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A Study of Apoptotic and Proliferative Activity in Endometrial Carcinoma by Immunohistochemistry

Dr.J.Nilavu[1]MD, Dr.G.Gayathri[2]MD, Dr. Hidhaya Fathima[3]MD,
[1,2]*Assistant Professor, Department of pathology, Govt Villupuram Medical,College, India*
[3]*Assistant professor, Department of pathology, Thirunelveli Medical college,India*
Corresponding Author: Dr.G.Gayathri

Abstract: *Endometrial cancer is a most common malignancy in developed countries. Among the various risk factors for endometrial cancers this study deals with certain genes such as Bcl-2 and Ki67 and its association with various endometrial carcinoma. Aims and Objectives- To determine the expression of Bcl-2 an antiapoptotic gene and Ki-67 a proliferative marker by immunohistochemistry. Materials and methods- 30 endometrial samples were collected from patients who presented with post menopausal bleeding and subjected to immunohistochemistry. Conclusion- Endometrial intraepithelial neoplasia showed 100% positivity for both Bcl-2 and Ki67 denoting the high mitotic activity and low apoptotic activity in this clonal neoplasm. This indicates the increased potential for these lesions to transform into frank malignancies.Bcl-2 expression decreases with increasing grade of the carcinoma whereas Ki-67 index increases. This indicates increased apoptotic and mitotic activity with higher grade carcinoma and negative correlation of the Bcl-2 and Ki67 expression.*

Keywords *– Endometrial carcinoma, EIN, Bcl-2, Ki67*

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I. Introduction

Endometrial cancers usually arises in postmenopausal women and 80% of them presents with postmenopausal bleeding. Endometrium which is hormone regulatory in function shows dynamic changes during the menstrual cycle. Numerous studies have proved that endometrium normally shows increased proliferative activity during the estrogenic phase and increased apoptotic activity during the menstrual phase. Effects of these proliferative and apoptotic activity are studied in the endometrial carcinoma and endometrial intra epithelial neoplasia using immunohistochemistry antibodies such as Ki67 a proliferative marker and Bcl-2 an anti- apoptotic marker. Based on these results the values of Bcl-2 and Ki67 was compared and analysed.

II. Aims and Objectives

2.1 Aim: The aim of the present study is to study the mitotic activity and apoptotic activity in endometrial intra epithelial neoplasia and endometrial carcinoma. This is studied immunohistochemically by applying Bcl-2 an anti- apoptotic marker and Ki67 a cell proliferative marker.

2.2 Objectives:

a. To determine the expression of Bcl-2 and Ki67 in EIN.
b. To determine the expression of Bcl-2 and Ki67 in endometrial carcinoma.

III. Materials and Methods

3.1 Endometrial samples of 30 patients with post menopausal bleeding was collected and reviewed.
3.2 Inclusion criteria included patients presenting with postmenopausal bleeding.
3.3 Exclusion criteria included patients with other known causes of bleeding such as polyp, adenomyosis, leiomyoma, coagulopathy etc..
3.4 Study period: Two years.
3.5 Study design: Prospective study
3.6 Procedure: Immunohistochemistry was performed on all these cases using both Bcl-2 and Ki67 as primary antibodies and high reactive polymer as secondary kit. Antigen retrieval was done in TRIS- EDTA buffer at ph 9 by using microwave. Brown staining of the cytoplasm in the glandular cells is taken positive for Bcl-2 and the nucleus is considered positive for Ki67**.** The mean percentage of positive glandular cells for both Bcl2 and Ki67 will be determined by counting 1000 cells in 10 randomly selected high power fields.

