Original Article

Bcl-2 May Contribute to Evolution of Endometrial Hyperplasia, but It Isn't a Factor in Subsequent Carcinogenesis

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ABSTRACT

Endometrial carcinoma is a prevalent disease in the Western world and is experiencing an upward trend in developing countries as well. Endometrial hyperplasia is regarded as a precancerous lesion. Apoptosis plays an important role in the neoplastic transformation of cells, with Bcl-2 serving as an anti-apoptotic cellular marker. It is possible that Bcl-2 may plays an important role in the development of endometrial carcinoma. The objective was to evaluate and compare the expression of the Bcl-2 markers accross the spectrum of normal endometrium endometrial hyperplasia and endometrial adenocarcinoma**.** A total of 50 cases were included in this study, comprising of 10 cases of normal endometrium, 10 cases of endometrial hyperplasia without atypia, 10 cases of atypical endometrial hyperplasia and 20 cases of endometrial adenocarcinomas. The cases were collected from January 2017 to June 2018. Immunohistochemical staining with Bcl-2 was performed and the results were subsequently analyzed. Bcl-2 staining demonstrated a notable increase in cases exhibiting with strong staining intensity, from 20% in normal endometrial tissue to 75% in cases of endometrial hyperplasia. However, there was a notable decline in the number of cases exhibiting with strong intensity Bcl-2 staining as the lesions progressed from endometrial hyperplasia to endometrial carcinoma (30% of the cases). The results were statistically significant (P =0.00309). However, there was no significant association was observed between staining and either atypical hyperplasia or endometrial carcinomas (p=0.429), or between the degree of carcinoma and staining (p=0.6903). Bcl-2 expression demonstrated an increase from cases of normal endometrium to endometrial hyperplasia, which supports the hypothesis that there is an increase in anti-apoptotic activity in endometrial hyperplastic lesions. The observed decrease in Bcl-2expression in endometrioid adenocarcinoma when compared to endometrial hyperplasia may indicate the involvement of alternative mechanisms of carcinogenesis, potentially beyond the failure of apoptosis.

Keywords: Bcl-2, Endometrium, Endometrial Hyperplasia, Endometrial Hyperplasia with Atypia, Endometrial Carcinoma

1. Introduction

Endometrial adenocarcinoma is prevalent form of cancer among women in Western countries (1). Uterine cancer was the most prevalent gynecological malignancy in the United States; as it is in other developed countries. In each year there were more than 60,000 new cases and over 10,000 deaths from the disease (2). Endometrial hyperplasia is defined as the proliferation of irregularly shaped and sized glands within the endometrium, accompanied by an increase in the gland- to- stroma ratio (3). The unopposed estrogen influence; in the absence of progesterone leads to the exuberant proliferation of the glands in endometrial hyperplasia (4). The World Health Organization (WHO) differentiates between two categories of endometrial hyperplasia: hyperplasia without atypia and atypical hyperplasia/ endometrioid intraepithelial neoplasia (EIN) (5). Benign endometrial hyperplasia is defined by the WHO classification as "an exaggerated proliferation of endometrial glands of irregular size and shape, with an associated increase in the gland to stroma ratio compared with proliferative endometrium, but without significant cytological atypia" (5,6). Atypical hyperplasia (AH) has been identified as a potential precancerous lesion (7). Atypical endometrial hyperplasia /endometrioid intraepithelial neoplasia (EIN) is defined as "cytological atypia superimposed on an endometrial hyperplasia" (6,8). Endometrial hyperplasia represents a precancerous lesion of well-differentiated (type 1) endometrial carcinoma (4). Nevertheless, the pathogenetic relationship between these conditions has not been subjected to the same degree of analysis as that which has been conducted in the case of cervical neoplasia (4). Any disturbance in the regulation of cellular functions, including cell proliferation and differentiation, can contribute to the development of neoplastic lesions. Subsequently, abnormalities in apoptosis regulation may prove to be a significant factor in this process (9). Bcl-2 is a protein that is located on the mitochondrial membrane, endoplasmic reticulum, and nuclear membrane (9). It exerts an anti-apoptotic function at the cellular level. The normal cycling endometrium has been observed to express Bcl-2, with recent studies indicating that Bcl-2 is strongly expressed in endometrial hyperplasia, with and without atypia (10). The development and progression of endometrial carcinomas are significantly influenced by anti-apoptotic markers (10). The objective of the present study was to observe and compare the expression of Bcl-2 in normal endometrium, endometrial hyperplasia and endometrial adenocarcinoma.

