

Immunohistochemical Expression of Bcl-2 and Ki-67 in Non-neoplastic and Neoplastic Endometrial Lesions- A cross-sectional study

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Abstract

Introduction Dynamic morphological changes occur in the female endometrium during the menstrual cycle. To keep tissue homeostasis, there should be a balance between apoptotic and mitotic activity in the normal menstrual cycle. Ki-67 is a known marker of cell mitotic activity, while Bcl-2 is an anti-apoptotic gene that controls apoptosis. When an imbalance in expression occurs, it is indicative of tumorigenesis.

This study was conducted with an aim to correlate the immunohistochemical expression of Bcl-2 and Ki-67 in non-neoplastic and neoplastic endometrial lesions with histomorphological features.

Objectives: The objective of the study was to classify non-neoplastic and neoplastic endometrial lesions on the basis of histomorphology and to correlate Bcl-2 and Ki-67 expression in non-neoplastic and neoplastic endometrial lesions.

Methodology: A cross-sectional study was carried out in a tertiary center, Bareilly, UP, for one year, which included 60 histopathologically confirmed cases of non-neoplastic and neoplastic endometrial lesions. A tissue microarray was prepared. Further, immunohistochemistry was then applied as per standard protocol. Calculations were made regarding staining percentage and intensity.

Results: In non-neoplastic endometrial lesions, Ki-67 showed the highest mean score in proliferative endometrium (8.5). In neoplastic endometrial lesions, endometrial carcinoma (11.5) had a substantially higher Ki67 mean score than hyperplasia. In non-neoplastic endometrial lesions, Bcl2 showed a higher mean score in endometrial polyp (10), and in neoplastic endometrial lesions, nonatypical hyperplasia (11.4) had higher mean score and least in endometrial carcinoma (8).

Conclusion: It has been discovered that Bcl-2 and Ki-67 are accurate indicators of disease progression and may provide a new indication for treatment and monitoring.

Keywords: Bcl-2; Ki-67; Hyperplasia; Endometrium

1. Introduction

The endometrium has a remarkable potential for regeneration and responds to an ovarian hormone in a cyclical pattern, causing monthly menstruation.¹ To keep tissue homeostasis, there should be an inhibitory mechanism to balance the growth, which is apoptosis or programmed cell death, to control the menstrual cycle. Hence, apoptosis, or programmed cell death, is essential for controlling the menstrual cycle.¹

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The endometrial lesions are divided into non-neoplastic and neoplastic conditions, in which non-neoplastic conditions include proliferative, secretory, atrophic, endometritis, endometrial polyp, disordered proliferation, and neoplastic conditions have a range of benign, premalignant, and malignant neoplasms of the endometrium. endometrial hyperplasia and endometrial carcinoma.² Now, endometrial hyperplasia is classified into non-atypical hyperplasia and atypical hyperplasia also known as EIN (endometrial intraepithelial neoplasia).¹

Bcl-2 family plays a significant part in the process of apoptosis, often known as programmed cell death. One of them, a proto-oncogene called Bcl-2, prolongs cell life by inhibiting apoptosis. Hence, it suggests proliferation. The discrepancy between proliferation and apoptosis plays an important role in the emergence of endometrial lesions such as hyperplasia, polyps, and carcinoma.² The Bcl2 family has more than 20 members, and the majority of them mediate apoptosis. The main members of proteins are Bcl-2, Bcl-x, and Mcl-1, which are involved in inhibiting apoptosis.¹

The second marker for our study is Ki-67, which is a nuclear protein, commonly known as pKi-67, and is produced by the MKI67 gene. It only occurs in proliferating cells and is directly associated with cellular growth.³

The role of Bcl-2 and Ki-67 expression in various malignancies has been well studied but the available literature for endometrial lesions is limited and also shows heterogeneous and inconsistent results, so this study aids in the identification of novel Bcl-2 marker with Ki-67 marker to select patients for targeted therapy and improve treatment plans.

The aim of the study was immunohistochemical expression of Bcl-2 and Ki-67 in non-neoplastic and neoplastic endometrial lesions

2. Material and methods

The study was cross-sectional carried out in Department of Pathology, Rohilkhand medical college and hospital Bareilly, UP for the duration of one year. All histopathologically confirmed cases of non-neoplastic and neoplastic endometrial lesions were included in this study.

