Stereological analysis of terminal villi of the placentas of
pregnant woman with sideropenic anemia

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Abstract
Iron deficiency, causing maternal sideropenic anemia, is one of the most frequent nutritive disorder that develops during the pregnancy. We collected 30 placentas from anemic mothers and 30 placentas from mothers belonging to the control group. Terminal villi (magnification 10x) and terminal villi capillaries (magnification 40x) were stereologically analyzed and numerically determined. In the placentas from anemic mothers we noted the values a) terminal villi: volume density 0.43 mm3, surface density 24.13 mm-1, total volume 185.57 cm3 and total surface 10.27 m2; b) capillaries of terminal villi: volume density 0.53 mm3 and total volume 224.18 cm3. In the placenta from mothers belonging to the control group we observed the following values a) terminal villi: volume density 0.44 mm3, surface density 23.27 mm-1, total volume 200.17 cm3 and total surface 10.15 m2; b) capillaries of terminal villi: volume density 0.42 mm3 and total volume 197.00 cm3. Compared with the control group anemic mothers’ placentas have a significant higher values of surface density of terminal villi (p<0.05), volume density (p<0.01) and absolute volume (p<0.0001) of terminal villi capillaries, and significant lower values of absolute volume of terminal villi (p<0.05). In anemic mothers’ placentas, the total volume of terminal villi changes disproportionately to the total surface of terminal villi with statistically significant increase of terminal villi capillaries compared with control group.

KEY WORDS: placenta, terminal villi, pregnancy anemia, stereology

INTRODUCTION

The placenta is a highly specialized organ during pregnancy that allows regular fetal growth and development. The labyrinth layer of the placenta is the only site for nutrients, gases and wastes exchange between the maternal and fetal circulations from middle of gestation to term [1]. Placental morphology and cellular architecture have all the necessary potential factors for oxygen delivery from the mother to the fetus [2].

Pregnancy hypoxia develops when expecting mothers are anemic or living at high altitude [3]. Pregnancy anemia usually develops because of the lack of iron and still is a major health problem with serious consequences for mother and developing fetus [4]. For quick and rough estimate of hematological status of pregnant woman, it is sufficient to control hemoglobin (Hb) and hematocrit (Htc) values, adjusted based on increase of blood level during the pregnancy [5].

Morpho-functionally, most of the terminal villi are specialized, with sinusoid-type capillaries in close contact with anuclear regions of syncytiotrophoblast, and significant increasing the proportion of terminal villi endothelial cells compared with the stromal cells [6]. The syncytiotrophoblast is a specialized multinuclear epithelial tissue that functions as a transporting epithelium and endocrine/paracrine organ, delivering nutrients to the fetus and producing hormones that sustain pregnancy [7]. Hypoxia in the placenta is associated with vascular remodeling, metabolic changes, and cell undergoes mitochondrial dysfunction, oxidative and endoplasmic reticular stress [8].

The placenta regulates fetomaternal exchange. During the pregnancy it adapts to support increasing fetus’ needs. The placenta that grows in a hypoxic condition develops morphological changes that influence placenta’s exchange and hemodynamic processes [9]. Pregnancy anemia may result with placental hypertrophy [10] or, if it is combined with malnutrition, can result with restriction of placental growth and development of small, hypotrophic placenta [11].

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The aim of our research was to determine quantitative parameters (volume density, total volume, surface density and total surface of terminal villi, as well as volume density and absolute volume of terminal villi's capillaries) of anemic mother's placentas and compare them to parameters of placentas of pregnant woman with the regular pregnancy and delivery.

MATERIALS AND METHODS

Samples

A total of 60 placentas from term delivery pregnancies were examined. The placentas were divided into two groups: the anemic group, 30 placentas from pregnancies complicated with sideropenic anemia, but without any other risk factor or disease that could affect the course and outcome of pregnancy, and the control group, 30 placentas from pregnancies without signs of anemia and any other risk factor for pregnancy disorder. Research criteria: single baby pregnancies, spontaneous (vaginal) and term delivery (from 37 to 42 gestational weeks), maternal age from 20 to 35 years, hemoglobin lower than 110 g/l in the first and the third trimester and lower than 105 g/l in the second trimester of pregnancy; hematocrit lower than 0.33 in the first and the third trimester and lower than 0.32 in the second trimester of pregnancy.

Procedures

The amnion and umbilical cords were removed from placentas, and then weighed and volume determination based on the amount of displaced liquid. Samples were taken through the thickness of the entire organ, fixed in 10% formalin solution, cut into 8μm thick slices and stained with hematoxylin and eosin. Using multipurpose test system M 42, terminal villi were analyzed on magnification 10x and terminal villi's blood vessels on magnification 40x, in the placentas of anemic pregnant woman (Figure 1, A and B) and placentas from healthy pregnant woman (Figure 1, C and D).

Anemic mother's placentas have 8% lower values of total volume of terminal villi but 8% higher values of terminal villi's surface in 1 cm³ of placental tissue. Anemic mother's placentas have 20% higher values of terminal villi's capillaries volume density and 12% higher values of terminal villi's capillaries total volume compared to the control group.