2. Materials and Methods

This study included endometrial biopsy, curettage and hysterectomy specimens received at the Department of Pathology at our tertiary care institution between January 2017 to June 2018.The study was conducted in a prospective manner. The study included cases of normal endometrium, endometrial hyperplasia and endometrial endometrioid carcinomas diagnosed from samples of endometrial curettings and hysterectomy specimens were included in our study. Samples with inadequate material and cases of non-epithelial tumors of the endometrium were excluded from the study. Immunohistochemical analysis (IHC) was conducted on 50 endometrial samples, comprising 10 normal endometrium, 20 cases of endometrial hyperplasia (10 of hyperplasia without atypia and 10 cases of atypical hyperplasia), and 20 endometrial carcinomas. The specimens were subsequently fixed in 10% formalin. The paraffin-embedded sections, measuring 3–5 microns in thickness, were stained with Haematoxylin and eosin (H&E) for the purpose of studying their histopathological features. The selection of the samples for immunohistochemical examination was based on the histological diagnosis according to the WHO histological classification. Accordingly, a, representative paraffin block was taken for each case. Immunohistochemical staining was conducted for Bcl-2 (Clone 124, DAKO), in accordance with the manufacturer's instructions. Tonsil tissue section samples were employed as positive control

2.1. Evaluation of Immunostaining for Bcl-2

Expression of Bcl-2 was localized in the cytoplasm of the glandular cells. The grading of Bcl-2 was conducted by in accordance with the staining intensity in the glandular cells in comparison to the positive control tonsil tissue (Table 1).

2.2. Statistical Analysis

The data were entered into an Excel spreadsheet and analyzed using SPSS 23 software. The level of significance between IHC (Bcl-2) staining and various parameters was determined using Pearson's chi-square test. A p-value of less than 0.05 was considered to indicate a statistically significant result.

3. Results

The relevant gross and microscopic characteristics, the International Federation of Gynecology and Obstetrics (FIGO) grading and the immunohistochemical scoring for Bcl-2 were entered into a proforma. Subsequently, the data were recorded in a master chart and subjected to analysis. Of the 20 cases of normal endometrium analyzed, the majority were classified as proliferative endometrium (70%), while the remaining cases were categorized as secretory endometrium (30%). The ages of the patients ranged from 30 to 70 years. Cases of endometrioid carcinoma were graded according to the FIGO grading system: FIGO 1 (5% or less of solid growth), FIGO 2 (6-50% solid growth), and FIGO 3 (>50% solid growth). The majority of endometrioid carcinoma cases belonged to FIGO grade 1 (12 out of 20 cases), followed by an equal number of FIGO grade 2 and FIGO grade 3 cases. The evaluation of Bcl-2 was conducted by grading the intensity of cytoplasmic staining in the glandular epithelial cells, ranging from score 0 (absent staining) to score 3 (strong intensity). Bcl-2 was observed as a brown cytoplasmic stain, and positive staining was observed in 100% of the cases (Figure 1).

Table 1: Intensity Scoring of Bcl-2 (11, 12)

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

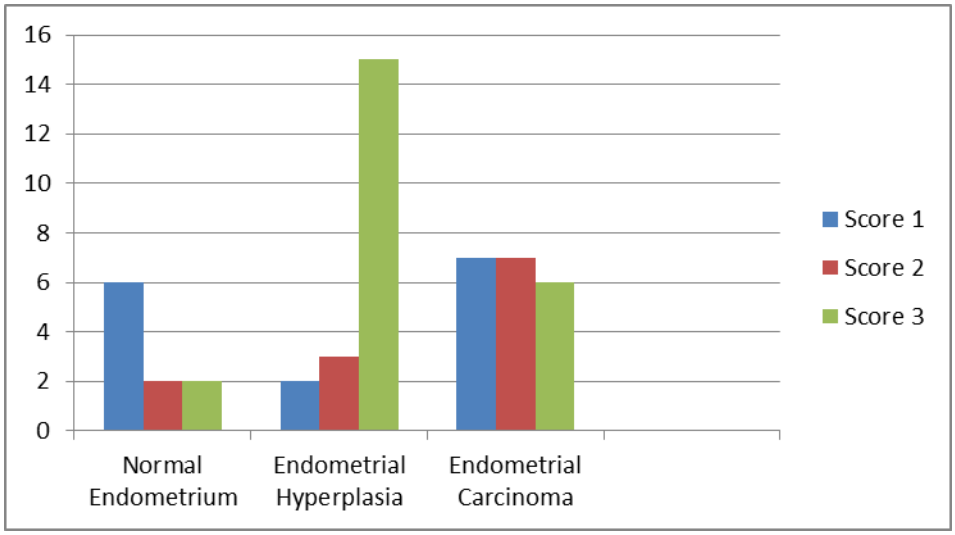

Figure 1: Distribution of Bcl-2 staining in relation to type of endometrial tissue

