Original Research Article

Analysis of immunohistochemical markers (Bcl-2 and Ki-67) expression in various endometrial lesions among women presenting with abnormal uterine bleeding

ABSTRACT

Background: Recently, Abnormal uterine bleeding (AUB) is on the rise and the underlying mechanism has been associated with the proliferation and apoptosis of certain markers. This study assessed the expression of anti-apoptotic marker (Bcl-2) and proliferative marker Ki-67 in cyclical and abnormal endometrium..

Methods: This was a cross-sectional study conducted among women with the clinical history of AUB, adequate clinical details and adequate endometrial specimens received in the form of endometrial curettage and endometrial biopsy. Immunostaining for Bcl – 2 and Ki – 67 markers were done on cyclical and abnormal endometrial lesions followed by scoring based on number of cells and intensity of staining.

Results: About 200 specimens were included in this study. On IHC, the Bcl-2 expression was highest in proliferative phase with a mean value of 14.96, followed by endometrial carcinoma (13.71), Disordered proliferative endometrium (DOPE) (12.44) and mean of hyperplastic pattern was 10.04 with the lowest mean score of 3.47 in secretory phase. Ki-67 expression was highest in endometrial carcinoma with a mean value of 15.43, followed by hyperplastic pattern of 14.93, proliferative phase of 14.00 and mean value of DOPE was 10.04. The expression of Ki-67 was significantly decreased in secretory phase with a mean score of 2.67.

Conclusion: There was an increased expression of both Bcl-2 and Ki-67 in the proliferative phase while it was minimal in secretory phase in cyclical endometrium. When Bcl-2 and Ki-67 staining were assessed in abnormal endometrium, there was an increased proliferative activity and increased anti-apoptotic activity in disordered proliferative endometrium, hyperplasia and endometrial carcinoma.

***Keywords**: Abnormal uterine bleeding, Endometrial lesions, Immunohistochemistry, Bcl-2 and Ki-67 expression*

1. INTRODUCTION

In perimenopausal women, abnormal uterine bleeding (AUB) is the most prevalent cause of hysterectomy and one of the most common gynaecologic symptoms. It accounts for over

70% of all gynecological appointments in the peri- and post-menopausal age group [1]. AUB is signalized by many symptoms that includes heavy menstrual bleeding (HMB), inter-menstrual bleeding and the combination of both heavy and prolonged menstrual bleeding [2].

In 2011, the International Federation of Gynecology and Obstetrics (FIGO) has classified the aetiological factors into PALM (structural entities) and COEIN (non-structural entities) system [3]. Also, the need for proper evaluation in AUB is necessary to categorize the patients for appropriate management according to the cause and to exclude atypical hyperplasia and carcinoma [4]. Thereby, the management is aimed at the improvement of symptoms and to improve better quality of life in women.

Recently, AUB is on the rise and the underlying mechanism has been associated with the proliferation and apoptosis of certain markers [1]. The disparity between proliferation of cells and apoptosis is considered to be one of the underlying pathogenesis in both benign and malignant endometrial diseases. Hence, the evaluation of hormonal response in normal endometrium and benign, hyperplastic and malignant lesions of endometrium is significantly important in characterizing malignant potential [5]. Several studies have examined the role of apoptosis in both endometriosis and normal. Increased apoptosis in AUB has been reported by researchers, and it is thought to be a morphological indicator of aberrant endometrial development [1].

In this study, we determined the histopathological features of endometrium presenting with the history of abnormal uterine bleeding and the expression of anti-apoptotic marker Bcl-2 and proliferative marker Ki-67 in cyclical and abnormal endometrium.

2. METHODOLOGY

This was a cross-sectional study conducted at the Department of Pathology, SRM medical college and research centre, Tamil Nadu, India from March 2017 to June 2018. Patients with clinical history of AUB, adequate clinical details and adequate endometrial specimens received in the form of endometrial curettage and endometrial biopsy were included in this study. Endometrial curettage was done using the sharp edge of the curette and scrapings from anterior, posterior and lateral borders were obtained. Those who do not have adequate clinical details and autolysed tissue were excluded. This study was carried out after getting approval from the Institutional Scientific committee and Ethics Committee of registration no. 1136/IEC/2017. Written informed consent was obtained from all the patients included.

