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Correlation of Bcl-2 and Ki 67 expression with stage of Endometrial carcinoma-Is there a link

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ABSTRACT

Introduction: There is increasing incidence of endometrial carcinoma in developing countries, with it being the second most common malignancy of the female genital tract. The Bcl-2 gene is an important component of the Bcl-2 family of proteins which play a central role in regulating cell death. Ki 67 is widely used as a cell proliferation marker to grade tumours. The objective of this study to understand the correlation between Bcl-2 and Ki-67 expression and the clinical stages of endometrioid endometrial carcinoma

Materials and Methods : A retrospective analysis was conducted on 20 endometrial carcinoma cases. Immunohistochemical staining of Bcl-2 and Ki-67 was performed and expression levels were scored. The expression levels were evaluated and correlated with tumor staging according to the TNM staging.

Results : 10 cases were of stage 1, 08 cases were of stage 3 and only 2 cases belonged to stage 2. All cases were 100% positive for both Bcl-2 and Ki 67 staining. There was a trend of decreasing Bcl-2 staining score with increasing stage of carcinoma which was found to be statistically significant (p=0.0044). Association between increasing stage of endometrial

carcinoma and increased degree of Ki67 staining was also found to be statistically significant. (p=0.0194).

Conclusion: The correlation of Bcl-2 and Ki-67 with endometrial carcinoma stages underscores their potential as prognostic indicators. Further studies with larger cohorts are warranted to validate these findings and explore their implications in clinical practice.

Key Words: Bcl-2. Ki-67, Immunohistochemistry, Endometrial Carcinoma

INTRODUCTION

There is increasing incidence of endometrial carcinoma in developing countries, with it being the second most common malignancy of the female genital tract. It is classified into two types: namely type 1 and type 2 carcinomas. Endometrial carcinomas are seen in the older age group, with a median age of presentation of 65 years. [1] Genetic predisposition as well as high body mass index and unopposed estrogen exposure play a role in its genesis.80% of endometrial carcinomas falls in the type 1 category and show endometrioid differentiation. Compared to type 2 carcinomas , these tumours have a good prognosis and is usually of a lower grade. Type 2 carcinomas are rare compared to type 1 carcinomas. They carry a poor prognosis and are more aggressive in nature. [2]

Endometrial carcinoma is classified as type I and type II endometrial carcinoma. Type I endometrioid tumors are the most common tumors and 80% of the tumor belongs to this type. It occurs typically at 65 years of age or less and is low grade, present at an early stage with a good prognosis. These tumors usually are hormone dependent. The occurrence of type II tumors is rare compared to type I tumors. They are high grade, aggressive in nature with poorer prognoses [1-3]

Immunohistochemical markers are of prime importance , in this era of targeted therapy. They play a significant role in therapeutic and prognosis of endometrial carcinoma. [3]

The Bcl-2 gene is an important component of the Bcl-2 family of proteins which play a central role in regulating cell death. [4] Regulation of balance between cell proliferation and cell death are very critical for normal and neoplastic tissue homeostasis. In the normal endometrial, the expression of Bcl-2 appears to be under control of hormonal levels.(5) Bcl-2 displays a cyclic pattern of expression, in the normal endometrium. Its expression is maximal in the glandular

epithelium, in the mid proliferative phase and disappears by the secretary phase. [6]The deregulated expression of Bcl-2 is said to play a role in evolution of different malignancies [4].Studied regarding Bcl-2 expression in endometrial hyperplasias and carcinomas are limited and have often revealed contrasting results. The exact mechanism of Bcl-2 in these lesions still remain unclear. [7,8]

Ki-67 is one of the cell proliferation markers expressed in the nucleus of proliferating mammalian cells. [9] It is therefore widely used as a cell proliferation marker to grade tumours[9]. Ki-67 also co-localizes with satellite NA and is found in protein complexes that bind to satellite DNA. [10] Ki-67 is a substrate of the cyclin-dependent kinase CDK1 and is hyperphosphorylated in mitosis, which may regulate its expression and / or localisation. [10]

