# bcl-2 Expression in Complete Hydatidiform Mole

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## Abstract

**Objective:** The objective of this study was to investigate the role of apoptosis in complete hydatidiform mole (CHM) by comparing apoptotic activity in CHM and normal placenta, using bcl-2 expression as the index of apoptotic activity.
**Materials and Methods:** Placental tissue samples were retrospectively analyzed from 15 patients with CHM and 11 healthy women undergoing first trimester termination of pregnancy. Diagnosis were confirmed histopathologically. After application of bcl-2, a classic avidin-biotin-peroxidase method and DAB chromogen were used for immunohistochemical analysis. Staining for bcl-2 was interpreted as positive (cytoplasmic staining) or negative (no staining reactivity). This immunopositivity was recorded separately for the different cellular components— cytotrophoblasts, syncytiotrophoblasts and intermediate trophoblasts. Results for syncytiotrophoblasts were recorded semiquantitatively and analyzed by chi-square test.
**Results:** In tissue samples from patients with CHM, bcl-2 immunoreactivity occurred solely in the syncytiotrophoblasts and was significantly stronger than that found in normal placentas (11/15 moderate staining, 4/15 strong staining vs. 4/11 mild staining, 7/11 moderate staining; $p<0.01$). Normal placentas showed –comparatively weak immunoreactivity in cytotrophoblasts as well as in syncytiotrophoblasts.
**Conclusion:** This study confirms previous findings and supports the contention that overexpression of bcl-2 oncoprotein may be important in the pathogenesis of CHM and the expression of bcl-2 is inversely correlated with the apoptotic index. We suggest that bcl-2 proteins play a role in the proliferation of the syncytiotrophoblasts in CHM by suppressing apoptosis.

**Keywords:** gestational trophoblastic disease, complete hydatidiform mole, pathogenesis, apoptosis, bcl-2

## Özet

### Komplet Mol Hidatidiformda bcl-2 Ekspresyonu

**Amaç:** Bu çalışmanın amacı, apoptotik aktivite indeksi olarak bcl-2 ekspresyonunun kullanılmasıyla, komplet mol hidatidiform (KMH) ve normal plasentalarda apoptotik aktiviteyi karşılaştırarak, KMH'de apoptozisin rolünü araştırmaktır.
**Materyal ve Metot:** KMH'li 15 hasta ve ilk trimestr gebelik terminasyonu yapılan 11 sağlıklı kadının plasental doku örnekleri retrospektif olarak incelendi. Tanılar histopatolojik olarak doğrulandı. bcl-2 uygulanmasından sonra, immünohistokimyasal ölçüm için klasik avidin-biotin-peroksidaz metodu ve DAB kromogen kullanıldı. bcl-2 boyanması, pozitif (sitoplazmik boyanma) veya negatif (boyanma yok) olarak yorumlandı. Sitotrofoblastlar, sinsityotrofoblastlar ve intermediate trofoblastlardaki immünopozitiflik belirlendi. Sinsityotrofoblastlar için sonuçlar, semikantitatif olarak değerlendirildi ve ki-kare testi ile analiz edildi.
**Sonuçlar:** KMH hastalarının doku örneklerindeki bcl-2 immünoreaktivitesi, yalnızca sinsityotrofoblastlarda normal plasentadakinden daha belirgin olarak bulundu (11/15 orta, 4/15 güçlü boyanmaya karşı, 4/11 hafif, 7/11 orta boyanma, $p<0.01$). Normal plasentalar, hem sinsityotrofoblastlarda hem de sitotrofoblastlarda, zayıf olmakla birlikte, immünoreaktivite gösterdi.
**Tartışma:** Bu çalışma, önceki bulguları doğrulayarak, bcl-2 onkoprotein overekspresyonunun KMH patogenezinde önemli olabileceğini ve bcl-2 ekspresyonunun apoptotik indeksle ters korelasyon gösterdiğini desteklemektedir. KMH'de bcl-2 proteinlerinin apoptozisi baskılayarak sinsityotrofoblastların proliferasyonunda rol oynadığı düşüncesindeyiz.

