The possible protective role of BCL-2 in recurrent abortion.

Haider Sabah Kadhim *MBChB, MSc, PhD.*

Abstract

Background: Recurrent abortion is a worldwide problem, with undefined causes. Apoptosis could play a major role in the process.
Objective: Detect the expression of Bcl-2 protein at the materno-fetal interface in patients with recurrent pregnancy loss (RPL).
Methods: Immunohistochemistry analysis of Bcl-2 protein using paraffin embedded sections of curate samples obtained from 40 women divided into three groups: 24 women with recurrent abortion, 10 women with abortion for the first time, and 6 women with induced abortion.
Results: The mean value of the expression of Bcl-2 protein was (57.9± 1.4), which is significantly higher than that of the second group (39± 1.9), and the third group (47.5± 2.4).
Conclusion: High expression of Bcl-2 protein in women with recurrent abortion may have a protective role in preventing placental apoptosis that leads to failure of pregnancy.
Keywords: Bcl-2, recurrent pregnancy loss (RPL).

Introduction

Apoptosis plays important roles in placentation and embryonic development [1]. The cells undergoing apoptosis have characteristic structural changes in the nucleus and cytoplasm. Expressions of apoptotic regulatory molecules, such as Fas, Fas ligand, P53, and the proteins of Bcl-2 family, have been reported in human placenta [2, 3]. Bcl-2 and P53 are two of the key players in the apoptotic signaling cascades. Bcl-2, a proto-oncogene first discovered in human follicular lymphoma [4], is involved in the inhibition of apoptosis and the survival of a variety of cell types [5]. Bcl-2 protein is located in the membranes of endoplasmic reticulum, nuclear envelope, and mitochondria. Over-expression of Bcl-2 suppresses apoptosis by preventing the activation of

Dept. Medical Microbiology, College of Medicine, Al-Nahrain University.
Address Correspondence to: Dr. Haider Sabah Kadhim .
E- mail: haider_kadhim@yahoo.com
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caspases that carry out the process. P53 is well known as a tumor suppressor. It is a transcription factor that induces apoptosis mainly through inducing the expression of a batch of redox-related genes [6] and the down-regulating Bcl-2 [7].

The expression of Bcl-2 in human placenta has been studied [1, 8]. However, the cellular distribution in the implantation site at early stage of pregnancy has not been reported.

Apoptosis is the physiological process by which excess or dysfunctional cells are eliminated during development or normal tissue homeostasis, and nowhere is it more dramatic than in the reproductive system. For example, apoptosis occurs cyclically in human nonpregnant endometrium, throughout pregnancy in mouse [9] and human deciduas [1], human placenta [10], amnion epithelial cells [11, 12], rat cervical SMCs during pregnancy [13], and mammary glands during weaning [14, 15]. At least two pathways activate apoptosis [16]. The first is a mechanism that involves activation of a group of tumor necrosis

factor receptors, such as Fas (ligand-receptor pathway). The second (exogenous stimulus pathway) is a parallel, mitochondria-dependent route activated by physiological stimuli (lack of growth factors, changes in hormonal environment, hypoxia, and hypoglycemia) and/or environmental stimuli (exposure to cytotoxic compounds, radiation, and viral infection) that is transmitted independently of surface membrane receptors and is governed by BCL2 family proteins [17].

Despite the remarkable nature of uterine growth during pregnancy, little information is available regarding the mechanisms that initiate and regulate this growth. Furthermore, no data precisely maps the contribution of hyperplasia, hypertrophy, and apoptosis to uterine growth from early to late pregnancy. The role of cell apoptosis in myometrial growth is largely unknown [17]. Therefore, the goal of the present study was to gain insight regarding the role of Bcl-2 in early pregnancy, whether this role is protective or not.

Patients and methods

In this study, forty (40) patients were collected from Al-Kadhimya teaching hospital and Al-Elwiya teaching hospital, and then divided into three groups:

Group A: 24 pregnant ladies presented with abortion during the first trimester, with history of previous 3-6 consecutive first trimester abortions, with no medical diseases (like autoimmune diseases), nor family history of genetic diseases or uterine anomaly, also all of them were confirmed by laboratory tests to be negative for acute infection with CMV, rubella and toxoplasmosis.

Group B: 10 pregnant ladies presented with abortion during the first trimester and had at least three previous normal pregnancies with no previous abortion, and no history of any other medical illness.

Group C: 6 pregnant ladies with elective termination of pregnancy in the first trimester for maternal diseases (illness not related to apoptosis) under approved consent of two senior gynecologists and a physician. This group considered as control group. Curate samples of the maternal-fetal interface were taken from all these women at the end of evacuation curate operation then embedded in paraffin and confirmed by a pathologist, and then subjected for immunohistochemistry technique using DAKO cytomation detection kit (Denmark).

