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Expression of BCL-2 and KI-67 in Cyclical Endometrium and in Endometrial Hyperplasia

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The Present study attempts to determine the relationship between proliferation and the inhibition of apoptosis in normal endometrium and hyperplasia, using monoclonal antibodies against the proliferation marker, Ki-67 and the anti-apoptotic protein, Bcl-2 and to determine the expression of B-cell lymphoma 2 (Bcl – 2) and Ki – 67 in cyclical endometrium in proliferative and secretory phase. The Present study includes the expression of Bcl – 2 and Ki – 67 in endometrial hyperplasia.

Keywords: Endometrium; apoptosis; Bcl – 2; Ki-67; hematoxylin; biopsy and xylene.

1. INTRODUCTION

An excess of estrogen relative to progestin, if sufficiently prolonged or marked, can induce exaggerated endometrial proliferation (hyperplasia), which is an important precursor of endometrial carcinoma [1]. There are several lines of evidence that a diagnosis of Endometrial hyperplasia (EH) may precede the development of endometrial cancer (EC) and that the two, share common predisposing risk factors. The incidence of EH is roughly three times higher than EC and certain atypical forms of EH are considered to represent direct precursor lesions to endometrioid EC [2].

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The importance of unopposed estrogen stimulation for the development of endometrial hyperplasia and subsequently adenocarcinoma [3] is well documented. However, the histogenetic mechanisms for the development of different endometrial lesions such as hyperplasia, polyps and adenocarcinoma have not been fully characterized to date.

The endometrium shows dynamic morphological changes during menstural cycle. These changes include – cell proliferation with high mitotic activity, secretory changes, and shedding of endometrial cells regulated by ovarian sex steroids [4]. Thus there should be a balance between cell growth (mitosis) and its inhibitory mechanism (apoptosis) in order to maintain tissue homeostasis and for the regulation of the menstrual cycle. The imbalances between proliferation and apoptosis are involved in the development of endometrial lesions such as hyperplasia, polyps, and carcinoma [5,6].

In the reproductive years, the endometrium is characterized by cyclical growth, shedding and regrowth in response to estrogen and progesterone secretion by the ovaries. Endometrium alters continuously depending on the levels of estrogen and progesterone [7].

The proliferative phase of the menstrual cycle, the endometrium does not differ significantly every day hence dating is not possible in the proliferative phase [8]. But after ovulation, the histological appearances in the secretory phase is considered relatively specific from day to day and hence accurate dating of secretory phase endometrium is possible [9]. Normally the endometrial cycle is 28 days, even though the length varies between women and even in the same woman. This is due to variation in the duration of the proliferative phase [10]. The secretory phase is usually constant and lasts for 14 days from the time of ovulation to the onset of menstruation [11].

In the reproductive years, the endometrium is divided into 2 regions, namely the superficial functionalis layer - stratum spongiosum and the basalis layer - stratum basale [12]. Stratum spongiosum exhibits the greater degree of hormonal responsiveness, while the basalis is unresponsive, and its histology does not vary greatly during the menstrual cycle [13].

Proliferation as measured by the expression of the proliferation marker, Ki-67, is distinctly related to estrogen receptor content in normally cycling endometrium6. Apoptosis, or programmed cell-death, is a complex process, in which bcl-2 has an important role. Bcl-2 is a protooncogene preventing apoptosis [14] and thereby prolongs cell survival. Bcl-2 expression has been found to be hormone-regulated in the endometrium [15], because it closely follows estrogen receptor (ER) levels [16], with peak activity in proliferative phase when maximal ER expression occurs [17].

Analyzing the expression of these markers in cyclical endometrium and in hyperplasia and with carcinoma will enable in identifying the specificity and sensitivity of these markers in determining the malignant potential [18] as well as in the treatment modalities specific against these markers. The Present study attempts to determine the relationship between proliferation and the inhibition of apoptosis in normal endometrium and hyperplasia, using monoclonal antibodies against the proliferation marker, Ki-67 and the anti-apoptotic protein, Bcl-2 and to determine the expression of B-cell lymphoma 2 (Bcl – 2) and Ki – 67 in cyclical endometrium in proliferative and secretory phase.

2. MATERIALS AND METHODS

This prospective, cross-sectional study, was done in the Department of Pathology, Sree Balaji Medical College and Hospital, Chromepet, Chennai, from March 2017 to June 2018.

