Original Article

Pathology Section

Expression of Bcl-2 and Ki-67 in Endometrial Hyperplasia and Disordered Endometrium: A Cross-sectional Study

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ABSTRACT

Introduction: Endometrial hyperplasia affects both premenopausal and postmenopausal women and is a pathological condition that ranges from mild, reversible glandular proliferations to direct precursors of cancer. B-cell Lymphoma 2 (Bcl-2), an antiapoptotic gene involved in the regulation of apoptosis, and Ki-67, a proliferation marker, serve as indicators for cell proliferation from endometrial hyperplasia to endometrial carcinoma.

Aim: To examine the expression of Bcl-2 and Ki-67 in endometrial hyperplasia and disordered endometrium.

Materials and Methods: A cross-sectional study was conducted in the Department of Pathology at MVJ Medical College and Research Hospital, located in Hoskote, Karnataka, India, over a period of two years, from September 2020 to September 2022. All endometrial samples and hysterectomy specimens were included. Immunohistochemical staining was performed using Bcl-2 and Ki-67 in disordered proliferative endometrium and endometrial hyperplasia, and the staining and intensity were graded. Statistical analysis was performed, with qualitative variables presented as percentages and quantitative variables presented as Mean±Standard Deviation (SD). A p-value <0.05 was considered statistically significant.

Results: The mean age of the study participants was 49.36 years. A total of 33 cases were included in the study, with 15 cases of disordered proliferative endometrium and 18 cases of endometrial hyperplasia. Bcl-2 expression in the 15 cases of disordered proliferative endometrium showed a score 4 positivity in 2 (13.3%) cases, score 8 positivity in 3 (20%) cases, and score 12 positivity in 10 (66.7%) cases, with a mean score of 10.13. Ki-67 expression in the 15 cases of disordered proliferative endometrium showed a score 4 positivity in 10 cases (66.7%), score 8 positivity in 4 (26.7%), and score 12 positivity in 1 (6.67%) case, with a mean score of 5.6.

Conclusion: The overexpression of Bcl-2 in endometrial hyperplasia is considered to be an indicative of progression towards frank malignancy. Ki-67, the proliferation marker, is used to determine the progression of endometrial hyperplasia to carcinoma.

INTRODUCTION

Abnormal Uterine Bleeding (AUB) is the most common cause of gynaecological evaluation. Endometrial hyperplasia affects both pre and postmenopausal women, accounting for approximately 15% of cases of AUB. Upto 5% to 15% of hyperplasia without atypia and more than 30% of atypical hyperplasia progress to carcinoma [1,2]. Disordered endometrium is due to a hormonal imbalance with increased levels of oestrogen and low levels of progesterone, commonly encountered in pre and postmenopausal women. This hormonal imbalance eventually precedes the development of endometrial hyperplasia and endometrial carcinoma [3]. In India, the estimated incidence of endometrial carcinoma is 4.3 per 100,000 women.

Apoptosis acts as an inhibitory mechanism to counterbalance excessive proliferation and maintain endometrial tissue homeostasis [1]. Bcl-2 is an antiapoptotic gene involved in the regulation of apoptosis, and Ki-67 is a recognised indicator of cell mitotic activity [1]. The delicate balance between apoptosis and mitotic activity is necessary for the tissue homeostasis of normal endometrium [3]. The present study aimed to investigate the expression of Bcl-2 and Ki-67 in disordered proliferative endometrium and endometrial hyperplasia. The study is needed to prevent the progression of endometrial hyperplasia and disordered proliferative endometrium to endometrial carcinoma and also for early diagnosis and treatment.

MATERIALS AND METHODS

A cross-sectional study was conducted in the Department of Pathology at MVJ Medical College and Research Hospital, located in Hoskote, Karnataka, India, over a period of two years, from September 2020 to September 2022. Biopsies and hysterectomy specimens received from the Obstetrics and Gynaecology Department and diagnosed as disordered proliferative endometrium and endometrial hyperplasia on histopathology were included in the study. The study was approved by the Institutional Ethics Committee (IEC) under letter number IEC Reg. No MVJMC&RH/PG/Synopsis/49/2020-21. A total of 33 cases were included, with 15 cases of disordered proliferative endometrium and 18 cases of endometrial hyperplasia.

Inclusion criteria: All endometrial samples and hysterectomy specimens were included. Disordered proliferative endometrium, hyperplasia without atypia, and atypical hyperplasia of all age groups were included in the study.

