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# STUDY ON EXPRESSION OF Bcl-2 IN CYCLICAL ENDOMETRIUM, ENDOMETRIAL HYPERPLASIA AND ENDOMETRIAL CARCINOMA

Pathology

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## ABSTRACT

**INTRODUCTION:** Abnormal uterine bleeding (AUB) is a clinical entity that has been defined as presence of irregular menstrual bleeding without diagnosing any particular organic lesion. It is considered to be a diagnosis of exclusion, without any pathologic entity4. Even though numerous causes has been found out for the abnormal uterine bleeding in women, the endometrial hyperplasia and carcinoma remains the most common causes5. The importance of ruling out or treating these conditions is due to high incidence of mortality and morbidity encountered with the patients having theseconditions. Many studies have revealed that the endometrial hyperplasia act as precursor for the development of the endometrial carcinoma, in particular endometrioid type of endometrial carcinoma[1,2].

**AIM:** In this study, an anti-apoptotic marker Bcl-2 has been used. Bcl-2 expression has been immunohistochemically assessed in various endometrial tissue such as cyclical endometrium, endometrial hyperplasia and endometrial carcinoma.

**OBJECTIVES:** To determine the expression of Bcl-2.In cyclical endometrium in the proliferative and secretory phases, endometrial hyperplasia, endometrial carcinoma.

**CONCLUSION:** In hyperplasia, Bcl-2 expression is increased in an increasing fashion from simple hyperplasia to complex hyperplasia without atypia and to complex hyperplasia with atypia. This is as a result of unopposed effect of the estrogen in the hyperplastic endometrial tissue[1,2]. Findings of this study reveals that Bcl-2 can be used as a potential therapeutic agent.

## KEYWORDS

Bcl-2, endometrial hyperplasia, endometrial carcinoma, Targeted therapy

## INTRODUCTION:

Abnormal uterine bleeding is a clinical entity that has been defined as presence of irregular menstrual bleeding without diagnosing any particular organic lesion. It is considered to be a diagnosis of exclusion, without any pathologic entity[4]. Pathologic texts however do not recognize such terms like AUB, and different lesions have been reported to cause irregular menstrual bleeding which ranges from something as innocuous as hyperplasia of the endometrium to more serious conditions such as hyperplasia with atypia, with final end of the spectrum being endometrial carcinoma. AUB affects approximately more than 10% of women those are in the age group of 18-45 years[5]. Even though numerous causes has been found out for the abnormal uterine bleeding in women, the endometrial hyperplasia and carcinoma remains the most common causes[5]. The importance of ruling out or treating this condition is due to high incidence of mortality and morbidity encountered with the patients having Endometrial hyperplasia and carcinoma. Many studies have revealed that the endometrial hyperplasia act as precursor for the development of the endometrial carcinoma[1,2], in particular endometrioid type of endometrial carcinoma. In this study, an anti-apoptotic marker Bcl-2 has been used. Bcl-2 expression has been immunohistochemically assessed in various endometrial tissue such as cyclical endometrium, endometrial hyperplasia and endometrial carcinoma. Unlike in other studies, the myometrial invasion and tumor grading are not included in this study. In cyclical endometrium which has been taken for the study includes both the proliferative and secretory endometrium. Considering various studies Bcl-2 expression is high in proliferative endometrium as it is an anti-apoptotic marker and there is reduced apoptosis in proliferative phase, whereas in secretory phase it is almost negative in mid and late secretory phase and mild focal positivity in case of the early secretory phase[6]. Likewise, in hyperplasia condition the expression of the Bcl-2 increases in accumulative frequency from simple hyperplasia to complex hyperplasia without atypia and then complex hyperplasia with atypia[3]. This remains the mainstay of this study as the hyperplasia is the precursor of endometrial carcinoma, if we are able to diagnose the complex hyperplasia with or without atypia at this stage those patients can be treated with anti Bcl-2 protein and progression of the disease can be halted.

