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Gestational Diabetes Related Changes in The Immunohistochemical Expression of Thrombomodulin in Term Human Placenta

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Abstract

Background: Gestational Diabetes Mellitus (GDM) known as an intolerance for glucose that identified during pregnancy, it leads to many macroscopic and microscopic placental changes, thus are responsible for increases fetal perinatal illness and mortality. Thrombomodulin (TM) is a known indicator for the endothelial damage, when it is expresses as an essential substance of protein C anticoagulant system, that act like a receptor for thrombin to cease change of the fibrinogen to fibrin and activates the aggregation of platelet and thrombous formation. This study evaluates the effect of gestational diabetes on the immunohistochemical expression of thrombomodulin in term human placenta assessed by Aperio Scope image analysis. Results showed macroscopic thrombi formation increased in GDM in comparison to control, and significant reduction in the immunohistochemical expression of thrombomodulin in GDM compared to control, this indicate that GDM is a hypercoagubility state that lead to significant thrombi formation, and consumption of thrombomodulin as one of the mechanisms that prevent thrombus formation and consequently preserve endothelial integrity.

Key words: Gestational diabetes, Thrombomodulin, Human placenta, Endothelial dysfunction, Thrombi.

Introduction

Human placenta is a highly vascularized organ with essential exchange function at maternofetal interface (1). It impacts the effects of mother situation on the foetus that make it a target organ for different pregnancy related pathologies (2). Thus, placental study helps to understand foetal development process in response to several mother conditions like GDM (3). Gestational diabetes mellitus known as an intolerance of glucose that occurs with the beginning of pregnancy (4), it is gives rise to many placental modifications, that related with a different structural and histological changes in placenta , that leads to a serious functional changes that restrictive the development and wellbeing of the fetus (5).

The endothelial cells takes an essential role in the vascular homeostasis maintaining, by releasing certain substances that regulating the thrombosis, vasodilatation, and fibrinolysis (6). The interaction between the tight junction and interendothelial adherens are important in the

homeostatic function (7), the lumenal surface in the normal endothelium expresses a molecules that work on reduction of thrombosis mechanisms and platelets regulation, that prevent thrombus formation and forming an anticoagultry surface in the blood flow paths, and one of these essential anticoagulants is the thrombomodulin (8).

Thrombomodulin is an endothelial cell membrane bound anticoagulant protein with an original and direct cellular effects (9), it is mostly found in the endothelial cells lumenal surface as well as coating the endothelium in all vascular beds (8). Furthermore it is known as an indicator for the endothelial damage, when it is expresses as an essential substance of protein C anticoagulant system (10). Some stimuli, such as tissue factor, arranged the cascade of coagulation and thrombin production as the coagulant executor. As a response to the production of thrombin, the endothelium thrombomodulin act like a receptor for thrombin to ease its ability of change the fibrinogen to fibrin and activates the aggregation of platelet (11).

Materials and Methods

The study involved 30 sample of term human placenta, that all delivered by elective caesarian sections, The age of the mother ranged from 20 to 40 years old. Samples were divided into two groups: Control and GDM group a 15 sample for each. Fresh placentae of each group were prepared for: macroscopic, general histological examination with H&E routine stain, Samples were fixed in 10% formalin overnight, then proceeded to dehydrated in ethanol: 70%,80%, 90%, 100%, cleared in xylene, infiltrated with paraffin and blocking according to (12), Sections were placed on glass slides that stained for ordinary H&E, and on positively charged slides for immunohistochemistry with Anti-Thrombomodulin , the slides were assessed by Aperio scope image analysis (version 12.4), data were assessed by Excel Microsoft 2010, as mean and standard deviation, values evaluated by unpaired t-test, and significant value selected at $p \leq 0.05$.

