Endothelin-1 Expression in Placental Tissue Vasculature in Normal Vaginal Delivery and Cesarean Sections

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Abstract

The placenta is a temporary organ required for the development of embryo and fetus. It allows the physiological exchange between the fetus and the mother. Endothelin is a human protein has three isoforms, endothelin-1, -2, and -3. Endothelin-1 (ET- 1) is the most potent and long-lasting vasoconstrictor known. Endothelin has two receptors, ETA and ETB, ETA receptors are found on the external surface of the vascular smooth muscle cells of blood vessels, and binding of endothelin to ETA increases vasoconstriction.Objectives: To study the histochemical distribution of vasoactive agent (endothelin -1) in placental tissue after normal vaginal delivery and elective caesarean section, this might be a determinant of the onset of parturition. The current study includes studying forty-two placentas (21NVD&21CS) with an eccentric cord insertion were obtained from a healthy pregnant female (with no hypertension, diabetes mellitus, or gynecological diseases or any other major diseases). The placental tissues were histologically prepared for paraffin sections. Staining procedure includes histochemical stain for endotheline-1 using goat polyclonal IgG antibody against endothelin-1 as primary antibody and biolinylated as secondary antibody. An immunostaining score according to the graduated intensities of the reaction product was defined and scored blindly by two investigators who scored Staining intensity (-, +, ++, +++, ++++). The median intensity of ET-1 was highest in placenta delivered by normal vaginal delivery (++++) and lowest in those by cesarean section (+). The normal vaginal delivery group of placentas was associated with statistically significant higher median ET-1 stain intensity compared to that of cesarean section group. In-conclusion: ET-1 activity in placental tissue is significantly higher in normal vaginal delivery group.

Key words: Endotheline1; Placental histochemistry; Immunocytochemistry; Human placenta

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Introduction

The placenta is a temporary organ required for the development of embryo and fetus. It allows the physiological exchange between the fetus and the mother. It is composed of cells derived from two genetically distinct individuals [1]. Endothelin is a human protein has three isoforms, endothelin-1, -2, and -3. Endothelin-1 (ET- 1) is the most potent and long-lasting vasoconstrictor known, being 100 times more potent than noradrenaline. Mature ET-1 is a 21-amino acid peptide, and it is the main member of the endothelin peptide family. Endothelin has two receptors, ETA and ETB. ET-1 and -2 bind to ETA and ETB, while ET-3 only binds to ETB. The immunoreactivity of ET-1 is localized to endothelial cells of capillaries of placental microvilli, small- and medium-sized arteries and veins as well as placental syncytiotrophoblast [2]. ETA receptors are found on the external surface of the vascular smooth muscle cells of blood vessels, and binding of endothelin to ETA increases vasoconstriction [3, 4], while ETB receptors are found predominantly on the luminal surface of the endothelial cell, but may also preside in the vascular smooth muscle cells in some vascular beds [5, 6].

ET-1 is produced by the vascular endothelium from a 39 amino acid precursor, through the actions of an endothelin converting enzyme (ECE) found on the endothelial cell membrane. Once ET-1 is released, it binds to receptor on the target tissue through ETA and ETB receptor. Both receptors are coupled to a Gq-protein and the formation of IP3. Increased IP3 causes calcium release by the sarcoplasmic reticulum, which causes smooth muscle contraction (7). The G protein-coupled receptors (GPCRs) are membrane proteins that traverse the plasma membrane seven times In their potential therapeutic value is very limited [8]. The following figure 1 below which describes histology of human placental chorionic villus.

The aim of the study is to determine immunohistochemical changes in vasoactive agent (endothelin -1) distribution of placental tissue after normal vaginal delivery and elective caesarean section.