Stereological analyses of terminal villi showed a) anemic mother's placentas had significantly lower terminal villi's surface density and significantly higher terminal villi's total volume compared to the control group; b) volume density as well as total surface of terminal villi did not differ significantly (Table 1).

Terminal villi's blood capillaries of anemic mother's placentas showed significant increase in 1 cm³ of placental tissue as well as in the total volume of placentas compared to the control group (Table 2).

DISCUSSION

It is a widely held belief that hypoxia is responsible for the change in anemic mothers' placentas and the placentas usually respond to hypoxia by increased capillary branching [13], increased trophoblast proliferation, but decreased fusion into syncytiotrophoblast [14]. Hypoxic injury disrupts the syncytial architecture and results with the increased density of syncytial

RESULTS

This research presents the results of stereological analysis of 60 placentas that have been divided into two groups: 30 placentas of anemic mothers and 30 placentas of the control group. Tissue sections, embedded in paraffinum, were cut on slides of 8μm thickness and stained with hematoxylin and eosin. Using multipurpose test system M 42, terminal villi were analyzed on magnification 10x and terminal villi's blood vessels on magnification 40x, in the placentas of anemic pregnant woman (Figure 1, A and B) and placentas from healthy pregnant woman (Figure 1, C and D).

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Statistical Analysis

Student t-test was used to determine the statistical significance of the results. Hypotheses were tested at level of significance α = 0.05 i.e., differences between samples was considered significant when p < 0.05.

### Table 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Anemia mean (SD)</th>
<th>Control mean (SD)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vtv (mm³)</td>
<td>0.43 (0.08)</td>
<td>0.44 (0.07)</td>
<td>0.05</td>
</tr>
<tr>
<td>Vtv (cm³)</td>
<td>185.57 (44.56)</td>
<td>200.17 (52.1)</td>
<td>0.05</td>
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<tr>
<td>Sdv (mm-1)</td>
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<td>Sdv (m²)</td>
<td>10.27 (2.93)</td>
<td>10.15 (2.72)</td>
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### Table 2

<table>
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<tr>
<th>Parameter</th>
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<th>Control mean (SD)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vtdv (mm³)</td>
<td>0.53 (0.11)</td>
<td>0.42 (0.12)</td>
<td>0.01</td>
</tr>
<tr>
<td>Vtdv (cm³)</td>
<td>224.18 (66.59)</td>
<td>197 (65.84)</td>
<td>0.001</td>
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knots and vasculosyncytial membrane thickness that promotes the release of soluble syncytial factors [2]. Study conducted on animal models, showed that iron-deficiency anemia in pregnant rats increase the expression of hypoxia and inflammatory markers in the placenta [15]. Despite the fetal resistance to maternal deficiency, any stress that alters placental development and function may have consequences for the developing fetus [16]. The placenta does not respond in a single way to hypoxia because of its large reserve capacity, and hypoxic lesions may not always result in poor fetal condition or outcome. On the other hand, very acute, in utero, hypoxic events, followed by prompt delivery, may not be associated with placental pathology, and many poor perinatal outcomes can be explained by an etiology other than hypoxia [17].

Sideropenic anemia is the most common type of nutritive anemia in pregnancy [18]. It develops because of iron deficiency. Other types are rare, and caused by folic acid and vitamin B12 deficiency, and some types of hemoglobinopathies [19]. Iron deficiency anemia accounts for 75% of all anemia in pregnancy [20]. According to WHO recommendations, there are three types of maternal anemia in pregnancy mild: (Hb 109-100 g/l), moderate (Hb 99-70 g/l) and severe (Hb<70 g/l) [21].

Pregnancy anemia is one of the risk factors for abnormal placental growth, that lead to development of either hypertrophic or hypotrophic placenta [22]. Stereology applied to placental section allows minimal-biased and economical quantification of the three-dimensional structure of the placenta from molecular to whole-organ level [23]. According to data, volume density of terminal villi counts from 0.41 mm³ at older pregnant women [24] to 0.62 mm³ in the regular pregnancy [25]. Placentas from mother with pre-eclampsia showed significant increase of terminal villi density but significant decrease of villi’s blood vessels [26,27]. In our research, volume density of terminal villi of anemic mothers’ placentas count 0.43 mm³, without significant difference, and our results are similar to values of older pregnant woman [24].