Results and Discussion

Macroscopic changes in GDM include a significant increase in placental central thickness with mean value (2.14±0.299 cm) compared to control group that measured about (1.4±0.327 cm) at $p \leq 0.05$ (Table1), as well as the presence of large thrombi on the maternal surface (Figure1) considered as one of the most recorded observations of most GDM samples. This study showed a variety of the histological changes in GDM placenta including; shrinkage in the villi size with relatively wide intervillous spaces, the presence of extravillous and intervillous fibrin deposition that obliterating villous vascular patterns, with difficultly to distinguish the trophoblastic tissue layers, narrowing in fetal capillaries of terminal villi, thickening wall and basement membrane of most terminal vessels, and a significant increasing in syncytial knots count compered to control group at different microscopic magnifications. The immunohistochemical expression of thrombomodulin in control group showed a widely distributed expression along the trophoblastic tissue, surrounding the villi and along the endothelial lining of the vascular structure that found within villous core. Also a batches of the strong positivity were expressed within the villous parenchymal connective tissue core (Figure2), where the mean positivity of the control group was 296981.2 ± 113810.7 (Figure2), that was significantly reduced with a mean value 22233.3±10941.6 in GDM (Table2), which showed a highly significant reduction in comparison to control group at p-value≤0.05 (Table3).

Table (1): Measurements of central thickness and widest diameter in control and GDM human placenta groups.

parameters	Control group	GDM group	P-value
Central thickness(cm)	1.4 ± 0.327	2.14 ± 0.299	**$2.68*10^{7}$**
Widest diameter(cm)	22.28 ± 0.924	22.933 ± 1.764	**0.107**

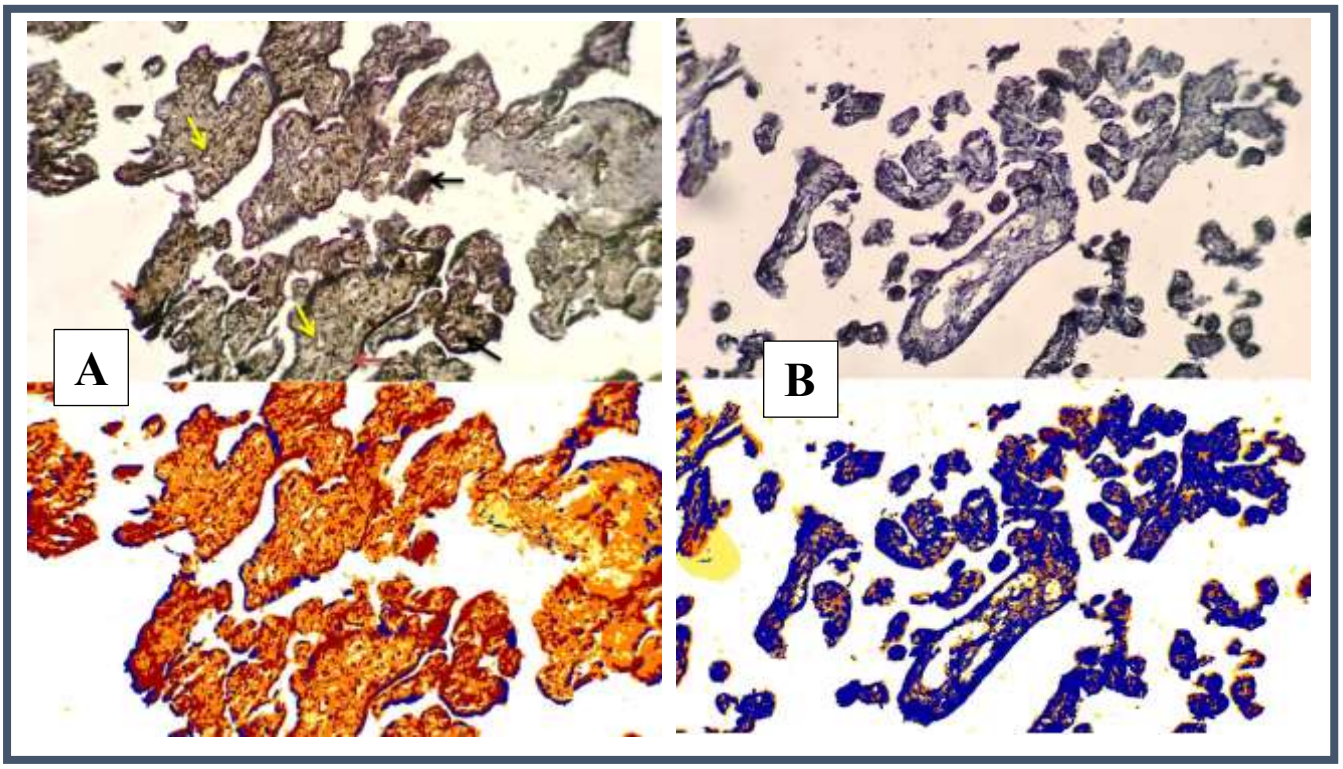
Table (2) Syncytial knots counting in control and GDM groups, at two different magnifications (10X and 40X) under light microscope.

