Stereological Analysis of Human Placenta in Cases of Placenta Previa in Comparison with Normally Implanted Controls

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Abstract

Background: placenta previa (PP) is an obstetric complication that can affect maternal and fetal morbidity and mortality. Its prevalence is rising due to cesarean sections. There is no quantitative data of placenta in PP. In this study, quantitative parameters of placenta in cases with PP in comparison with normally implanted controls were investigated.

Methods: In this quasi experimental study, placentas from pregnancies with PP and normally implanted controls (n=10) were obtained from women who underwent cesarean section. Three full-thickness columns of each placenta were sampled using systematic uniform random sampling (SURS). Columns were cut into slices and slices were sectioned with 4 μm thickness. SURS selected sections were stained by Masson's trichrome. Stereological analysis was done on 8-10 SURS microscopic fields of each section. Absolute volume and volume density of chorionic villi, intervillous space, syncytiotrophoblast, fibrin and blood vessels in chorionic villi were estimated in both groups. Statistical analysis was done using Mann Whitney-U test and significant level was set at p<0.05.

Results: There was a significant reduction in total volume and volume density of fibrin deposits on the surface of chorionic villi (p<0.05), and a significant increment in total volume and volume density of chorionic villous blood vessels in PP group in comparison with C group (p<0.05).

Conclusion: Results showed that impairment in situation of implantation in PP can cause significant changes in the structure of placenta. These changes probably can be influential on the evolution and survival of fetus.

Keywords: Histology, Placenta previa, Placenta, Stereology.
To cite this article: Heidari Z, Sakhavar N, Mahmoudzadeh-Sagheb H, Ezazi-Bojnourdi T. Stereological Analysis of Human Placenta in Cases of Placenta Previa in Comparison with Normally Implanted Controls. J Reprod Infertil. 2015;16(2):90-95.

Introduction

The human placenta is a wonderful interface between the developing embryo and mother. It is essential for fetal nourishment, growth and development. Placental chorionic villi constitute the major embryonic component of the placenta. It seems that any changes in the chorionic villi and its vessels might impose serious side effects on the developing fetus (1, 2).

Placenta previa (PP) is described as a placenta that is implanted over or very near the internal cervical os. It is an important cause of maternal mortality and morbidity and results in an approximately threefold increased maternal mortality ratio. Preterm delivery as a result of PP is a major cause of perinatal death. According to birth certificate data of the United States in 2003, PP complicated almost 1 in 300 deliveries. Advanced maternal age (over 35 years), prior cesarean delivery, multiparity, smoking, infertility treatment and male fetus are some risk factors of PP (3, 4). It has been shown that neonatal mortality rate in pregnancies complicated by PP increased threefold

and fetal anomalies increased 2.5-fold in comparison with normally positioned placentas (5). Practically, cesarean section is necessary in all women with PP. Because of the poorly contractile nature of the lower uterine segment, there may be uncontrollable postpartum bleeding (6). Placental bed biopsies at cesarean delivery in women with PP showed that there were increments of myometrial spiral arterioles with trophoblastic giant-cell infiltration (7).

In recent years, microscopic measurements have been increasingly used both in biological researches and treatments. During the last two decades, stereology has been considerably improved, so that it is the first choice for obtaining three-dimensional data from two-dimensional profiles. The modern stereology by efficient and design-based methods permits the quantitative description of morphology. Applications to placentas in normal and abnormal pregnancies have proved of great value for challenging earlier misconceptions and interpretation of the growth, morphogenesis, adaptation and functioning at the whole-organ level (8).

In histological methods, we are always faced with two-dimensional sections or their microscopic images. Stereology, based on mathematics and statistics, makes it possible to estimate three-dimensional data from two-dimensional profiles of an object (9). In histology, it helps in measurement of parameters including number, size, surface area and volume by two-dimensional tissue sections (10). As an advantage, it provides the opportunity of generalizing data to entire structure, by proper and systematic sampling of the sections. There is no precise data concerning structural changes of placenta in PP. In the present study, the stereological changes of placenta in PP patients in comparison with healthy controls were investigated.

Methods

In this quasi experimental study, placentas from full-term mothers with PP (PP) were obtained immediately after cesarean section in Imam Ali Hospital, Zahedan, Iran. The control group included normal placentas from healthy full-term mothers with no pregnancy complications who had been age and gestational age matched with PP group (n=10). All placentas were delivered by caesarean sectioning. Mothers with fetal malformations and chronic diseases, smokers and ad-dicted mothers were excluded from the study. Maternal parameters including age, weight, height, gestational age, number of pregnancies, number of abortions, number of deliveries, socio-economic status and the history of obstetric and gynecologic diseases, and fetal parameters including sex, birth height and weight and also placental parameters including size and weight were recorded in the information form. The study was approved by the ethics committee of Zahedan University of Medical Sciences (No: 91-911) and written informed consent was obtained from all participants.