After conventional processing, two paraffin sections of 5 µm thickness were stained by H & E for histopathological study. In addition, 4 µm sections were cut from paraffin block tissue and taken on a glass slide coated with Aminopropyltriethoxysilane (APES) for immunostaining of Bcl – 2 and Ki – 67 markers on cyclical, DOPE, hyperplastic and malignant endometrial lesions were done and scoring was based on number and intensity of staining. All the slides were studied under low power and high power light microscopy (Leica DM750).

Statistical analysis was done by using SPSS software of version 17.0. The percentages and mean was used to represent categorical variables. Association between various histopathological patterns and expression of IHC markers (Bcl-2 and Ki-67) were assessed using ANOVA. The P value of less than 0.05 was considered as statistically significant.

3. RESULTS

In our study, among 200 cases, IHC was done on 170 cases by excluding endometrial polyp, atrophic endometrium, endometritis and pill endometrium. The IHC was done on cyclical, DOPE, hyperplastic and malignant lesions to evaluate the progression of disease stage. The expression of Bcl-2 in various endometrial lesions show a significant p value of 0.0001. Bcl-2 expression was highest in proliferative phase with a mean value of 14.96, followed by endometrial carcinoma (13.71), DOPE (12.44) and hyperplastic pattern of 10.25. The expression of Bcl-2 was significantly decreased in secretory phase with a mean score of 3.47 (Table 1). Furthermore, the expression of Bcl-2 in various endometrial lesions was shown in Figure 1.

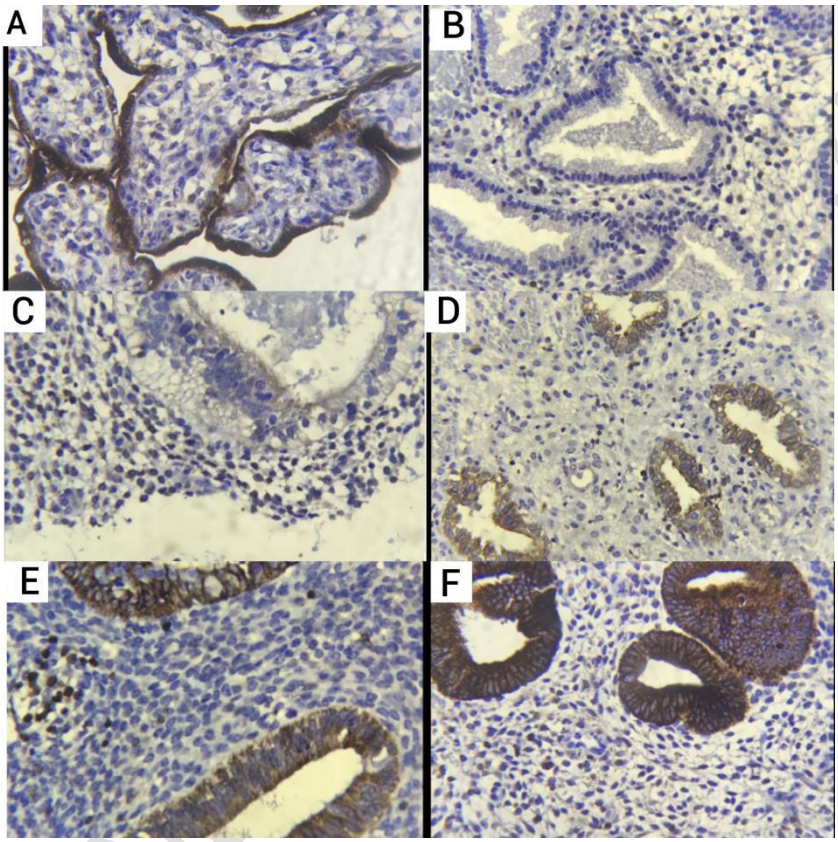

Figure 1. Bcl-2 expression showing A) Positive control Placental tissue B) (0) scoring of intensity C) (1+) scoring of intensity D) (2+) scoring of intensity E) (3+) scoring of intensity F) (4+) scoring of intensity (IHC, 400X)