## AIM:

To assess the mitotic activity in cyclical endometrium, endometrial hyperplasia and endometrial carcinoma by using Bcl-2 immunohistochemistry so that targeted therapy using anti-Bcl-2 can be given in endometrial hyperplasia and carcinoma. Various literature concludes the hyperplasia of endometrium is one of the precursor lesion of the endometrial carcinoma[3]. This study is done to elicit the overexpression of the Bcl-2 in hyperplastic condition, so that these protein can be targeted for therapy in patients with endometrial hyperplasia which shows positivity for this protein and can be helpful in the better prognosis and survival of the patients.

## OBJECTIVES:

To study the expression of Bcl-2 in cyclical endometrium in the proliferative and secretory phases.

To study the expression of Bcl-2 in endometrial hyperplasia and endometrial carcinoma.

## MATERIAL & METHODS:

The present study has been conducted with 60 patients presenting with AUB in the department of Obstetrics and Gynaecology who has undergone either Dilatation &Curettage or hysterectomy. Patients with clinically and radiologically detectable lesions in the uterus of any origin or patients with any kind of hormonal therapy were not included in the study. After histopathological reporting using H&E sections, the cases were separated and used for IHC staining of Bcl-2. The IHC staining for Bcl-2 will be performed using Anti Bcl – 2 oncoprotein and high Reactive Polymer as secondary kit. Antigen retrieval was done by using microwave in Tris- EDTA buffer at pH 9. Staining was performed with DAB as chromogen. Brown staining of the cytoplasm in glandular cells is considered as positive for Bcl-2. In this study, intensity and proportion of staining were calculated as below.

## SCORING OF Bcl-2:

Proportion of stained cells in the endometrial glands were evaluated manually by counting 10 consecutive high power fields (40X magnification) and assigning one of the following categories:

In this study, Bcl-2 staining less than <10% of cells is considered as negative, Bcl-2 staining of glandular epithelial cells ranging from 11-50% is considered as the weak positive and the proportion of cells showing positivity more than 50% is considered as strong positivity. The staining intensity varies from equivocal or weak to strong between the cases[3]. The number of cases taken in the each category and the number of cases showing weak and strong positivity has been compared. (Table 1)

Table 1: Proportion scoring for Bcl-2[3]:

| Score | Proportion of positive cells |
|---|---|
| 0 | <10% |
| 1+ | 11-50% |
| 2+ | >50% |

**STATISTICALANALYSIS**

Statistical analysis was carried out using SPSS version 21.0 (IBM SPSS, US) software with Regression Modules installed. ANOVA and Bonferroni test were conducted in the positive cases among the groups of our area of interest. Statistical significance was considered when the p value is <0.05 and statistical significance was noted between the groups.

**RESULTS:**

A total of 60 endometrial samples were studied for Bcl-2 expression which included 10 cases (17%) of proliferative endometrium, 10 cases (17%) of secretory endometrium and 25 cases (41%) of Hyperplasia and 15 cases (25%) of endometrial carcinoma. (Table 2)

**Table 2: Distribution of cases**

| S.No | Endometrial lesions | Sample size | Percentage |
|---|---|---|---|
| 1 | Proliferative phase | 10 | 17% |
| 2 | secretory phase | 10 | 17% |
| 3 | Endometrial hyperplasia | 25 | 41% |
| 4 | Endometrial carcinoma | 15 | 25% |
| | Total | 60 | 100% |

**AGE DISTRIBUTION:**

Out of 60 cases taken for the study 31(52%) of cases were below the age group of 45 years, 20(33%) of cases were in the age group of 45-60 years, 9(15%) of cases were in the age group of above 60. (Table 3)

**Table 3 : AGE DISTRIBUTION OF CASES**

| S. No | Age | Number of cases (60) | Percentage |
|---|---|---|---|
| 1 | <45 years | 31 | 52% |
| 2 | 45-60 years | 20 | 33% |
| 3 | >60 years | 9 | 15% |
| | Total | 60 | 100% |

