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Exploring the Impact of BCL-2 and P53 on Apoptosis in Spontaneous Abortion

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Abstract

Apoptosis, a form of programmed cell death, is regulated by both pro-apoptotic factors (such as p53) and anti-apoptotic factors (such as Bcl-2). During pregnancy, apoptosis plays an important role in maintaining the balance for the proper development of the placenta. However, spontaneous abortions are often associated with cellular dysfunction, which may result from excessive apoptosis. This study aimed to investigate how pro-apoptotic and anti-apoptotic factors interact and influence the occurrence of spontaneous abortion. This study involved the collection of 30 placentas from women aged 35 to 40 years who had experienced spontaneous miscarriages, which were compared to 20 placentas from women who underwent on-demand abortions, serving as a control group. Tissue sections were treated with monoclonal antibodies targeting BCL-2 and p53, and analyzed using the H score. Results are presented as mean ± standard deviation (SD). Independent and paired Student's t-tests were used to compare continuous variables. The analysis of pro-apoptotic factors showed a higher expression of p53 in the miscarriage group (230.33 ± 63.80) compared to the control group (131.22 ± 36.04). In contrast, the H score for the anti-apoptotic factor Bcl-2 was significantly higher in the control group (167 ± 92.73) compared to the miscarriage group (120.20 ± 100). The increased expression of pro-apoptotic factors, along with the reduced levels of anti-apoptotic factors, appears to contribute to pregnancy loss and spontaneous abortion.

Keywords: p53, Spontaneous abortion, Bcl-2, Apoptosis

Introduction

Placental development involves several stages of cell division and differentiation. Apoptosis, a key process during this development, is responsible for the remodeling of trophoblastic tissue in healthy pregnancies [1]. These apoptotic processes are present at the maternal-fetal interface and play a role in both physiological and pathological conditions [2].

Originally, apoptosis was defined as a strictly physiological process. However, recent research has revealed that apoptosis can also be linked to pathological conditions. Apoptosis is energy-dependent (ATP), and due to the discovery of other non-apoptotic processes requiring gene activation and energy, the term "programmed cell death" is now used with greater caution in the context of apoptosis [3].

Apoptosis can contribute to miscarriage, particularly when genetic abnormalities or developmental issues lead to the termination of a non-viable pregnancy. However, miscarriage can result from other causes, and further investigation is required to pinpoint the specific mechanisms at play.

Both the extrinsic and intrinsic apoptotic pathways converge at caspase-3 activation, known as the execution pathway [4]. In the study of carcinogenesis, it has been demonstrated that multiple genes are involved in this complex, multi-level process. p53, a tumor suppressor gene, regulates the cell cycle and plays a protective role by halting the cell cycle to repair DNA. If repair is not possible, p53 induces apoptosis [5].

Bcl-2 proteins primarily regulate mitochondrial apoptotic processes, controlling the release of cytochrome C and

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mitochondrial membrane permeability through mechanisms that are not fully understood [6-9].

The increasing rate of spontaneous abortions, linked to multiple factors, has motivated research aimed at providing better solutions for these patients. While earlier studies focused on issues like insufficient luteal support, cytogenetic abnormalities, and endometrial factors, they have not fully explained how changes in cellular signaling pathways contribute to miscarriages. Recurrent miscarriage (RM) is believed to be caused by issues in placental proliferation and apoptosis. This study aims to explore how pro-apoptotic and anti-apoptotic factors interact and influence the occurrence of spontaneous abortion.

Materials and Methods

This study included 30 placental samples from women aged 35 to 40 years who experienced spontaneous miscarriages with no identifiable medical cause. A control group consisted of 20 placentas from women in the same age range who underwent elective medical abortions. All participants were selected consecutively from those admitted to the Gynecology Department at The Emergency Clinical County Hospital Arad between 2021 and 2022.

Immunohistochemical analysis was performed on 4 µm thick slices of formalin-fixed, paraffin-embedded tissue using an Autostainer Link 48 (Agilent Technologies, Santa Clara, CA, USA). Paraffinized slides were subjected to target retrieval, followed by staining using a DAB (3,3′-diaminobenzidine) detection kit. Hematoxylin served as the counterstain. The sections were incubated with monoclonal antibodies against BCL2 (clone 124) and p53 (clone DO-70, Agilent Technologies, Santa Clara, CA, USA). Positive control slides from appendix tissue were included in each case, and negative control was used by omitting the primary antibody.

Two pathologists, blinded to the study group assignments, independently evaluated the specimens using the H score method. The intensity of staining (rated from 0 to 3) was multiplied by the percentage of positive cells, and the scores were summed, with a potential range from 0 to 300. A score of less than 1% positive cells indicated a poor outcome. Imaging and measurements were captured using a Leica DM3000 LED microscope with Leica Biosystem's LAS EZ software. Statistical analysis was performed using paired and independent Student's t-tests, with a p-value of less than 0.05 considered statistically significant.