Among cases with normal endometrium, only two cases showed a staining intensity of 3+. The majority of cases displayed low staining intensity (6 out of 10 cases). Two cases had moderate staining. Notably, all cases with strong intensity belonged to the proliferative endometrium category. In contrast, all cases of secretory endometrium (3 cases) showed only weak (1+) staining with Bcl-2 (Figure 2). This was in striking contrast to the staining observed in proliferative endometrium, where only a single case showed weak (1+) staining intensity. Fifteen cases of endometrial hyperplasia exhibited strong cytoplasmic staining with Bcl-2 (Figure 3). Subsequently, three cases demonstrated moderate 2+ staining, while only two exhibited 1+ staining intensity. It is noteworthy that all cases exhibiting a staining intensity of 3+ were instances of hyperplasia without atypia. Among the ten cases of atypical endometrial hyperplasia, three exhibited moderate (2+) staining (Figure 4), and two displayed weak staining. The analysis of Bcl-2 staining in cases of endometrial carcinoma revealed that six cases displayed strong intensity staining. Among the remaining cases, an equal number (7 cases each) exhibited 1+ and and 2+ staining intensity, respectively (Figure 5). No discrepancy was observed between the staining patterns of atypical endometrial hyperplasia and endometrial carcinoma (p=0.429). Furthermore, no significant correlation was identified between Bcl-2 expression and age (p=0.72). The majority of cases(12 out of 20 cases) were classified as FIGO grade 1,, with four cases displaying strong staining intensity. Two cases of Grade 2 carcinoma also exhibited strong staining, while all Grade 3 cases showed 1+ staining. No statistically significant difference was observed when comparing staining between the grades of carcinoma (p=0.6903). However, the comparative analysis of Bcl-2 staining across the three groups was statistically significant (p=0.003098).

4. Discussion

Endometrial carcinoma represents the fourth most common malignancies affecting women, accounting for 7% of all cases in the USA alone (13). In India, it is the third most common gynecological malignancy (14). Endometrial carcinomas exhibit a high degree of biological diversity (15). The deregulation of apoptotic pathway factors, including p53, Fas/FasL, and Bcl-2, as well as survival pathways (phosphatidylinositol-3-kinase [PI3K]/AKT), and signaling pathways of hormone receptors (progesterone receptor [PR]), have been identified as crucial mechanisms underlying chemotherapy resistance as well (13). A substantial body of evidence from clinicopathological studies have supported the neoplastic potential of endometrial hyperplasia (16). Up to 40% of cases of atypical hyperplasia are observed to coexist with carcinoma, and many exhibit the same genetic alterations linked with endometrioid adenocarcinomas (6). However, hyperplasia without atypia is characterized by a paucity of mutations and is associated with a diminished propensity for malignant transformation (6).

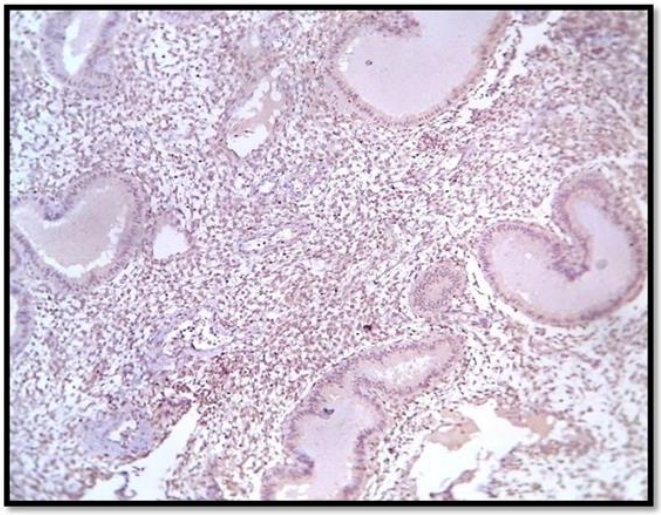

Figure 2. depicts a photomicrograph of immunohistochemical staining using a Bcl-2 antibody, exhibiting a weak intensity of staining (1+) in normal secretory endometrium (Bcl-2,10x).