Studies have shown increasing Ki67 positivity with increasing severity of endometrial lesions i.e., from endometrial hyperplasia to endometrial carcinoma.[11,12]

At the St. Gallen Consensus Conference, it was decided that a threshold of ≥ 20% can be taken as high Ki 67 expression level in the case of breast cancer. [13] There have been various studies reporting different cut-off values of Ki67 index which can be used for post operative management in carcinoma breast. [14,15] The risk of recurrence in endometrial cancer is determined by staging, histological subtype, grading , lymph node status and degree of myometrial invasion. Increasing number of studies have shown that Ki 67 has an important prognostic and predictive value in the case of endometrial cancer. [16]

MATERIALS AND METHODS

The present study was conducted from January 2017 to June 2018 in our tertiary care institution. Institutional ethical clearance and patients informed consent was obtained prior to the study. Cases diagnosed as endometrioid enomdetrial carcinoma were included. Non epithelial and Non endometrioid malignancies of the endometrium were excluded from the present study. All the relevant clinical, laboratory , and radiological findings of the patient was recorded. Following

surgical procedure , hysterectomy specimens were sent to the department of pathology. 20 cases diagnosed as endometrial carcinoma (endometrioid type) were included here. Tumours were staged according to AJCC recommendations (8^{th} ed.) (Table 1).

Immunohistochemical staining was performed for Bcl-2 (Clone 124, DAKO) and Ki67(Clone Mib-1, DAKO), following the manufacturer's instruction. Positive control tissue sections of tonsil (for Bcl-2 and Ki67) were used.

Assessment of Bcl-2 expression was scored based on intensity of staining (Table 2). Assessment of Ki-67 was performed using proportion of positive cells (percentage positivity) (Table 3). Relevant data was recorded in Microsoft Excel and analysis performed using SPSS 23 software. Fishers exact test was used to analyze level of significance between level of Bcl-2 and Ki 67 expression with regard to stage of tumours. P value of <0.05 was considered significant.

Table 1: TNM Staging of Endometrial Carcinoma [17]

T- Primary tumour

TNM	FIGO stages	Surgical-pathologic findings
TX	-	Primary tumour cannot be assessed
T0	-	No evidence of primary tumour
Tis*	-	Carcinoma in situ (preinvasive carcinoma)
T1	I[a]	Tumour confined to corpus uteri
T1a	IA[a]	Tumour limited to endometrium or invading less than one half of the myometrium
T1b	IB	Tumour invades one half or more of the myometrium
T2	II	Tumour invades stromal

		connective tissue of the cervix but does not extend beyond uterus
T3 and / N1	III	Local and /or regional spread as specified below
T3a	III A	Tumour invades the serosa of the corpus uteri or adnexae (direct extension or metastasis)
T3b	III B	Vaginal or parametrial involvement (direct extension or metastasis)
N1	IIIC	Metastases to pelvic and/or para-aortic lymph nodes
	IIIC1	Metastasis to pelvic lymphnodes
N2	IIIC2	Metastases to para-aortic lymph nodes with or without metastasis to pelvic lymphnodes
T4	IVA	Tumour invades bladder/or bowel mucosa
M1	IV B	Distant metastasis (excludes metastasis to vagina, pelvic serosa or adnexae

Regional lymph nodes (N)

TNM	Surgical-pathologic findings

NX	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastasis
N1	Regional lymph node metastasis to pelvic lymph nodes

Distant metastasis (M)

TNM	Surgical-pathologic findings
M0	No distant metastasis
M1	Distant metastasis (includes metastasis to inguinal lymph nodes, intraperitoneal disease, or lung, liver, or bone metastases; it excludes metastasis to para-aortic lymph nodes, vagina, pelvic serosa, or adnexa)

Table 2 **: Intensity Scoring of Bcl-2** [18]

Score	**Intensity of staining**
0	Absent staining
1	Weak staining
2	Moderate staining
3	Strong staining

Table 3 : Proportion scoring of Ki- 67 [19]

Scoring	Proportion of positive cells

0	< 5%
1+	5-25%
2+	25-50%
3+	50-100%

RESULTS

The ages of all patients ranged from 50 years to 70 years with a mean age of 58.2 years. The mean tumour size was 4.5 cm. A total of 20 cases of endometrioid endometrial carcinoma were included in this study. 10 cases were of stage 1, 08 cases were of stage 3 and only 2 cases belonged to stage 2. Immunohistochemical staining was analyzed for markers Bcl-2 and Ki-67.