The study was conducted following the Declaration of Helsinki and was approved by the Resident Laboratory Ethics Committee (protocol code 06/31 March 2020).

Results and Discussion

50 women participated in this study, of which 30 were in the experimental group (those who experienced spontaneous miscarriages) and 20 were in the control group (those who had medical abortions). No significant differences were found between the two groups regarding maternal age, BMI, or gestational age at the time of abortion **(Table 1)**.

Table 1. Maternal demographic data

Maternal demographics	Study group (n = 30)	Control group (n = 20)	P-value
Age of mother (years ± SD)	37.5 ± 3.1	38.7 ± 2.4	0.7
Gestational age at abortion	10.6 ± 2.0	10.3 ± 2.9	0.4
BMI (kg/m²)	24.1 ± 2.3	22.5 ± 1.6	0.4

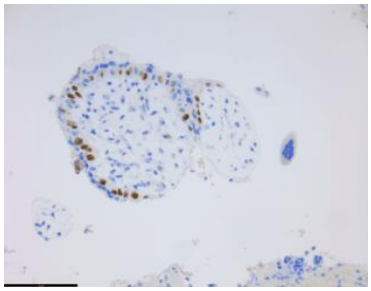
The analysis focused on the expression of p53 and Bcl-2 in the placental tissue to understand the balance between pro-apoptotic and anti-apoptotic factors. The results revealed a significant increase in p53 expression in the study group (230.33 ± 63.80) compared to the control group (171.22 ± 36.04), as shown in **Table 2**. Histopathological and immunohistochemical analyses provided important insights into the apoptotic processes involved in spontaneous abortion. High p53 expression, observed in spontaneous abortion cases, suggests an increase in pro-apoptotic activity.

Table 2. p53 and Bcl-2 expression values

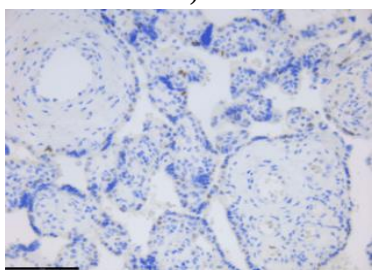
Parameter	Study group (n = 30)	Control group (n = 20)	P-value
p53 H-score ± SD	230.33 ± 63.80	171.22 ± 36.04	0.003
Bcl-2 H-score ± SD	120.20 ± 100	167 ± 92.73	0.023

The expression of Bcl-2 was found to be significantly lower in the study group (120.20 ± 100) compared to the control group (167 ± 92.73), suggesting that reduced anti-apoptotic activity may contribute to miscarriage. This difference in expression was also reflected in the histological images, which showed weaker Bcl-2 expression in the study group.

The statistical analysis confirmed the significance of these findings, with the T-test results indicating that the differences between the two groups for both p53 and Bcl-2 expression were statistically significant **(Figures 1-3)**.



a)



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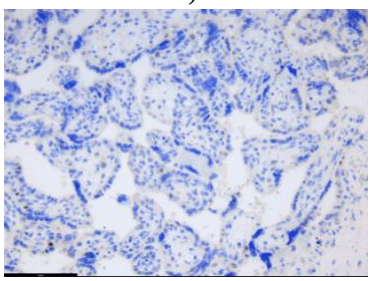

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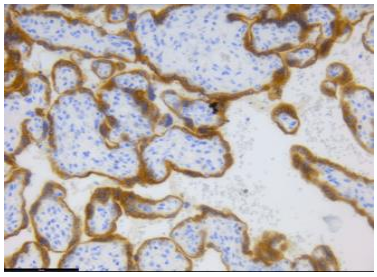

d)

Figure 1. a) higher expression of apoptotic protein (p53) in the trophoblast cells of the chorionic villi of RM cases, b) scattered cells that are positive for p53 in sporadic abortion cases, c) low expression of anti-apoptotic protein (Bcl-2) in the trophoblast cells of the chorionic villi of RM cases, and d) high expression of Bcl-2 in sporadic abortion cases.