The study material included 50 endometrial biopsy samples and endometrium from the Total Abdominal Hysterectomy (TAH) & bilateral salphingoopherectomy (BSO) specimens received by the Department of Pathology. These samples were studied for Bcl-2 and Ki-67expression which included 20% (n=10) of proliferative endometrium, 20% (n=10) of secretory endometrium, 30% of hyperplasia without atypia and 30% of atypical endometrium.

Inclusion criteria:

1. Women aged between 20 - 50 years.
2. 30 specimens of endometrial hyperplasia diagnosed by histopathology.
3. 20 specimens with normal endometrium confirmed by histopathology.

The cases that deemed to fit in the inclusion criteria were identified and sections of 3 -5 microns in thickness from the 10% neutral

buffered formalin fixed parrafin embedded blocks were made and hematoxylin [19] and eosin staining was done. For immunohistochemistry [20], 2 sections of 3-micron thickness are cut and taken on charged slides each case and incubated at 60º-70ºC for 1 hour.

Haematoxylin and Eosin Staining: [21]

The procedure was done according to Bancroft et al. 2008.

Immunohistochemistry Staining Method [21]:

Method: Two step indirect technique.

Principle: Antigens in tissues and cells are detected by a two-stage process the binding of the primary antibody to specific epitopes and the subsequent detection of this binding by a colorimetric reaction

The procedure was done according to Bancroft et al. 2008.

Interpretation:

Bcl – 2: Bcl2 positivity will be indicated by cytoplasmic positivity [22,23] in glandular and stromal cells. But in my study, only the positive cells in the glandular epithelium was considered [24]. A section from tonsil shows higher grade positivity and was used as a control for analyzing the intensity [25].

In case of Bcl - 2, both positivity and intensity are considered [26]. The number of cells positive for Bcl-2 were counted in 100 epithelial cells and repeated in 10 high power field – HPFs [27] and then expressed as a percentage. The intensity of the staining in the positive epithelial cells is graded as:

Grade 1 - Mild
Grade 2 - Moderate
Grade 3 - Strong
Grade 4 - Very strong

Ki – 67: The positive expression of Ki-67 was defined as presence of brown-yellow granules in cell nuclei or in both cell nuclei and cytomembrane [21,25,28-30].
A section from tonsil was used as control for Ki – 67 expression.

Since the intensity was found to be uniform in all the cells, only positivity is calculated. The positive nuclei were counted in 100 epithelial cells and repeated in 10 HPFs and the total number of positive cells was expressed as a percentage of 1000. Then they are graded as follows based on their positivity:

1 - 25% - Grade 1
26 - 50% - Grade 2
51 - 75% - Grade 3
76 to 100% - Grade 4.

Statistical Analysis of various parameters:

When the results were statistically analysed between various parameters, using SPS software, by comparing the mean differences of different diagnostic groups, significant correlations were observed between PE and HWA, SE and AH in Bcl – 2 expression. When the mean differences of Ki 67 expression was correlated, no significant p value was found out.

When Pearson chi square test was applied to obtain the p value between the Ki 67 expression and Bcl – 2 expression in various groups, p value was found to be not significant. (0.211, 0.227, 0.823, 0.959 for proliferative endometrium, secretory endometrium, hyperplasia without atypia and atypical hyperplasia respectively).

Significant p value was not obtained due to smaller sample size. More clinical trials may be needed to understand the role of Bcl - 2 and Ki - 67 in genomic therapies and thereby could aid in treating the precursor lesion, endometrial hyperplasia and thereby preventing it progressing to carcinoma.

3. RESULTS

A total of 50 endometrial samples were studied for Bcl-2 and Ki-67expression which included 20% (n=10) of proliferative endometrium, 20% (n=10) of secretory endometrium, 30% of hyperplasia without atypia and 30% of atypical endometrium.

The mean percentage of positive cells expressing Bcl - 2 in the glandular epithelium of various histologic types studied in my group are 64.2% in proliferative endometrium, followed by hyperplasia without atypia – 48.4%, atypical hyperplasia - 29.6% and then by secretory endometrium with 11%.