Patients and Methods

The current study includes studying a forty-two placentas (21NVD&21CS) with an eccentric cord insertion were obtained from a healthy pregnant female (with no medical history of hypertension, diabetes mellitus, or gynecological diseases or any other major diseases), nonsmoker. These placentas after NVD and elective CS were collected in the obstetric ward of AL- Hilla teaching hospital. The placentas were examined after being expelled, grossly to ensure that they have an eccentric cord insertion and have no abnormality or infarction, then

the placental membranes were cut off and the umbilical cord were cut at 2 cm from its insertion. Then the largest diameter of the placenta measured from the fetal side that passing through the area of cord insertion was excised in a form of ribbon of 1 cm width.

Preparation of the paraffin sections:

The placental tissues were histologically prepared for paraffin section according to 10, 11 as follows:

Fixation, dehydration, clearing, impregnation, embedding, sectioning, dewaxing, hydration, staining and mounting.

Immunostaining for endothelin-1

Staining procedure includes the following steps [12, 13]:

1. The slides that are previously prepared are deparaffinize.
2. Immerse slid in (Retrieval solution) sodium citrate Buffer, pH 6.0. Heat at 95 c° for 10 minutes. Allow slides to cool in room temperature for 20 minutes.
3. Wash in deionized water three times for 2 minutes.
4. Apply enough Hydrogen peroxide to cover specimen. And incubate for 5 to 10 minutes. Wash in buffer
5. Incubate specimens for 20 minutes in 1-3 drops of serum block.
6. Apply enough primary antibodies, goat polyclonal IgG antibody against endothelin-1, Incubate for 2 hours. Wash in buffer
7. Incubate specimens for 30 minutes in 1-3 drops of biolinylated secondary antibody. Wash in buffer
8. Incubate specimens for 30 minutes in 1-3 drops of HRP streptavidine complex. Wash in buffer
9. Add 1-3 drops of HRP substrate to each slid. Develop until light brown staining is visible, 30 second - 10 minute
10. Counter stain with hematoxylin 5-10 minutes. And immediately wash with several change of deionized H_2O.
11. Dehydrate section by ethanol and them xylene.
12. Immediately add 1-2 drop of permanent mounting media and cover with class cover slip. The slide then observed under light microscope.

Analysis of endothelin-1 reactivity:

Semi quantification of antigen expression was evaluated under the light microscope at 400X magnification. An immunostaining score according to the graduated intensities of the reaction product was defined and scored blindly by two investigators who scored Staining intensity as follow:

- : Indicated no staining (<10 cells per field)
+ : Weak (10-25 cells per field)
++ : Moderate (25-50 cells per field)
+++: Strong (50-75 cells per field)
++++: Very strong stain intensity (>75 cells per field) [14]

Results

ET-1 reactivity intensity in cesarean section & normal vaginal delivery:

The immunohistochemical analysis in this study included endothelial and smooth muscle cells. The median intensity of ET-1 was highest in placenta delivered by normal vaginal delivery (++++) and lowest intensity in those by cesarean section group (+) see figure (2).The normal vaginal delivery group of placentas was associated with statistically significant higher median ET-1 stain reactivity compared to that of cesarean section group. The difference was statistically significant (P <0.05).as shown in (table1).

Endothelin-1-like immunohistochemical intensity was present in virtually all the capillary endothelium. The staining was specific for endothelin.

Table 1.

Show the difference in median tissue ET-1 stain reactivity between placenta delivered by normal vaginal delivery and cesarean section. The median reactivity is significantly higher in NVD group in comparison with CS group.

*CS= cesarean section, **NVD=normal vaginal delivery

Name of test	CS*	NVD**	Mann-Whitney U-test	P.value
-	220	100	82.500	0.0036
+	240	160		
++	60	480		
+++	0	240		
++++	0	100		
Total	520	1080		
Median	+	++++		
Mean rank	14	23.75		

.*CS= cesarean section, **NVD=normal vaginal delivery

Although the ET-1 reactivity is seen in the luminal endothelial cells but it is more evident in the outer surface of smooth muscle cells of the blood vessel and syncytiotrophoblast as well in the normal vaginal delivery group cells, while in those of cesarean section group, the immunohistochemical reactivity is distributed all over the tissue and approximately it is equivocal and weak.

Table 2.

