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STUDY OF BCL2 EXPRESSION IN ENDOMETRIAL HYPERPLASIA AND ENDOMETRIAL CARCINOMA

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ABSTRACT

Endometrial cancer in one among the most common cancers affecting women. Endometrial hyperplasia is the precursor lesion of endometrioid endometrial carcinoma. Apoptosis and related proteins play an important role in the development of endometrial carcinoma. This study is done to compare the expression of BCL 2 in Endometrial hyperplasia and Endometrial carcinoma in biopsy specimens, with normal endometrium as control, and therefore to assess its role in carcinogenesis and as a therapeutic target. This study included a total of 118 samples of endometrial tissues, with 47 samples of carcinoma of endometrium, 13 cases of atypical

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hyperplasia, 34 cases of hyperplasia without atypia, and 24 samples of normal endometrial tissue. Representative tissue sections were studied by two pathologists and a final diagnosis was made, following which immunohistochemistry was performed using BCL-2 monoclonal antibody. The results were analysed statistically and comparison of BCL2 expression in Endometrial hyperplasia and endometrial carcinoma was determined by the Chi-square test. It was seen that BCL 2 expression increased from normal endometrium (62.5%) to endometrial hyperplasia without atypia (88.2%) and atypical hyperplasia (100%). Thereafter, a fall in BCL2 expression was observed in increasing grades of Endometrial Carcinoma (55.3%). The result was statistically significant (p=0.005). This supports that, increase in anti-apoptotic activity is associated with hyperplastic lesions.

KEYWORDS: BCL2, Endometrial hyperplasia, Endometrial carcinoma

INTRODUCTION

Endometrial carcinoma (EC) is the sixth most common cancer diagnosed in women worldwide, making it one of the most common cancers. India stands third in terms of overall mortality rate in 2022 and fifth among those with the greatest occurrence.[1] It is the third most prevalent genital tract cancer in women in India.[2]

Type I EC is low grade and is oestrogen-related. Endometrioid carcinoma is the prototype of this tumor. They are frequently linked to endometrial hyperplasia, and typically have a better prognosis. Type II ECs are oestrogen-independent and are more aggressive histological subtypes. Serous and clear cell carcinomas are two prototypes of this tumour. They are associated with endometrial atrophy and hence, more common in post-menopausal women. Type II tumours are linked to a worse clinical outcome.

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Endometrial Hyperplasia are of two types. EH Without Atypia (Benign EH) is less concerning and has a lower risk of progressing to cancer and is characterized by simple or complex glandular proliferation without cellular atypia. Atypical Endometrial Hyperplasia is more significant and carries a high risk to progress into endometrial carcinoma. They exhibits abnormal cell features, like nuclear atypia with increase in mitotic activity (2,3).

Apoptosis is an important mechanism in maintaining cell homeostasis, dysregulation of which can significantly contribute to cancer development 008. BCL-2 or the B-cell lymphoma -2, a proto-oncogene, regulates apoptosis and dysfunction of this gene can lead to carcinogenesis. Dysregulated BCL-2 expression due to mutations or other mechanisms is a triggering event in the pathogenesis of endometrial malignancies, contributing to tumour progression and survival of malignant cells.0012

This study aims to study BCL 2 expressions in hyperplasia and carcinoma of endometrium, using normal endometrium as a control, to assess its role in disease progression and its value as a potential therapeutic target.

MATERIALS AND METHODS

This study was conducted in the Department of Pathology, Sree Gokulam Medical College and Research Foundation, Thiruvananthapuram. Hysterectomy and endometrial biopsy specimens diagnosed as Endometrial hyperplasia and Endometrial carcinoma in 2023 and 2024 were included. Biopsy specimens from 24 normal endometrium are taken as control group. Representative sections of the endometrial lesion were taken and processed in automated tissue processor.

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These tissues were embedded in paraffin blocks which were sectioned in the microtome in about 3 micrometre thickness.

Haematoxylin and eosin staining of these sections were done and histopathological examination was done and examined by two pathologists.

In case of any inter observer variability, the slide was reviewed by a senior pathologist to arrive at a final diagnosis.

Following which, immunohistochemistry was performed with BCL-2 monoclonal antibody. The interpretation staining pattern and staining intensity of BCL2 is given in Table 1 and 2 respectively.