Table 1. Distribution of cases

S. no.	Histologic Group	No. of cases	Percentage
1.	Proliferative endometrium	10	20 %
2.	Secretory Endometrium	10	20 %
3.	Hyperplasia without atypia	15	30 %
4.	Atypical Hyperplasia	15	30 %
Total		50	

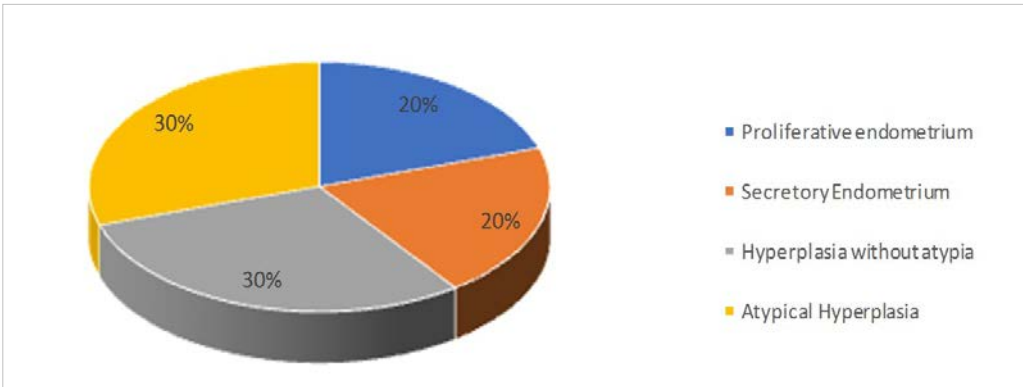

Chart 1. Distribution of cases

Table 2. Expression of Bcl – 2 in various endometrial lesions

S. no.	Histologic group	*n**	Bcl – 2 Intensity of staining				Negative	Mean %**
			Grade 1	Grade 2	Grade 3	Grade 4		
1	Proliferative Endometrium	10	4	2	3	1	-	64.2
2	Secretory Endometrium	10	4	1	-	-	5	11
3	Hyperplasia without atypia	15	5	4	5	1	-	48.4
4	Atypical Hyperplasia	15	6	6	1	-	2	29.6

** No. of cases*

*** mean percentage of number of Bcl – 2 positive cells in each histologic type*

Table 3. Expression of Ki-67 in various endometrial lesions

S. no.	Histologic group	*n**	Ki – 67 positivity grade				Negative	Mean %**
			Grade 1	Grade 2	Grade 3	Grade 4		
1	Proliferative Endometrium	10	1	9	-	-	-	29.80
2	Secretory Endometrium	10	10	-	-	-	-	3.6
3	Hyperplasia without atypia	15	8	6	1	-	-	25.53
4	Atypical Hyperplasia	15	6	7	2	-	-	32.4