Detection of Bcl2 done by Immunohistochemistry, the procedure includes briefly; 5µm thickness tissue sections on positively charged slides were deparafinized in xylen then rehydrated in a series of ethanol concentrations. And then, 2-3 drops of peroxidase block were applied onto the tissue sections a step which is followed by application of the primary antibody (anti-Bcl2 protein) (DAKO Denmark), then the secondary antibody was added, followed by application of the hoarse reddish peroxidase (HRP) conjugate, and then its substrate DAB chromogen. Sections were counterstained with hematoxyline, sections dehydrated and mounted to be finally examined under the microscope. The expression of Bcl2 was measured by counting the number of positive decidual and trophoblastic cells, which gave a brown cytoplasmic staining under the light microscope (figure 1). The extent of the immunohistochemistry signal in the villi was determined in 10 fields (X100 magnification). In each field the total

number of villi were counted and the extent of nuclear staining of the cytotrophoblast and syncytiotrophoblast in a given villous was counted and simplified as percent, the percentage of positively stained villi was calculated for each case by taking the mean of the percentages of the positively stained villi in the 10 fields [18]. The scorer was blinded to the clinical diagnosis of the tissues at the time of assessment, and tissues were independently assessed by two observers.

Negative controls were obtained by omitting the monoclonal antibody (Anti-Bcl2) and using phosphate buffer saline to verify the signal specificity.

Statistical Analyses

The program SPSS was used to determine the difference in the expression of Bcl2 protein among the three groups. Values of $p<0.001$ were considered as statistically significant. A correlation had been done by using Pearson correlation depending on frequency of abortion as group A more than 1 abortion, and group B one abortion.

Results

The results of immunohistochemistry for Bcl-2 in group A, those with recurrent abortion in 1st trimester, had a mean reading of 57.9% with standard deviation 1.4 see table (1). It is significantly higher than the mean results of group C the group considered as control that was 47.5% with standard deviation 2.411. The mean results of group B, those with one abortion only, was 39% with standard deviation of 1.94 and it was significantly lower than group C and A.

Table 1: The mean percentage results of immunohistochemistry of Bcl-2

Groups	*Number*	*Bcl-2 Mean $\pm$ STD*	*P-value*
Group A	24	57.9 ± 1.416	
Group B	10	39.0 ± 1.944	0.001
Group C	6	47.5 ± 2.411	

Group A: Recurrent abortion.
Group B: One abortion.
Group C: Control.

The correlation was done by using Pearson correlation depending on frequency of abortion as group A had more than 1 abortion and group B had only one abortion, and results showed a significant correlation as in table 2.

Table 2: The Pearson correlation depending on frequency of abortion between group A and group B

Bcl2 expression	Frequency of Abortion	Pearson Correlation	p-value
Group A N=24	>1		
		0.602	<0.001
Group B N=10	1		
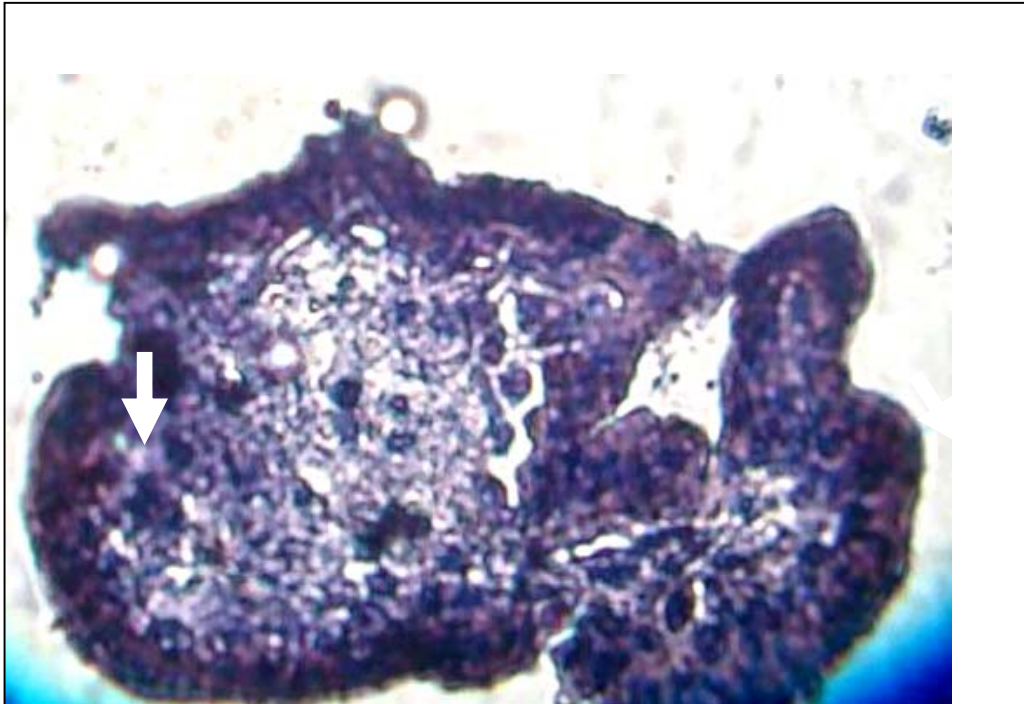
Figure 1: Detection of Bcl-2 protein by immunohistochemistry in women with pregnancy loss. The expression was diffuse heterogeneous brown cytoplasmic staining involving the trophoblasts, both cyto- and synsytiotrophoblasts in the three groups of women but the percentage of villi involved is higher and more diffusely stained in the recurrent loss group. Light microscope magnification power (X400).

