Evaluation of Intraplacental Villous Microvascular Density and Vascular Surface Area in Pregnancy Induced Hypertension and Its Correlation to Newborn Body Weight

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Abstract

Background Preeclampsia is a major problem in obstetric practice; it is considered one of the largest causes of maternal and perinatal morbidity and mortality, and one of the most important causes of intrauterine growth restriction and low birth weight.

Objectives To evaluate the intraplacental villous microvascular density and vascular surface area in pregnancy induced hypertension in term placentae, and its effect on newborn body weight.

Methods A sample of 50 placentae divided into 25 normal term placentae (Control group) and 25 term placentae of pregnancy induced hypertension (preelampsia) considered as the (Test group). Fresh placental tissues were taken from the peripheral placental area, processed to paraffin blocks, stained with CD-34 (clone QBEnd-10) (DAKOCYTOMATION), assessment of the vascular density, and vascular area with image j software.

Results Significant increase in vascular density, with a significant reduction in vascular area seen in placentae of preeclampsia compared to control group at p ≤ 0.05, in addition fibrosis with vascular degeneration and stenosis were evident in terminal villi, lead to avascular terminal villi in preeclampsia compared to control group. Significant reduction in newborn body weight in preeclampsia compared to control at p ≤ 0.05.

Conclusions These results suggested that placenta adapt its structure to maintain its function, this adaptation reflected as an increase in vascular density that consequently occur as uteroplacental perfusion reduces due to maternal vasospasm, and the placenta becomes ischemic as gestation progresses due to hypoxia that affect terminal villi vasculature. Fibrin deposition, vascular degeneration, thickened vessels wall, and stenosis those together reduce the vascular area; these changes accordingly were reflected on newborn body weight in preeclampsia.

Key words Preeclampsia, vascular density, vascular area, placenta.

List of abbreviation: PE = Pre-eclampsia.

Introduction

Preeclampsia (PE) is a heterogenic multisystemic disorder, of a sudden occurrence during the second half of pregnancy (1). It is generally defined as the development of hypertension and proteinuria after 20 weeks of gestation in a previously normotensive woman (2). The precise etiopathogenesis of PE remains to be a subject of extensive research, but it is believed that it is likely to be multifactorial, nevertheless, it is accepted that it is the presence of the placenta rather than the fetus, which is responsible for development of PE. The onset, severity, and progression of PE are significantly affected by the maternal response to placental derived factors and proteins (3).

Normal placental development requires continuous process of trophoblastic invasion of maternal endometrium as well as of
vasculature. The interstitial trophoblastic invasion causes proper anchorage to the endometrium whereas endovascular invasion leads to uterine spiral artery remodeling ultimately causing increased maternal blood flow and hence proper uteroplacental perfusion. The disease is associated with an increase in the vascular resistance at fetoplacental interface and associated with changes seen in the fetal stem arteries. Pregnancy complications like hypertension are reflected in placenta in a significant way both macroscopically and microscopically. Several studies have shown that utero-placental blood flow is decreased in pregnancy induced hypertension due to maternal vasospasm, that leads to fetal hypoxia and accordingly it may lead to fetal distress and fetal death. Placental cells expressing CD-34 were mainly restricted to the embryonic vessels of placenta, blood in fetal vessels and vessel walls. So it is used to identify placental vascular structures within placental villi.

The present study aimed to record the changes on the vascular density and vascular area in the placental villi from mothers with pregnancy induced hypertension, and its effect on birth weight of the new born babies in comparison to normotensive mothers. Since placenta is the mirror of maternal and fetal status, it could reflect the changes due to maternal hypertension.

Methods
A sample of 50 human placentae delivered by normal vaginal delivery, collected from the Obstetric and Gynecological Department at AL-Imamain Al-Kadhimain Medical City, with gestational age (38-40) weeks, taken after patient informed consent that had been signed by all participants, those samples fall into two groups:

1. Test group, include (25 placentae) delivered by mothers complaining from PE, which is defined according to International Society for the Study of Hypertension in Pregnancy characterized by blood pressure greater than 140/90 mmHg measured on two occasions with 6 hours apart and protein urea more than 300 mg/24 hours in a previously normotensive women as defined by the at 35 week of gestation.

2. Control group that include the other 25 placentae that delivered to mother with normotensive and no protein urea.

All pregnancies with known vascular diseases as essential hypertension, and diabetes were excluded.

Tissue preparation
Immediately after delivery, placentae were cleaned from blood, five small tissue pieces were taken from the peripheral placental region (full depth cores of placental tissue), placed in 10 % neutral buffered formaldehyde as the proper fixative that is most commonly used in immunohistochemistry protocols, kept in the fixative for 3 days, cord and membranes were carefully removed, tissues proceed to paraffin blocks using routine histological techniques, 4 µm thickness sections taken from mid placental thickness to show villi in mid plane of intervillous space and placed on positively charged slides.