**No. of cases*

*** mean percentage of number of Ki - 67 positive cells in each histologic type*

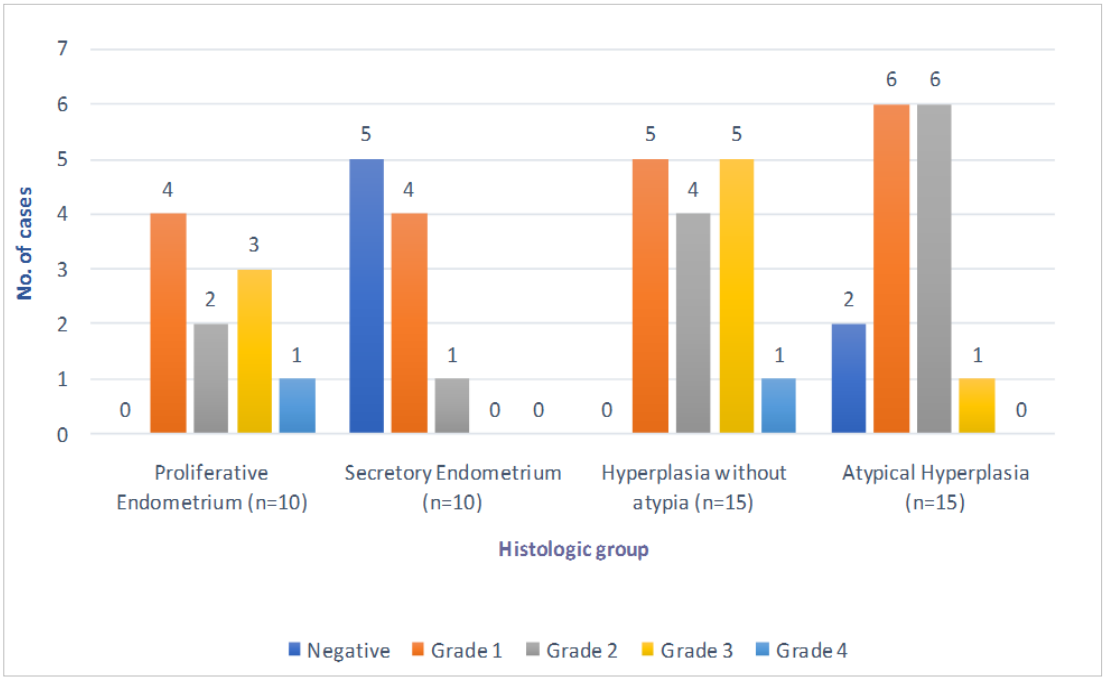

Chart 2. Expression of Bcl – 2 in various endometrial lesions

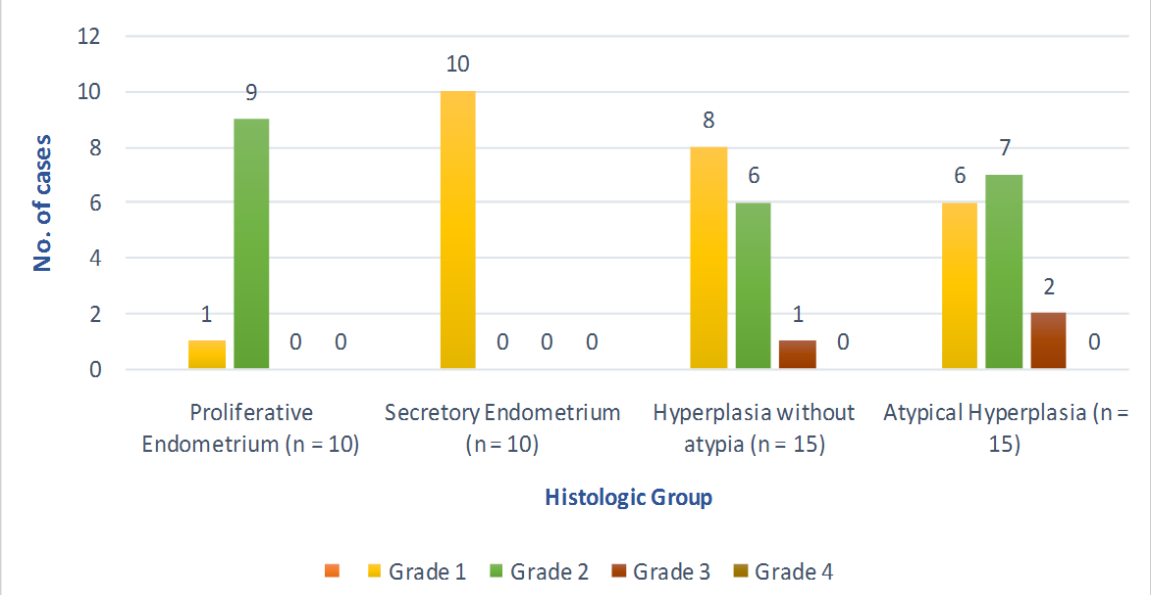

Chart 3. Expression of Ki-67 in various endometrial lesions

Table 4. Mean percentage of positivie cells for Bcl- 2 and Ki- 67 in various histologic groups

S. no.	Histologic group	Sample size	Bcl – 2	Ki – 67
1	Proliferative Endometrium	10	64.2	29.80
2	Secretory Endometrium	10	11	3.6
3	Hyperplasia without atypia	15	48.4	25.53
4	Atypical Hyperplasia	15	29.6	32.4