3. Methodology

This study was conducted at tertiary care center after taking approval from the Institutional Ethics Committee (IEC) and informed consent from the patient. The specimens were fixed in 10% buffered formalin and subjected to gross and microscopic examination of H&E slides, and a tissue microarray was prepared. Further Immunohistochemistry was then applied as per standard protocol. Calculations were made regarding staining percentage and intensity. The positive number of cells for Bcl-2 expression was counted in 100 epithelial cells and repeated in 10 HPFs.⁴

- Grade 0 – Absent
- Grade 1 –Mild
- Grade 2 – Moderate
- Grade 3 – Strong
- Grade 4 – Very strong

Positive control: Tonsil was used as a positive control for Bcl-2 whose staining was always grade 4.⁴

The positive number of cells for Ki-67 expression were counted in 100 epithelial cells and repeated in 10 HPFs and then positive cells for Ki-67 expressions were calculated using the percentage of 1000. These cells were graded on the basis of their positivity as follows.⁴

- <5% - Grade 0
- 5-25% - Grade 1
- 25-50% - Grade 2
- 50- 75% - Grade 3
- 75-100% - Grade 4

Positive control: Tonsil was taken as a control for Ki-67 expression.¹

Weighted score=Percentage of Positive cells (PP%)×Staining intensity(SI).

The proportion score was always maintained at grade 4 since the Bcl-2 staining was consistent across all glandular epithelial cells. Since cells were often strongly stained by Ki-67, grade 4 was maintained for the intensity score. Therefore, 4 was maintained constant for both Bcl-2 and Ki67 so the weighted score for Bcl-2 was based on intensity, whereas Ki-67 was based on percentage. The score was obtained for both Bcl2 and Ki67 by multiplying by percentage and staining intensity i.e. $4 \times \text{grade} = \text{score}$

- $4 \times 0 = \text{score } 0$
- $4 \times 1 = \text{score } 4$
- $4 \times 2 = \text{score } 8$
- $4 \times 3 = \text{score } 12$
- $4 \times 4 = \text{score } 16$

These scores were added, and the result was divided by the sample size to determine the mean score.⁴

4. Results

In the study, 60 cases of endometrial lesions were included. Out of 60 cases, 41 cases (68.33%) of non-neoplastic lesions and 19 cases (31.67%) of neoplastic lesions were included. Out of eight cases of endometrial cancer in this study, six (75%) were well-differentiated, with two (25%) being poorly differentiated.

In this study, Ki67 for the non-neoplastic lesion, maximum Ki67 expression was seen in proliferative endometrium (100%), followed by disordered proliferation (66.6%), and in neoplastic endometrial lesions, Ki-67 expression was 85.7% cases in nonatypical hyperplasia and 100% positivity showed in atypical hyperplasia and carcinoma.

In non-neoplastic endometrial lesions, the highest score of Ki67 was seen in proliferative endometrium (8.5), followed by disordered proliferation (3.5). In neoplastic endometrial lesions highest score of Ki67 was seen in carcinoma (11.5), followed by atypical hyperplasia (8), and the least expression was seen in nonatypical hyperplasia (5.1).

Table 1 Expression of Ki67 in Various Nonneoplastic and Neoplastic Endometrial Lesions.

S.no	Types of endometrial lesions	No. of cases (n=60)					Total no of Positive cases (%)	Mean score
		Negative	Positive					
			Score 4	Score 8	Score 12	Score 16		
1	Proliferative endometrium (n=8)	0	3	2	2	1	100%	8.5
2	Secretory endometrium (n=8)	3	5	0	0	0	62.5%	2.5
3	Atrophic endometrium (n=6)	4	2	0	0	0	33.3%	1.3
4	Endometritis (n=6)	3	3	0	0	0	50%	2
5	Endometrial polyp (n=4)	2	2	0	0	0	50%	2
6	Disordered proliferation (n=9)	3	4	2	0	0	66.6%	3.5
7	Non atypical endometrial hyperplasia (n=7)	1	3	3	0	0	85.7%	5.1
8	Atypical hyperplasia (n=4)	0	2	1	0	1	100%	8
9	Endometrial carcinoma (n=8)	0	0	3	3	2	100%	11.5
	Total	16	24	11	5	4	-	-

In our study, both neoplastic and non-neoplastic cases showed positivity for Bcl-2 in 100% cases with different intensities, except secretory endometrium (62.5%) and atrophic endometrium (66.6%). In nonneoplastic endometrial

lesions, the mean score of Bcl-2 expression was highest in endometrial polyps (10), followed by disordered proliferation (9.3%), and proliferative phase (8.5). In neoplastic endometrial lesions, Bcl2 showed maximum expression in nonatypical hyperplasia (11.4), followed by atypical hyperplasia (9), and slightly decreased in endometrial carcinoma (8).

Table 2 Expression Of Bcl2 in Various Nonneoplastic and Neoplastic Endometrial Lesions.