Table 1. Expression of Bcl-2 in various endometrial lesions

Histopathological Diagnosis	N	Negative	Weighted Score				Mean Score	ANOVA	P value
			4	8	12	16			
Proliferative phase	46	0	0	1	10	35	14.96	48.089	0.0001
Secretory phase	30	14	6	10	0	0	3.47		

Disordered proliferative phase	27	0	1	4	13	9	12.44		
Hyperplasia	60	0	10	20	16	14	10.25		
Adenocarcinoma endometrium	7	0	0	0	4	3	13.71		

The expression of Ki-67 in various endometrial lesions show a significant p-value of 0.0001. Ki-67 expression was highest in endometrial carcinoma with a mean value of 15.43, followed by, hyperplastic pattern of 14.93, proliferative phase of 14 and DOPE of 13.78. The expression of Ki-67 was significantly decreased in secretory phase with a mean score of 2.67 (Table 2). In addition, the expression of Ki-67 in various endometrial lesions was shown in Figure 2. On comparing the mean score of Bcl-2 and Ki-67 in cyclical endometrium, Bcl-2 score is marginally increased than ki-67. In DOPE, hyperplasia and malignancy ki-67 score was significantly higher than Bcl-2 mean score (Figure 3).

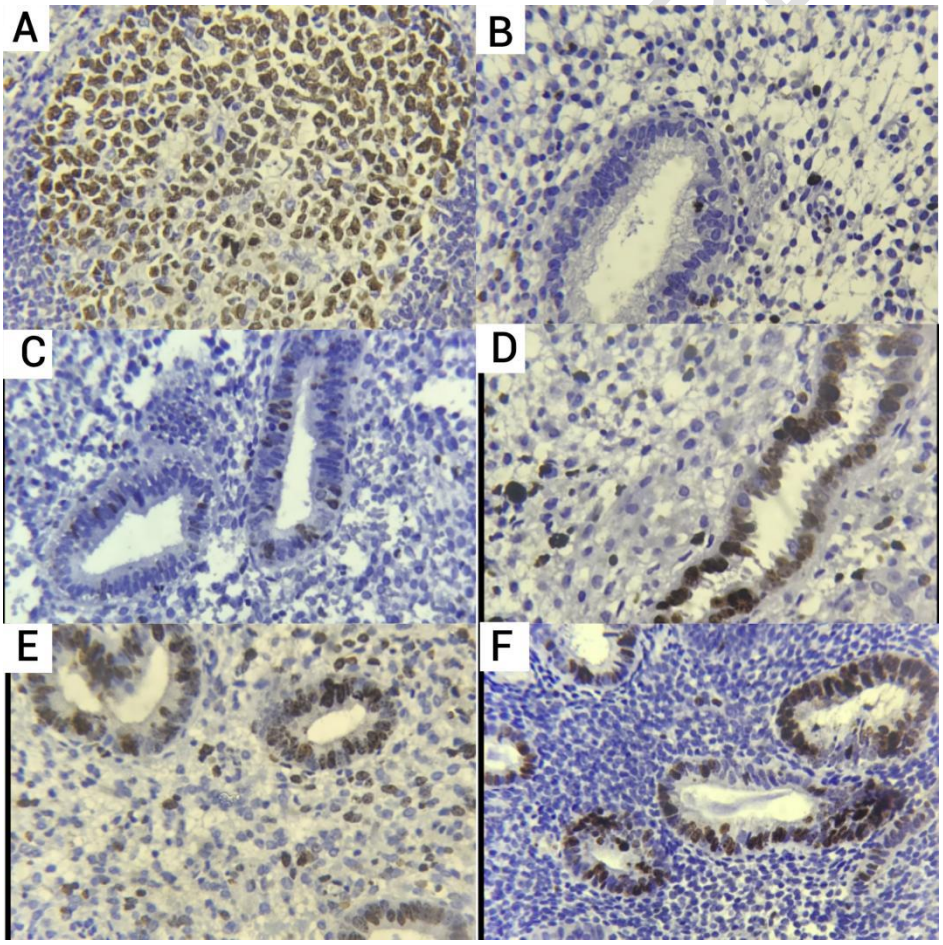

Figure 2. Ki-67 expression showing A) Positive control Tonsillar tissue B) (0) scoring of intensity C) (1+) scoring of intensity D) (2+) scoring of intensity E) (3+) scoring of intensity F) (4+) scoring of intensity (IHC, 400X)

Table 2. Expression of Ki-67 in various endometrial lesions

Histopathological Diagnosis	N	Negative	Weighted Score				Mean Score	ANOVA	P value
			4	8	12	16			
Proliferative phase	46	0	6	0	5	35	14	17.051	0.0001
Secretory phase	30	15	10	5	0	0	2.67		
Disordered proliferative phase	27	0	2	4	4	18	13.78		
Hyperplasia	60	0	0	0	16	44	14.93		
Adenocarcinoma endometrium	7	0	0	0	1	6	15.43		

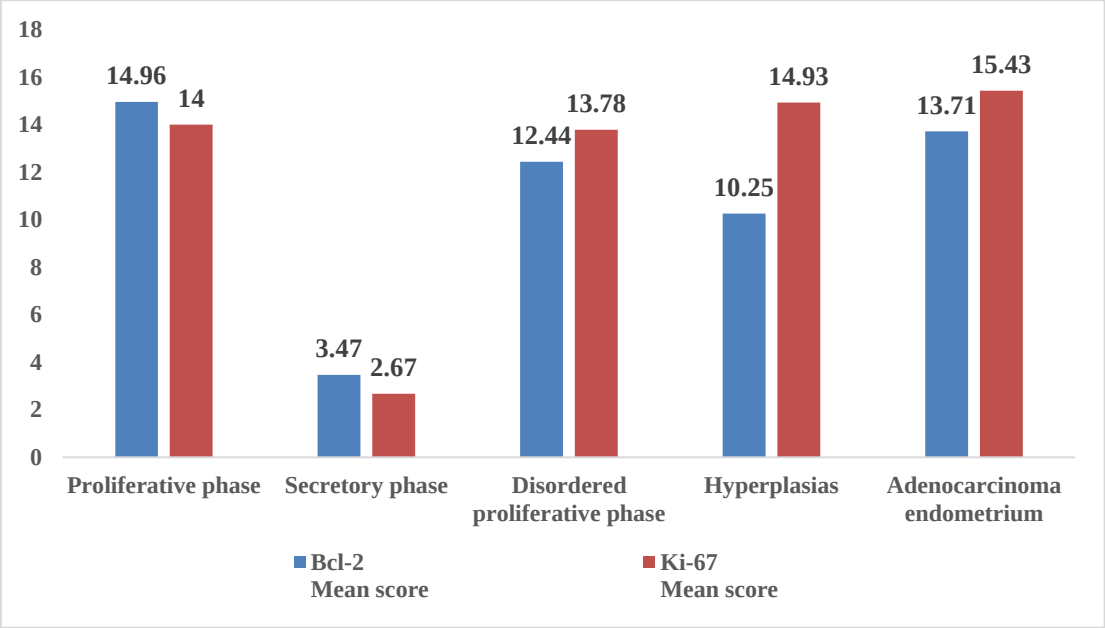

Figure 3. Comparison of Bcl-2 and Ki-67 in various endometrial lesions

4. DISCUSSION

In this study, IHC was performed among 170 cases after excluding endometrial polyp, atrophic endometrium, endometritis and pill endometrium. Further, the IHC was done on cyclical, DOPE, hyperplastic and malignant lesions to assess the progression of disease stage.

