE	Count	6	18	24
		% within GROUP	15.0%	45.0%	30.0%
	MILD	Count	20	5	25
		% within GROUP	50.0%	12.5%	31.3%
	MODERATE	Count	12	0	12
		% within GROUP	30.0%	.0%	15.0%
	STRONG	Count	2	0	2
		% within GROUP	5.0%	.0%	2.5%
Total		Count	40	40	80
		% within GROUP	100.0%	100.0%	100.0%

Table 4 – VEGF staining in trophoblastic cells of placentas of diabetic mother and normal mother

			GROUP		
			DIABETES	NORMAL	Total
VEGF IN TROPHOBLAST	WEEK INTENSITY(0.5)	Count	12	3	15
		% within GROUP	30.0%	7.5%	18.8%
	MODERATE INTENSITY(1)	Count	15	16	31
		% within GROUP	37.5%	40.0%	38.8%
	STRONG INTENSITY(2	Count	13	21	34
		% within GROUP	32.5%	52.5%	42.5%
Total		Count	40	40	80
		% within GROUP	100.0%	100.0%	100.0%

Table 5 - VEGF staining in endothelial cells of placentas of diabetic mothers and normal mothers

			GROUP		
			DIABETES	NORMAL	Total
VEGF IN ENDOTHELIAL CELLS	NEGATIVE (0)	Count	27	1	28
		% within GROUP	67.5%	2.5%	35.0%
	WEEK INTENSITY(0.5)	Count	8	1	9
		% within GROUP	20.0%	2.5%	11.3%
	MODERATE INTENSITY(1)	Count	1	16	17
		% within GROUP	2.5%	40.0%	21.3%
	STRONG INTENSITY(2	Count	4	22	26
		% within GROUP	10.0%	55.0%	32.5%
Total		Count	40	40	80
		% within GROUP	100.0%	100.0%	100.0%

VI Discussion

Diabetes is now-a-days one of the leading metabolic disorder in the world which has the effect on all the organs of the body , including the temporary organ like placenta and thus can have adverse fetal outcome and thus is of serious concern[9]. The increased placental weight and thickness in diabetes may be because of reactionary hyperglycemia in fetuses of diabetic mothers which leads to compensatory hyperplasia of the villous structure and fetal macrosomia. Another factor which leads to villous hyperplasia could be because of vascular compromises in diabetes mellitus which causes low oxygen tension in chorionic villous blood[10]**.** Weight of the newborn baby depends directly on the environment it experienced during the intrauterine life. In case of gestational diabetes mellitus, glucose crosses the placental barrier and causes fetal hyperglycemia which in turn stimulates the pancreatic islet cells and leads to fetal hyperinsulinemia, and as insulin itself is an anabolic hormone can lead to fetal morbidity and mortality, mainly in association with fetal macrosomia[11]. Burstein et al in the year 1963, observed that placentas from the diabetic patients had marked increase in syncytial knotting[12]**.** In 2014, Rafah Hady Lateef Al-Mamori in his study showed that there was an increased number of syncytial knots in the terminal villi in the placentas of diabetic mothers controlled by insulin[13]**.** Villous edema is defined as accumulation of fluid in the interstitium of the villi with disruption and replacement of intravillous cellular architecture. As hyaluronic acid molecules have the property to retain water, it was concluded that, the presence of abnormal deposits of mucopolysaccharides in the villous stroma can lead to the appearance of the true villous edema in placentas of diabetic mothers[14]**.** In the year 2011, Vineeta Tewari et al, In 2012, Lavinia Gheorman et al, and in 2014, Rafah Hady Lateef Al-Mamori in their respective studies showed that there was an increased incidence of villous edema in the placentas of diabetic patients as compared to normal patients.[15,16,13]

Fibrosis of the stem villi is a normal phenomenon in the placenta and it is a good indicator of placental maturation. Fibrosis usually starts at about 15th week post-menstruation, usually around the stem vessels and completes a few weeks before term. Stromal fibrosis is considered abnormal when it is not restricted to the stem villi. It has been speculated that, in diabetic patients there is an increased villous stromal oxygen partial pressure, in the face of inadequate uptake by the fetal capillaries, which stimulates the synthesis of collagen[17]**.** In the year 2015, Soad A. Treesh et al also mentioned in their study, an increase in the incidence of villous stromal fibrosis which was demonstrated with the help of Masson Trichrome stain[18]**.**