TABLE 1: BCL2 STAINING PATTERN

Brown staining in cytoplasm of >10% endometrial glandular cells	POSITIVE
Brown staining in cytoplasm of less than 10% of endometrial glandular cells or absence of staining	NEGATIVE

Table 2: INTENSITY OF STAINING PATTERN

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BCL 2 EXPRESSION IN CELLS	STRENGTH OF STAINING
Negative score / 0	No staining
Positive score 1 +	Slight staining in some or most cells
Positive score 2+	Moderate strong staining
Positive score 3+	Strong staining in almost all the cells

Microsoft Office Excel for handling and preparing data and SPSS were used for analysis. Percentage proportion was calculated. The association between BCL2 expression in endometrial hyperplasia and carcinoma was evaluated by chi-square test. p value less than 0.05 were considered as significant

OBSERVATIONS AND RESULT

This study included a total of 118 samples of endometrial tissues, with 47 samples of carcinoma of endometrium, 13 cases of atypical hyperplasia, 34 cases of hyperplasia without atypia, and 24 samples of normal endometrial tissue

The age of the patients ranged from 30 years to 78 years. The mean age of presentation was 52 years. Maximum samples were in the age group 41 to 60 years. Highest percentage of endometrial carcinoma, was noted in age group 41 to 60 years. However, the percentage of EC in the age group more than 60 years, was 76.2% with 16 out of 21 cases, presenting with various symptoms.

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In the present study, 46.6% of samples were endometrial curetting and 53.4% samples were hysterectomy specimens. On comparing the expression of BCL2 with the two types of specimens, high positivity was noted in Endometrial curetting (81%) compared to Hysterectomy specimens (60%). The association was found to be statistically significant(p=0.003)

COMPARISON OF BCL2 EXPRESSION IN TYPES OF ENDOMETRIAL HYPERPLASIA

On comparing the expression of BCL2 in endometrial hyperplasia, 30 out of 34 cases of endometrial hyperplasia without atypia showed positive expression (88.2%), whereas all the 13 cases of atypical hyperplasia were BCL2 positive. Thus, the expression of BCL2 was increased from hyperplasia without atypia to atypical hyperplasia cases. But the comparison failed to achieve significance. (p=0.363).

COMPARISON OF BCL2 EXPRESSION IN NORMAL ENDOMETRIUM, ENDOMETRIAL HYPERPLASIA AND ENDOMETRIAL CARCINOMA

62.5% samples of normal endometrium were positive for BCL2 expression in our study. Out of 47 cases of endometrial hyperplasia ,43 cases were BCL 2 positive (91.5%) and out of the 47 cases of endometrial carcinoma, only 55.3% cases demonstrated BCL2 positivity. Thus, the expression of BCL2 increased from normal endometrial samples to endometrial hyperplasia. Thereafter a fall in expression was noted in cases of endometrial carcinoma. This result, on statistical analysis, was found to be significant. (p=<0.001).

On correlating with BCL2 expression with normal, hyperplasia without atypia, atypical hyperplasia and carcinoma, positivity was noted in 62.5% of normal endometrium, 88.2% cases

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of hyperplasia without atypia, 100% cases of atypical hyperplasia and 55.3% cases of endometrial carcinoma. Here, we could find that, expression of BCL2 increases from normal through hyperplasia without atypia, to atypical hyperplasia. But expression decreased in carcinoma cases. The comparison was statistically significant (P=0. 005).

COMPARING THE INTENSITY OF BCL2 EXPRESSION IN THE STUDY POPULATION

On comparing the intensity of BCL2 Expression, a greater number of normal endometrium were negative, and followed by 1+ or weak positivity (29.2%). Strong or 3+ positivity was observed only in 12.5 % samples.

In endometrial hyperplasia without atypia, maximum number of cases showed strong /3+ positivity (35.3%) whereas, in atypical hyperplasia maximum cases (46.2%) showed 2+ positivity (Figure 1). Strong positivity / 3+ was noted in 38.5 % cases.