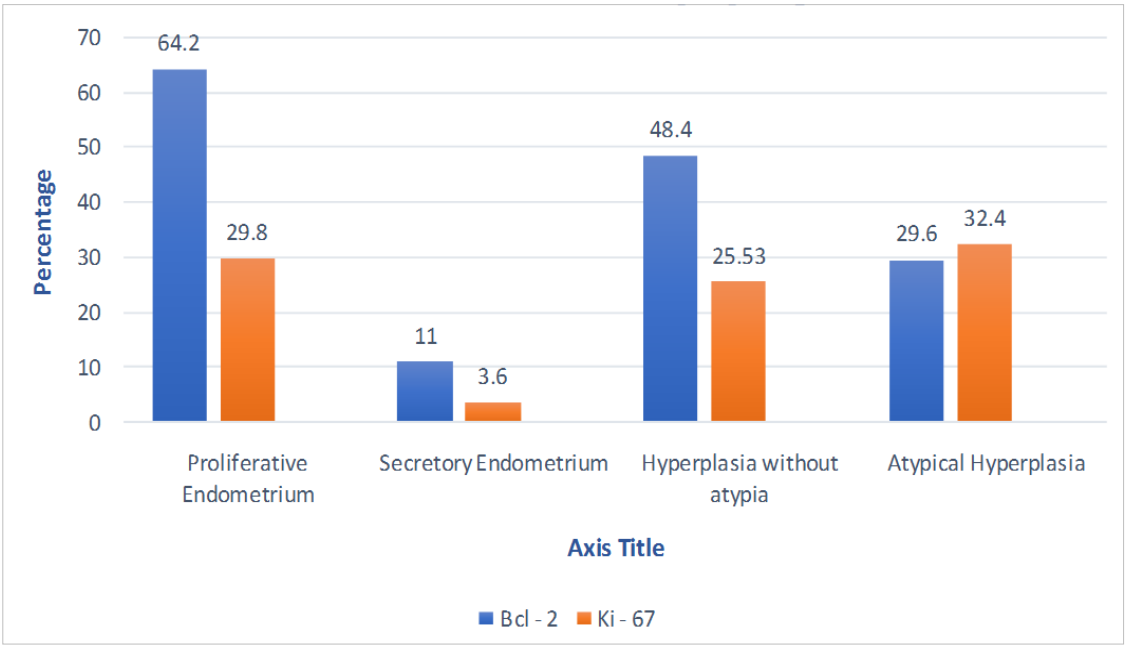

Chart 4. Mean percentage of positivie cells for Bcl- 2 AND Ki- 67 in various histologic groups

Table 5. Bcl- 2 positive cell ratio in various histologic groups

S. no.	Histologic group	Bcl – 2 positive cases		Mean percentage	Standard deviation
1	Proliferative Endometrium	10/10	100 %	64.2	14.85336
2	Secretory Endometrium	5/10	50 %	11	13.24974
3	Hyperplasia without atypia	15/15	100 %	48.4	17.69100
4	Atypical Hyperplasia	13/15	86 %	29.6	18.38011

Table 6. Ki - 67 positive cell ratio in various histologic groups

S. no.	Histologic group	Ki - 67 positive cases		Mean percentage	Standard deviation
1	Proliferative Endometrium	10/10	100 %	29.80	4.51664
2	Secretory Endometrium	10/10	100 %	3.6	2.71621
3	Hyperplasia without atypia	15/15	100 %	25.53	14.33212
4	Atypical Hyperplasia	15/15	100 %	32.4	13.48968

Table 7. Expression of Ki – 67 and Bcl – 2 in normal cyclical endometrium

Grade	Proliferative Endometrium (n = 10)		Secretory Endometrium (n = 10)	
	Bcl – 2	Ki -67	Bcl - 2	Ki -67
1	4	1	4	10
2	2	9	1	-
3	3	-	-	-
4	1	-	-	-

Table 8. Expression of Ki – 67 and Bcl – 2 in hyperplastic endometrium

Grade	Hyperplasia without atypia (n = 15)		Atypical Hyperplasia (n = 15)	
	Bcl – 2	Ki -67	Bcl – 2	Ki -67
1	5	8	6	6
2	4	6	6	7
3	5	1	1	2
4	1	-	-	-

Table 9. Test statistics of Pearson correlation used for comparison of Bcl – 2 and Ki – 67 expression in various HP patterns

	Proliferative Endometrium	Secretory Endometrium	Hyperplasia without atypia	Atypical Hyperplasia
Pearson Correlation	0.433	0.420	-0.063	0.015
p-value	0.211	0.227	0.823	0.959

Table 10. Ki – 67 and Bcl – 2 staining scores (Mean differences of different diagnostic groups)

Diagnostic group compared	p value Ki – 67 Score	p value Bcl – 2 Score
Proliferative endometrium		
Secretory Endometrium	.000	.000
Hyperplasia without atypia	.879	.024
Atypical Hyperplasia	.984	.000
Secretory endometrium		
Proliferative endometrium	.000	.000
Hyperplasia without atypia	.000	.000
Atypical Hyperplasia	.000	.009
Hyperplasia without atypia		
Proliferative endometrium	.879	.024
Secretory endometrium	.000	.000
Atypical Hyperplasia	.712	.003
Atypical Hyperplasia		
Proliferative endometrium	.984	.000
Secretory endometrium	.000	.009
Hyperplasia without atypia	.712	.003