Fibrinoid is a non-cellular homogenous eosinophilic material seen in placenta. Fibrinoid necrosis also referred as intravillous fibrinoid is a fibrinoid patch that replaces the villous stroma predominantly, the chorionic villi[19]**.** In the year 2012, Lavinia Gheorman et al noticed an increased incidence of fibrinoid necrosis in 47% of diabetic cases[20]**.**

Liebhart, in the year 1971, noted that there was marked thickening of basement membrane in diabetic placentas. There as on for this thickening of basement membrane was probably because the secretory products

of trophoblastic cells constitute the basal lamina[21]. The only study in the literature which has given descriptive analysis of the PAS staining, was that of Tewari et al in the year 2011[15]**.** In the present study only few cases showed strong and moderate staining in diabetic group, as compared to the study done by Tewari et al but the difference between the diabetic group and control group was significant and thus present study showed there was basement membrane thickening in diabetic group because of increased glycogen deposition. The accumulation of glycogen in the placentas of diabetic mothers occurs in marked contrast to other tissues, such as maternal liver, from which glycogen disappears. Glycogenesis and glycogenolysis occurring in the muscle and the liver are under the control of insulin, which regulates the activity of phosphorylase and glycogen synthase. However, in diabetic mothers the glycogen accumulation in the placenta is not dependent on insulin and is related to the extent of maternal hyperglycemia. The increased capacity of placental cells for glucose uptake in diabetes could be related to the expression of GLUTs (glucose carrier transporter isoforms), especially GLUT1[15]**.** In the year 1996, Carmeliet et al observed in their study that VEGF and its receptors are essential for the development of embryonic vasculature as embryonic death can result from the loss of even a single VEGF allele[22]. VEGF is a potent inducer of endothelial cell proliferation, activation and migration. L. Pietro et al in the year 2010 found out that VEGF was generally detected in muscle cells and vascular endothelial cells of the intermediate villi, in the cytoplasm of the syncytiotrophoblast, in the mesenchymal cells and in the capillary endothelial cells and cytotrophoblastic cells in the basal decidua proximity to the maternal vessels. The women with gestational diabetes showed somewhat different pattern of staining of VEGF[23]. In the present study the expression of VEGF was reduced at both the sites in diabetic patients with the trophoblastic cells showing strong positivity in 32.5% of cases, and the fetal endothelial cells showing strong positivity in only 10% of cases.

VII Conclusion

Diabetes mellitus represents the most common metabolic complication of pregnancy and is associated with increased maternal and foetal morbidity and due to recently adapted western lifestyle; people are more prone for diabetes and related complication. Histopathology along with immunohistochemistry using various new developing markers, would be of great help for fetal well being in diabetic mothers, and might help in preventing potential negative outcomes in pregnancy, moreso if we are able to do study these changes in vivo, which can be a future perspective.