Among the results of different researchers, Reshetnikova et al. [11] indicate that total volume of chorionic villi (terminal and others) of the placentas of the mother with sideropenic anemia counts 157.80 cm³ and is significantly lower compared to the control group counting 234.5 cm³. Other researchers indicate that the total volume of placental villi seems to increase significantly compared to the control group [10]. The placenta is potentially subjected to stressful conditions during delivery, and sample collection may be delayed by routine postpartum inspection by clinical staff [28]. The results of morphometrical analysis of placental tissues are partly contradictory because numerous factors can influence the results of the stereological analysis such as number of analysed placentas and possible relation of maternal anemia and malnutrition [22] or pregnancy at high altitude [14]. Placental hypertrophy is related to mild or moderate anemia thus the placental enlargement seems to be a uniform physiological compensatory mechanism [29]. Our research indicate that absolute volumes of terminal villi of the anemic mothers’ placentas count 185.57 cm³, and thus it is significantly lower compared to control group where it count 200.17 cm³. A long-lasting hypoxia,
related to duration of anemia (during each trimester of pregnancy), seems to disrupt the compensatory mechanisms of placenta and cause the shrinkage of terminal villi. A hypoxic environment during long-lasting hypoxia in sideropenic anemia can cause shape deformation of terminal villi, capillaries branch extensively and form fist-like terminal villi with short multiple invaginations that enlarge terminal villi’s surface [29]. Among rare data considering terminal villi’s surface density in preplacental hypoxic environment we contrive that Huang et al. [10] claims that the terminal villi’s surface density does not significantly differ between placentas of anemic pregnant women compared with the control group.

In our research, stereological analysis showed that surface density of terminal villi in anemic mothers’ placenta is 24.13 mm$^2$ and in control group 22.27 mm$^2$, with statistically significant difference. Such results may be explained by a characteristic shape of terminal villi. Because of increased number of capillaries, villi become deformed, with multiple invaginations [29], and thus increased share of terminal villi’s surface in organ volume.

Specific pregnancy condition, such as pregnancy diabetes or multiple pregnancies increase the placental mass [3]. The placental surface increase in hypoxic condition in anemic mother and pregnancies at high altitude. Described placental enlargement may result in increase syncytial knots’ ripping off, that undisolved in maternal blood may undergo necrosis and lead to preeclamptic symptoms [3]. Severe maternal anemia, with hemoglobin lower than 70g/l results in decrease of placental villi’s surface, and increase of blood vessels’ surface compared to the control group 30. Total surface of all chorionic villi in anemic mothers’ placenta is 9.50 m$^2$ [11], and in healthy pregnant woman it counts from 11.04 m$^2$ [24] to 14.90 m$^2$ [11]. Terminal villi’s surface at term pregnancy counts 10.34 m$^2$ [24].

During our research, terminal villi were analyzed as a separate variable. The results showed that in the placentas of anemic mothers terminal villi’s total surface is 10.27 m$^2$ and in control group 10.15 m$^2$, with no significant difference among researched groups. Our results are partly diverse with the results of other researchers, especially considering that we did not represent the values of other villi’s total surface as cited authors [10,11,30]. On the other hand, pregnant women were not severely anemic with extremely low hemoglobin values, lower than 70g/L, that may be an explanation why the significant shrinkage of terminal villi total volume did not occur.

We concluded that the anemic mothers’ placentas develop changes of terminal villi’s total volume, but not the surface, and our data are consistent with the belief that the anemic mothers’ placentas show anisomorphic changes i.e., the surface of villi change disproportionately with the volume of the villi and vice versa [9].

Transplacental oxygen transport is one of the numerous placental villi’s functions, but is also a cause that influences growth and differentiation of villi more than any other factor [7]. The placenta from maternal anemia show signs of chronic preplacental hypoxia that causes prevalence of branching angiogenesis with intense trophoblast knotting as a result of increased tangential sectioning across irregular villous surface [29]. According to our research data, there is statistically significant increase of volume density of terminal villi’s capillaries in placentas of mothers with sideropenic anemia where it counts 0.53 mm$^2$ and compared to the control group that counts 0.42 mm$^2$. The total volume of terminal villi’s capillaries in our research is 22.4.18 cm$^3$ and is significantly higher compared to the control group (197.00 cm$^3$). Placenta modulates to hypoxia by increased branching and number of capillaries and thinning the placenta’s barrier [31] and during sideropenic anemia, the terminal villi capillaries undergoes adaptation that results in volume increase. Placental villi exposed to low oxygen partial pressure may show an increase of fetal capillaries, even there are no exact evidence of capillary proliferation and changes are result of capillary dilatation [32].

Hypoxia during pregnancy anemia impacts structure and developing of placental blood vessels, particularly the capillaries of terminal villi, the most important structure required for fetomaternal exchange. Therefore, terminal villi have been analyzed as an independent variable, to give the knowledge of placentas’ compensatory pathways.

**CONCLUSION**

Our research indicates that mild and moderate maternal sideropenic anemia lead to significant morphological and structural changes in terminal villi. Improved changes have been signed as anisomorphic, we noted the significant reduction of the total volume of terminal villi but the total surface did not differ significantly. The capillaries of terminal villi showed the significant increase both volume density and total volume in the placentas of mothers with sideropenic anemia compared with the control group.

**DECLARATION OF INTEREST**

The authors declare no conflict of interest.

**REFERENCES**


[2] Sankar KD, Bhanu PS, Kiran S, Ramakrishna BA, Shanthi V. Vasculosyncytial membrane in relation to syncytial knots
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