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Expression of Bcl-2 and Ki-67 in Cyclical Endometrium and in Endometrial Hyperplasia – An Analysis

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Abstract:*Abnormal Uterine Bleeding affectsabout 5 to 15% of women in reproductive age, in developing countries including India. [1] The investigation and treatment of patients with Abnormal Uterine Bleeding has been hampered by lack of proper investigatory protocol and targeting the appropriate mechanism. In the present study, identification of the role of bcl-2, an anti-apoptotic marker and Ki-67, a proliferative marker in endometrial biopsies is performed. In the present study, Bcl-2 and Ki-67 showed ascending expression in hyperplastic states. This indicates that as Hyperplasia progresses towards atypia, there is decreased apoptosis and increased proliferative activity.Thus, induction of apoptosis by blocking the expression of bcl-2 in patients with simple hyperplasia, may halt pathological progression to complex hyperplasia and atypia and further carcinoma. By evaluating the bcl-2 and Ki-67 expression using immunohistochemistry in cyclical and hyperplastic endometrium, the role of these proteins and identification of a dynamic etiology could be standardized. Thereby, therapy targeting these proteins and the genes encoding them could be elucidated for treating patients with Abnormal Uterine Bleeding.*

Keywords*: endometrium, Bcl-2, Ki-67, endometrial hyperplasia*

I. Introduction

In developing countries, like India Abnormal Uterine bleeding affects about 5 to 15% of women in reproductive age and it is increased in older women. In U.S. about 5 to 10% of cases in clinical outpatient setting were found to have Abnormal Uterine Bleeding. [1] One among the causes of Abnormal Uterine bleeding is endometrial hyperplasia. Endometrial hyperplasia is defined as a proliferation of glands of irregular size and shape with an associated increase in the gland/ stromal ratio compared with proliferative endometrium. [2] The human endometrium reveals dynamic morphological changes during menstrual cycle in reproductive women. Bcl-2 is an anti-apoptotic gene and Ki-67 is a recognized indicator of cell proliferation. Present study aims to study the balance between apoptotic and mitotic activity of the endometrium in cyclical endometrium and endometrial hyperplasia. This is performed by applying immunohistochemicalreagents including Bcl-2 and Ki-67 in formalin fixed paraffin embedded endometrial biopsy specimens. Correlation between Bcl-2 and Ki-67 expression were compared and analyzed in fifty endometrial biopsy samples. The results obtained would provide insight into therapeutic options targeting genes for Bcl-2 and Ki-67 in addition to conventional hormonal therapy for patients with abnormal uterine bleeding.

II. Aim And Objectives

2.1 Aim: The aim of the present study is to study the apoptotic and mitotic activity in endometrium, the balance of which is essential for the regulation of normal endometrial development and regression, shedding during menstrual cycle. This is studied immunohistochemically by applying Bcl-2, an anti-apoptotic marker and Ki-67, a cell proliferation marker in normal cyclical endometrium and endometrial hyperplasia.

2.2 Objectives:
a. To determine the expression of Bcl-2 and Ki-67 in cyclical endometrium in the proliferative andsecretory phases.
b. To observe the expression of Bcl-2 and Ki-67 in endometrial hyperplasias.

III. Materials And Methods

3.1 Endometrial biopsy samples of 30 patients with Abnormal Uterine Bleeding were collected and compared with 20 persons considered as control group who had appeared for screening test as a part of infertility workup with normal menstrual cycle, after informed consent.
3.2 Inclusion criteria included patients of reproductive age group with history of abnormal uterine bleeding for a period of 6 months or above.