All cases were 100% positive for both Bcl-2 and Ki 67 staining. With regard to Bcl-2 staining 06 cases of stage 1 carcinoma displayed score 3 staining. Both cases of stage 2 carcinoma showed score 2 staining and in case o stage 3 carcinoma, majority of the cases (06/08) showe score 1 staining. This trend of decreasing Bcl-2 staining score with increasing stage of carcinoma was found to be statistically significant (p=0.0044).

With respect to Ki 67 expression, 04 cases each of stage 1 carcinoma showed 1+ and 2+ Ki 67 staining respectively, with only two case showing 3+ staining. One case each of stage 2 carcinoma showed 2+ and 3+ Ki 67 staining. Majority of cases of stage 3 carcinoma displayed strong 3+ Ki 67 staining (06/08) and only two cases showed 2+ staining. Association between stage of endometrial carcinoma and degree of Ki67 staining was also found to be statistically significant. (p=0.0194).

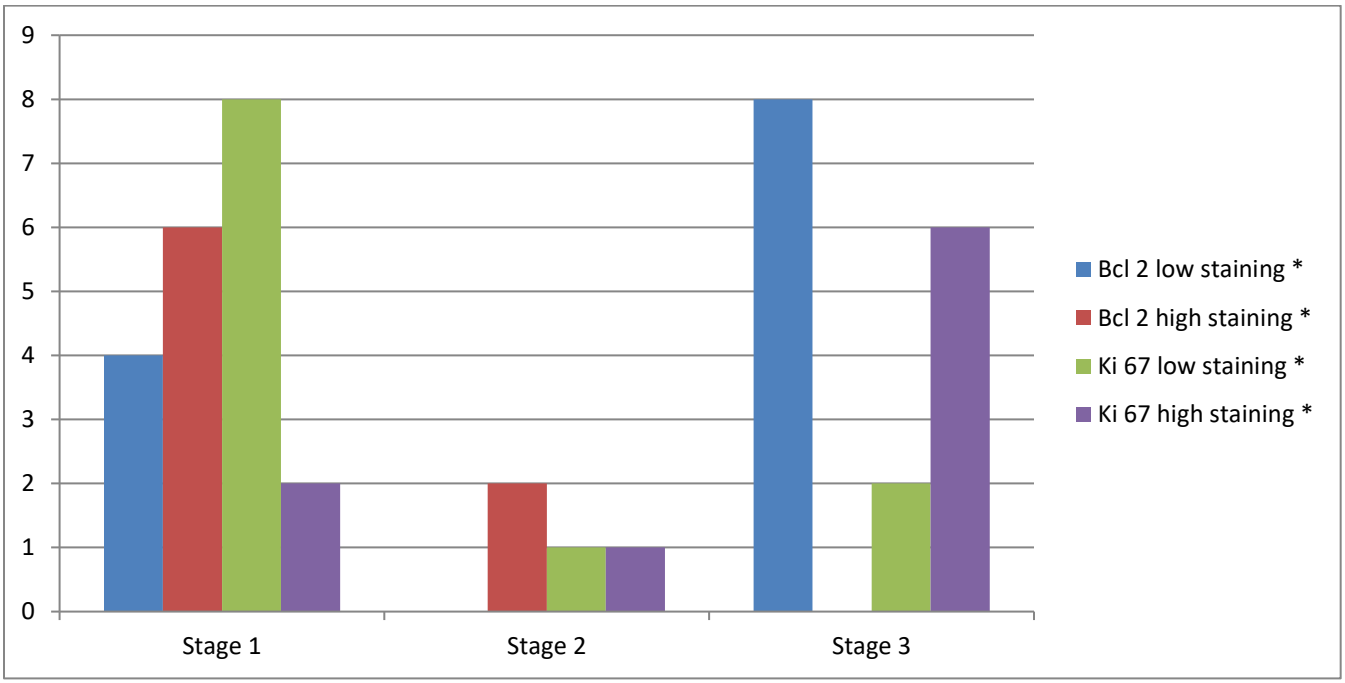

Figure 1: Association of endometrial carcinoma stage with Bcl-2 and Ki 67 expression