The placentas were removed by a standard method. Immediately after the childbirth, the umbilical cord was clamped to ensure placental blood drainage. The placental weight was measured using a digital scale of 0.01 gram precision, the placental thickness (t) was measured using a digital caliper with 0.01 *mm* precision in three points and then the mean was applied for estimation of the total placental volume. The placental surface area was determined using Cavalier`s point-counting method by this formula: A=ΣP. A (p), where A is estimation of the surface area, ΣP is the sum of the number of points landing on the surface of placenta, A (p) is the area associated with each point in stereological grid. Then, total volume of each placenta was estimated using this formula, V=A.t (11), where V is estimation of the volume, A is surface of placenta and t is the mean thickness of placenta.

Then using the systematic uniform random sampling (SURS), three full-thickness columns of tissues from each placenta were selected and fixed in modified Lillie`s solution. Then the columns were cut to 5 *mm* slices and 5-7 SURS selected slices from each column were processed by routine histological method and embedded in paraffin wax. The tissue slices were serially sectioned to 4 *μm* thickness. Next, 5-7 SURS selected sections of each slice were stained with Masson's trichrome technique. In each section, 8-10 SURS selected microscopic fields were analyzed. The images of the selected fields were transferred to a computer using a digital photomicroscope. Next, the stereological grid containing organized points were superimposed on the tissue images and the volume density of placental villi, intervillous space, villous surface fibrin, villous core vessels, and syncytiotrophoblast were estimated as previously described using this formula:

Vv=P (part)/P (ref)

Where Vv is volume density, P (part) and P (ref) are the number of test points falling in each desired structure profiles and in the reference space, respectively. In order to estimate the absolute volume of a part, the volume density of that part is multiplied by the total placental volume (11, 12).

All stereological analysis was done by two expert histologists on blind coded sections. Data analysis was performed using SPSS-18 software and comparison between the groups was done using nonparametric statistical test of Mann-Whitney U test and p<0.05 was taken as the significant level.

Results

The difference in mean age, gestational age, gravid and parity of patients in PP and control groups was not statistically significant (Table 1). Male/female ratio of newborns was similar in both groups (7/3). The average placental weight in PP group had 18% increment compared to C group, but this difference was not statistically significant. The umbilical cord diameter in PP group was reduced and the reduction was statistically significant compared to C group (p<0.05). The absolute and relative volume of chorionic villi in PP group decreased compared to the C group but it was not

Table 1. Demographic and obstetric characteristics of placenta previa and control group

Parameters	Placenta previa (n=10)	Control (n=10)
Age (year)	29.0 (6.5)	24.0 (2.3)
Gravida	4.5 (3)	2.0 (1.2)
Parity	2.5 (2.5)	1.0 (1.2)
Gestational age (week)	36.5 (2.3)	38.3 (1)

Data presented as Median (IQR)

statistically significant. The absolute volume of fibrin in PP group had a 47.6% reduction compared with the C group and it was statistically significant (p=0.01). The relative volume of fibrin in PP group had a 44% reduction compared with the C group and this reduction was statistically significant (p<0.05). The absolute volume of blood vessels in PP group increased almost 54% compared with the C group and this increase was statistically significant (p<0.05). The relative volume of blood vessels in PP group had almost 64% increment compared with C group and this difference was statistically significant (p<0.05). Also the absolute and relative volume of the syncytiotrophoblast in PP group increased compared with C group, but the increase was not statistically significant (Table 2).

Table 2. Comparison of stereological parameters of placenta in patients with placenta previa compared with the control group

Stereological parameters	Placenta previa (n=10)	Control (n=10)	p-value
Placental weight (*g*)	545.0 (120)	405.0 (162.5)	0.059
Placental volume (*cm³*)	431.0 (117.6)	414.0 (203.7)	0.971
Cord diameter (*mm*)	9.2 (2.4)	13.0 (4.8)	0.042
Intervillous space			
Total volume (*mm³*)	171.0 (57.4)	157.0 (28)	0.130
Volume density (*%*)	40.0(2.3)	34.0 (12)	0.211
Chorionic Villi			
Total volume (*mm³*)	249.0 (95.8)	275.0 (178)	0.55
Volume density (*%*)	60.1 (2.4)	66.0 (11)	0.134
Fibrin			
Total volume (*mm³*)	8.6 (6.2)	16.0(7.5)	0.01
Volume density (*%*)	1.8 (1.4)	4.0 (1.1)	0.006
Blood vessels			
Total volume (*mm³*)	147.4 (62.2)	81.0 (86.8)	0.032
Volume density (*%*)	36.1 (3.6)	19.0 (9.5)	0.002
Syncytiotrophoblast			
Total volume (*mm³*)	69.8 (35.8)	53.0 (38)	0.523
Volume density (*%*)	16.1 (2.5)	13.0 (2)	0.149

Data presented as Median (IQR)

Discussion

In the present study, the volume of blood vessels of placental villi significantly increased and the volume of villous surface fibrin decreased in PP group compared to the C group. Although no quantitative and stereological studies have been carried out yet on microscopic parameters of the placenta in PP patients, this study provided some quantitative information for cases of PP. Some stereological studies have been accomplished on other diseases affecting placenta, such as pre-eclampsia (13), anemia, hypoxia, diabetes and gestational diabetes (14).

