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# IMMUNOHISTOCHEMICAL STUDY OF THE HUMAN PLACENTAL VASCULATURE AFTER NORMAL VAGINAL DELIVERY AND ELECTIVE CESEAREAN SECTION

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## ABSTRACT

**Background:** 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. Endothelins 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:** study the histochemical distribution of vasoactive agent (endothelin -1) in placental tissue after normal vaginal delivery and elective caesarean section which might be a determinant of the onset of parturition.

Method: 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 hypertension, diabetes mellitus, or gynecological diseases or any other major diseases).the placental tissues were histologically prepared for paraffin section. 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 (-, +, ++, +++, ++++)

**Results:** the median intensity of ET-1 was highest in placenta delivered by normal vaginal delivery (++++) and lowest in 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

**Conclusion:** ET-1 activity in placental tissue is significantly higher in normal vaginal delivery group.

## KEYWORDS

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 (Junqueira and Cameiro, 2005).

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. Endothelins 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 syncytiotrophoblasts (Wang and Zhao, 2010).

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 (Bobik et al., 1990 and Lüscher et al., 1993), 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 (Clozel et al., 1992 and Deng et al., 1995).

ET-1 is produced by the vascular endothelium from a 39 amino acid precursor, big ET-1, 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 of these 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 (Richard, 2009).

## THE AIM OF THE STUDY

To determine histochemical changes in vasoactive agent (endothelin -1) distribution of placental tissue after normal vaginal delivery and elective caesarean section which might be a determinant of the onset of parturition.

## MATERIALS 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. Then the largest diameter of the placenta measured from the fetal side that passing through the area of cord insertion (the umbilical cord were cut at 2 cm from its 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 Bancroft and Stevens (1987) and Baker et al. (1998) as follows:

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

## IMMUNOSTAINING FOR ENDOTHELIN-1:

Staining procedure includes the following steps: (Cuello, 1993and Daniel et al., 1997).

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.

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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 antibody, 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 H2O.
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 STAINING:

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)
(José et al., 2001).

## RESULTS:

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

(The histochemical 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 cesarean section group (+) see figure (1.1).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. The difference was statistically significant (P <0.05).as shown in table (1.1)

although the staining intensity is seen in the lumenal endothelial cells but it is more notified in the smooth muscle cells outside the vessel of the normal vaginal delivery group, while in those of cesarean section group, is distributed all over the tissue.

The histochemical staining intensity is proportionated to vasoactivity whether vasoconstriction or production of the endothelin itself

## DISCUSSION

ET-1 is the principal vasoactive substance which is involved in the regulation of the fetoplacental circulation and may subserve specific trophoblastic functions( Mondon et al., 1993). It is suggested that endothelin may act as a circulating hormone during pregnancy and labor in both maternal and fetal circulations ( Iwata et al., 1991). ET-1 also act as growth factors and seem to be involved in fetal development (Stjernquist M. 1998).

Similar findings were reported by Usuki et al.,(1990) 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 (José et al., 2001) and ultimately may potentiate the oxytocin response of myometrium in pregnant woman to initiate the normal parturition process (Guillermo et al., 1995) 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.

The current study expresses the higher intensity of endothelin 1 n those of NVD group placentas than CS group which agreed with previous studies and suggestions.

**Table (1): Show the difference in median tissue ET-1 stain intensity between placenta delivered by normal vaginal delivery and cesarean section.**

| Name of test | CS* | NVD** | Mann-Whitney U-test | P.value |
|---|---|---|---|---|
| - | 220 | ١٠٠ | 82.500 | 0.0036 |
| + | 240 | ١٦٠ | | |
| ++ | 60 | ٤٨٠ | | |
| +++ | 0 | 240 | | |
| ++++ | 0 | 100 | | |
| Total | 520 | ١٠٨٠ | | |
| Median | + | ++++ | | |
| Mean rank | 14 | 23.75 | | |

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

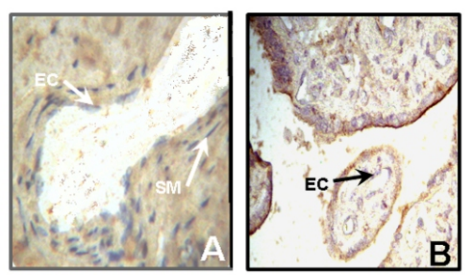


**Figure (1:1)** 400x Immunohistochemical staining for ET-1 expression in chorionic villi of placenta delivered by: (SM: smooth muscle cell, EC: endothelial cell)
(A) Cesarean section.
(B) Normal vaginal delivery.

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