*(Bcl-2 low staining (score 1 and score 2), Bcl-2 high staining (Bcl-2 score 3); Ki 67 low staining (score 1+ and score 2+), Ki 67 high staining (score 3+)

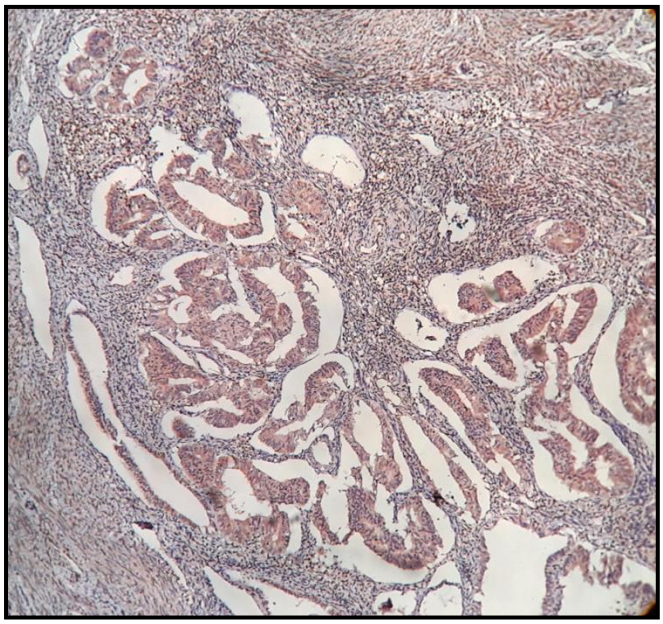

Figure 2: Photomicrograph of Bcl-2 score 2 staining in endometrioid endometrial carcinoma (Bcl-2, 10x)

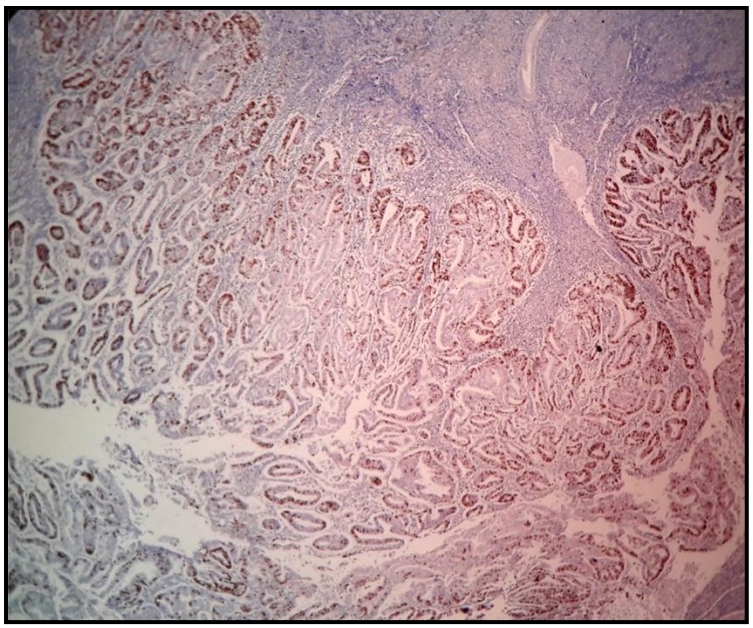

Figure 3: Photomicrograph of Ki 67 3+ staining in endometrioid endometrial carcinoma (Ki-67, 10x)

DISCUSSION

The results of this study demonstrate a significant decrease in Bcl-2 expression correlated with the advancing stages of endometrioid endometrial carcinoma as well as a significant increase of Ki 67 expression with increased stages of carcinoma.