syncytial knots count		
Groups	**10X**	**40X**
Control	15.7±5.289	2.64±1.159
GDM	47.1±12.537	13.23±3.747
P-value	**$4.64*10^{14}$**	**$1.18*10^{21}$**

Table (3): Mean number of strong positive pixel of anti-thrombomodulin antibody in control and GDM human placenta groups, and its statistically significance.

Group	**Control**	**GDM**
Mean ± SD	296981.2±113810.7	**22233.3±10941.6**
P-value	**$3.71149*10^{21}$**	

Figure (1): Macroscopic image showed the presence of large thrombi in GDM human placentas.



Figure(2): A tissue sections of human term placenta under light microscope (10X); (A) Control group, showing the immunohistochemical expression of (TM) with use of Aperio image scope, that showed a widely positive (TM) staining along villi trophoblastic tissue (red arrows), along the endothelial lining (yellow arrows) of the vascular structure that found within villous core, and a batches of the strong positivity were expressed within the villous parenchymal connective tissue core (black arrows). **(B) GDM group**, showing the immunohistochemical expression of (TM) with use of Aperio image scope, that showed a highly significant reduction in (TM) immune-staining comparison to control group.

Discussion:

Gestational diabetes is a common complication during pregnancy in which increase the amount of blood glucose to the baby and placenta, thus placental changes occur in growth, structure and function. These changes are in accordance to glycemic control spectically in the time of placental development, in this study patients in gestational diabetes were on diet control only. One of the most important placental changes that was noticed in GDM placenta is the increase in placental size and central thickness compared to the control placenta, this due to impairment in placental function as GDM is a metabolic disorder affect placental sufficiency leading to increase in size and its depth.

(3) mentioned the increasing in placental diameter signifies an increase in the exchange area of placenta, while the central thickness increasing signifies an increase of the blood vessels sprouting in order leads to increased placenta efficiency. Another study by (13) explain that the increasing in placental size is part of a compensatory mechanism for ineffectiveness of maternal oxygen-hemoglobin exchange in diabetic women. As well as the presence of large thrombi on the maternal surface of GDM placentas. This could be due to changes in coagulations due to endothelial damage, this agreed with (14)(15)(16) who mentioned that GDM is a state ,and this is an adaptive situation to prevent the hemorrhage as mentioned by (17). The increased susceptibility to thrombus formation is due to platelets activation and blood coagulation function and fibrinolytic system (18)(19)(20). GDM placenta showed reduction in terminal villi proportion in relation to control group with relative widening in intervillous space, increase in fibrin deposition this could related to metabolic changes exerted by blood glucose level this agreed with (21) who found that in GDM hyperglycemia is a procoagulatory, procontractile, proangiogenetic and proinflammatory factor act on vascular system dysfunction.(13) mentioned presence of villous immaturity, mural thrombosis, ischemia, infarction, presence of massive perivillous fibrin and villous fibrinoid depositions.

A relative thickening in the basement membrane of placental barrier was observed in placental section of GDM compared to that of control group, this may be due to metabolic effect of

hyperglycemia on endothelial cells and basement membrane, similar observations have been correlated about the thickening in the wall and the basement membrane of the villi vessels and by (22) who reported that all the vessels in diabetic placentae had a thicker walls, while in normal placentae the wall showed more thinning (thin placental barrier), and this differentiation in the placental barrier thickness effect on the efficiency of exchange process between mother and fetus, when nutrient and oxygen transfer decreased with the wall thickness increasing of the placenta barrier and vice versa. Also (3) study reported that the basement membrane thickening was a highly significant microscopic findings, and the recent data highlighted that Varity due to the associated between the Mucopolysaccharide and glycogen deposits with the trophoblastic activity reduction and this have a direct relations with the glycemic control.