3.3 Exclusion criteria included patients with clinically and radiologically detectable lesions in the uterus including polyp, adenomyosis, leiomyoma, coagulopathy, pure ovulatory dysfunction and other endocrine abnormalities. Patients already on Hormone replacement therapy and Oral contraceptive pill usage were also not included in the present study.
3.4 Study period: two years
3.5 Study design: prospective study
3.6 Positive control: Placental tissue was taken as positive control for bcl-2 whose staining was always grade 4.
3.7 The phase of the menstrual cycle was determined by the menstrual history and histopathological analysis for dating on the endometrium. Out of twenty patients with normal menstrual history, 12 patients were in proliferative phase and 8 patients were in secretory phase.
3.8 Endometrial hyperplasia was categorized as per Kurman[3] classification. This included 15 patients of simple typical hyperplasia, 9 patients of complex typical hyperplasia and 6 patients of complex atypical hyperplasia.
3.9 Procedure: Immunohistochemistry was performed on all the above slides using bcl-2 and Ki-67 as primary antibody 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.
3.10 Brown staining of the cytoplasm in glandular cells is considered as positive for bcl-2.Brown staining of the nucleus is considered as positive for Ki-67. Both intensity and proportion of staining were calculated as below.

SCORING OF Bcl-2 AND Ki-67[4, 5, 6, 7]

Table 1: Grading of Bcl-2& Ki-67 with intensity of staining

0	Absent staining
1+	Weak staining
2+	Moderate staining
3+	Strong staining
4+	Very strong staining

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

Table 2: Proportion scoring for bcl-2 & Ki-67

Score	Proportion of positive cells
0	< 5%
1	5 – 25 %
2	26 – 50 %
3	50 – 75 %
4	75 – 100%

Weighted score = Intensity score X Proportion score.

Bcl-2 stains uniformly all glandular epithelial cells so proportion score is always kept as grade 4. Ki-67 usually stains cells with strong intensity so intensity score is kept as grade 4. So for both Bcl-2 and Ki-67, 4 is kept constant.Hence, Bcl-2 weighted score is based on intensity and Ki-67 weighted score is based on proportion.Mean score is calculated by adding the weighted score and dividing it by the sample size.
Mean score = Totalscore/ sample size. [4.5, 6, 7]

IV. Observation

Table-3.Distribution of cases

S.No	Endometrial lesions	Sample size	Percentage
1.	Proliferative phase	12	24%
2.	Secretory phase	8	16%
3.	Hyperplasia	30	60%
	Total	50	100%

A total of 50 endometrial samples were studied for Bcl-2 and Ki67 expression which included 12 cases (24%) of proliferative endometrium, 8 cases (16%) of secretory endometrium and30 cases (60%) of hyperplasias.

Table-4.Expression of Bcl-2 in various endometrial lesions.

S.NO	Endometriumlesions	Sample size	negative	Weighted Score4	Weighted score8	Weighted Score12	Weighted Score16	mean score
1.	Proliferative phase	12	-	3	4	4	1	9
2.	Secretory phase	8	5	2	1	-	-	2
3.	Hyperplasia	30	7	8	7	5	3	6.5
	Total	50						

Mean score was 9 for bcl-2 in proliferative phase, 2 in secretory phase and 6.5 in endometrial hyperplasia.

Table-5. Expression of Ki67 in various endometrial lesions

S.No	Endometrial lesions	Sample size	negative	Weighted Score4	Weighted Score8	Weighted Score12	Weighted Score16	Mean score
1.	Proliferative phase	12	4	7	1	-	-	3
2.	Secretory phase	8	6	2	-	-	-	1
3.	Hyperplasia	30	8	12	5	3	2	5.2
	Total	50						

Ki-67 showed maximum mean score in hyperplasia (5.2) followed by proliferative phase (3) and decreased expression in secretory phase (1).

Table-6. Comparison of Bcl -2 and Ki-67 in variousendometrial lesions

S.NO	**Endometrial lesions**	**Bcl-2 mean score**	**Ki67 mean score**
1.	Proliferative phase	9	3
2.	Secretory phase	2	1
3.	Hyperplasia	6.5	5.2

On comparing the mean score of Bcl – 2 and Ki-67 in proliferative phase, bcl-2 score is three fold higher than Ki67 mean score. In Secretory phase Bcl-2 istwofold higher than Ki-67 and in hyperplasia Bcl-2 is marginally higher than Ki-67 mean score.

Table-7. Expression of Bcl-2 and Ki67 in cyclical endometrium

S.No	Endometrial phase	Sample size	Bcl-2 mean score	Ki67 mean score
1.	Proliferative phase	12	9	3
2.	Early Secretory phase	3	5.33	2.66
3.	Mid secretory phase	2	0	0
4.	Late secretory phase	3	0	0

In comparing Bcl-2 and Ki-67 expression in cyclical endometrium, both Bcl-2 and Ki67 expression was high in the proliferative phase and showed decreased expression in the early secretory phase. In the mid and late secretory phase both markers showed immunonegativity.