The binding sites and its immunohistochemical reactivity of endothelin-1 expression in NVD & CS groups. (+: weak, ++: moderate reactivity, +++: strong reactivity).

Binding sites for endothelin-1	NVD group	CS group	P value
Smooth muscle	++	+	≤0.05
Endothelial cells	+	+	≥0.05
Syncytiotrophoblast	+++	+	≤0.05
Cytotrophoblast	+	++	≥0.05

Figure 1.

Histological illustration of the placental villus components [9].

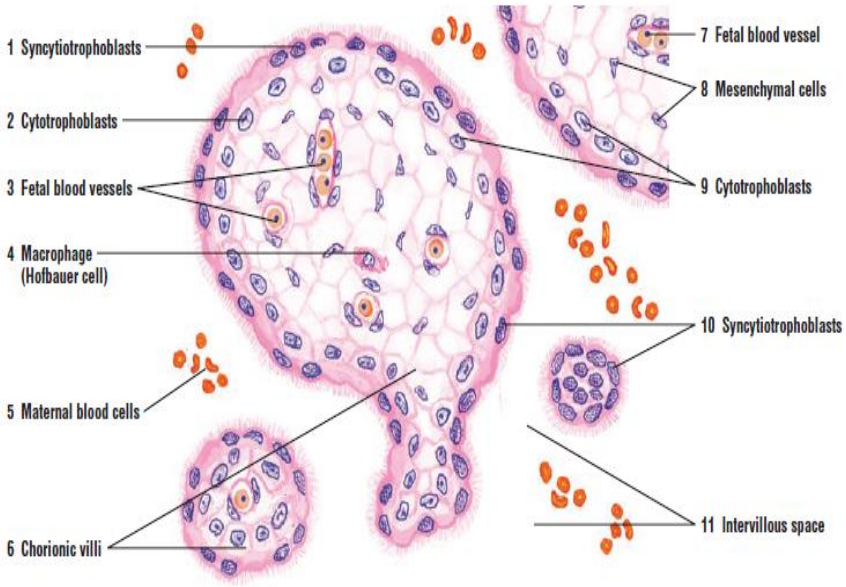
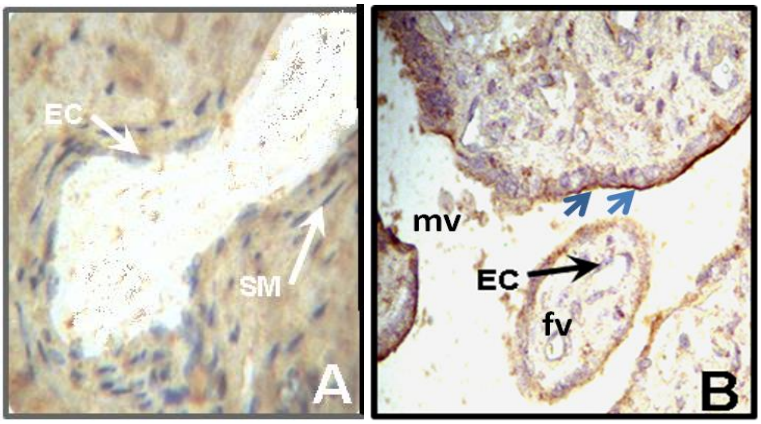


Figure 2.

400X Immunohistochemical staining for ET-1 expression in chorionic villi of placenta delivered by: (A) Cesarean section. (B) Normal vaginal delivery, (SM: smooth muscle cell nucleus, EC: nucleus of endothelial cell of fetal vessel, mv: maternal vessel, blue arrows: syncytiotrophoblast cells).