3.7 Scoring of Bcl-2 and Ki67: Brown staining of the cytoplasm in the glandular cells is taken positive for Bcl-2 and the nucleus is considered positive for Ki67**.** The mean percentage of positive glandular cells for both Bcl2 and Ki67 will be determined by counting 1000 cells in 10 randomly selected high power fields.
Grading[1,2,3,4,5] of Bcl-2 and Ki67 is based on both positivity and intensity of staining. Positivity for both Bcl-2 and Ki67 will be scored as
grade1 = <25%
grade 2= 25-50%
grade 3= 50-75%
grade 4= 75 – 100%
Immunostaining intensity will be scored as
Grade 1 = mild
Grade 2 = moderate
Grade 3 = strong
Grade 4 = very strong
Weighted score= positivity * intensity.
Bcl-2 stains uniformly all glandular epithelial cells, so number of cells showing positivity is always kept as grade 4. Ki67 staining cells of very strong intensity only are counted as positive. So intensity is always kept as grade 4. Bcl-2 is graded, mainly based on the intensity and Ki67 is graded mainly based on positivity and both are multiplied by 4. Thus maximum score is 16, and both Bcl-2 and Ki67 is given a score out of 16.

IV. Observation

Table -1 Expression of Bcl-2 in various types of endometrial carcinoma

s.no	Endometrial Carcinoma	Sample size	Negative	Score 4	Score 8	Score 12	Score 16	Mean score
1.	Grade 1	20		1	9	3	2	7.2
2.	Grade 2	2					2	16
3.	Grade 3	3	3					-
4.	Serous intraepithelial carcinoma	1	1					-
5.	Serous adenocarcinoma	1	1					-
6.	EIN	3			2	1		9.3

Bcl-2 score was 7.2 in grade 1 endometrial carcinoma, 16 in grade 2 and completely negative in grade 3 carcinoma, Serous intraepithelial neoplasia, Serous adenocarcinoma. Endometrial intraepithelial neoplasia showed a mean score of 9.3.

Table -2 Expression of Ki67 in various types of endometrial carcinoma

s.no	Endometrial Carcinoma	Sample size	Negative	Score 4	Score 8	Score 12	Score 16	Mean score
1.	Grade 1	20	-	10	6	4	0	6.8
2.	Grade 2	2	-	-	-	-	2	16
3.	Grade 3	3	3	-	1	2	-	12
4.	Serous intraepithelial carcinoma	1	1	-	-	-	-	-
5.	Serous adenocarcinoma	1	-	-	1	-	-	8
6.	EIN	3	1	-	-	-	2	10.6

Ki67 showed up a mean score of 6.8 for grade 1 endometrial carcinoma, 16 for grade 2 endometrial carcinoma and 12 for grade 3 endometrial carcinoma. EIN showed up a mean score of 10.6.

Table 3 Comparison of Bcl-2 and Ki67 in various endometrial tumours

s.no	Endometrial Carcinoma	Sample size	Mean score Bcl-2	Mean score Ki67
1.	Grade 1	20	7.2	6.8
2.	Grade 2	2	16	16
3.	Grade 3	3	0	12
4.	Serous intraepithelial carcinoma	1	0	0
5.	Serous adenocarcinoma	1	0	8
6.	EIN	3	9.3	10.6

Bcl-2 showed up a mean score of 7.2 for Bcl-2 and 6.8 for Ki67 in grade 1 endometrial carcinoma. In grade 2 carcinoma they showed a equal positivity of 16. In grade 3 tumours ki67 showed up a mean score of 12

whereas Bcl-2 showed only negative staining. Both were negative for Serous intraepithelial carcinoma. Serous adenocarcinoma showed a score of 8 for Ki67, whereas negative for Bcl-2.

Table 4 Expression of Ki67 in endometrial Carcinoma

S. NO	Endometrial lesions	Sample size	Ki67 mean index
1.	Endometrial adenocarcinoma grade -1	20	54%
2.	Endometrial adenocarcinoma grade-2	2	78%
3.	Endometrial adenocarcinoma grade-3	3	60%
4.	Serous intraepithelial carcinoma	1	0
5.	Serous adenocarcinoma	1	42%

V. Discussion

A total of 30 cases of endometrial carcinoma and EIN were studied which included 20 cases of grade 1 endometrial carcinoma, 2 cases of grade 2 endometrial carcinoma ,3 cases of grade 3 endometrial carcinoma, 1 case of serous intraepithelial neoplasia, 1 case of serous adencarcinoma and 3 cases of EIN.