VIII Acknowledgement

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References

[1]. Hamilton WJ and Boyd JD,Observation on the human placenta,Proc R Soc Med, 44, 1951, 489-96.
[2]. M. Gauster, G. Desoye , M. Tötsch and U. Hiden,The Placenta and Gestational Diabetes Mellitus, CurrDiab Rep, 12, 2012,16–23.
[3]. Desoye G and Sylvie H,The human placenta in gestational diabetes, Diabetes Care, 30(2), 2007, 120-126.
[4]. Demir, R., Kaufmann, P., Castellucci, M., Erbengi, T. and Kotowski, A. ,Fetalvasculogenesis and angiogenesis in human placental villi,ActaAnat, 1989, Vol. 136, No.3, pp. 190-203, ISSN 0001-5180.
[5]. Jirkovská M, The Morphology of Villous Capillary Bed in Normal and Diabetic Placenta. In: Zheng J (editor), Recent Advances in Research on the Human Placenta [Internet].,Croatia (Europe); Intech open science (Cited March 7, 2012).
[6]. Verma R, Mishra Sand Kaul JM, Cellular changes in the placenta in pregnancies complicated with diabetes, Int J Morphoogyl, 28(1), 2010, 259-64.
[7]. Dutta DC,Medical and surgical illness complicating pregnancy, Text book of obstetrics, 6th edition. Calcutta: New Central Book Agency, 2004.
[8]. Hanson U and Persson B, Outcome of pregnancies complicated by type 1 insulin-dependent diabetes in Sweden: acute pregnancy complications, neonatal mortality and morbidity, Am J Perinatol, 10(4), 1993Jul, 330-3.
[9]. Kaveeshwar S A and Cornwall J, The current status of diabetes mellitus in India. The Australasian Medical J,7(1), 2014, 45-48.
[10]. Calderon IM, Damasceno DC, Amorin RL, Costa RA, Brasil MA and Rudge MV,Morphometric study of placental villi and vessels in women with mild hyperglycemia or gestational or overt diabetes, Diabetes Res ClinPract, 78(1), 2007 Oct, 65-71.
[11]. Scott M. Nelson, Philip M. Coan, Graham J. Burton, and Robert S. Lindsay,Placental Structure in Type 1 Diabetes, Relation to Fetal Insulin, Leptin, and IGF-I. Diabetes, 58, 2009 Nov, 2634-2641.
[12]. Burstein R, Berns AW, Hirata Y and Blumenthal HT,A comparative histoand immunopathological study of the placenta in diabetes mellitus and in erythroblastosisfetalis. Am J ObstetGynecol, 86, 1963 May 1, 66-76.
[13]. Lateef Al- Mamori RH, Morphopathology of human placenta in diabetic pregnancy, J BiolAgricul Healthcare, 4(3), 2014, 99-104.
[14]. Wasserman L, Shlesinger H, Abramovici A, Goldman JA and AllaloufD, Glycosaminoglycan patterns in diabetic and toxemic term placentas, Am J ObstetGynecol, 1;138(7 Pt 1), 1980 Dec, 769-73.
[15]. Tewari V, Tewari A and Bhardwaj N, Histological and histochemical changes in placenta of diabetic pregnant females and its comparision with normal placenta, Asian Pacific J Tropical Disease, 1(1), 2011, 1-4.
[16]. Gheorman L, Pleşea IE and Gheorman V, Histopathological considerations of placenta in pregnancy with diabetes, Rom J MorpholEmbryol, 53(2), 2012, 329-36.
[17]. Faye-Petersen OM, Heller DS and Joshi VV, Handbook of placental pathology, New Delhi, India, Taylor & Francis, 2005, p. 85-90.

[18]. Treesh SA and Khair NS,Histological Changes of the Human Placenta in Pregnancies Complicated with Diabetes, Journal of Cytol Histol, 6, 2015, 307.
[19]. Fox H, Morphological pathology of the placenta. In: Gruenwald P (ed), The placenta and its maternal supply line: effects of insufficiency on the fetus. Lancaster, Medical Technical Publications, 1975. p. 25–51.
[20]. Khaskhelli LK, Memon S, Goswami P and Ban S, Change in Normal Morphology of Placenta and Its Possible Effects on Fetal Outcome in Diabetic Mothers as Compared to Non-Diabetic Mothers., JLUMHS, 12(1), 2013 Jan-Apr, 49-54.
[21]. Liebhart M,The electron-microscopic pattern of placental villi in diabetes of the mother, Acta Med Pol, 12(2), 1971, 133-7.
[22]. Carmeliet P, Ferreira V, Breier G, Pollefeyt S, Kieckens L, Gertsenstein M, et al, Abnormal blood vessel development and lethality in embryos lacking a single VEGF allele, Nature, 1996 Apr 4, 380(6573):435-9.
[23]. Pietro L, Daher S, Rudge MV, Calderon IM, Damasceno DC, SinzatoYK, et al,Vascular endothelial growth factor (VEGF) and VEGFreceptor expression in placenta of hyperglycemic pregnant women, Placenta., 31(9), 2010 Sep, 770-80.

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