Table-8. Expression of Bcl-2 and Ki67 in Hyperplasia

S. No	Type of endometrium	Sample size	Bcl-2 mean score	Ki67 mean score
1.	Simple hyperplasia without atypia	15	4.7	2.54
2.	Simple hyperplasia with atypia	-	-	-
3.	Complex hyperplasia without atypia	9	6	3.33
4.	Complex hyperplasia with atypia	6	14.6	9.33

In comparing Bcl-2 and Ki-67 in endometrial hyperplasia, Bcl-2 mean score was twofold higher than Ki-67 in Simple Hyperplasia without atypia. In Complex hyperplasia without atypia, Bcl-2 mean score was twofold higher than Ki-67 and in Complex hyperplasia with atypia, Bcl-2 mean score was marginally increased than Ki-67.

V. Discussion

A total of 50 endometrial samples were studied for Bcl-2 and Ki-67 expression which included 12 cases (24%) of proliferative endometrium, 8 cases (16%) of secretory endometrium and 30 cases (60%) of endometrial hyperplasia. Regarding Bcl-2 expression there was uniform cytoplasmic staining of the glandular cells and weak staining of the stromal cells. In the present study, only the positivity of the glandular cells were considered as positive. In Ki 67 nuclear staining of the glandular epithelial cells were considered as positive.

It was observed that in cyclical endometrium, there was increased expression of both Bcl-2 and Ki-67 in the proliferative phase. In the secretory phase, early secretory endometrium had mild expression of Bcl-2 and Ki-67 while in the mid and late secretory endometrium there was immunonegativity for both the markers. These patterns of expression correlated with the previous studies. [8, 9, 10, 11]

Comparison was done between Bcl-2 and Ki-67 in proliferative phase. It was observed that Bcl-2 was three fold higher than Ki-67 in Proliferative phase.

Regarding secretory phase, Bcl-2 was twofold higher in early secretory phase, while both markers were negative in mid and late secretory phase.

Thus, both the anti-apoptotic activity (Bcl-2) and proliferative activity (Ki-67) was high in the proliferative endometrium, whereas the secretory phase showed increased apoptotic activity(Bcl-2 decreased) and decreased Proliferation (Ki67 decreased).

In hyperplastic endometrium, the Bcl-2 score showed increased expression in ascending order of frequency from simple hyperplasia to complex hyperplasia and complex hyperplasia with atypia. This indicates the hyperplastic states which are under the influence of unopposed estrogenic stimulation, have more anti-apoptotic activity. Ki-67 also showed similar pattern of expression. In complex hyperplasia with atypia there was increased expression than the other two states. This correlates with increased premalignant potential in complex hyperplasia with atypia than in benign hyperplastic states.

With respect to Bcl-2 staining the previous studies [12, 13, 14, and 15] done in the hyperplastic endometrium showed focal or less intense staining than the proliferative endometrium. Similar finding was observed in the present study.

In comparing Bcl-2 staining and Ki-67 in Hyperplasia, Bcl-2 expression was twofold higher when compared to Ki-67 staining. Thus, indicating that anti-apoptotic activity is higher when compared to proliferative activity.

Table-9: Review of Literature pertaining to cyclical endometrium

S.No.	Author	Total number of patients	Bcl-2 expression in proliferative phase	Bcl-2 expression in secretory phase	Ki 67 expression in proliferative phase	Ki67 expression in secretory phase
1.	Mertens HJMM et al [9]	30	Increased	Decreased	Increased	Decreased
2.	Vaskivuo et al[10]	39	Increased	Decreased Very low / absent in menstrual phase	Increased	Decreased
3.	A.Gompel et al[11]	49	Peaked in proliferative phase	Disappeared with onset of secretory phase	-	-