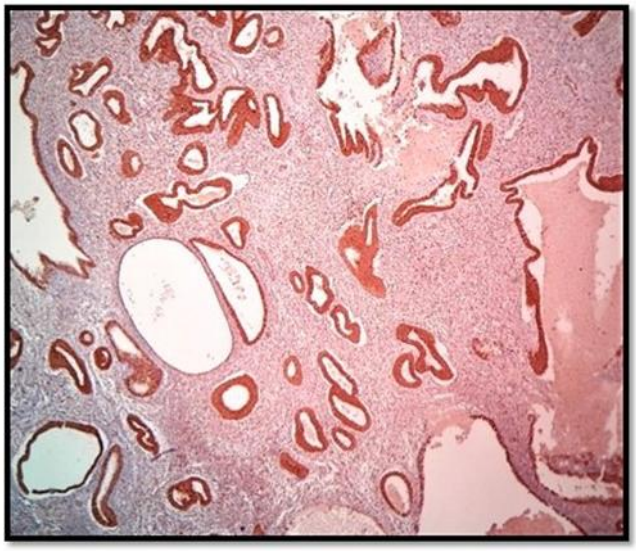

Figure 3. The photomicrograph illustrates the immunohistochemical staining of the Bcl-2 antibody, which exhibits a pronounced intensity of staining (3+) in the context of endometrial hyperplasia without atypia (Bcl-2,10x).

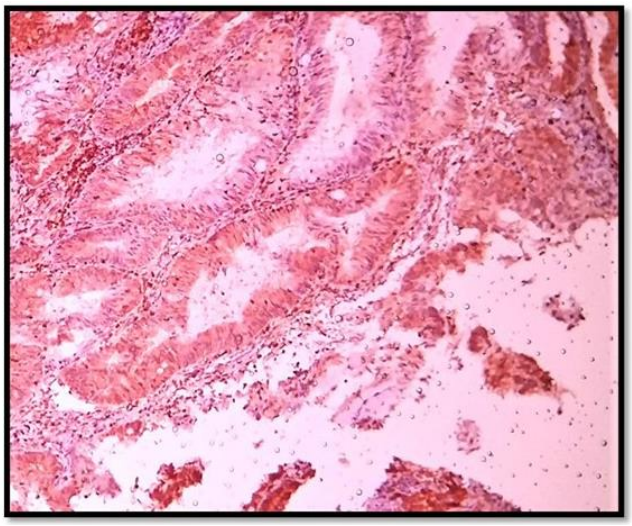

Figure 4. depicts the results of an immunohistochemical staining procedure utilizing a Bcl-2 antibody. The image illustrates a moderate intensity of staining (2+) within the endometrial glands in a case of hyperplasia with atypia (Bcl-2, 10x).

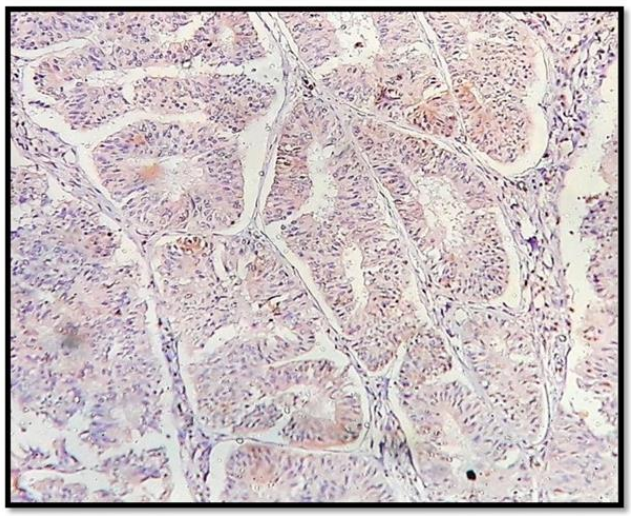

Figure 5. depicts a photomicrograph of an immunohistochemical staining utilizing a Bcl-2 antibody. The staining exhibits a weak intensity (1+) in the context of endometrioid endometrial carcinoma (Bcl-2, 10x).