As well (23) reported that the wall thickness in villi vessels were one of the most present changes in the diabetic placenta in comparison with normal women, as a result of endothelial proliferation and basement membrane thickening. Thickened basement membranes, was reported by (24) and the immature villi in GDM are associated with decrease in microvilli on the syncytiotrophoblast surface (25). Villous edema is a prominent feature in intermediate and terminal villi of GDM group compered to control group this may related to impairment in villous maturity and vascular dysfunction leading to disturbance in vascular exchange. This agreed with (26) who observed structural abnormalities as villous immaturity with decreasing in its number with increasing of intervillous fibrin deposition , in addition to vascular inflammation, and thickening of villi basement membranes; all cause impairment in fluid return to the vascular spaces giving rise to villous edema.

Fibrin deposition was found in wide areas within the villi and in the intervillous space in GDM placenta compared to limited intervillous fibrin deposition in control group, this may related to vascular impairment due to villous immaturity and this agreed with who mentioned the metabolic disorder in GDM associated with increase in fibrin deposition (27)(28). Syncytial knots increment in GDM indicate syncytial apoptosis as mentioned by (29) to provide a way to decrease barrier thickness to overcome the placental dysfunction in GDM.

Thrombomodulin immunohistochemical expression in this study showed an obvious significant variation between control and GDM placental group, when the control group showed a widely (TM) distribution along the villi trophoblastic tissue, as well as surrounding the endothelial lining the vascular structure that found within villous core. In addition a some batches of the strong positivity were expressed within the villous parenchymal connective tissue core, this distribution was reported by previous study (30) that TM is expressed in the cells that are in direct contact with the vascular system , cells of the stem villi stroma as myofibroblasts and pericytes ,and the adventitia surrounding the arteries of the stem villi.

The coagulation activity increases during normal gestation, this leads to increment in anticoagulation as thrombomodulin to preserve placental vasculature, however thrombotic changes were relatively higher during pregnancy with gestational diabetes, this lead to consumption of antithrombotic factors which were reduced in women with GDM. This agreed with (14) who reported that coagulation activity increased during normal pregnancy as a result of the height level in coagulation factor, also (30) added that the increase of TM expression in the stem villi is due to adaptive response to control and regulate the fetal blood flow in the villous tree in normal gestation.

Our results showed that the immunohistochemical expression of thrombomodulin in the GDM was significantly reduced compered to control group. This could be due to the fact that thrombomodulin is one of the most important surface components of the vascular endothelium integrity, that affected by any defect occurring in the endothelium such as in gestational diabetes, and this leads to decrease in its levels in the tissues within the endothelial damaged in placenta vasculature, as well as coagulation and clots formation that consume the anticoagulation.

(31) reported that excessive level of glucose in GDM lead to endothelial damage, leading to angiogenesis and mitochondrial deficiency. In addition to (32) who reported that the cellular transport mechanism which ease to glucose processing that line with the vessels leads to uncontrolled glucose level in diabetes, as well as might resulting in blood clotting formation lead to capillaries damages.

While (33) study pointed out that endothelial cells have antithrombotic activity by providing a surface that discourages the attachment of cells and clotting proteins surface receptors, such as thrombomodulin. It was stated in a study conducted by (34) the placental endothelial dysfunction that occur in preeclampsia was correlated with decrease in the thrombomodulin

immunohistochemical expression that seen in placental villous capillaries, syncytiotrophoblast and villous core this due to increase in the coagulation and increase in the fibrin deposition in endothelial cells and syncytiotrophoblast.

In other hand (30) study suggested that the endothelial damage of the stem villi arteries is characterized by a decrease in TM expression in preeclampsia, and TM expression was negative in the endothelial cells but positive in myofibroblasts and pericytes of placenta stroma, while he added that placentas with increased TM showed decreased in the syncytial knots. And that disagreed with our findings as in gestational diabetes there were a decrease in TM expression and increase in the syncytial knots in the tissue sections of GDM placentas, this occur in response to disturbance in blood glucose levels which induce endothelial damage that activate thrombotic cascade and collectively lead to reduction in tissue thrombomodulin, and reduced vascular dimensions this agreed with (21) who considered hyperglycemia in GDM is a proconstricting and procoagulatory factors.

