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Original Article

Placental apoptosis in recurrent miscarriage

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Abstract Apoptosis is an interactive and dynamic biological process involved in all phases of embryogenesis. We aimed to study the effect of placental apoptosis on recurrent miscarriage (RM). Placental tissue samples were collected from 40 women with RM (study group) and 30 women with sporadic spontaneous abortion (control group). Samples were prepared and stained immunohistochemically with markers for both the apoptotic protein (p53) and anti-apoptotic Bcl-2 antibodies. Our results showed that expression of the apoptotic (p53) protein was significantly increased in the placental tissues of the RM group ($p = 0.003$). By contrast, the expression of anti-apoptotic (Bcl-2) antibodies was significantly increased in the placental tissues of the control group ($p = 0.025$). We concluded that placental apoptosis plays a crucial role in pregnancy continuation. However, increased p53 expression in placental tissue in early pregnancy could negatively affect pregnancy continuation.

Introduction

Recurrent miscarriage (RM) is a common pregnancy complication. It is defined as three or more consecutive pregnancy losses that occur before the fetus reaches viability, i.e., prior to 24 gestational weeks [1]. The etiology of RM is variable, where unbalanced placental apoptosis plays an important role [2]. Normal placental development undergoes several sequences of cell division and differentiation, followed by invasion of the embryonic trophoblast cells into the decidua and remodeling of the vasculature to increase blood flow into both the placenta and the fetus. The placenta then goes through a series of tissue remodeling with apoptotic changes, which result in regular loss of trophoblast cells [3]. Apoptotic changes have been detected in the maternal–fetal interfaces of the placenta during normal as well as complicated pregnancies [4]. Additionally, apoptosis promotes maternal immune tolerance to the paternal antigens expressed by the trophoblast cells [5,6].

Apoptosis is an interactive and dynamic biological process involved in all phases of embryogenesis. It comprises elimination of undesirable cells to maintain normal tissue

Conflicts of interest: The author declares no conflicts of interests.

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function. Apoptosis could be activated through either intrinsic (mitochondrial mediated) or extrinsic (death receptor mediated) apoptotic pathways [7]. In intrinsic pathways, mitochondrial response to cellular stress, such as DNA damage, usually initiates apoptotic signals. P53, a tumor suppressor protein, could activate the mitochondrial pathway, which in turn could activate pro-apoptotic Bcl-2 family members. In addition, certain death receptors and the mitochondrial pathway may act to amplify signals triggered by the death receptor pathway, suggesting that crossover can occur between the two pathways [8,9].

In our study, we investigated the effect of placental expression of apoptotic protein as well as anti-apCoptotic antibodies on pregnancy continuation.

Patients and methods

Patients

After obtaining approval from the Hospital Ethics Committee, this prospective case—control study was carried out between April 2016 and October 2016 in the Obstetrics and Gynecology Department of Al-Azhar University Hospitals, Cairo, Egypt. It included 40 women with RM as a study group and 30 women with sporadic spontaneous miscarriage (spontaneous abortion of undetected cause, after having at least one normal pregnancy) as a control group. Members of the control group were matched for age with those with RM. Written informed consent was obtained from all women who were enrolled in the study.

Placental samples were taken from all cases by dilatation and evacuation without any prior pharmaceutical induction and within the first 24 h post-diagnosis. The following data were collected from all women enrolled in the study: age, parity, body mass index (BMI), and number of previous abortions and maternal diseases. Next, a thorough clinical examination was performed, which aimed to exclude common disorders already known as aggravating factors for an increased risk of abortion. Following the evacuation, specimens from both groups were fixed in 10% neutral-buffered formalin, routinely processed, embedded in paraffin wax, and sectioned and mounted onto APES coated slides.

Immunohistochemical staining

An immunohistochemical assay for anti-apoptotic antibody Bcl-2 and apoptotic protein p53 expression were performed on formalin fixed, paraffin-embedded tissue sections using the peroxidase labeled avidin—biotin method. Commercially available antibodies for both the Bcl-2 antibody [100: SC-509] and the p53 antibody [(BP 53.12): sc-81168] for apoptotic cells (Santa Cruz Biotechnology Inc, Santa Cruz, CA, USA) were used to recognize these antibodies, according to the manufacturer's instructions.

Sequential slides with 4 μm-thick tissue sections were first dewaxed followed by hydration in a sequential treatment of xylene, ethanol, and water. Then, the slides were incubated in heated citrate buffer (0.01 M citric acid, pH 6.0) to retrieve the antigens. Endogenous peroxidase activity and non-specific bindings were blocked with 3% H_2O_2. The slides were then incubated with primary antibodies (dilution 1:200) overnight at 4 °C, followed by the addition of a biotinylated secondary antibody and streptavidin—horseradish peroxidase. The peroxidase reaction was developed with 3,3′-diaminobenzidine (DAB; Sigma Chemical Co.), which resulted in a brown-colored product. Finally, the slides were counterstained with hematoxylin, dehydrated in alcohol, and mounted.

Data registration and statistical analysis

Both the number and the optical density of the immunopositive Bcl-2 and p53 cells were evaluated. Digital images of 10 randomly selected, high-power fields [hpf] (×400) were both captured and analyzed using a Carl Zeis microscope and Zen Image software (2012, blue edition). Computerized image analysis (Optical Density) was used to accurately measure the strength of the immunohistochemical reactions instead of visual analysis, with the reactions designated as either (+, or weak), (++ or moderate), or (+++ or strong reaction). The data were presented as a mean ± standard deviation (SD). Continuous variables were compared using the paired and independent student t-tests. Values of $p < 0.05$ were considered statistically significant.

Result

Maternal demographic data

Seventy women were enrolled in this study: 40 women with RM (study group) and 30 women with sporadic spontaneous miscarriages (control group). Both groups showed no significant differences with respect to age ($p = 0.7$), parity ($p = 0.9$), BMI ($p = 0.4$), and gestational age ($p = 0.4$) at the time of miscarriage; however, the number of abortions was significantly higher among the study group than among the control group ($p = 0.001$) (Table 1).

Apoptotic p53 protein and anti-apoptotic Bcl-2 expression

Apoptotic changes in placental tissues were examined by detecting the expression of the p53 protein in comparison to expression of the anti-apoptotic (Bcl-2) antibody. In the placental tissues of the RM group (Fig. 1A), the mean number of apoptotic/p53 immunopositive cells was significantly higher than that of the control group (Fig. 1B) (mean = 39 ± 8.831761 vs. 18 ± 6.839428) respectively ($p = 0.003$) (Table 2). By

Table 1 Maternal demographic data; where BMI = Body Mass Index.

	Study group (n = 40)	Control group (n = 30)	P value
Maternal age (years ± SD)	22.5 ± 3.2	23.7 ± 2.1	0.7
Parity	2.7 ± 1.4	2.6 ± 2.4	0.9
BMI (kg/m^2)	24.1 ± 3.3	23.8 ± 2.6	0.4
Gestational age at time of abortion	12.6 ± 2.1	11.3 ± 3.3	0.4
Number of abortions	**5.1 ± 0.7**	**0.00**	**0.001**

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