Bcl-2 provides a survival advantage to cells through its anti-apoptotic activity (9). Recent studies have shown strong expression of Bcl-2 in endometrial hyperplasia, with and without atypia (10). Its role in endometrial carcinoma remains unclear and further studies are needed to understand this matter (10). In the present study, an analysis of Bcl-2 staining was performed based on the intensity of cytoplasmic staining. All 50 cases exhibited positive staining for Bcl-2. In cases of normal endometrium, the lowest number of cases displayed strong staining intensity, while the remaining cases showed weak and moderate staining intensity. Comparable results were reported by (12). Taylor LJ et al. and other previous studies (10, 12). In another study, P. Havelka studied the expression of Bcl-2 and apoptosis in normal and artificial endometrial cycles (17). The researchers observed an increase in the 5^{th} to 7^{th} day following ovulation. The apoptotic index demonstrated a progressive increase as menstruation approached, both in spontaneous and artificial cycles. Furthermore, the authors also proposed that the reduction in Bcl-2 expression is associated with a decline in the presence of estrogen (ER) and progesterone (PR) receptors during the secretory phase of the endometrial cycle (17). Taylor LJ et al stated that during the proliferative phase, low levels of apoptosis are necessary during the proliferative phase to maintain the endometrial function, despite the fact that the endometrium is constantly proliferating and thickening. This could explain why the expression of the anti-apoptotic protein Bcl-2 is comparatively higher in the proliferative phase (10). Similarly (12), have commented on this similar finding in their finding noting, that increased apoptosis and decreased mitotic activity occur during this phase due to the preparation of the secretory endometrium for subsequent endometrial breakdown (12). Our findings in this study, with respect to Bcl-2 staining, showed, that the intensity of Bcl-2 increased from normal endometrium to endometrial hyperplasia, but reduced comparatively in cases of endometrial carcinoma. Sustained overexpression of Bcl-2 may be associated with unopposed estrogen as evidenced by the increased staining in endometrial hyperplasia. Bcl-2 being an anti-apoptotic protein, may disrupt the apoptotic process in endometrial cells, thereby promoting cell survival and increasing the chance of acquiring newer and more diverse mutations in oncogenes and/or tumor suppressor genes. This, in turn, could lead to carcinogenesis (18). It has also points been suggested that that Bcl-2 may play a pivotal role in the early (estrogen-dependent) stages of carcinogenesis (18). The reduced expression in endometrial carcinomas may indicate mechanisms other than anti-apoptosis for tumor growth and progression. Similar findings have been reported in other studies as well (16). Tomasz Banas reported lower expression of Bcl-2 in high- grade endometrial carcinomas compared to grade 1 and 2 carcinomas (19). Kokawa et al. performed a study on the Bcl-2 and BAX expression in 40 cases consisting of normal endometrium, endometrial hyperplasia and carcinoma. They showed that Bcl-2 expression was decreased in atypical hyperplasia as compared to hyperplasia without atypia, and the carcinoma cases showed only weak immunostaining with Bcl-2. They felt that this result could mean that Bcl-2 is essential in the generation of endometrial hyperplasia without atypia. The ratio of Bcl-2 to BAX decreased in endometrial carcinomas, which may indicate increased apoptosis during the progression from endometrial hyperplasia with atypia to carcinoma (20). Sakuragi et al found that cytoplasmic Bcl-2 was less frequently expressed with the deeper myometrial invasion of the tumor (21). Theodore H Neimann et al. found similar results in their study with decreased Bcl-2 expression in atypical hyperplasia and carcinoma compared to endometrial hyperplasia without atypia. The few cases of atypical hyperplasia and endometrial carcinoma that showed Bcl-2 expression, showed focal reactivity with reduced intensity. They proposed that Bcl-2 may not have a consistent role in carcinogenesis. Also, the finding of absent reactivity in the majority of cases of atypical endometrial hyperplasia and carcinoma, could be due to cellular mechanisms that decreased Bcl-2 expression in the same (16). Mohamed Laban reported decreased expression of Bcl-2 expression in moderately and poorly differentiated carcinomas (22), which was similar to the findings of our study. Bcl-2 may act as an initiating factor in the transformation of normal to hyperplastic endometrium, but may not directly affect the biological nature of endometrial carcinoma. Prospective studies with a larger sample sizes are

warranted to ascertain the precise role of Bcl-2 in the endometrium.

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Authors' Contribution
Study Concept and Design: AKK, RT, KU
Acquisition of Data: AKK
Analysis and Interpretation of Data: AKK, RT
Drafting of Manuscript: AKK, RT
Critical revision of manuscript for important intellectual content: KU
Statistical Analysis: AKK, RT
Administrative, technical and material support: AKK, RT,KU.

Ethics
The study was approved by the Institutional Ethics Committee. Informed consent was obtained from all study participants.

Conflict of Interest
The authors declare that they have no conflict of interests.

Data Availability
The data that support the findings of this study are available on request from the corresponding author.

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