(35) reported that plasma levels of soluble thrombomodulin were increased and it is a significantly higher in 79 patients' serum with diabetes mellitus. As well as a study of (36) also reported that the soluble TM releasing into the bloodstream lead to a reduction in TM expression on cell surfaces and that can be attributed to endothelial dysfunction, when he was evidenced that the release of soluble TM is a result of the endothelial damage in the preeclampsia patients. (37) study showed that TM terminates the pro-thrombotic actions of thrombin, providing an enzyme cofactor activates protein C which is an important antithrombotic enzyme. In addition to a study that conducted by (38) showed that the disturbances of the TM-protein C antithrombotic action is noticed in many diseases associated with vascular disturbance, such as venous thrombosis, myocardial ischemia, disseminated intravascular coagulation, and stroke (39) and (40) studies.

In addition to (41) study who reported that TM expression can determine the extent of microvascular thrombus formation, and its role in protection against thrombosis and ischemia. As well as the previous clinical data that in (42) study, that suggested the increase that happen in the TM level in plasma of diabetic patient indicate vascular endothelial injury which inversely related to protein C activity, and this strongly related to microangiopathy and diabetic neuropathy. The reduction in terminal villi capillary dimensions seen in casting results can attributed to vascular changes in GDM that lead to reduction in TM, as in diabetic microangiopathy endothelial hyperplasia, capillary thinning and closure, thrombosis, all were reported as features of diabetic microangiopathy (43).

The underlining mechanism for the local causes in reduction in TM immunohistochemical expression could attributed to metabolic and inflammatory mechanisms that change endothelial anticoagulant activity and TM expression, one of the theories is accumulation of activated glucose end product on endothelial surface render it more vulnerable for coagulation and reduced TM expression (44). Other reason for reduction in TM expression is presence of tissue hypoxia, as in GDM could occur due to glycemic control and hypercoagulable state that impede blood supply , this was reported by (45) as tissue hypoxia reduced TM tissue expression. Other cause in reduction in TM expression is attributed to hyperglycemia as mentioned in microvasculature related to peripheral nerves in diabetic patients as mentioned by (43) which lead to changes in endothelial surface features and reduced in TM expression and activation of coagulation process**.**

Conclusion

Gestational diabetes acts as a strong stimulus for vascular thrombosis due to the effect of glucose imbalance of endothelial cells, this considered as a proconstricting effect of gestational diabetes on placental terminal villi capillaries, also as a pro coagulating factors that leads to thrombosis and down regulation of placental thrombomodulin expression, which can be consider as a bio marker for endothelial injury in GDM.

References

1. Babawale MO, Lovat S, Mayhew TM, Lammiman MJ, James DK, Leach L. GDM junct. 2000;
2. Carrasco-Wong I, Moller A, Giachini FR, Lima V V, Toledo F, Stojanova J, et al. Placental structure in gestational diabetes mellitus. Biochim Biophys Acta (BBA)-Molecular Basis Dis. 2020;1866(2):165535.
3. Anjum S. Histomorphological Alterations of Placenta in Normal vs Gestational Diabetes Mellitus Subjects: A Case-control Study. Int J Anat Radiol Surg. 2021;10(December 2015):20–5.