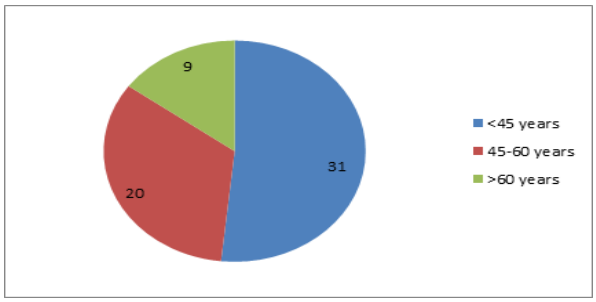


**TABLE 4:AGE DISTRIBUTION AMONG THE GROUPS:**

| S.NO | Age of the patient | Cyclical endometrium | | Endometrial Hyperplasia | Endometrial carcinoma |
|---|---|---|---|---|---|
| | | Proliferative phase | Secretory phase | | |
| 1 | <45 years | 8 (80%) | 10 (100%) | 12 (48%) | 1 (7%) |
| 2 | 45-60 years | 2 (20%) | - | 12 (48%) | 6 (40%) |
| 3 | >60 years | - | - | 1 (4%) | 8 (53%) |
| | Total | 10(100%) | 10(100%) | 25(100%) | 15(100%) |

The age distribution among the cases are statistically significant with mean values of 57 years for endometrial carcinoma cases, 48 years for simple hyperplasia, 39 years for complex hyperplasia without atypia and 41 years for complex hyperplasia with atypia.

**Expression of bcl-2 in various endometrial lesion :**

Out of 60 cases taken in the study, 16 cases shows negativity (27%), 27 cases shows weak positivity (45%) and 17 cases shows strong positivity (28%).

In proliferative phase total number of cases taken was 10, out of which 5 cases showed weak positivity (50%) and 5 cases shows strong positivity (50%). None of the proliferative endometrium was immunonegativefor bcl 2. They showed equal distribution for weakly positive staining (n = 5) and strongly positive staining (n = 5).

In secretory phase, 10 cases were studied, out of which 6 cases showed negativity (60%) and 4 cases showed weak positivity (40%) , there are no strong positive cases in the secretory phase. It can be concluded that secretory phase of endometrium is more prone to immonegativity for bcl 2.

In cases of endometrial hyperplasia, 25 cases were studied, out of which 6 cases showed negativity (24%), 9 cases showed weak positivity (36%), 10 cases showed strong positivity (40%). In endometrial carcinoma, 15 cases were studied, out of which 4 cases were negative (27%), 9 cases were weakly positive (60%) and 2 cases were strongly positive (13%). (Table 4)

**Table 4: Expression of bcl-2 in various endometrial lesion**

| s.no | Endometrial lesion | Sample size | Negative (0) | 1+ | 2+ |
|---|---|---|---|---|---|
| 1 | Proliferative phase | 10 | - | 5(50%) | 5(50%) |
| 2 | Secretory phase | 10 | 6(60%) | 4(40%) | - |
| 3 | Hyperplasia | 25 | 6(24%) | 9(36%) | 10(40%) |
| 4 | Endometrial carcinoma | 15 | 4(27%) | 9(60%) | 2(13%) |
| | Total | 60 | 16 | 27 | 17 |

**Expression of bcl-2 in hyperplasia:**

Out of 25 endometrial hyperplasia studied, 6 cases showed negativity (24%) for Bcl-2, 9 cases showed weak positivity (36%) and 10 cases showed strong positivity(40%).

In simple hyperplasia without atypia, 15 cases were taken out of which 3 cases showed negativity (20%), 7 cases showed weak positivity (47%) and 5 cases (33%) shows strong positivity.

In complex hyperplasia without atypia, 5 cases were taken for the study, out of which 2 case show negativity (40%) , 1 cases showed weak positivity (20%) and 2 cases showed strong positivity (40%).