**p value not significant, p>0.05 – not significant.*

Table 11. Analysis of Bcl – 2 expression of hyperplasias in various studies

Study	My study results		Kokawa[65] et al		Nunobiki[66] et al	
Type	**N**	**%**	**N**	**%**	**N**	**%**
Hyperplasia without atypia	15	48.4	9	36.2	30	60
Atypical Hyperplasia	15	29.6	7	16.3	30	36.96

4. DISCUSSION

A total of 50 endometrial samples were studied for Bcl-2 and Ki67 expression which included 20% of proliferative endometrium, 20% of secretory endometrium, 30% of hyperplasia without atypia, 30% of atypical hyperplasia (Table 1).

The mean percentage of positive cells expressing Bcl - 2 in the glandular epithelium of various histologic types studied in my group are

64.2% in proliferative endometrium, followed by hyperplasia without atypia – 48.4%, atypical hyperplasia - 29.6% and then by secretory endometrium with 11%.

The mean percentage of Ki – 67 positive cells was the highest in the atypical hyperplasia with 32.4%, followed by the proliferative endometrium group – 29.80%, 25.53% in hyperplasia without atypia group and a minimal 3.6% in the secretory endometrial samples.

Cyclical Endometrium:

Bcl-2 in Cyclical endometrium:

The scoring for Bcl-2 was restricted to the positive cells in the epithelial compartment. The cells stained uneven, mostly in the peripheral margin of the epithelial cell cytoplasm [31]. Some cells showed perinuclear and granular staining in the cytoplasm. Proliferative endometrium showed positivity for bcl -2 with varying intensities but all cases were positive, whereas only 50% of cases showed positivity for Bcl-2 in the secretory endometrium. Even the mean percentage of positive cells in secretory endometrium was low accounting only 11%. The mean percentage of positive cells in the glandular epithelium of proliferative endometrium was more - 64.2%.

With the onset of secretory activity, bcl-2 epithelial positivity was restricted to the basal portions of the endometrium, disappearing in the late part of this phase. The grade ranged from 0 to 2. Cells showing secretory vacuoles were negative, whereas cells still showing proliferative activity were bcl-2 positive. The pattern of staining within the cells was granular or in clumps. As pre- decidualization changes occurred, intermittent stromal positivity (lymphocytes) was seen around the glands [32].

When analysed with the articles which studied the Bcl-2 expression in normal endometrium such as Gompel et al. [15], X J Tao et al. [16], Mertens H J MM et al. [17], Morsi et al. [25] and T. E. Vaskivuo et al. [28], my results were consistent with these studies.

Samples of normal endometrium randomly distributed throughout the cyclical endometrium and they found that Bcl-2 staining predominated in glandular cells peaked at the end of the proliferative phase and disappeared at the onset of secretory activity. X J Tao et al. [16] also observed that Bcl-2 immunoreactivity was maximal during the proliferative phase and decreased in the secretory phase.

In a study by Helena Mertens et al. [17], 30 endometrial samples of ovulatory cyclical endometrium studied, Bcl2 expression was high in the proliferative phase and decreased significantly in the secretory phase, especially in the glandular epithelial cells.

In the study by Morsi et al. [25], 18 samples of proliferative endometrium and 19 samples of secretory endometrium (SE) were included and Bcl- 2 expression showed a same pattern of predominance (n = 100%) in PE and decreased (n = 47.4%) in SE.

T. E. Vaskivuo et al. [28] analysed 39 endometrial samples and concluded that Bcl-2 expression is increased in the proliferative phase and decreased in the secretory phase being very low or absent in the secretory and menstrual phases.

Ki - 67 in Cyclical endometrium:

The proliferative activity was high in proliferative endometrium with an average of 29.80% and lowest in secretory endometrium with a mean percentage of 3.6%.G. T. Gurda et al. [29] analysed 11 cases of proliferative endometrium and 19 cases of secretory endometrium for Ki – 67 expression and in their study, 56% ± 22% for PE and 2.6% ± 3.8% for SE. In the study by Morsi et al. [25], the Ki – 67 expression in PE was 29.7% and in SE, it was 2.5%. Morsi et al. [25] included 18 cases of proliferative endometrium and 19 cases of secretory endometrium in his study.