Exclusion criteria: Cases with scanty endometrium and inadequate fixation were excluded from the study.

Methodology: Hyperplasia was classified based on the recent 2020 World Health Organisation (WHO) classification of tumours of the Female Genital Tract (FGT) [4]. Bcl-2 and Ki-67 staining were performed on all cases using the avidin-biotin peroxidase method. Antigen retrieval was done using a pressure cooker for 15-20 minutes in Tris Ethylenediamine Tetraacetic Acid (EDTA) buffer (pH 9). Endogenous peroxidase blocking was performed by adding Hydrogen Peroxide (H_2O_2) to the section for five minutes. Evaluation of Bcl-2 (clone 124) and Ki-67 (clone KS5) (PathInsitu) was done as the primary antibody, and a high-reactive polymer was used as the secondary kit. The evaluation was done manually by counting at

least 100 cells in 10 high-power fields. A section from the tonsil was used as a control for both Bcl-2 and Ki-67 expression [5,6].

Evaluation of Bcl-2 staining: Brown staining of the cytoplasm in glandular cells is considered as positive for Bcl-2.

Evaluation of Ki-67 staining: Brown staining of the nucleus is considered as positive for Ki-67.

Grading of Bcl-2 and Ki-67: The Percentage of Positive cells (PP) and Staining Intensity (SI) were used for scoring.

Scoring of PP%:

- 0 for <5%
- 1 for 5-25%
- 2 for 26-50%
- 3 for 50-75%
- 4 for >76%

Scoring of Staining Intensity (SI):

- 0: Absent staining
- 1: Weak
- 2: Moderate
- 3: Strong/intense staining
- 4: Very strong staining

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

The Bcl-2 stains uniformly in all glandular epithelial cells, so the proportion score is always kept as grade 4. Ki-67 usually stains cells with strong intensity, so the intensity score is kept as grade 4. Thus, for both Bcl-2 and Ki-67, 4 is kept constant. The mean score was calculated by adding the weighted score and dividing it by the sample size [5,6].

Mean score=total score/sample size.

STATISTICAL ANALYSIS

The collected data was entered into Microsoft Excel. Tables and charts will be generated using Microsoft word. Qualitative variables will be presented using percentages, while quantitative variables will be presented using mean scores.

RESULTS

A total of 33 endometrial samples were studied for Bcl-2 and Ki-67 expression. Among them, 45.45% (n=15) were disordered proliferative endometrium, A total of 16 (48.48%) cases were hyperplasia without atypia, and 2 (6.06%) were atypical hyperplasias. The study included 10 cases in the reproductive age group (18-40 years), 18 cases in the perimenopausal age group (41-50 years), and five cases in the postmenopausal age group (>50 years).

Regarding Bcl-2 expression, out of the 15 cases of disordered proliferative endometrium, 2 (13.3%) cases showed score 4 positivity, 3 (20%) showed score 8 positivity, and 10 cases (66.7%) showed score 12 positivity, with a mean score of 10.13. Among the 16 cases of hyperplasia without atypia, 2 (12.5%) cases, showed score 8 positivity, 11 cases (68.75%) showed score 12 positivity, and 3 (15.75%) showed score 16 positivity, with a mean score of 12.25. The two cases of atypical hyperplasia each showed 50% positivity in score 8 and 16, with a mean score of 10.0. The expression of Bcl-2 is shown in [Table/Fig-1,2a,b].

Regarding Ki-67 expression, the total 15 cases of disordered proliferative endometrium showed score 4 positivity in 10 cases (66.7%), score 8 positivity in 4 (26.7%) cases, and score 12 positivity in 1 (6.67%) case, with a mean score of 5.6. Among the 16 cases of hyperplasia without atypia, eight cases showed 50%
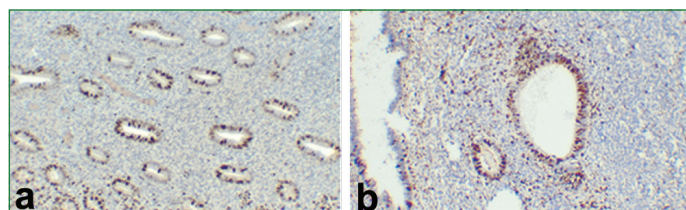
S. No.	Endometrial lesions	Sample size	Score 4 (<25%) n (%)	Score 8 (25-50%) n (%)	Score 12 (50-75%) n (%)	Score 16 (>75%)	Mean score
1)	Disordered proliferative endometrium	15	2 (13.3%)	3 (20%)	10 (66.7%)	-	10.13
2)	Hyperplasia without atypia	16		2 (12.5%)	11 (68.75%)	3 (18.75%)	12.25
3)	Atypical hyperplasia	2	-	1 (50%)	1 (50%)	-	10
	Total	33					