In my study the expression of Bcl-2 is maximum in grade-1 and grade-2 tumors and is immunonegative in poorly differentiated carcinoma and serous adenocarcinoma. This loss of Bcl-2 expression is associated with increased apoptotic activity[6,7]. In my case serous adenocarcinoma shows increased apoptotic bodies and this correlates with the immunonegativity for Bcl-2.

Ki67 expression showed maximum expression in grade 2 and grade 3 tumours and its expression decreased in grade 1 endometrioid adenocarcinoma. This denotes that mitotic activity progressively increases with grade of the tumor. Ki-67 mean index was 54% for grade1 carcinoma and for other cases Ki-67 index was 78% for grade 2 carcinoma, 60% for grade 3 carcinoma, 42% in serous adenocarcinoma and was immunonegative for serous endometrial intraepithelial carcinoma. Thus Ki67 index increases with the grade of the tumour and also correlates with expression of serous adenocarcinoma which is around 40% according to literature[8]. It expression is increased in high grade serous adenocarcinoma. Thus my result correlates with most of the previous studies[9,10.]

Bcl-2 expression decreases with increasing grade of the tumour whereas Ki-67 index increases. This indicates increased apoptotic and mitotic activity with higher grade carcinomas.

3 cases of EIN was studied and both Bcl-2 and Ki-67 showed maximal expression compared to all endometrial lesions. Although the sample size is low, all the 3 cases of EIN showed 100% positivity for both Bcl-2 and Ki-67. This indicates that both anti- apoptotic activity and proliferative activity are high in the EIN which is monoclonal proliferation of neoplastic cells. This denotes increased propensity for the EIN lesions to evolve in to cancer[11].

VI. Conclusion

Endometrial intraepithelial neoplasia showed 100% positivity for both Bcl-2 and Ki67 denoting the high mitotic activity and low apoptotic activity in this clonal neoplasm. This indicates the increased potential for these lesions to transform into frank malignancies.

Bcl-2 expression decreases with increasing grade of the tumor whereas Ki-67 index increases. This indicates increased apoptotic and mitotic activity with higher grade carcinoma and negative correlation of the Bcl-2 and Ki67 expression.

This negative correlation means, the imbalance between the apoptotic and mitotic activity is responsible for the development of neoplastic conditions. The failure of apoptotic mechanism to remove the damaged and mutated cells combined with the proliferating ability of the neoplastic cells is responsible for the tumorogenesis.

Increasing the number of cases on future research will give more precise results enabling targeted therapy for these genes.