**Anahtar sözcükler:** gestasyonel trofoblastik hastalık, komplet mol hidatidiform, patogenez, apoptozis, bcl-2

## Introduction

Gestational trophoblastic disease (GTD) is a heterogeneous group of diseases, characterized by abnormally proliferating trophoblastic tissues (1). The three tropho-blasts— cytotrophoblast, syncytiotrophoblast and intermediate trophoblast— contribute to the substance of the placenta. The most common type of GTD is hydatidiform mole, which can be of two types: "complete", containing no fetal tissue and demonstrating excessive circumferential trophoblastic hyperplasia around the abnormal villi; and "partial", containing some fetal tissue, usually markedly abnormal, and hydropic villi with focal cytotrophoblastic growth. Hydatidiform moles may develop into invasive moles (10-15%) or persistent GTD (10-30%) (2). Complete moles have a small risk of malignant transformation into choriocarcinomas (2). The pathogenesis of GTD is not fully understood (3).

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Apoptosis has been found to play a crucial role in the pathogenesis and prognosis of many human diseases. Apoptosis describes the morphological processes leading to controlled cellular self-destruction (4). The apoptotic mode of cell death is an active and defined process which plays an important role in the development of multicellular organisms and in the regulation and maintenance of the cell populations in tissues under physiological and pathological conditions.

The bcl-2 family is a group of proteins that play a major role in the control of cell death and survival and are critical participants in the execution of the cell death mechanism. The bcl-2 gene family seems to act as a regulator of the apoptotic pathway (5). The two most important apoptosis-regulating proteins of this family are most likely bcl-2 and bax. The former is a member of the anti-apoptotic family and the latter of the pro-apoptotic family. Together they probably act as a rheostat for the cell death program (5, 6).

Expression of the apoptosis gene bcl-2 has been shown to have an inverse correlation with the apoptotic index (AI), suggesting that bcl-2 is likely the genetic regulator of apoptosis in GTD (7).

In this study we investigated the expression of bcl-2 in normal human placenta and complete hydatidiform mole (CHM).

## Materials and Methods

***Samples***

Formalin-fixed, paraffin-embedded molar tissue and normal first-trimester placental tissue samples were retrospectively collected from the archival files of the Pathology Department. Sections of the samples were stained with hematoxylin-eosin and histopathologically reviewed by the same expert pathologist (İ.Ö.). Diagnosis of CHM was based on histopathological examination of the molar tissue, showing characteristically abnormal proliferation of trophoblastic tissue, lack of an identifiable foetus, chorionic villi with generalised hydatidiform swelling, and diffuse trophoblastic hyperplasia resulting from abnormal fertilisation. A total of 15 CHM samples were selected. Placenta samples were taken from 11 healthy women undergoing first-trimester elective termination of pregnancy, with live fetus, and without any first trimester bleeding. The diagnosis of normal placental structure confirmed by histopathology.

***Immunohistochemical Analysis***

Biopsy samples obtained from complete hydatidiform mole patients were fixed in 10% formalin, routinely processed and embedded in paraffin. Five micrometer-thick serial sections were obtained by rotary microtome and transferred onto adhesive slides. The sections were dried in the autoclave at 50°C for 16 hours. Then they were deparaffinized and dehydrated by immersion into xylene twice for 10 minutes and into alcohol four times for 5 minutes. Rehydratation was carried out by washing with distilled water for 2 minutes and immersion in tris buffered saline for 5 minutes. The specimens were then incubated in 3% H2O2 for 5 minutes to inhibit activation of endogenous peroxidases and then transferred into tris buffered saline for 5 minutes. After application of bcl-2 (DAKO; PDM016, U.S.A.) for 30 minutes, they were washed with tris buffered saline. A classic avidin-biotin-peroxidase method and DAB chromogen (20 minutes) was then used for immunohistochemical analysis of bcl-2. A tonsil specimen was used as positive control for bcl-2. Mayer's hematoxylin was used as counterstain and slides were examined by light microscopy.