4. Ehlers E, Talton OO, Schust DJ, Schulz LC. Placental structural abnormalities in gestational diabetes and when they develop: A scoping review. Placenta [Internet]. 2021;116(April):58–66. Available from: https://doi.org/10.1016/j.placenta.2021.04.005
5. Placental structure in gestational diabetes mellitus. Biochim Biophys Acta - Mol Basis Dis [Internet]. 2020;1866(2):165535. Available from: https://doi.org/10.1016/j.bbadis.2019.165535
6. Drozdz D, Łatka M, Drozdz T, Sztefko K, Kwinta P. Thrombomodulin as a new marker of endothelial dysfunction in chronic kidney disease in children. Oxid Med Cell Longev. 2018;2018.
7. Walsh TG, Murphy RP, Fitzpatrick P, Rochfort KD, Guinan AF, Murphy A, et al. Stabilization of brain microvascular endothelial barrier function by shear stress involves VE-cadherin signaling leading to modulation of pTyr-occludin levels. J Cell Physiol. 2011;226(11):3053–63.
8. Martin FA, Murphy RP, Cummins PM. Thrombomodulin and the vascular endothelium : insights into functional , regulatory , and therapeutic aspects. 2023;(4).
9. Chao T-H, Tsai W-C, Chen J-Y, Liu P-Y, Chung H-C, Tseng S-Y, et al. Soluble thrombomodulin is a paracrine anti-apoptotic factor for vascular endothelial protection. Int J Cardiol [Internet]. 2014;172(2):340–9. Available from: https://www.sciencedirect.com/science/article/pii/S0167527314001971
10. Kubisz P. Endothelial and platelet markers in diabetes mellitus type 2. World J Diabetes. 2015;6(3):423.
11. Watanabe-kusunoki K, Nakazawa D, Ishizu A, Atsumi T. Thrombomodulin as a Physiological Modulator of Intravascular Injury. 2020;11(September):1–12.
12. Chong WC, Wu R, Tu Y. A Study on Tissue Processing. Int J Innov Interdiscip Res Issue [Internet]. 2012;3(April):2012. Available from: https://www.researchgate.net/publication/299602601
13. Daskalakis G, Marinopoulos S, Krielesi V, Papapanagiotou A, Papantoniou N, Mesogitis S, et al. Placental pathology in women with gestational diabetes. Acta Obstet Gynecol Scand. 2008;87(4):403–7.
14. Yoon H-J. Coagulation abnormalities and bleeding in pregnancy: an anesthesiologist's perspective. Anesth pain Med. 2019;14(4):371–9.
15. Dong C, Gu X, Chen F, Long Y, Zhu D, Yang X, et al. The variation degree of coagulation function is not responsible for extra risk of hemorrhage in gestational diabetes mellitus. J Clin Lab Anal. 2020;34(4):1–6.
16. Lee J, Eley VA, Wyssusek KH, Kimble RMN, Way M, van Zundert AA. Rotational thromboelastometry (ROTEM®) in gestational diabetes mellitus and coagulation in healthy term pregnancy: A prospective observational study in Australia. Aust New Zeal J Obstet Gynaecol. 2022;62(3):389–94.
17. Gresele P, Fuster V, Lopez JA, Page CP, Vermylen J. Platelets in hematologic and cardiovascular disorders: a clinical handbook. Cambridge University Press; 2007.
18. Liu B-Y, Jian Y-L, Zhong M, Yu Y-H, Wang Q, Zhang J. Value of coagulation function and fibrinolytic system assessment in patients with gestational diabetes mellitus. Nan Fang yi ke da xue xue bao= J South Med Univ. 2007;27(1):35–7.
19. Bekdemir H, Berberoglu Z, Gorar S, Dellal D, Aktas A, Aral Y. Hemostatic changes in gestational diabetes mellitus. Int J Diabetes Dev Ctries. 2015;35:502–6.
20. Gorar S, Alioglu B, Ademoglu E, Uyar S, Bekdemir H, Candan Z, et al. Is There a Tendency for Thrombosis in Gestational Diabetes Mellitus? J Lab Physicians. 2016;8(02):101–5.
21. Meng Q, Shao L, Luo X, Mu Y, Xu W, Gao C, et al. Ultrastructure of placenta of gravidas with gestational diabetes mellitus. Obstet Gynecol Int. 2015;2015.
22. Sawy NA El, Mohammad ;, Iqbal S, Alkushi AG. Histomorphological Study of Placenta in Gestational Diabetes Mellitus Estudio Histomorfológico de la Placenta en Diabetes Mellitus Gestacional. Int J Morphol. 2018;36(2):687–92.
23. Mahmoud EA, Al-Bakri NA, Qasim BJ. Histopathological changes of placenta in pregnant women complicated with pregestational diabetes. J Pharm Sci Res. 2018;10(11):2952–9.

