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Compromised chorionic villous vascularization in idiopathic second trimester fetal loss

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ABSTRACT

Background: For normal fetal growth and development a well-developed chorionic villous vascularization is essential.
Aim: The aim of this study is to investigate whether idiopathic second trimester fetal loss is associated with an underdeveloped chorionic villous vascularization.
Methods: 38 placentas after late miscarriage, classified as idiopathic fetal loss (IFL, n = 16) or as fetal loss due to intrauterine infection (IUI, n = 22) were collected. After CD34 immunohistochemical staining the villous stromal area, number of villous vessels, vascular area and vascular area density (central, peripheral and total) were measured in randomly selected immature intermediate villi.
Results: The mean gestational age was 19 + 4 weeks for the IFL group and 20 + 6 weeks for the IUI group. After controlling for gestational age, we found no differences in fetal weight, placental weight, villous stromal area, number of vessels and central vascular features. The mean peripheral vascular area and peripheral vascular area density were, after adjusting for gestational age, reduced in the IFL group.
Conclusion: Idiopathic second trimester fetal loss is associated with a reduced peripheral chorionic villous vascularization. We hypothesize that in these cases, placentation is already disturbed in first trimester of pregnancy, leading to a reduced materno–fetal interface in second trimester, thus to early postplacental fetal hypoxia and fetal death.

1. Introduction

The incidence of a late miscarriage, defined as a spontaneous pregnancy loss between 12 and 24 weeks gestation, is approximately 1–2% [1,2]. The following causes for late miscarriage are described: intrauterine infection, uterine abnormalities, cervical incompetence, antiphospholipid syndrome and congenital malformations [2–6]. These mechanisms only explain a proportion of the late miscarriages. It is suggested that, in idiopathic late miscarriage, an inadequate placental development could play a role [7,8].

In the first trimester of pregnancy, chorionic villous vascularization is formed by means of vasculogenesis and angiogenesis resulting in an intensive network of peripherally located mesenchymal capillaries at the end of the first trimester [9–12]. With increasing gestational age the materno–fetal diffusion distance will decrease due to margination [9,13]. This is a process in which the intravillous capillary position becomes closer to the villous surface due to decrease of villous stromal area and increase of capillary diameter. This results in a thin vasculosyncitial membrane, important for an optimal materno–fetal exchange of oxygen and nutrition [9,14].

As compared with controls, in second trimester terminated aneuploid pregnancies a reduced villous vascularization with fewer peripheral capillaries has been observed [15,16]. This could lead to an underdeveloped vasculosyncitial membrane, and thus to a reduced materno–fetal exchange. Based on these observations we hypothesized that the reduced villous vascularization could be the underlying cause of intrauterine fetal growth restriction and intrauterine fetal death in aneuploid, but also in euploid pregnancies. The aim of our study is to investigate whether second trimester idiopathic fetal loss is associated with reduced chorionic villous vascularization.

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2. Methods

2.1. Patient selection

We performed a retrospective study at the Liverpool Women's Hospital National Health Service Trust. The study was approved by the Local Research Ethics Committee. Placental tissue of spontaneous or, in case of intrauterine fetal death, induced miscarriages between 16 and 24 weeks gestation from January 2002 to December 2005 were retrieved and examined. We excluded twin pregnancies, late fetal loss due to congenital malformations, pregnancies with an intrauterine device in situ, women with antiphospholipid syndrome or uterine anomalies, pregnancies with an unknown gestational age and fetal loss with a retention time (time between the intrauterine fetal death and the delivery) of 2 or more weeks. The latter was based on the fact that chorionic villous vascularization is not influenced by prolonged retention time [17].

The cases were divided into two groups: (I) cases with idiopathic fetal loss not caused by an intrauterine infection (IFL, n = 16) and (II) cases with a live fetus at onset of labour with histopathological findings of intrauterine infection (IUI, n = 22).

The diagnosis of intrauterine infection was based on histopathological findings of a perinatal pathologist (McP). A staging and grading system for maternal and fetal inflammatory response was used; stage S0 no inflammation, stage S1 early inflammation (acute subchorionitis or early acute chorionitis), stage S2 intermediate inflammation (acute chorioamnionitis), stage S3 advanced inflammation (necrotizing chorioamnionitis), grade G1 mild and grade G2 severe [18]. Intrauterine infection was defined as at least S1G2 maternal inflammatory response or with fetal inflammatory response (S1G1) involvement. Cases of fetal death prior to labor and stillbirths, not due to intrauterine infection (S0G0 and S1G1), in whom the viability of the fetus at the time of miscarriage was not known, were assigned to the IFL group. The following patient characteristics were noted: gestational age, gravidity, parity, fetal and placental weight. Gestational age was determined by first trimester crown-rump length (CRL) measurement in all cases.