Table-10: Review of Literature pertaining to endometrial hyperplasia

Author	Observation in simple hyperplasia	Observation in complex hyperplasia	Observation in complex atypical hyperplasia	Observation in endometrial carcinoma	Others
Theodore H. Niemann et al[12]	-	100% expression of bcl-2	25% expression of bcl-2	34% expression of bcl-2	-
Robert et al[13]	Ki67 expression was much lower in simple hyperplasia	Ki67 mean index was high in complex hyperplasia	Ki67 had lower expression in complex atypical hyperplasia than in complex hyperplasia	-	Least score of Ki67 was observed in atrophic endometrium
Morsi, Hassan et al[14] 107 patients		High expression of bcl-2 and ki-67 in complex hyperplasia			Ki67 showed increased expression as the grade of endometrial carcinoma progressed. Bcl-2 reacted only weakly in grade I endometrial carcinoma
Olga B. Ioffe[15]	Bcl-2 expression was increased in simple hyperplasia compared to proliferative endometrium	Bcl-2 expression decreased in complex hyperplasia		Bcl-2 expression decreased in endometrial carcinoma	
Olga B. Ioffe[4]	Ki67 expression was decreased in simple hyperplasia	Ki67 expression decreased in complex hyperplasia		Ki67 index was increased in endometrial carcinoma	

VI. Conclusion

Bcl-2 expression was uniformly high in the proliferative endometrium than in endometrial hyperplasia. In secretory phase, the expression of Bcl-2 diminished markedly. This indicates that there is anti-apoptotic activity in the proliferative endometrium due to the influence of estrogen.As hyperplasia progresses towards atypia, there is decreased apoptosis and increased proliferative activity.

Ki67 expression was high in hyperplastic states compared to the proliferative endometrium indicating increased mitotic activity in these abnormal states thereby depicting the premalignant status of hyperplasia.

Comparison between Bcl-2 and Ki67 showed that it was three fold higher in proliferative endometrium, which indicates higher anti – apoptotic activity than proliferative activity which drastically decreased in secretory endometrium. Comparison between Bcl-2 and Ki67 in Hyperplastic states indicate that Bcl-2 staining was

twofold higher than Ki-67 expression, thus reflecting that the anti-apoptotic activity, under the influence of estrogen is higher in Hyperplasia.

Tumorsdeploy various strategies to limit or evade apoptosis that frequently involves the intervention of Bcl-2 pathway. Our study indicates that Bcl-2 expression increased progressively from simple hyperplasia towards atypical hyperplasia, indicating escape from apoptosis. Overall, the above findings suggest that Bcl-2, might prove to be a potential therapeutic target in Bcl-2 positive endometrial hyperplasia patients.

It is to be hoped that in future similar studies with larger sample size and more clinical trials may be needed to understand the role of Bcl-2 and Ki-67 more precisely. Further, it is believed that targeting the genes encoding Bcl-2 and Ki-67 in genomic therapies could aid in treating Abnormal Uterine Bleeding due to endometrial hyperplasia and halt it's progression to carcinoma.

Images

Fig-1 : Proliferative phase showing cytoplasmic Positivity of Bcl – 2 grade-1, 10X

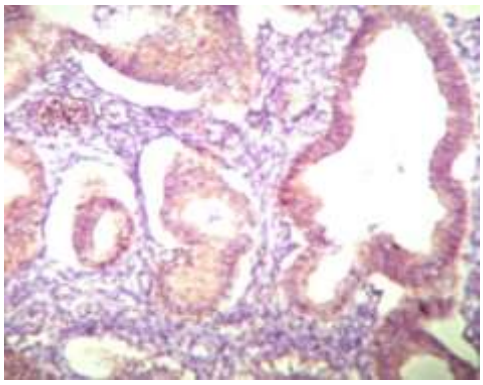

Fig-2 : Proliferative phase showing cytoplasmic positivity of Bcl-2 grade -2, 10X