In the present study, no statistically significant difference was shown in the placental villi volume and intervillous spaces between PP and C groups. As mentioned above, there is no precise data concerning structural changes of placental villi in PP. Mayhew et al. studied the placentas of preeclampsia patients (PE) by stereological method and found no significant difference between PE and control groups in terms of placental villi volumes (13). But the studies of Ducray et al. using an image analysis method on PE patients' placentas indicated that there was an increase of placental villi volume and also significant reduction of intervillous space volume (15).

In the present study, the weights of placenta increased in PP group compared with the C group, but this difference was not statistically significant. Papinniemi showed higher placental-to-birth weight ratios in comparison to the controls in PP patients and achieved the same results (16).

The volume of syncytiotrophoblast increased in PP group compared with the C group, but this difference was not statistically significant. Biswas performed placental bed biopsies at cesarean delivery in women with PP and controls, and reported that the syncytiotrophoblast increased significantly in PP group compared to the control group (7). Increment of syncytiotrophoblast may affect fetal growth and development because of more thickness of blood-placental barrier.

In this study, the volume of blood vessels increased in PP group compared with the control group. In Biwas's study, myometrial spiral arterioles increased in previa group (7). Maly studied the placentas of preeclampsia patients (PE) by the stereological method and found that in PE group the volume of blood vessels was less than the control group. As we know, PP is a protective factor for PE and reduces the rate of PE (17), thus the opposite results in PP could be justifiable. It has

been shown that the age and parity of the mother can affect the number and vascularity of placental villi. Apart from blood flow, the activity of various growth-promoting hormones, such as insulin-like growth factor or placental growth factor, plays a role in supporting normal placental development (18). As means of age and parity of our PP patients were similar to the control group, the growth factor probably can cause vascular volume differences. This could be an important subject for future research in this field.

In PP, the placental bed is situated in the lower segment of the uterus. Severe post-partum hemorrhage during cesarean section due to PP is still one of the leading causes of maternal mortality (19). In lower segment implantation, the muscles surrounding the placental bed are inadequate for contraction, retraction and thus bleeding ensues (20). More blood vessels in PP employ more blood vessels in placental bed and it seems that angiogenic factors that affect placenta probably could promote angiogenesis in placental bed; this could explain sever postpartum bleeding in PP cases.

In the present study, the volume of villous surface fibrin decreased in PP group compared to the C group. Minor perivillous fibrin deposition is almost always present in term placentas (21). Villous surface fibrin deposit is derived from the maternal blood in the intervillous space and an immunological basis for the activation of this process has been proposed. The turbulence of maternal blood within the intervillous space results in maternal platelet adherence to the villous syncytiotrophoblast with subsequent thrombosis. The areas of syncytiotrophoblast covered with fibrin deposits are cut off from their oxygen supply and thus undergo ischemic necrosis (22).

Reduction of fibrin deposit in PP cases may be due to vascular and antithrombotic changes. Further research is necessary in this issue. It has also been reported that Nitabuch's fibrinoid layer is thinned or missed in PP (23). Normally in a pregnancy, the placenta attaches to the uterine wall and is separated from the uterus by Nitabuch's fibrinoid layer. Under normal circumstances, as the placenta develops, it grows only within the endometrium and is clearly separated from the myometrium and the decidua basalis by Nitabuch's layer. This separation is important, because under normal circumstances, after the childbirth, the placenta is cleanly separated from the uterus at this level and the maternal endometrial blood ves-

sels invaded by the trophoblasts rapidly contract, controlling maternal placental bed hemorrhage (24). This process fails in PP and commonly leads to severe post-partum hemorrhage in these cases. It has been shown that abnormal decidua formation at the time of placental implantation, specifically imperfect development of the fibrinoid (Nitabuch's) layer is more common when there is an abnormal site of placental implantation like previa (25). Kanfer studied the placentas of PE using stereological method and showed that the volume of villous surface fibrin increased in PE group compared to the controls. They stated that antifibrinolytic potential increased in pregnancy-induced hypertension and preeclampsia. This change and the association of the highest PAI-2 placental concentrations with the lowest concentrations of thrombomodulin, may contribute to prethrombotic state and to the excessive placental perivillous fibrin deposition observed in these situations (25-27). The studies of Ducray et al. using an image analysis method on PE patients' placentas also indicated that there was an increase of placental volume of villous surface fibrin (15, 27). According to the protective effect of PP on PE, the opposite results are justifiable.

Conclusion

According to the results of this study, it can be concluded that PP imposes some changes in placental structures including increment of blood vessels of chorionic villi and a decrease in villous surface fibrin volume, all of which might eventually lead to placental malfunction. Thus, it can be concluded that impairment in situation of implantation in PP can cause significant changes in the structure of placenta that may be influential on the evolution and survival of fetus.

Acknowledgement

Conflict of Interest

The authors declare no conflict of interest.

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