[Table/Fig-1]: Expression of BCL-2 in various endometrial lesions.
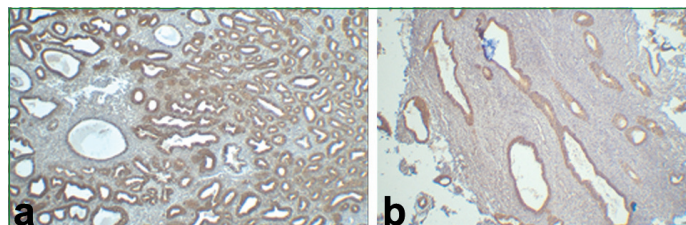
[Table/Fig-2]: a) Bcl-2 showing score 16 positivity in hyperplasia without atypia; b) Bcl-2 showing score 12 positivity in atypical hyperplasia (10x).

positivity in score 4, 5 (31.25%) cases showed score 8 positivity, and 3 (18.75%) showed score 12 positivity, with a mean score of 6.75. In atypical hyperplasia, two cases showed 100% positivity in score 12, with a mean score of 12.0. The expression of Ki-67 is shown in [Table/Fig-3a,b,4].

[Table/Fig-3]: a) Ki-67 nuclear positivity showing score 12 in hyperplasia without atypia (10x); b) Ki-67 positivity showing score 12 positivity in atypical hyperplasia (40x).

S. No.	Endometrial lesions	Sample size	Score 4 (<25%) n (%)	Score 8 (25-50%) n (%)	Score 12 (50-75%) n (%)	Score 16 (>75%)	Mean score
1)	Disordered proliferative endometrium	15	10 (66.7)	4 (26.7)	1 (6.67)	-	5.6
2)	Hyperplasia without atypia	16	8 (50)	5 (31.25)	3 (18.75)	-	6.75
3)	Atypical hyperplasia	2	-	-	2 (100)	-	12.0
	Total	33					

[Table/Fig-4]: Expression of Ki-67 in various endometrial lesions.

When comparing the mean scores of Bcl-2 and Ki-67 in disordered proliferative endometrium, the Bcl-2 score was two times higher than the Ki-67 mean score. Bcl-2 showed a two-fold higher score compared to Ki-67 in hyperplasia without atypia. Ki-67 mean score was higher than Bcl-2 in atypical hyperplasia.

DISCUSSION

A total of 33 endometrial samples were studied for Bcl-2 and Ki-67 expression. The spectrum of lesions included 15 (45.45%) of disordered proliferative cases, 48.48% (n=16) of hyperplasia without atypia, and 2 (6.06%) cases of atypical hyperplasia. A similar study conducted by Morsi HM et al., recorded a higher number of cases (107), with 6 (5.60%) cases being disordered proliferative endometrium and 20 (18.69%) being hyperplasia [7]. The normal endometrial cycle maintains a delicate balance

of proliferation and apoptosis to maintain tissue homeostasis. An imbalance between proliferation and apoptosis can lead to various endometrial lesions such as disordered proliferations and hyperplasia progressing to carcinomas. In the present study, authors evaluated the expression of Bcl-2 and Ki-67 markers to explore their potential diagnostic and prognostic applications in endometrial cancer.

Regarding Bcl-2 expression, present study showed a decreasing order of frequency, with the highest mean score observed in hyperplasia without atypia (12.25), followed by disordered proliferative endometrium (10.13), and least score seen in atypical hyperplasia (10.0). In the present study, disordered proliferative endometrium demonstrated a mean Bcl-2 score of 10.13, which was slightly lower than that of hyperplasia without atypia (12.25). This indicates that antiapoptotic activity and cell survival are increased in hyperplasia, consistent with previous studies conducted by Arjunan A et al., Shalini P et al., and Morsi HM et al., [5-7]. These findings suggest that the risk of progression from disordered proliferative endometrium to hyperplasia still persists [5-7].

The expression of Bcl-2 in hyperplasia without atypia showed a higher mean score of 12.25 compared to atypical hyperplasia (10.0). Similar observations were noted in studies conducted by Morsi HM et al., Kokawa K et al., and Nunobiki O et al., [7-9]. Laban M et al., observed an increase in apoptosis when progressing from normal endometrium to hyperplasia and further to cancer, while the antiapoptotic rate decreased when progressing to atypia [10].