In complex hyperplasia with atypia, 5 cases were studied out of which 1 case showed weak positivity (20%) and 3 cases showed strong positivity (60%). There was one immunonegative cases (20%) in the complex hyperplasia with atypia. (Table 5)

**Images of cyclical Endometrium and Endometrial Hyperplasia: (H&E, Bcl-2)**

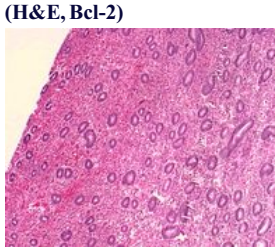

Figure 1A: proliferative endometrium (H&E) in scanner view (4X)

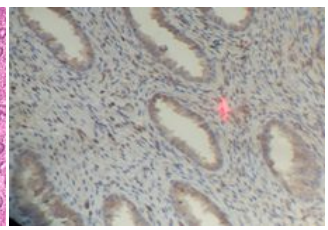

Figure 1B : proliferative endometrium showing Bcl-2 expression In low power view (10X)

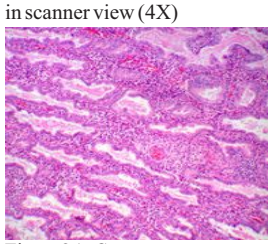

Figure2A: Secretory Endometrium (H&E) in 10X

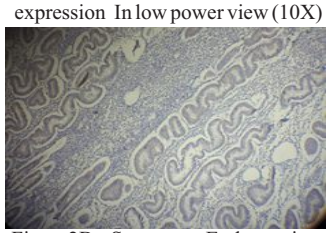

Figure2B: Secretory Endometrium showing Bcl-2 expression in 10X

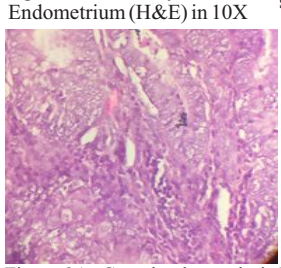

Figure 3A: Complex hyperplasia with atypia in 40X (H&E)

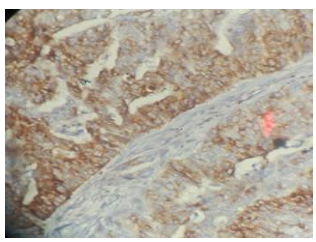

Figure 3B: Complex hyperplasia with atypiashowing Bcl-2 expression in 40X

**Table 5: Expression of bcl-2 in hyperplasia**

| s.no | Type of endometrium | Sample size | Negative | 1+ | 2+ |
|---|---|---|---|---|---|
| 1 | Simple hyperplasia without atypia | 15 | 3 (20%) | 7 (47%) | 5 (33%) |
| 2 | Complex hyperplasia without atypia | 5 | 2 (40%) | 1 (20%) | 2 (40%) |
| 3 | Complex hyperplasia with atypia | 5 | 1 (20%) | 1 (20%) | 3 (60%) |
| | Total | 25 | 6(24%) | 9(36%) | 10(40%) |

## DISCUSSION:

In this present study, a total of 60 cases of endometrial samples were studied. Immunohistochemistry expression on Bcl-2 was evaluated based on proportion of positivity of glandular cells and the intensity of the staining. From the given 60 cases, cyclical endometrium consisted the majority (n = 20), out of which half the cases (n = 10) comprised the proliferative pattern of endometrium and the other half being the secretory pattern (n = 10). Endometrial hyperplasia represented 25 cases (n = 25) which encompassed the following pathologic entities such as simple or complex hyperplasia with / without atypia. In previous studies, it had been found that the IHC expression of bcl 2 does not vary between these entities of hyperplasia to merit them with individual groups of its own. The other end of the spectrum, endometrial carcinoma comprised of 15 cases (n = 15), which again encompassed endometrial carcinomas of Type I as classified by the WHO. Bcl-2 expression was evaluated on the following - the glandular cells showing cytoplasmic staining were considered to be positive which also included uniformity of the staining pattern, otherwise it was not included in the criteria of positivity and the stromal cells also showed weak staining, which did not alter the results in this study since the major criteria of the pathologic entity included in this study deals with the pathologic alterations in the endometrial glands.Out of 60 cases taken in the study, 16 cases shows negativity (27%), 27 cases shows weak positivity (45%) and 17 cases shows strong positivity (28%). In proliferative phase, they showed equal distribution for weakly positive staining (n = 5) and strongly positive staining (n = 5) for bcl 2, which correlates with the previous study by Gompel et al1 , Mertens HJMM et al[9], Vaskivuo et al[10]. In secretory phase, 10 cases were studied, out of which 6 cases showed negativity (60%) and 4 cases showed weak positivity (40%) ,all the positive cases were in the early secretory phase and there are no strong positive cases in the secretory phase which also correlates with the study by Gompel e[1] al1,Mertens HJMM et al[9], Vaskivuo et al[10]. It can be concluded that secretory phase of endometrium is more prone to immune negativity for Bcl-2.