From the data in table 1 and figure 2, one can describe the following findings:

Immunohistochemical reactivity is present virtually in all parts of fetal placental tissue, namely the syncytiotrophoblast, cytotrophoblast and endothelial cell lining of the fetal blood vessels, although there are distinct differences between these locations as follows (table 2)

• The median endothelin-1 immunoreactivity is more evident in the syncytiotrophoblast cells in NVD group (+++) than CS group(+),The difference was statistically significant (P <0.05), as shown in (figure 2 B)

• The median endothelin-1 immunoreactivity in cytotrophoblast cells in NVD group is less evident than the CS group. The difference was statistically non-significant (P ≥0.05)

• The endothelin-1 immunoreactivity is less evident in endothelial cells and non-significant difference in both groups as shown in (figure 2 A& B)

• There is a significant difference in the endothelin-1 immunoreactivity of smooth muscle cells in NVD & CS groups, evidently more in NVD group.

Discussion

ET-1 is the principal vasoactive substance which is involved in the regulation of the fetoplacental circulation and may sub serve specific trophoblastic functions, Endothelins are 21-amino acid peptides involved in regulating vascular homeostasis. Endothelin-1 (ET-1) being the most potent vasoconstrictor currently known [15,16]. It is suggested that endothelin may act as a circulating hormone during pregnancy and labor in both maternal and fetal circulations [17]. ET-1 also acts as growth factors and seems to be involved in fetal

development; endothelin-1 (ET-1) stimulates the secretion of vascular endothelial growth factor (VEGF) [18, 19].

Similar findings were reported by 20. who showed that the plasma concentration of ET-1 increased gradually during normal pregnancy. As pregnancy advances, the level becomes higher after 29 weeks of gestation. The plasma ET-1 during labor pain was higher than that in third trimester of pregnancy without labor pain. These results suggest that ET-1 might play an important role in uterine contraction and thus participation in labor.

Production of ET-1 by trophoblast may contribute to regulation of vascular tone 14 and ultimately may potentiate the oxytocin response of myometrium in pregnant woman to initiate the normal parturition process [21] i.e., more production of ET-1 in the preterm period (before 38 weeks) may predict the mode of termination of current pregnancy whether by NVD or CS. Hence, the higher ET-1immunostaining intensity was observed and detected in the NVD group generally.

The current study expresses the higher intensity of endothelin 1 in those of NVD group placentas than CS group which agreed with previous studies and suggestions. The finding that endothelin-1 is produced locally in the human placenta completes the criteria for an autacoid system. We now know that the endothelial cells lining blood vessels and capillaries in the placenta make endothelin-1 in vivo. Endothelin-1 produced in placental capillaries may enhance vascular permeability, this evidence, coupled with the previous demonstrations that endothelin-1 constricts fetoplacental vasculature by acting on specific high-affinity receptor sites, completes the requirements for a tissue-active autacoids system. The presence of this constrictor system in the human fetoplacental circulation raises the important question of its possible physiological role in the regulation of blood flow and vascular permeability in the human fetoplacental vascular bed. Endothelin-1 may play a role in the constriction of umbilical vessels at the time of delivery [22].

The figure 2A, has more evident finding that support the idea of high production of endothelin-1 in vivo near term of pregnancy since the increased immunoreactivity in syncytiotrophoblast cells seen in the same figure, which might indicate high production rate of endothelin-1 by the syncytiotrophoblast cells in NVD group and conclusively might have role in the initiation of normal vaginal delivery as a mode of labor unless there are other unexpected causes to shift the delivery into an emergency caesarean section [23]. The immunohistochemical reactivity in the current study is proportionated to vasoactivity whether vasoconstriction or production of the endothelin in vivo.

Ethical Approval

The study was approved by the Ethical Committee.

Conflicts of Interest

The authors declare that they have no competing interests.

Authors' Contributions

Both authors shared in conception, design of the study, acquisition of data, and manuscript writing, the critical revising and final approval of the version to be published.

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Obstacles

Unavailability of the antibodies kit for endothelin-1 in the local market, push us to wait for long time before the products arrived.

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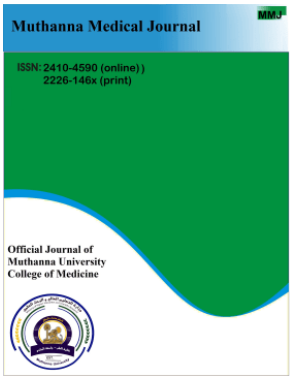

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