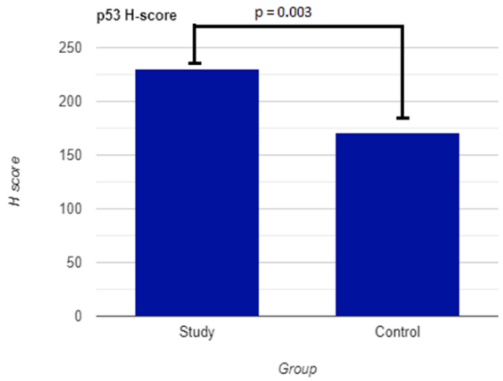

Figure 2. p53 Study and control group values

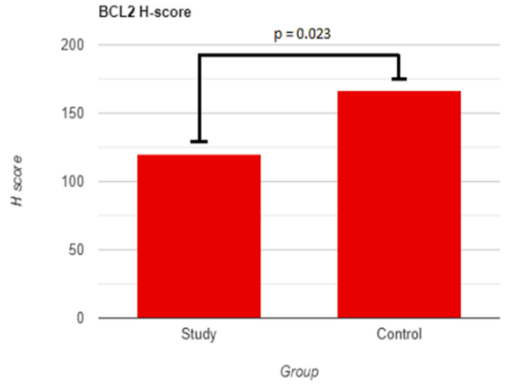

Figure 3. BCL2 study and control group values

Apoptosis, or programmed cell death, is a complex biological process involving multiple stages, each regulated by specific mechanisms. The balance between cell growth, differentiation, and maintenance of cellular functions can influence apoptosis. Recently, various pathological conditions, such as ischemic injuries, autoimmune diseases, and cancers, have been associated with disruptions in this process [3].

Embryonic development also depends on a delicate balance between cell division and cell death, and any disruption in this balance can result in fetal loss [10]. In this study, the focus was on the expression of p53 and Bcl-2 to assess the apoptotic and anti-apoptotic pathways in placental tissues from cases of spontaneous miscarriage. When comparing the placental tissue from the study group with the control group, a significant increase in p53, a pro-apoptotic protein, was observed, while the expression of Bcl-2, an anti-apoptotic protein, was notably reduced. These findings align with previous research, which also observed elevated p53 levels in spontaneous abortions [11, 12].

The data revealed that the H score for p53 expression was over 50% higher in the study group compared to the control group, whereas Bcl-2 expression was about 30% lower in the study group. A reduction in Bcl-2 can interfere with vital processes such as cell differentiation and proliferation, which are necessary for proper fetal development, thus contributing to spontaneous abortion. Through immunohistochemistry, we observed an inverse relationship between p53 and Bcl-2 expression. This research helps shed light on the mechanisms involved in spontaneous abortion, which often has multiple causes. During normal development, Bcl-2 shows diffuse cytoplasmic expression, with a brown color visible due to DAB chromogen binding to the antigen-antibody complex.

Some studies suggest that apoptosis may be reversible in certain conditions, particularly in its early stages, especially when p53 induces apoptosis. Early repair of damaged DNA in the process can reverse apoptosis [3]. Furthermore, apoptosis plays a significant role in tumor development and treatment responses [13, 14]. The Bcl/Bax gene family is essential in protecting cells from apoptosis, with Bcl-2 expression leading to a reduction in pro-apoptotic signals and an increase in cell proliferation—critical during embryonic development and organ formation.

The results of our study are consistent with other research that found a higher expression of the Bax gene in early spontaneous abortion compared to voluntarily terminated pregnancies. The Bax gene triggers p53 expression, initiating apoptosis through the mitochondrial pathway. Chronic endometritis, linked to infertility and miscarriage, has not been extensively studied concerning apoptosis. Recent studies suggest it may be useful to screen infertile patients for this condition [15, 16].

In the case of miscarriage, an imbalance between pro-apoptotic factors like p53 and anti-apoptotic factors like Bcl-2 could contribute to pregnancy loss. Elevated p53 levels can lead to excessive apoptosis of trophoblast cells, which are essential for placental function and pregnancy maintenance. On the other hand, reduced Bcl-2 expression can make these cells more susceptible to apoptosis. The delicate balance between these proteins plays a crucial role in miscarriage, as disruptions can lead to the loss of trophoblast cells, causing the pregnancy to fail.

Our study showed a clear inverse relationship between p53 and Bcl-2 expression. The study group exhibited higher p53 levels and lower Bcl-2 levels compared to the control group, supporting the hypothesis that increased apoptosis can contribute to spontaneous abortions. The statistical significance of our findings reinforces their importance. However, our study's limitations include its unicentric and prospective nature, with a limited sample size, which may impact the generalizability and statistical power of the results.

Conclusion

Conditions like chronic endometritis and endometriosis are closely linked to infertility and spontaneous abortion due to an increase in apoptotic processes. Identifying biomarkers that can indicate the shift from normal, homeostatic apoptosis to disease-related pathological apoptosis is crucial for improving prognosis and enabling personalized treatments in the future.

A reduced presence of pro-apoptotic factors is tied to the viability of the fetus, supporting the mechanisms that promote maternal immune tolerance. The analysis of pro-apoptotic (p53) and anti-apoptotic (Bcl-2) factors in the study group revealed an elevated expression of p53. This highlights a clear inverse relationship between the two factors: the study group had higher p53 levels, while Bcl-2 was lower in cases of spontaneous abortion compared to medically induced abortions. These findings support the hypothesis that increased apoptosis contributes to the occurrence of spontaneous abortion.

The overexpression of pro-apoptotic factors, coupled with the reduction of anti-apoptotic factors, seems to play a role in pregnancy failure and the onset of spontaneous abortion.

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