S.no	Types of endometrial lesions	No. of cases (n=60)					Total no of Positive cases (%)	Mean score
		Negative	Positive					
			Score 4	Score 8	Score 12	Score 16		
1	Proliferative endometrium (n=8)	0	3	2	2	1	100%	8.5
2	Secretory endometrium (n=8)	3	2	3	0	0	62.5%	4
3	Atrophic endometrium (n=6)	2	2	0	2	0	66.6%	5.3
4	Endometritis (n=6)	0	3	2	1	0	100%	6.6
5	Endometrial polyp (n=4)	0		2	2	0	100%	10
6	Disordered proliferation (n=9)	0	2	3	3	1	100%	9.3
7	Non atypical endometrial hyperplasia (n=7)	0	1	1	3	2	100%	11.4
8	Atypical hyperplasia (n=4)	0	1	1	2	0	100%	9
9	Endometrial carcinoma (n=8)	0	3	3	1	1	100%	8

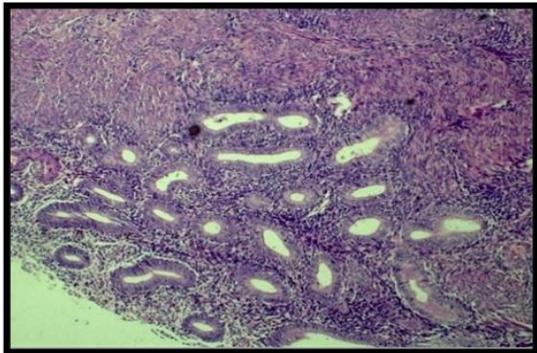


Figure 1a H&E (100X)

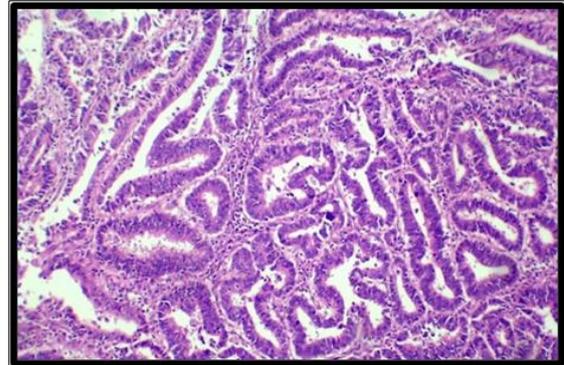
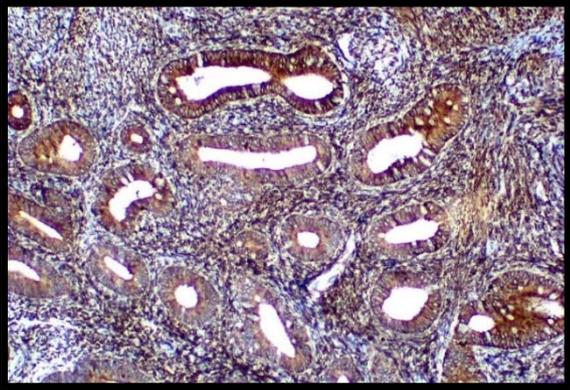
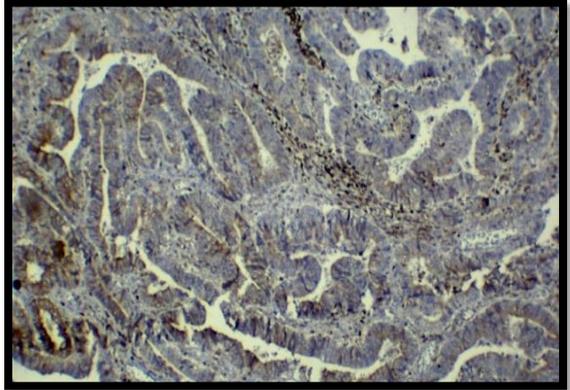
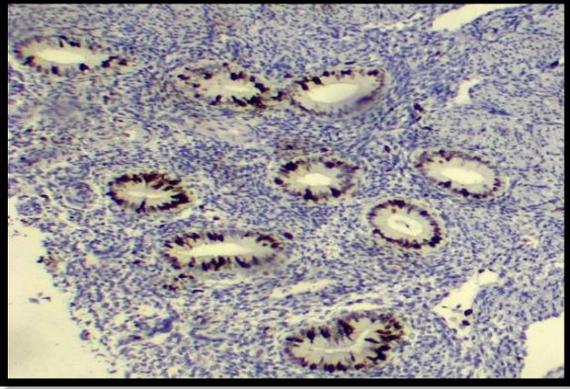
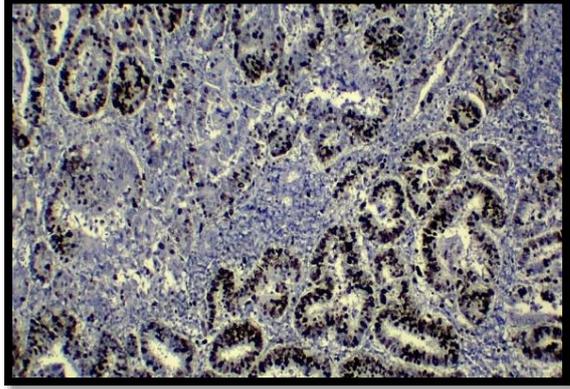