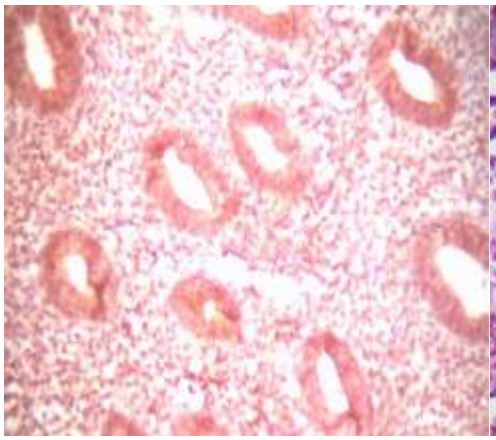

Fig-3 : Proliferative phase showing grade 3 Cytoplasmic positivity of Bcl-2,10X

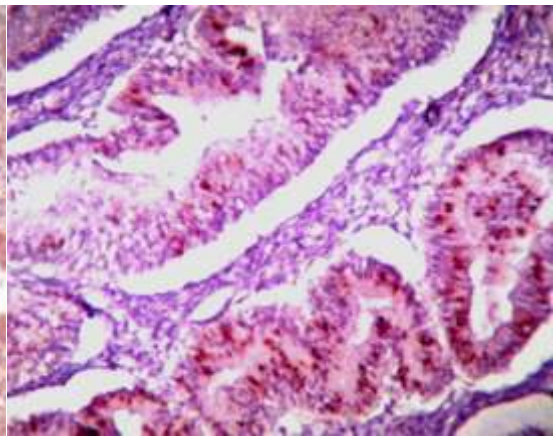

Fig-4 : Proliferative phase showing nuclear positivity of Ki 67, 10 X

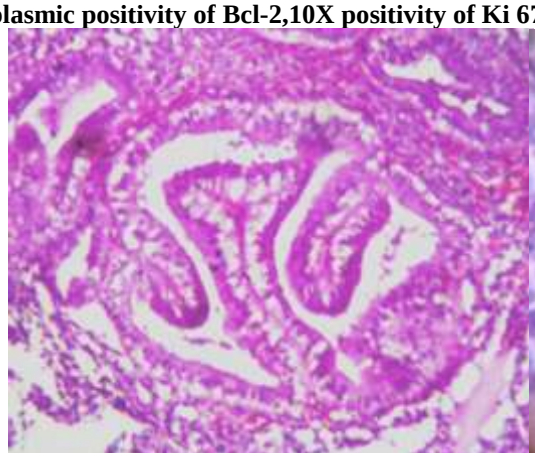

Fig-5: Early secretory phase showing Sub nuclearvacuoles, H &E,10X

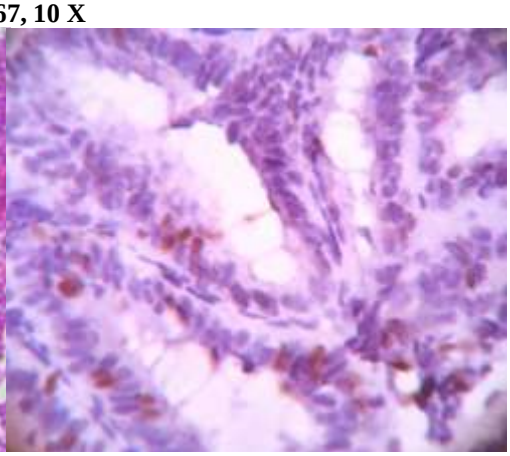

Fig-6:Secretory phase showing nuclear positivity for Ki67, 40 X

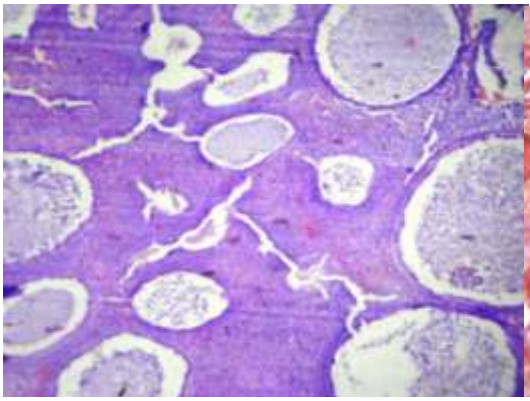
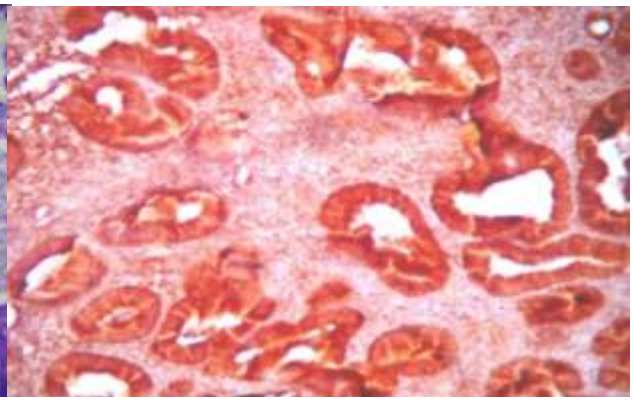