VII. Images

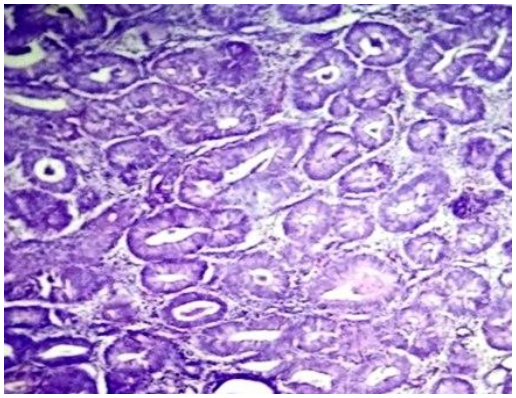

Fig 1 EIN showing glandular crowding(H&E**)**

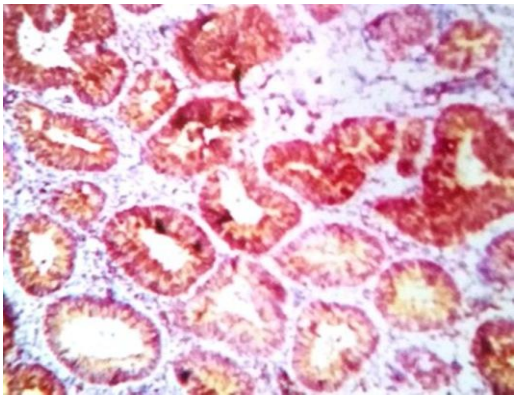

Fig 2 EIN showing gr-3cytoplasmic positivity of Bcl-2

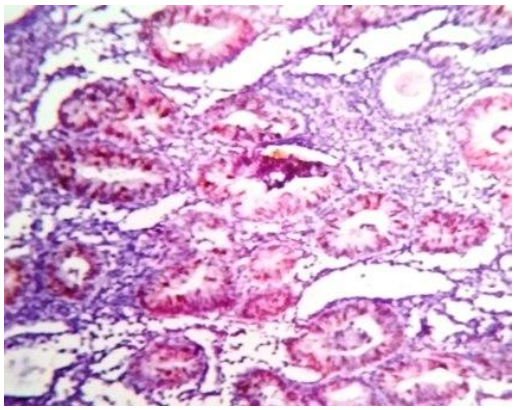

Fig 3 EIN nuclear positivity in glandular cells

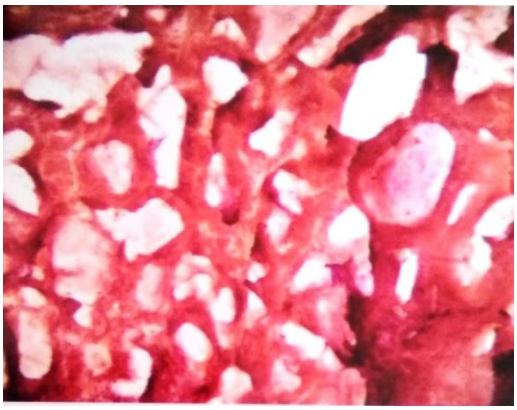

Fig 4 Grade 4Bcl-2 staining in well differentiated Ca

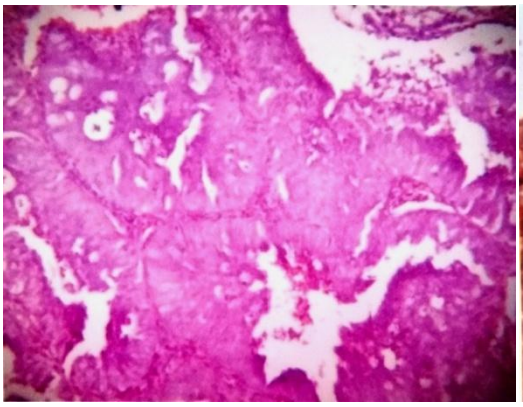

Fig 5 Moderately differentiated Ca (H & E)

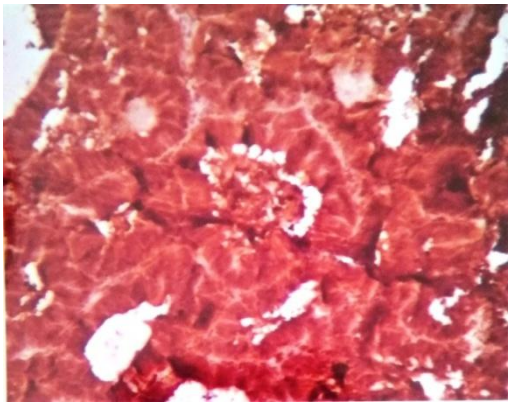

Fig 6 Bcl-2 gr 4 positivity in Moderately differentiated Ca

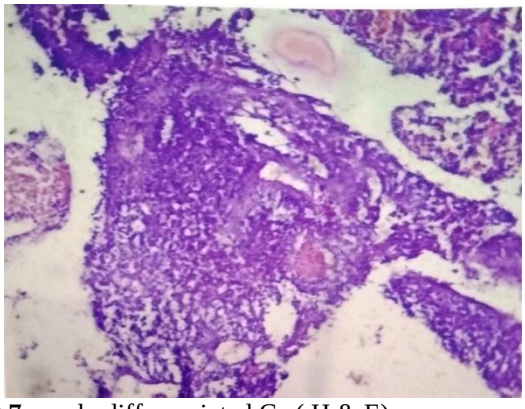

Fig 7 poorly differenciated Ca (H & E)