Figure 2a H&E (100X)

	
<p>Figure 1b Bcl2 grade 3 (IHC 100X)</p>	<p>Figure 2b Bcl2 grade 2 (IHC 100X)</p>
	
<p>Figure 1c Ki67 grade 1 (IHC:100X)</p>	<p>Figure 2c Ki67 grade 2 (IHC 100X)</p>
<p>Figure 1 Proliferative Endometrium (H&E AND IHC)</p>	<p>Figure 2 Well-differentiated Endometrial carcinoma (H&E AND IHC)</p>

5. Discussion

Visualisation of mitosis, apoptosis, and necrosis in endometrium is mostly based on morphology, but can also include immunohistochemistry or DNA labelling. Ki-67 reactivity leads to proliferation, but bcl-2 expression prevents apoptosis.⁵

In our study the mean Bcl2 score was 8.5 in proliferative, and 4 in secretory phase whereas the mean score of Ki67 was 8.5 in proliferative and 2.5 in secretory phase, thus both Bcl2 and Ki67 expression was high in proliferative phase and was mildly expressed in secretory endometrium similar study was conducted by Shalini P et al¹ analysed 50 endometrial samples and concluded that Bcl2 and Ki67 were increased in proliferative phase endometrium and decreased in secretory phase endometrium. This confirms earlier results that estrogen causes an increase in Bcl-2 expression at the start of the menstrual cycle (proliferative) endometrium, whereas progesterone reduces and promotes apoptosis towards the end of the menstrual cycle (secretory) endometrium.⁶

In the present study, the Bcl2 mean score of endometrial polyps was 10, and the Ki67 mean score was 2, which is similar to our study conducted by Taylor L J et al⁷ showed that Bcl-2 expression was significantly higher in the glandular epithelium of endometrial polyps than in proliferative endometrium.

We found that the Bcl2 and Ki67 mean score of disordered proliferation was 9.3 and 3.5, respectively, according to our study. Thus, a similar study was conducted by Bhati S et al⁸ that Bcl2 was more in disordered proliferative lesions as compared to Ki67.

In our study, we found the mean score of Bcl-2 and Ki67 was highest in hyperplasia, 10.54 and 6.18, followed by disordered proliferative endometrium, 9.3 and 3.5, respectively. A study conducted by Saranya N et al⁹ stated that the mean score of Bcl2 and Ki67 was highest in hyperplasia, 11.27 and 8.72, as compared to disordered proliferation, 8.57 and 8, respectively. This was similar to our study. This also suggests that in hyperplasia, antiapoptotic activity and cell survival were elevated.

The findings of present study suggest that Ki67 expression was higher in atypical hyperplasia (8) as compared to nonatypical hyperplasia (5.1) and Bcl2 mean score was higher in nonatypical hyperplasia (11.4) as compared to atypical hyperplasia (9) which was similar to our study. Shalini P et al¹ found that higher levels of Ki-67 expression seen in atypical hyperplasia (32.4) than hyperplasia without atypia (25.53) and Bcl2 mean was higher in hyperplasia without atypia (48.4) followed by atypical hyperplasia (29.6).

According to our study, we found that Ki67 was 100% positive in all cases of endometrial carcinoma. Ki67 expression was significantly higher in carcinoma as compared with all other endometrial lesions, which was similar to this study. Gurda GT et al¹⁰ found that the Ki-67 index increased gradually from 2.6% in Secretory endometrium to 17% in nonatypical hyperplasia, and 60% in endometrial cancer, respectively. This shows that Ki67 was higher in endometrial carcinoma.

This study demonstrates that Bcl2 showed 100% positivity in all endometrial carcinoma in different intensities. Bcl2 mean score of hyperplasia and endometrial carcinoma was 10.5 and 8, respectively. In our study, this showed that Bcl2 was high in hyperplasia as compared to endometrial carcinoma. Similar study was concluded by Risberg B et al¹¹ in which they discovered that endometrial hyperplasia had greater Bcl2 expression than carcinoma; thus these studies were concordant with this study.

6. Conclusion

The significance of Ki67 and Bcl2 as biomarkers in distinguishing between neoplastic and non-neoplastic endometrial lesions is highlighted by this study. Their patterns of expression help with targeted therapy, risk assessment, and early identification by revealing data about the biological behaviour of these lesions. When statistical validation and analysis are combined, these indicators become essential resources for improving clinical applications and research on endometrial disease.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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