Immunohistochemical staining
Include DAKO monoclonal mouse anti human CD34 class II Clone QBEnd-10 Code No. M7165 stain with labeled streptavidin Biotin technique. The staining include: Deparaffinization, rehydration, Heat induced epitope retrieval incubation in peroxide blocking reagent, application of primary antibody, and biotinylated linker antibody, incubation with streptavidin/peroxidase and with 3,3-.di- amino benzidine, counter stain in Mayers haematoxylin, and mounting.

Newborn body weight measurements
This is measured in grams immediately after delivery for the control and test groups’ babies at the premature infant unit at the Al-Imamain Al-Kadhimain Medical City.
Vascular counting
This done in sections that stained positive for CD-34 marker as the latter is expressed in vascular endothelial cells of the fetal capillaries, and blood cells within the vascular lumen. Vascularization was determined by counting of vascular density and surface area of vascular structures that stained positive for CD-34 by measuring their cumulative areas in each field (9).
Five random fields were selected from each placenta to measure the followings:

**Microvascular density:** done by counting the number of fetal blood vessels that stained positive with CD-34 (appear brown in color) in the placental villi, manual counting of vessels at high power fields sections at 400x magnification power, after background subtraction option in the selected fields to make the positively stained vessels stand out. Making marks with image J 1.49 (as dot in the center of the marked vessels) to avoid double counting of the vascular structures (Fig. 1A)

Image J 1.49 is a public domain Java image processing program, it can display, edit, analyze, process, save and print images, can read many image formats. It is multithreaded, so time-consuming operations such as image file reading can be performed in parallel with other operations.

The mean vascular surface area of fetal vessels (in pixels): done on slides stained positive with CD-34, with the use of software program image j 1.49, (Fig. 1B). It can calculate area and pixel value statistics of user-defined selections. Selections are of defined areas within an image. Area selections are created using the freehand selection tools. Area selections can be measured from drop down list (Analyze>Measure).

![Fig. 1B. Image j 1.49 window showed the tool bar, the positively stained vessels in placental villi that appear in brown color were manually selected by free hand selection, area was calculated by adding the cumulative areas of stained vascular structures measured in pixels. Image J 1.49 window for CD34 positive immunohistochemistry, 400x.](image)

Tissue sections measurements were examined using 400 x magnifications, area of interest were recorded Sony cyber shot digital camera.

**Statistical analysis**
The data were assessed by statistical package of social sciences (SPSS 17). The vascular measures would be compared between test group and control group using unpaired t-test. Newborn body weights were estimated for the test and control groups and compared using unpaired t-test. P value ≤ 0.05 considered statistically significant. All data were expressed as (mean ± SE).

**Results**
CD34 stain appear positive in control and test groups, its stain all vascular structures in placental villi even minute blood capillaries
that appear in brown color, including vascular wall and vascular lumen. Placental villi showed extensive perivillous fibrin deposition (Fig. 2A) and intervillous fibrin deposition, with vascular degeneration presenting progressive fibrosis, and avascular terminal villi (Fig. 2B); vascular stenosis also can be seen in terminal vascular structures fig. 3. Thickening in the wall of blood vessels were seen in PE compared to normal term placentae (Fig. 4).

Increase in the mean ± SE of vascular density and reduction in (mean ± SE) of vascular area was significantly seen in PE, compared to normal placentae at term at p ≤ 0.05 (Table 1 and Fig. 5).

Fig. 2. Placenta of pregnancy induced hypertension at term showed extensive perivillous fibrin deposition (A) (black arrow), and intervillous (B) fibrin deposition (black arrows), immunohistochemistry for CD34-that showed brown positive color of fetal capillaries in placental villi, at 400x.

Fig. 3. Terminal villi in Preeclampsia of term placenta showed a reduction the vascular area and absence in fetal vessels that stained in brown color positive for CD-34 immunohistochemistry, at 400x.

Fig. 4. Thickened wall placental vessels in Preeclampsia in positively stained vessels that showed brown color for CD-34 immunohistochemistry at 400x.

The decreased blood vessel lumen found in the mature intermediate villi and terminal villi, this may results in the complete absence of capillaries in the terminal villi in most of the placentae (Fig. 3-5). The new born birth weight ranged from 1800-2700 Gram in PE, compared to 2500-3500 Gram in control group, their mean ± SE was significantly reduced in PE compared to control group at p < 0.05 (Table 2).

This reduction in blood vessel density in placental villi in PE cases compared to control placentae is associated with fibrinization, stenosis and thickening in vessel wall of the villi.
Discussion
CD-34 showed an intense positive reaction in the vessels within the villous axis, tertiary villi, even minute capillaries were markedly demarcated, thus it allow us to precisely appreciate the vascular density and vascular area of these vessels. The CD34 protein is a member of a family of single-pass transmembrane sialomucin proteins that show expression on early hematopoietic and vascular-associated tissues (10).