**Images of Endometrial Carcinoma: (H&E, Bcl-2)**

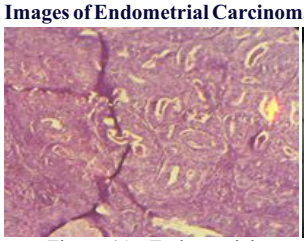

Figure 4A : Endometrial carcinoma(H&E) in 40X

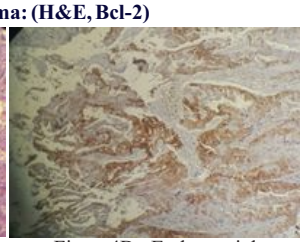

Figure4B : Endometrial carcinoma showing Bcl-2 expression in 40X

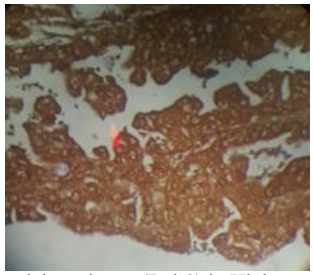

Figure5: Endometrial carcinoma(Bcl-2) in High magnification (40X)

In cases of endometrial hyperplasia, 25 cases were studied, out of which 6 cases showed negativity (24%), 9 cases showed weak positivity (36%), 10 cases showed strong positivity (40%). Immunopositivity for Bcl-2 in endometrial hyperplasia shows the tendency for strong expression of the aforementioned marker. Out of 25 endometrial hyperplasia taken for the study, 6 cases shows negativity (24%) for Bcl-2, 9 cases shows weak positivity (36%) and 10 cases shows strong positivity(40%). In simple hyperplasia without atypia, 15 cases were taken out of which 3 cases show negativity (20%), 7 cases shows weak positivity (47%) and 5 cases (33%) shows strong positivity. In complex hyperplasia without atypia, 5 cases were taken for the study, out of which 2 case show negativity (40%), 1 cases shows weak positivity (20%) and 2 cases showed strong positivity (40%), both simple and complex hyperplasia values does correlate with previous study by Mohamed Laban et al[7]. Signifying the strong positivity increase from simple hyperplasia to complex hyperplasia without atypia to with atypia which concurrent with study by Mohamed Laban et al[7] . In complex hyperplasia with atypia, 5 cases were studied out of which 1 cases show weak negativity (20%) and 3 cases showed strong positivity (60%). There was one immunonegative cases (20%) in the complex hyperplasia with atypia.

In endometrial carcinoma, 15 cases were studied, out of which 4 cases were negative (27%), 9 cases were weakly positive (60%) and 2 cases were strongly positive (13%) which correlate with the study by Mohamed Laban et al[7] and does not correlate with the study by Theodore H.Niemann et al[8]. This states the obvious fact that in the presence of action of estrogen stimulation which is primarily unopposed, leads to the pathological entity of endometrial hyperplasia by the increased inhibition of apoptosis as Bcl-2 is under the effect of oestrogen. As the spectrum of the hyperplasia progresses from hyperplasia without atypia to hyperplasia with atypia, it can be postulated that it is primarily due to an increased anti apoptotic activity followed by an inevitable increased proliferation.

## CONCLUSION:

Bcl-2 expression was high in the proliferative endometrium as expected due to anti-apoptotic activity in the this phase due to proliferation of glands under the influence of estrogen. In hyperplasia, Bcl-2 strong positive expression is increased in ascending order of frequency from simple hyperplasia to complex hyperplasia without atypia and to complex hyperplasia with atypia[6]. This is as a result of unopposed effect of the estrogen in the hyperplastic endometrial tissue. Above findings of this study reveals that Bcl-2 can be used as a potential therapeutic target in Bcl-2 positive endometrial hyperplasia patients, in treating Abnormal Uterine Bleeding and halt it's progression to carcinoma.

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