The results are summarized in figure 2 as it shows mean of the 3 groups
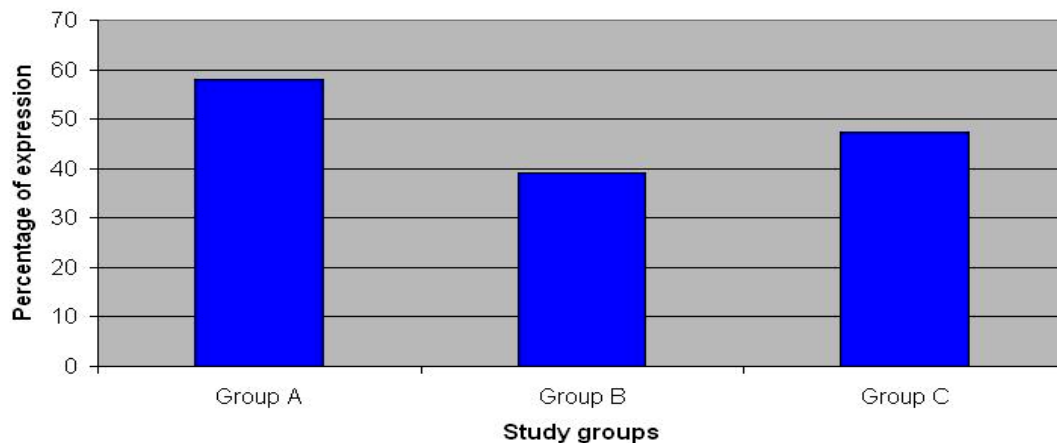
Figure 2: The mean percentage of Bcl-2 expression in the three groups under study

Discussion

In the present study, we found a significant increase in the expression of BCL2 protein at recurrent abortion as it may be a protective measure to counteract a probable increase of apoptotic proteins. It is reasonable to speculate that BCL2 proteins could be protecting myometrial SMCs from premature termination of the proliferative phase of uterine growth by preventing the development of true apoptosis in myometrial tissue, which is incompatible with the function of this organ. In group A, this was of no benefit as pregnancy loss took place recurrently. In the 2nd group, namely B, the level of Bcl-2 expression was low as the process of loss took place for the 1st time, hence the level was not as high as in the recurrent group. In the group C the condition represents the normal level of expression as it is an elective termination and no role of any of the pro- or anti-apoptotic proteins in it.

Despite the clear transformation that takes place in the myometrium and the possible importance of the different phases of uterine growth throughout pregnancy and labor, little is known about the causes and mechanisms of this transition from myometrial hyperplasia to hypertrophy. We believe that such transformation is triggered by the process called uterine conversion, an adaptive mechanism to accommodate the growing fetus [19]. During early pregnancy, the shape of the fetus is spherical, and maternal blood supply is abundant. The process of fetal growth continues until a critical time, specific to each species, when the conceptus reaches a maximum spherical radius and the uterine tissue is stretched. Tension is so great that it creates ischemia, resulting in circulatory stasis, which is detrimental to maternal blood flow. The conversion of embryo shape from a sphere to a cylinder, which requires only a few hours, causes a release of uterine tissue tension and reestablishment of the maternal blood supply throughout the uterus [20]. Notably, late gestation is accompanied by rapid growth of the fetus, and it also is marked by a second period of mechanical stretch, which ends at parturition. It is a reasonable hypothesis that uterine conversion can cause transient ischemia in a stretched myometrium that can lead to a hypoxic response in this tissue and activation of the intrinsic apoptotic-pathway [13].