Table 1. Variation in the vascular density and vascular area (in pixels) in normal term placentae and PE, with their statistical significance

<table>
<thead>
<tr>
<th>Vascular measures</th>
<th>Normal placenta</th>
<th>Pre-eclampsia</th>
</tr>
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<tbody>
<tr>
<td>VD</td>
<td>8±2.13</td>
<td>11.84±2.28*</td>
</tr>
<tr>
<td>VA (pixels)</td>
<td>121409±348.4</td>
<td>80506±237.9**</td>
</tr>
</tbody>
</table>

VD = vascular density, VA = vascular area* p = 0.008, ** p = 0.032

Table 2. Comparison of birth weight (in grams) of newborn between preeclampsia and control group

<table>
<thead>
<tr>
<th>Birth weight (gm)</th>
<th>Normal placenta</th>
<th>Pre-eclampsia</th>
</tr>
</thead>
<tbody>
<tr>
<td>mean ± SE</td>
<td>2924.35±260*</td>
<td>2343.6±237**</td>
</tr>
<tr>
<td>Range</td>
<td>(2500-3500)</td>
<td>(1800-2700)</td>
</tr>
</tbody>
</table>

* p = 0.005, ** p = 0.003

Pregnancies complicated by PE are reflected on the placenta both macroscopically and microscopically, although the placenta adapts well to the hypoxic condition in PE, these compensatory changes that occur are insufficient. Placental examination has clinical value in cases of PE and intrauterine growth retardation, both of which are associated with high perinatal morbidity and mortality accompanied with gross pathological changes in the placenta (11).

In the present study, comparing PE placentae to control placentae, the increase in vascular density; and reduction in vascular area of the PE placentae impedes normal placentation and pathologically results in massive microscopic changes in the placenta. Consequently, the resultant decreased perfusion could cause oxidative stress.

The cytotrophoblast cells invade into the uterine spiral arteries and transform them from small-caliber resistance vessels into high-caliber capacitance vessels capable of providing enhanced placental perfusion adequate for the growing fetus. For this transformation, a certain amount of hypoxia is needed to stimulate placental blood vessel formation. Until approximately 10 weeks of gestation, the embryo exists in a hypoxic environment with nutrients provided by the endometrial glands, however, prolonged durations of hypoxia or oxidative stress leads to poor placental
perfusion, which is the underlying pathogenesis of PE (12).

The reduction in vascular area measure found in placental villi that stained with CD-34 could be part of pathology involving the spiral artery that may extend to villous capillaries, since the invasion of uterine spiral arterioles by trophoblasts is limited to the superficial portions of the decidua, and 30-50% of these arterioles in the placental bed escape trophoblast remodeling (13,14).

It was found that the mean luminal diameter of uterine spiral arterioles in women with PE is less than one-third of the diameter of similar vessels from uncomplicated pregnancies (15). While the increase in vascular density could be due to stimulation of vasculogenesis due to hypoxia, that consequently occur as uteroplacental perfusion reduces, and the placenta becomes ischemic as gestation progresses (16,17).

This fetal hypoxia as lead to morphological and histological changes in the placenta, leading to PE or PE-associated intraterine growth retardation, which contributes to premature delivery and fetal death (18,19).

PE has also been related to an imbalance between pro- and anti-angiogenic factors in maternal circulation (20). Placental vasculature includes specialized blood vessels and supportive structures that permit gas exchange and nutrient transfer from the maternal circulation to the fetus. A functional balance of pro-angiogenic (placental growth factor and vascular endothelial growth factor) and anti-angiogenic factors (soluble forms-like tyrosine kinase-1 and s-endoglin) is important for optimal placental formation (21).

In pregnancy, the placenta is the predominant source of angiogenic factors and the imbalance seen in PE is considered as response to placental hypoxia. Whether the angiogenic imbalance in PE is a maternal response independent of off springsize, remains uncertain (22).

However, it has been speculated that PE is an adverse manifestation of the mother’s attempt to compensate for impaired fetal growth, and PE increases the risk of small gestational age in the offspring (23).

These results suggested that placenta adapt its structure to maintain its function, this adaptation reflected as an increase in vascular density that consequently occur as uteroplacental perfusion reduces due to maternal vasospasm, and the placenta becomes ischemic as gestation progresses due to hypoxia that affect terminal villi vasculature. Fibrin deposition, vascular degeneration, thickened vessels wall, and stenosis those together reduce the vascular area; these changes accordingly were reflected on newborn body weight in preeclampsia.

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Author contributions

Dr. Kamona collected the samples and did cross sectional study of placenta, Dr. Al-Amily writ the research; and Dr. Al-Marsoummi analyzed vascular surface area of fetal vessels that stained with CD-34, by using software program image j and doing statistical analysis by SPSS 17 statistical analysis software.

Conflict of interest

The authors declare no conflict of interest.

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References


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