A study by Helena Mertens et al. [17] in 2002 analysing expression of both Bcl - 2 and Ki – 67 in 30 cyclical endometrial samples also showed a similar pattern of increased Ki – 67 expression in PE and decreased in SE.

Similar pattern of Ki – 67 expression in the glandular epithelium was observed in the studies by Risberg et al. [30] and Vaskivou et al. [28].

Hyperplasia:

Bcl – 2 in Hyperplasia:

In my study, Bcl – 2 was found positive in all 15 cases of HWA, the mean percentage of positive cells expressing Bcl – 2 was 48.4% in various

grades of intensity. (Grade 1 to grade 4). Whereas in the 15 cases of atypical hyperplasia, 2 cases were negative for Bcl – 2 and the mean percentage of positive cells was 29.6% in grade 0 to grade 3. When compared with the expression of Bcl – 2 in proliferative endometrium (64.2%), it is observed that the expression of Bcl -2 demonstrates reduced Bcl-2 expression with a trend from normal endometrium (highest expression), through hyperplasia without atypia to atypical hyperplasia (lowest expression). Similar trend of Bcl-2 in the hyperplastic endometrium was observed in studies conducted by Vaskivuo et al. [28], Risberg et al. [30] and Sakuragi et al. [31] where Bcl-2 expression was highest in normal endometrium than the hyperplasias.

Studies by Morsi et al. [25], Kokawa et al. [31] and Nunobiki et al. [32] noted Bcl-2 expression to be higher in non- atypical hyperplasias compared with atypical hyperplasias.

The finding of decreased bcl-2 expression in atypical hyperplasia suggests a possible role for bcl-2 in promoting the malignant transformation of hyperplastic cells. As soon as nuclear atypia was observed, bcl-2 expression was difficult to detect. In addition, it has been shown in various studies that Bcl-2 overexpression plays an important role in epithelial tumor development.

Ki - 67 in Hyperplasia:

Ki – 67 was found positive in all the 30 cases of hyperplasia with a 25.53% and 32.4% as mean percentage of positivity in hyperplasia without atypia and atypical hyperplasia respectively. The positivity varied from 7% to 54% in non-atypical hyperplasia group and 18% to 64% in atypical hyperplasia group.

The expression of the Ki – 67 is the highest in atypical hyperplasia (32.4%), followed by, proliferative endometrium (29.80%), hyperplasia without atypia (25.53%) and is lowest in secretory endometrium (3.6%).

Morsi et al. [25] and Nunobiki et al. [32] in their studies observed stepwise increased expression of Ki – 67 from normal, simple or complex hyperplasia with or without atypia to endometrial adenocarcinoma.

Morsi et al. [25] studied 107 cases which included 18 cases of proliferative endometrium, 19 secretory endometrium, 15 postmenopausal endometrium, 6 cases of disordered proliferative endometrium, 12 cases of simple hyperplasia, 8 complex hyperplasia, and 29 endometrial adenocarcinomas for expression of Bcl – 2, Ki - 67 and M 30. In his study, strong reactivity was seen in cases of simple hyperplasia (with a mean percentage of 21.8%), in cases of complex hyperplasia (with a mean percentage of 19.8%), and in cases of disordered proliferative endometria (mean percentage of 19.5%). Gurda et al. [29] analysed 104 cases of endometrial lesions for Ki – 67 and in his study and the mean percentage of Ki – 67 positivity was 0.8% in SEM, 11% in nonatypical hyperplasia, 33% in atypical hyperplasia, and 73% in carcinoma. Thus, my study values correlates with the above studies.

5. CONCLUSION

Bcl – 2 being an anti apoptotic marker, and Ki – 67 being a proliferative index is expressed more in proliferative phase than in the secretory phase. Bcl – 2 expression is decreasing from hyperplasia without atypia to atypical hyperplasia and Ki - 67 expression is increasing from hyperplasia without atypia to atypical hyperplasia. This implies that there is decreased apoptosis and increased proliferative activity when the hyperplasia progresses towards atypia. Hence Bcl – 2 and Ki -67 may have some utility as a marker of EH progression and hence to endometrial carcinoma.

CONSENT AND ETHICAL APPROVAL

As per university standard guideline, participant consent and ethical approval have been collected and preserved by the authors.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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