Staining for bcl-2 was interpreted as positive (cytoplasmic staining) or negative (no staining reactivity). This immunopositivity was recorded separately for the different cellular components— cytotrophoblasts, syncytiotrophoblasts and intermediate trophoblasts.

The results of immunostaining of the syncytiotrophoblasts were analysed semiquantitatively. The percentage of positive cells (the intensity of staining) for bcl-2 were recorded as follows: (+) mild staining, (++) moderate staining, (+++) strong staining (Table 1).

**Table 1.** Staining intensity of syncytiotrophoblasts with bcl-2 in CHM and normal placental tissues

| bcl-2 staining intensity | Complete hydatidiform mole (CHM) (n=15) | Normal, first-trimester placenta (n=11) |
|---|---|---|
| Mild staining, (+) | — | 4 |
| Moderate staining, (++) | 11 | 7 |
| Strong staining, (+++) | 4 | — |

SPSS 10.0 program (Windows, Microsoft) and Mann-Whitney U test for demographic characteristics and chi-square test were used for comparison of the results of the immunostaining of the syncytiotrophoblasts. A value of $p<0.05$ was considered significant.

## Results

There were no differences in mean age, gestational age, gravidity or parity between the subjects ($p>0.05$). For CHM, all syncytiotrophoblasts stained for bcl-2 in the cytoplasm but there was no staining in the cytoplasm of cytotrophoblasts or intermediate trophoblasts, while for normal placenta, all syncytio- and cytotrophoblasts stained for bcl-2. The distribution of bcl-2 apoptosis markers in CHM and normal placenta is shown in Figures 1 and 2.

When compared with normal placenta, the expression of bcl-2 protein was significantly stronger in CHM ($p<0.05$). The staining intensity of syncytiotrophoblasts with bcl-2 in CHM and normal placental tissues is shown in Table 1.