Alterations in apoptotic regulatory proteins may contribute to tumor progression and resistance to therapy. Bcl-2, an anti-apoptotic protein, plays a crucial role in regulating cell survival, and its down regulation in endometrioid endometrial carcinoma suggests a shift towards increased apoptosis in more advanced tumor stages. [20] Previous studies have indicated that high Bcl-2

expression is associated with a more favorable prognosis in various cancers, including endometrial carcinoma (20). The decrease in Bcl-2 levels observed in our research correlates with findings by Wang et al. (2021), who noted that diminished Bcl-2 expression was linked to increased tumor invasiveness and a higher likelihood of metastasis. This indicates that as endometrial carcinoma progresses, the tumor cells may adapt by evading apoptosis, which could be mediated by other cellular pathways. [21]

The relationship between Bcl-2 and other apoptotic regulators, such as Bax and caspases, has been explored in previous literature.

Li et al. (2020) found that a decrease in Bcl-2 was associated with an increase in Bax, suggesting a potential mechanism by which cancer cells might enhance their apoptotic susceptibility. This dual regulation underscores the complex nature of apoptosis in endometrioid endometrial carcinoma . [22]

The prognostic implications of Bcl-2 expression in endometrial carcinoma are of importance. Our findings suggest that low Bcl-2 levels in advanced stages may serve as a marker for aggressive disease and poor prognosis. Mvondo et al. (2022) indicated that decreased Bcl-2 expression was predictive of reduced overall survival in endometrial cancer patients. The identification of Bcl-2 as a potential biomarker could aid in stratifying patients for targeted therapies. [23]

We also found a significant correlation between increasing Ki-67 expression and the advancing stages of endometrioid endometrial carcinoma . Ki-67, a well-established marker of cellular proliferation, serves as a critical indicator of tumor growth and aggressiveness. [24]Numerous studies have documented the relationship between Ki-67 expression and endometrial carcinoma. Lax et al. (2018) reported that elevated Ki-67 levels were linked to high-grade tumors and advanced stage disease. [24]

A meta-analysis by Zhang et al. (2020) found that high Ki-67 expression was associated with reduced overall survival in patients with endometrial cancer, indicating its potential as a prognostic biomarker. The implications of Ki-67 expression is significant, in terms of recurrence

and metastasis of endometrial carcinoma . [25] Lax et al. (2018) found that patients with Ki-67 expression levels exceeding 20% had a markedly increased risk of disease recurrence. [24]

Gaarenstroom et al. (2016) reported that a Ki-67 expression of ≥ 20% is predictive of poorer overall survival in patients with endometrial carcinoma. [26]

The mechanisms underlying increased Ki-67 expression in advanced endometrial carcinoma may be multifaceted. Mvondo et al. (2022), suggested that alterations in key regulatory pathways, such as the PI3K/Akt and MAPK signaling cascades, may lead to increased cell cycle activity and, consequently, elevated Ki-67 levels. [23]

The clinical implications of our findings are significant. The correlation between Ki-67 expression and endometrial carcinoma staging underscores the potential of Ki-67 as a valuable prognostic marker in clinical practice. Early identification of patients with high Ki-67 levels could prompt more aggressive treatment strategies, as these patients may be at greater risk for rapid disease progression. This is consistent with the conclusions of Gaarenstroom et al. (2016), who emphasized the importance of incorporating proliferation markers like Ki-67 into routine histopathological assessment for endometrial cancer. [26]

CONCLUSION

Our study points towards the fact that decreased Bcl-2 expression is associated with advancing stages of endometrioid endometrial carcinoma. Also there is a clear association between increasing Ki-67 expression and increasing stages of carcinoma. This highlights the role of Ki-67 as a potential prognostic biomarker .Further investigations, into the role of Bcl-2 and Ki 67 in endometrial carcinoma will be essential for developing more effective therapeutic strategies aimed at modulating apoptotic pathways in this malignancy.

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None

CONFLICTS OF INTEREST

None

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