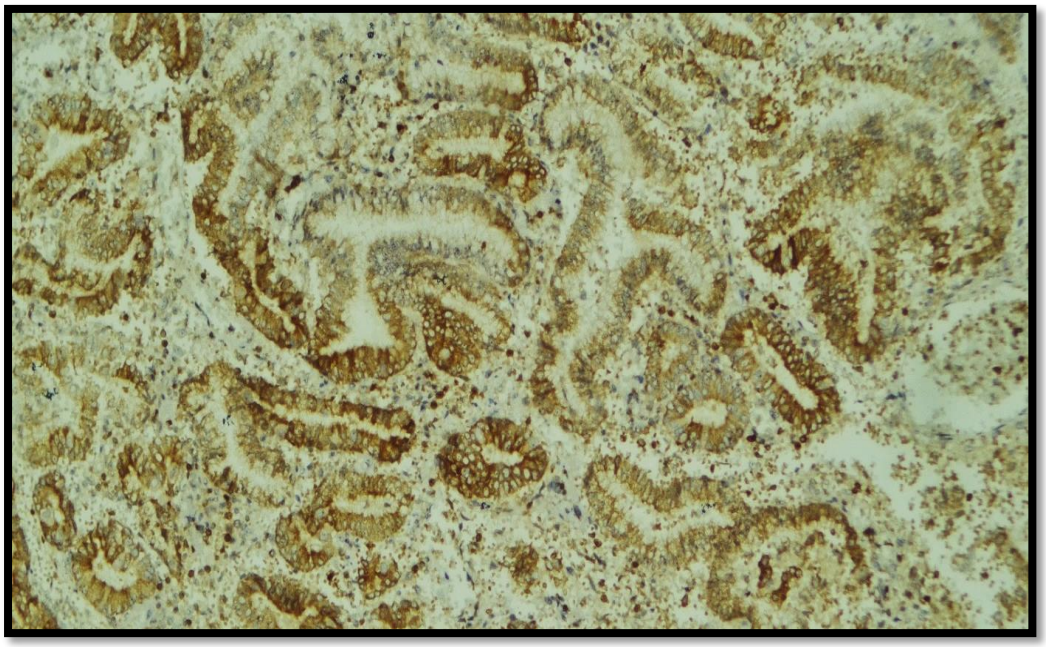

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FIGURE 1: Photomicrograph showing moderate positivity for BCL2 immunohistochemical expression in atypical hyperplasia (X40)

In endometrial carcinoma, where 44.7 % cases showed negative expression, 3+/ strong intensity of BCL2 was noted only in 10.6 % cases.

Hence, in our study, we observed an increase in strength of intensity of BCL2 expression from normal to hyperplasia without atypia, to atypical hyperplasia. The intensity decreased in carcinoma cases.

COMPARISON OF BCL 2 EXPRESSION IN GRADES OF ENDOMETRIAL CARCINOMA

In this study, 20 out of 47 cases of endometrial carcinomas were Grade I (42.5%), 12 cases were Grade II (25.5%) and 15 cases were Grade III (31.9%).

Strong intensity of BCL2 expression was observed in 25% Grade I cases, with maximum number (40%) of grade 1 showing 2+ /moderate intensity (Figure 2). Strong intensity / 3+ was not observed in grade II and grade III carcinoma (Figure 3). Maximum proportion of Grade II and Grade III carcinomas were Negative for BCL 2 expression.

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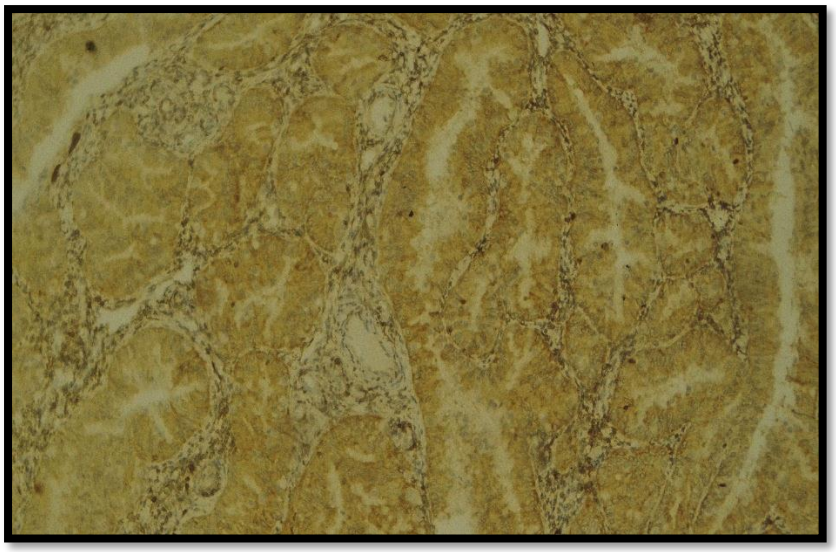

FIGURE 2: Photomicrograph showing moderate positivity for BCL2 immunohistochemical expression in grade I carcinoma endometrium (X40)