Fig-7: Simple hyperplasia showingFig-8: Simple hyperplasia showing grade 4
Cystically dilated glands. H & E, 10 XCytoplasmic positivity for Bcl-2, 10 X

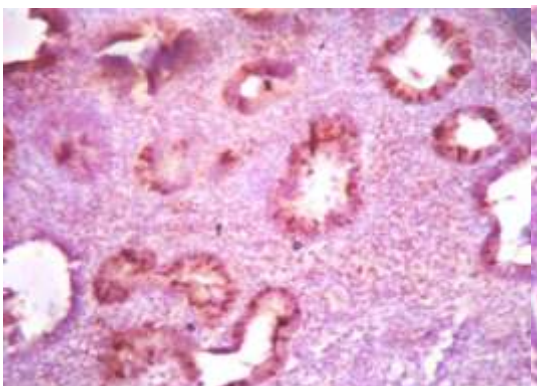
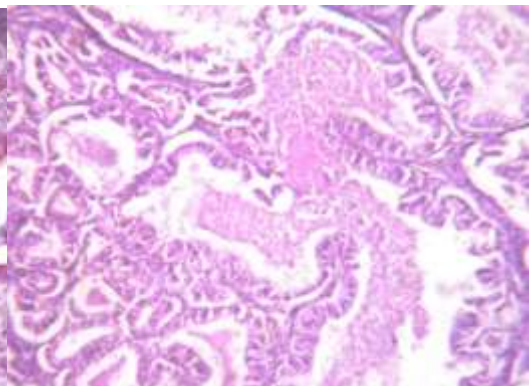

Fig-9: Simple hyperplasia showingFig-10: Complex hyperplasia showing
Nuclear positivity for Ki67, 10 XComplex branching glands, H&E, 10 X

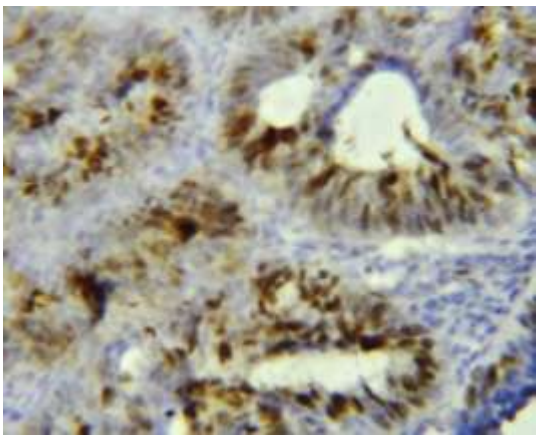
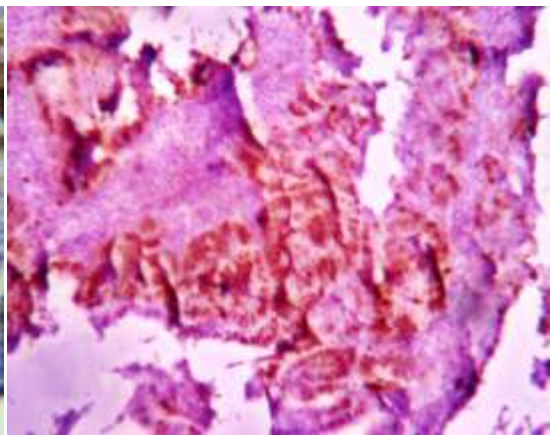

Fig-11: Complex hyperplasia with atypiaFig-12: Complex hyperplasia with atypia
Showing nuclearpositivityfor Ki67, 40Xshowing nuclear positivity for Ki 67, 10 X

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