In conclusion, our data may demonstrate that the myometrium undergoes an increase in the anti-apoptotic proteins from early pregnancy to prevent pregnancy loss. These changes could decide the fate of the pregnancy.

References

1. Kucera E, König F, Tangl T, Grosschmidt K, Kainz C and Sliutz G. Bcl-2 expression as a novel immunohistochemical marker for ruptured tubal ectopic pregnancy. Human Reproduction, 2001: Vol. 16, No. 6, 1286-1290.

2. Runic R, Lockwood CJ, Ma Y, Dipasquale B, Guller S. Expression of Fas ligand by human cytotrophoblasts: implications in placentation and fetal survival. J Clin Endocrinol Metab. 1996; 81:3119–3122.

3. Qiao S, Nagasaka T, Harada T, Nakashima N. p53, Bax and Bcl-2 expression, and apoptosis in gestational trophoblast of complete hydatidiform mole. Placenta. 1998; 19:361–369.

4. Tsujimoto Y, Cossman J, Jaffe E, Croce CM. Involvement of the bcl-2 gene in human follicular lymphoma. Science. 1985; 228:1440–1443.

5. Hockenbery DM, Zutter M, Hickey W, Nahm M, Korsmeyer SJ. BCL2 protein is topographically restricted in tissues characterized by apoptotic cell death. Proc Natl Acad Sci U S A. 1991; 88:6961–6965.

6. Polyak K, Xia Y, Zweier JL, Kinzler KW, Vogelstein B. A model for p53-induced apoptosis. Nature. 1997; 389:300–305.

7. Miyashita T, Krajewski S, Krajewska M, Wang HG, Lin HK, Liebermann DA,et al. Tumor suppressor p53 is a regulator of bcl-2 and bax gene expression in vitro and in vivo. Oncogene. 1994; 9:1799–1805.

8. Haidacher S, Blaschitz A, Desoye G, Dohr G. Immunohistochemical evidence of p53 protein in human placenta and choriocarcinoma cell lines. Hum Reprod. 1995; 10:983–988.

9. Mu J, Kanzaki T, Tomimatsu T, Fukuda H, Wasada K, Fujii E, et al. Expression of apoptosis in placentae from mice lacking the prostaglandin F receptor. Placenta 2002; 23:215–223.

10. Heazell A E P, Buttle H R, Baker P N and Crocker I P. Altered Expression of Regulators of Caspase Activity within Trophoblast of Normal Pregnancies and Pregnancies Complicated by Preeclampsia
Reproductive Sciences, 2008; 15(10): 1034 - 1043.

11. Lei H, Furth EE, Kalluri R, Chiou T, Tilly KI, Tilly JL,et al. III.. A program of cell death and extracellular matrix degradation is activated in the amnion before the onset of labor. J Clin Invest 1996; 98:1971–1978.

12. Kumagai K, Otsuki Y, Ito Y, Shibata MA, Abe H, Ueki M. Apoptosis in the normal human amnion at term, independent of Bcl-2 regulation and onset of labor. Mol Hum Reprod 2001; 7:681–689.

13. Shynlova O, Oldenhof A, Dorogin A, Xu Q, Mu J, Nashman N et al. Myometrial Apoptosis: Activation of the Caspase Cascade in the Pregnant Rat Myometrium at Midgestation. Biology of Reproduction 2006; 74, 839–849.

14. Suganuma N, Harada M, Furuhashi M, Nawa A, Kikkawa F. Apoptosis in human endometrial and endometriotic tissues. Horm Res 1997; 48(suppl 3):42–47.

15. Green KA, Streuli CH. Apoptosis regulation in the mammary gland. Cell Mol Life Sci 2004; 61:1867–1883.

16. Crighton D, Ryan KM. Splicing DNA-damage responses to tumor cell death. Biochim Biophys Acta 2004; 1705:3–15.

17. David G Breckenridge and Ding Xue. Regulation of mitochondrial membrane permeabilization by BCL-2 family proteins and caspases; Current Opinion in Cell Biology 2004, 16:647–652.

18. Hennessy A, Pilmore HL, Simmons LA and Painter DM. A Deficiency of placental IL-10 in preeclampsia. The Journal of Immunology. 1999; 163: 3491-3495.

19. Reynolds SRM. Patterns of uterine growth during pregnancy. In: Physiology of the Uterus, 2nd ed. New York: Paul B. Hoeber, Inc. 1949:218–234.

20. Kliman, HJ. Uteroplacental Blood Flow; the story of decidualization, menstruation, and trophoblast invasion. American Journal of Pathology. 2000; 157:1759-1768.