24. Weiner E, Barber E, Feldstein O, Schreiber L, Dekalo A, Mizrachi Y, et al. The placental component and neonatal outcome in singleton vs. twin pregnancies complicated by gestational diabetes mellitus. Placenta [Internet]. 2018;63:39–44. Available from: https://www.sciencedirect.com/science/article/pii/S0143400418300365
25. Akarsu S, Bagirzade M, Omeroglu S, Büke B. Placental vascularization and apoptosis in Type-1 and gestational DM. J Matern Neonatal Med [Internet]. 2017 May 3;30(9):1045–50. Available from: https://doi.org/10.1080/14767058.2016.1199676
26. Ehlers E, Talton OO, Schust DJ, Schulz LC. Placental structural abnormalities in gestational diabetes and when they develop: A scoping review. Placenta. 2021;116:58–66.
27. Nelson SM, Coan PM, Burton GJ, Lindsay RS. Placental structure in type 1 diabetes: relation to fetal insulin, leptin, and IGF-I. Diabetes. 2009;58(11):2634–41.
28. Liang X, Zhang J, Wang Y, Wu Y, Liu H, Feng W, et al. Comparative study of microvascular structural changes in the gestational diabetic placenta. Diabetes Vasc Dis Res. 2023;20(3):1–9.
29. Kareem HR. Research Article Age Related Changes in Human Placental Oxidative Stress and its Effect on Syncytial Apoptosis (Syncytial Knots Formation) 1. 2014;29(08):38–41.
30. Bosco C, Parra M, Barja P, Rodrigo R, Fernández V, Suarez M, et al. Increased immunohistochemical expression of thrombomodulin at placental perivascular myofibroblast in severe preeclampsia (PE). Histol Histopathol. 2005;20(4):1045–55.
31. Wang M, Li Y, Li S, Lv J. Endothelial Dysfunction and Diabetic Cardiomyopathy. Front Endocrinol (Lausanne). 2022;13(April):1–12.
32. Irshad Z, Xue M, Ashour A, Larkin JR, Thornalley PJ, Rabbani N. Activation of the unfolded protein response in high glucose treated endothelial cells is mediated by methylglyoxal. Sci Rep. 2019;9(1):1–14.
33. Yau JW, Teoh H, Verma S. Endothelial cell control of thrombosis. BMC Cardiovasc Disord [Internet]. 2015;15(1):1–11. Available from: http://dx.doi.org/10.1186/s12872-015-0124-z
34. Turner RJ, Bloemenkamp KWM, Bruijn JA, Baelde HJ. Loss of Thrombomodulin in Placental Dysfunction in Preeclampsia. Arterioscler Thromb Vasc Biol. 2016;36(4):728–35.
35. Shimano H, Takahashi K, Kawakami M, Gotoda T, Harada K, Shimada M, et al. Elevated serum and urinary thrombomodulin levels in patients with non-insulin-dependent diabetes mellitus. Clin Chim Acta. 1994 Mar;225(2):89–96.
36. Ayala-Ramírez P, Gámez T, Castro-Pontón DL, Silva J, García-Robles R, Olaya-C M. Expression of tissue factor and thrombomodulin in the placentas of pregnancies affected by early-onset and late-onset preeclampsia. J Obstet Gynaecol Res. 2021;47(7):2307–17.
37. Esmon CT. The protein C anticoagulant pathway. Arterioscler Thromb a J Vasc Biol. 1992;12(2):135–45.
38. Svensson PJ, Dahlback B. Resistance to activated protein C as a basis for venous thrombosis. N Engl J Med. 1994;330(8):517–22.
39. Snow TR, Deal MT, Dickey DT, Esmon CT. Protein C activation following coronary artery occlusion in the in situ porcine heart. Circulation. 1991;84(1):293–9.
40. Ninomia T, Wang L, Kumar SR, Kim A, Zlokovic B V. Brain injury and cerebrovascular fibrin deposition correlate with reduced antithrombotic brain capillary functions in a hypertensive stroke model. J Cereb Blood Flow Metab. 2000;20(6):998–1009.
41. Wong VLY, Hofman FM, Ishii H, Fisher M. Regional distribution of thrombomodulin in human brain. Brain Res. 1991;556(1):1–5.
42. Nawor P. Plasma thrombomodulin: Amarker for micro-vascular complications in diabetes mellitus. Vasa. 1996;25(3).
43. Hafer-Macko CE, Ivey FM, Gyure KA, Sorkin JD, Macko RF. Thrombomodulin deficiency in human diabetic nerve microvasculature. Diabetes. 2002;51(6):1957–63.
44. Sugimoto K, Nishizawa Y, Horiuchi S, Yagihashi S. Localization in human diabetic peripheral nerve of Nε-carboxymethyllysine-protein adducts, an advanced glycation endproduct. Diabetologia. 1997;40:1380–7.
45. Ogawa S, Gerlach H, Esposito C, Pasagian-Macaulay A, Brett J, Stern D. Hypoxia modulates the barrier and coagulant function of cultured bovine endothelium. Increased monolayer

permeability and induction of procoagulant properties. J Clin Invest. 1990;85(4):1090–8