In cyclical endometrium, there was increased expression of both Bcl-2 and Ki-67 in the proliferative phase whereas, in secretory phase, Bcl-2 and Ki-67 showed mild expression that can be explained due to onset of progesterone production during the secretory phase and increased oestrogen stimulation during the proliferative phase. These observations are similar to the studies done by Arjunan A et al.[6], Mertens et al.[7] and Vaskivuo et al.[8]. On comparison, it was observed that Bcl-2 was slightly higher than Ki-67 in both proliferative and secretory phase. It was observed that in DOPE, the mean score of Bcl-2 was 12.44 that was slightly higher than hyperplasia (10.25%). This observation was not in concordance with previous studies [9,10], that can be explained due to prolonged oestrogen exposure. Also, the expression of Ki-67 was increased in DOPE that was almost equivalent to proliferative phase. This observation was in concordance with previous studies [9,10] and indicates that cell proliferation was also increased in disordered proliferative phase.

In hyperplasia and malignant endometrium, the Bcl-2 score showed increased expression in ascending order of frequency from hyperplasia without atypia to atypical hyperplasia and malignancy. This indicates that hyperplastic states which are under the influence of unopposed oestrogenic stimulation, have decreased apoptotic activity. In a recent study, it was stated that Bcl-2 protein loss appeared as a highly specific marker of endometrial precursor lesion, with high diagnostic accuracy [11]. Thus, the finding of Bcl-2 protein loss in endometrial hyperplasia might be a novel indication for treatment and follow-up, especially when precancerous features are ambiguous at histological examination. Ki-67 also showed similar pattern of expression. In endometrial carcinoma, there was increased expression than the other two states. This correlates with increased premalignant potential in hyperplasia without atypia and hyperplasia with atypia. These observations were in accordance with Arjunan A et al.[6] and Morsi HM et al.[10].

On comparing Bcl-2 staining and Ki-67 in DOPE, hyperplasia and malignancy, ki-67 expression was higher when compared to Bcl-2 staining. Thus indicating proliferative activity was higher than anti-apoptotic activity. This observation was similar to Apoustolou et al.[9] However, Bcl-2 expression was higher than Ki-67 expression as noted in Arjunan A et al.[6] This was in line with the findings of the previous studies [12, 13]. Although Bcl-2 family proteins play a crucial role in the regulation of the cell proliferation, it might eventually lead to the development of endometrial hyperplasia and possibly neoplasia [14].

The lack of comparison between endometrial hyperplasia and disordered proliferative endometrium with normal endometrium and endometrial carcinoma was one of the study's limitations. It is hoped that future studies with a larger sample size and more clinical trials may be necessary to better understand the role of Bcl-2 and Ki-67. Additionally, it is thought that targeted genomic therapies that target the genes encoding Bcl-2 and Ki-67 could help treat abnormal uterine bleeding caused by endometrial hyperplasia and prevent its progression to carcinoma.

5. CONCLUSION

When Bcl-2 and Ki-67 staining were examined in DOPE, hyperplasia, and malignancy, the expression of Ki-67 and Bcl-2 staining was increased suggesting a definite progression of DOPE to hyperplasia that may in turn progress to malignancy. Henceforth, it is essential for the clinicians to follow up the patients with disordered phase of endometrium for further progression of the disease to hyperplasia and malignancy. This study emphasized that there was an increased expression of both Bcl-2 and Ki-67 in the proliferative phase whereas in secretory phase, both shown milder expression in cyclical endometrium because of the onset of progesterone production during the secretory phase and increased oestrogen

stimulation during the proliferative phase. Further studies are needed to understand the role of Bcl-2 and Ki-67 in tumor pathogenesis.

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