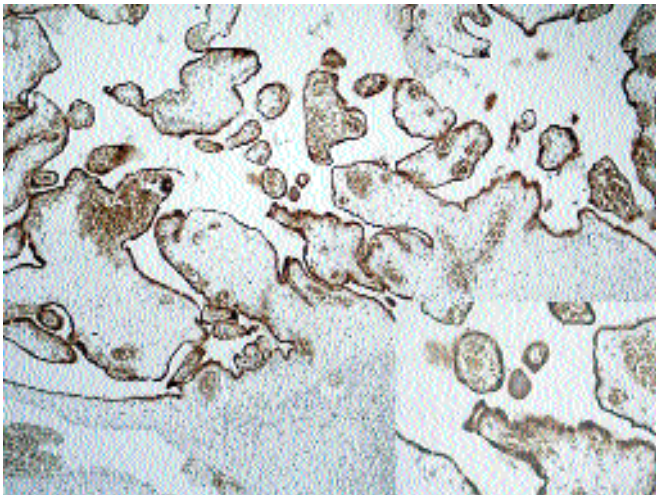

**Figure 1.** bcl-2 positivity in normal first trimester placenta (X100). Inset: (X200).

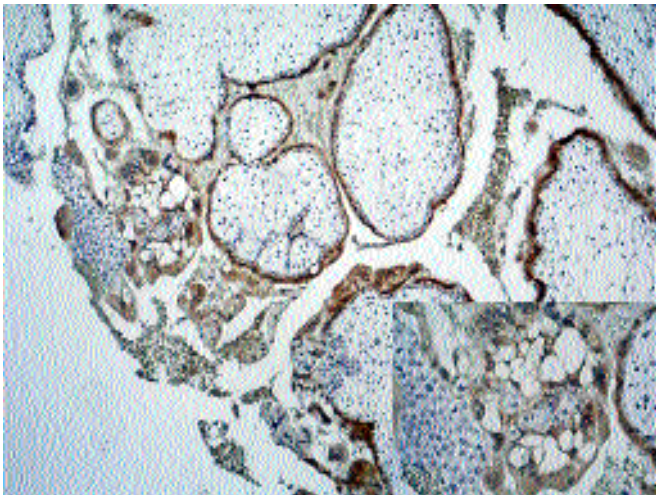

**Figure 2.** bcl-2 immunostaining pattern in the complete hydatidiform mole (X100). Nearly pan-syncytiotrophoblastic immunostaining of bcl-2 in trophoblastic tissue. Cytotrophoblasts and intermediate trophoblasts were negative for bcl-2. Inset: (X200).

## Discussion

GTDs are characterized by altered expression of several growth regulatory factors and oncogenes. While differences in expression of oncoproteins may be important to the development of GTD, the precise molecular changes that are critical to pathogenesis remain unknown (8).

Programmed cell death is a widespread phenomenon, occurring in all kinds of living organisms (9). Defects in apoptotic cell death regulation contribute to many diseases. The bcl-2 gene is a major regulator of apoptosis and belongs to a family of proteins that harbors both pro- and anti-apoptotic members. Of these, bcl-2 itself is an anti-apoptotic protein, exerting its influence by enhancing cell survival rather than stimulating cell division. An immunohistochemical assay for determining bcl-2 expression in archival tissues has been available for several years and has permitted significant insight into the role of this protein in the development and progression of diseases characterized by progressive cell accumulation.

Several studies have focused on the role of apoptosis in the pathogenesis of GTD. Complete moles and choriocarcinomas demonstrate high levels of apoptosis (and hence high levels of the pro-apoptotic protein bax and low levels of the anti-apoptotic protein bcl-2). It has been observed that bcl-2 accumulation was found predominantly in syncytiotrophoblasts of normal placenta, and cytotrophoblasts and intermediate trophoblasts did not express bcl-2 in all cases (13). The level of apoptosis correlates with the histological type of the gestational trophoblasts, and AI is higher in cytotrophoblasts in CHM (13). In contrast, normal placentas and partial moles have low levels of apoptosis and low bax/bcl-2 ratios (10, 11, 12, 13, 14). It has been suggested that bcl-2 oncoproteins may be important in the pathogenesis of CHM (1). The involvement of bcl-2 in GTD was reported in the study by Fulop et al. (8) which found significantly stronger expression of bcl-2 in the terminally differentiated syncytiotrophoblasts of complete moles and choriocarcinomas in comparison with normal placentas and partial moles (14). Wong et al. have examined the expression of both bcl-2 and bax in GTD by immunohistochemical methods (12). They found that the AI (i.e. percentage of apoptotic cells in the tissue) was significantly different among various categories of trophoblastic lesions and increased in the following order: normal placentas < spontaneous abortions < choriocarcinomas < hydatidiform moles (1). Thus, the expression of bcl-2 is inversely correlated with the AI. The fact that an increase in the bax/bcl-2 ratio was also observed in CHM suggested that it may contribute partly to the high level of apoptosis (13). bcl-2 expression is probably regulating apoptosis in normal placentas and GTD, whereas bax expression is not (12). The difference in AI and bcl-2 expression between non-molar placentas and hydatidiform moles offers a potential adjunctive diagnostic tool to distinguish the two entities (12).

Our study found that the expression of bcl-2 protein was significantly stronger in CHM when compared with normal placenta, suggesting that bcl-2 oncoproteins may play a role in the proliferation of the syncytiotrophoblasts but not in the proliferation of the cytotrophoblasts, intermediate cytotrophoblasts, and villous stromal cells in CHM.

We conclude that our results support the proposition that overexpression of bcl-2 oncoprotein may be important in the pathogenesis of CHM.

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