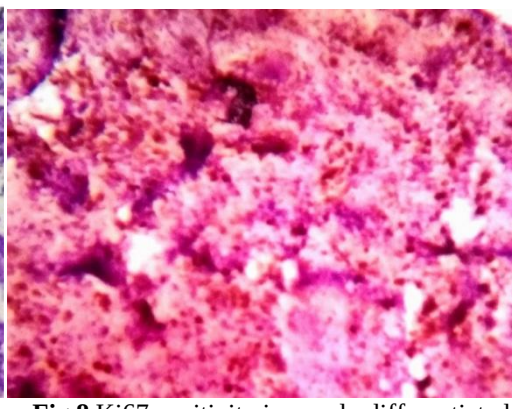

Fig 8 Ki67 positivity in poorly differentiated Ca

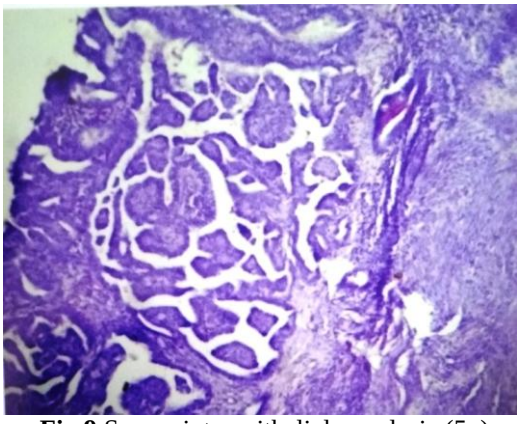

Fig 9 Serous intraepithelial neoplasia (5x)

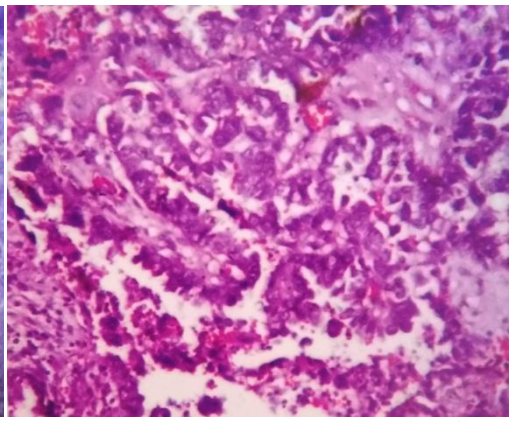

Fig 10 Serous Carcinoma (10X)

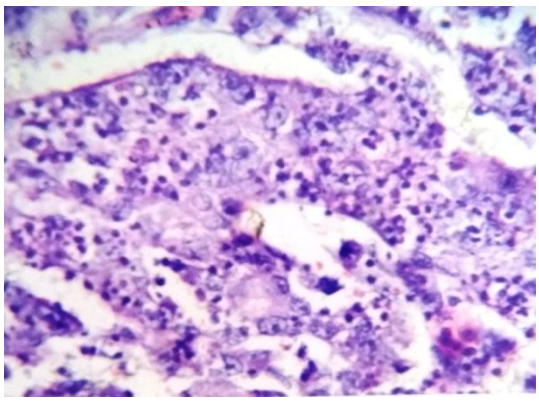

Fig 9 Serous Carcinoma (H &E) (40X)

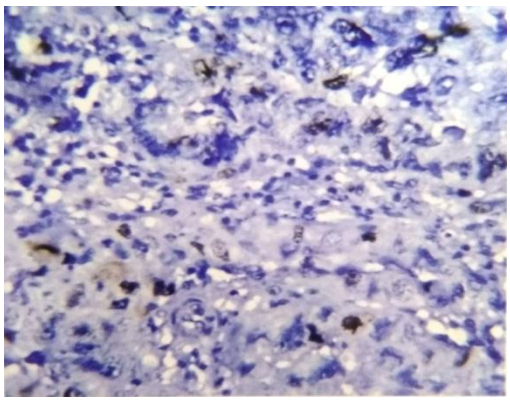

Fig 10 Ki-67 in Serous Carcinoma

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