2.2. Immunohistochemistry

In all cases paraffin blocks were retrieved and stained with hematoxylin and anti-CD34 antibody. Full depth cores of placental tissue sections of 5 μm thick were cut and mounted on polysine coated glass slides (VWR international, Leuven, Belgium). The paraffin embedded sections were de-waxed and re-hydrated through xylene and graded alcohols. Antigen retrieval was performed in 0.01 M citrate buffer (pH6.0) for 1 min using the pressure cooker method. Monoclonal mouse anti-CD34 antibody (DakoCytomatron clone QBEnd-10, diluted 1/100 in tris buffered saline and 0.5% bovine serum albumin) was applied for 30 min at room temperature. Blocking of endogenous peroxidase activity and detection of the primary antibody was performed according to the protocol described in the mouse Envision + HRP kit (Dako UK Ltd, Cambridgeshire, UK). Samples were counterstained for 30 s in filtered Harris Haematoxylin and permanently mounted in DPX solution (Themo Electron Corporation, Cheshire, UK).

2.3. Analysis of extent of vascularization

The slides were examined at 400× magnification by one trained observer (RO), blinded to group and duration of pregnancy. From each placenta, fifteen mesenchymal or immature intermediate villi were randomly chosen. The diameter had to be greater than 80 μm to rule out terminal villi, and stromal connective tissue fibres and perivascular smooth muscle had to be absent to rule out stem villi [19]. In a pilot study, performed by two trained observers, an interobserver error of less than 10% and an intraobserver error of less than 10% were found when 15 villi per observer were randomly selected; this was consistent with Lisman et al. [16].

The vessels in the 15 randomly selected villi were counted. The process of margination was illustrated by describing whether these vessels were located peripherally or centrally. A peripherally located vessel was defined as a vessel situated in direct contact with the syncitiotrophoblastic layer of the villus, as of such contributing to a vasculosyncytial membrane. A centrally located vessel was defined as a vessel without connection to the syncitiotrophoblastic layer [13].

The microscopic field was transported onto a screen using a colour camera mounted on a standard light microscope. Contours of the stroma of the villi and all included vessels were traced manually on the computer monitor with a mouse-controlled cursor using an on-screen magnification of 400× (Fig. 1A–C). Morphometrical measurements were performed using Eclipse Net (Version 1.20.0, laboratory-imaging ltd., Czech).

The following features were measured or calculated: the villous stromal area without the trophoblastic layer, the number of vessels per villus, vascular area (area of all vessels per villus) and vascular area density (percentage of villous stromal area occupied by vascular area). The vascular features were subdivided in centrally and peripherally located vessels.

2.4. Statistics

Patient characteristics were presented as means with standard error of the mean. Skewness was tested using Shapiro Wilk test. Variables with skewed distributions (total, central and peripheral vascular area) were logarithmically transformed and reported as back-transformed geometric means. Comparison between groups was made using t-test or Mann–Whitney U test when appropriate. Correlation was tested using Pearson correlation test. Differences in morphometrical measurements were analyzed using a general linear model adjusting for possible confounders. A probability value <0.05 was considered statistically significant in this pilot study. The statistical analysis was performed using SPSS for Windows, version 15.0.0 (SPSS Inc., Chicago, Il).

3. Results

In total, 38 placental tissues, divided in two groups, were examined. The IFL group (n = 16) consisted of nine women in whom, after confirmation of fetal death, labor was induced within a few days and of seven women with a spontaneous miscarriage in whom the viability of the fetus at the time of miscarriage was not known. In nine cases no maternal inflammatory response (S0G0) and in seven a mild inflammatory response (S1G1) without fetal inflammatory involvement was observed. These subgroups showed similar patient characteristics and morphometrical measurements.

The IUI group consisted of 22 women in whom the placental examination revealed a severe maternal and/or a fetal inflammatory response. In 15 cases there was advanced maternal inflammation (S3G2) and in 17 cases a fetal inflammatory response was observed.

Patient characteristics are summarized in Table 1. No significant differences in age, gravidity, parity and gestational age between the two groups were found. The results are presented in Table 2. After adjusting for gestational age, there were no differences between the two groups for both fetal weight and placental weight. The stromal surface area of the examined immature intermediate villi, the number of vessels, the vascular area and the vascular area density were, after adjusting for gestational age, similar between the two groups.

After subdivision of the vascular features in centrally and peripherally located vessels we did not observe a difference in the number of vessels, the central vascular area and the central vascular area density between the two groups (Table 3). The peripheral