Ki-67 expression: It was observed in a decreasing order of frequency, with the highest mean score seen in atypical hyperplasia (12.0), followed by hyperplasia without atypia (6.75), and least score recorded in disordered proliferative endometrium (5.6). The present study was consistent with the findings of studies conducted by Arjunan A et al., Shalini P et al., and Morsi HM et al., [5-7].

In disordered proliferative endometrium, the expression of Ki-67 showed a mean score of 5.6, which is lower than that of hyperplasia without atypia (6.75) and atypical hyperplasia (12.0). Comparison studies conducted by Morsi HM et al., Shalini P et al., and Arjunan A et al., also showed similar lower expression of Ki-67 in disordered proliferative endometrium compared to benign hyperplasias. Previous literature explains disordered proliferative endometrium as an intermediate stage between normal proliferative and benign hyperplastic endometrium, preceding the development of hyperplasias [5-7]. When comparing the Bcl-2 and Ki-67 staining, Bcl-2 showed descending expression from hyperplasia without atypia to atypical hyperplasia, with disordered proliferative endometrium in between. In contrast, Ki-67 staining showed increased expression in atypical hyperplasia compared to hyperplasia without atypia and disordered proliferative endometrium.

As hyperplasia progresses towards atypia, there is a decrease in apoptosis and an increase in proliferative activity. The present study aligns with previous studies conducted by Shalini P et al., and Arjunan A et al., where the Bcl-2 score diminishes from hyperplasia without atypia to atypical hyperplasia, and the Ki-67 score increases from hyperplasia without atypia to atypical hyperplasia. This suggests that there is reduced apoptosis and increased proliferative activity as hyperplasia advances towards atypia. Therefore, Bcl-2 and Ki-67 can be used as diagnostic markers to monitor the progression of endometrial hyperplasia into endometrial carcinoma [5,6]. Detailed information about the present study presented in [Table/Fig-5].

S. No.	Author	Place of study	Observation in disordered endometrium	Observation in hyperplasia without atypia	Observation in atypical hyperplasia
1	Present study (2022) 33 cases	Karnataka	Bcl-2- 66.7% (10)-score 12 Ki-67- 66.7% (10 cases)- score 4	Bcl-2- 68.75% (11 cases) score 12 Ki-67- 50% (8 cases)- score 4	Bcl-2- 50% (1 case) score 8 and 12 Ki-67- 100% (2 cases) positivity in score 12
2	Shalini P et al., (2021) 50 cases [6]	Chennai	-	Bcl-2- 33.3% (5 cases) - score 4 and score 12. Ki-67- 53.3 (8 cases)- score 4 40% (6 cases)- score 8	Bcl-2- 40% (6 cases)- score 4 and score 8. Ki-67- 46.6% (7 cases)- score 8 40% (6 cases)- score 4
3	Arjunan A et al., (2016) 50 cases [5]	Coimbatore	-	Bcl-2- 26.6% (8 cases)- score 4 23.33% (7 cases)- score 8 Ki-67- 40% (12 cases)- score 4	
4	Morsi HM et al., (2000) 107 cases [7]	Germany	Bcl-2- 50% (3 cases) score 8 and 12. Ki-67 mean percentage of 19.5%	Ki-67- 21.8% - complex hyperplasia (with a mean percentage of 19.8%)	-

[Table/Fig-5]: Observation of BCL-2 and Ki-67 in various studies [5-7].

Limitation(s)

The limitations of the present study included the lack of comparison between endometrial hyperplasia and disordered proliferative endometrium with normal endometrium and endometrial carcinoma.

CONCLUSION(S)

The expression of Bcl-2 decreases from hyperplasia without atypia to atypical hyperplasia, while Ki-67 expression increases from hyperplasia without atypia to atypical hyperplasia. This indicates a reduction in apoptosis and an increase in the proliferative index as hyperplasia progresses towards atypia. Therefore, Bcl-2 and Ki-67 can be useful markers for assessing disordered proliferative endometrium and endometrial hyperplasia that is progressing towards carcinoma. Consequently, Bcl-2 and Ki-67 markers can be considered as diagnostic and prognostic immunohistochemical markers.

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• Was informed consent obtained from the subjects involved in the study?   NA
• For any images presented appropriate consent has been obtained from the subjects.   NA