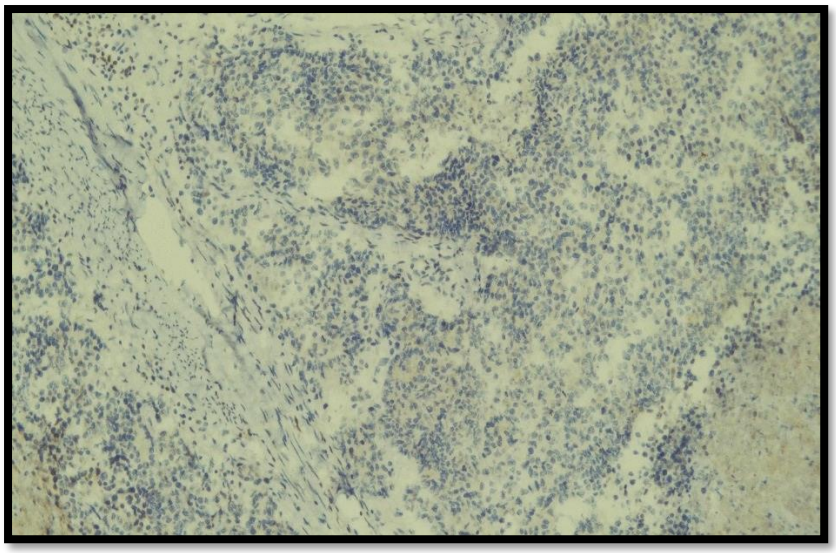

FIGURE 3: Photomicrograph showing negative BCL2 immunohistochemical expression in grade III endometrial carcinoma (X40)

DISCUSSION:

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Tissue homeostasis in higher organism is maintained by balancing cellular proliferation and cell death. Apoptosis or programmed cell death Type I and autophagy or programmed cell death type 2 are the two important mechanisms by which homeostasis in maintained.[4]

Thus, defects in apoptosis play an important role in pathogenesis of tumours , which allow the neoplastic cells to survive beyond their lifespan.[5]

BCL2, a proto-oncogene, is involved in cell death regulation and survival of cells without affecting proliferation of cells. Over- expression of BCL-2 is implicated in many blood cell malignancies, non-neoplastic epithelia, and in number of carcinomas.

Apoptotic pathways are triggered by endometrial cells in human endometrium as the menstrual cycle progress from secretory phase to menstruation.[6]

Expression of this gene is maximum in the proliferative endometrial glands and thereafter decreases as it enters the secretory phase in endometrial tissue under normal conditions.[6,7]

In the present study, 62.5% samples of normal endometrium were positive for BCL2 expression.

Laban et al [8] demonstrated in his study of BCL 2 expression that BCL2 expression increase from normal endometrium to ,endometrial hyperplasia without atypia and then to atypical hyperplasia.

Niemann et al[9] conducted a study in endometrium to study the expression of BCL2 in hyperplasia as well as carcinoma using immunohistochemistry , noting that, staining pattern observed in atypical hyperplasia and carcinoma was focal and less intense compared to the reactivity observed in proliferative phase of normal endometrium

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Similar observations were noted in a study conducted by Simin Samani et al[10], that BCL 2 expression showed increase in positivity from normal endometrium (60%) to 76.2% in endometrial hyperplasia, following which a fall in BCL2 positivity was noted.

Similar findings were observed in our study. We could find that, expression of BCL2 increases from normal through hyperplasia without atypia, to atypical hyperplasia. But expression decreased in carcinoma cases. The comparison was statistically significant (P=0. 005).

Also, in our study, we observed an increase in strength of intensity of BCL2 expression from normal to hyperplasia without atypia, to atypical hyperplasia. The intensity decreased in carcinoma cases.

In his study, Laban et al [8] noted that , with decreasing differentiation, the staining intensity also decreased. This was similar to the results obtained in our study. But, the study failed to attain statistical significance (p=0.29).

In a study done by Lech Chyczewski et al[11] in Poland, the researcher found a significant correlation between the grade of endometrial carcinoma and staining pattern of BCL2. They observed that in type I carcinoma the staining decreased as follows: 88.4% in stage I ,20.8% in stage II and 11.1% in stage III. The result was statistically significant with p value less than 0.001. This was similar to observations in our study.

CONCLUSSION

BCL 2 expression increased from normal endometrium, through endometrial hyperplasia without atypia, to atypical hyperplasia. This suggest that, loss of apoptosis is an important factor involved in hyperplastic transformation of endometrium.

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Endometrial hyperplasia, being the precursor lesion of endometrioid carcinoma, targeted therapy against BCL 2, may be used to prevent tumorigenesis.

Decrease in staining pattern in high grade carcinoma, suggests a loss of control of cellular homeostasis or the presence of an alternate or additional mechanisms involved in tumour progression in high grade carcinoma. As this finding was statistically significant, loss of BCL 2 expression, can be considered as a marker of aggressiveness in carcinoma cases.

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