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Role of Bcl-2 expression in the diagnosis of endometrial hyperplasia and carcinoma

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ABSTRACT
In comparision to normal or hyperplastic endometrium the expression of Bcl-2 is lower in carcinoma of the endometrium. In the development of endometrial carcinoma the loss of Bcl-2 function either by mutational or other mechanisms is an early event. In this study we evaluated the expression of Bcl-2 gene in hyperplastic and malignant endometrium to assess its diagnostic implications. In women presenting with abnormal uterine bleeding (AUB), endometrial biopsy sections were routinely stained with H&E stain and immunohistochemical staining for Bcl-2 expression was performed. 278 cases of endometrial hyperplasias and carcinomas showed a decreased staining of Bcl-2 as the lesion progresses from simple to atypical hyperplasia to carcinoma. Hence, Bcl-2 expression decreases as the lesion progresses from benign to frank malignancy and loss of Bcl-2 function is an early event in the pathogenesis of endometrial carcinogenesis.

I. INTRODUCTION
Endometrial diseases are the commonest gynaecological disorders worldwide which affects women.[1] In the peri-menopausal and postmenopausal age group they constitute around 70.0% of all gynaecologic consultancies.[2] The endometrial diseases usually present with abnormal uterine bleeding (AUB) which is the most common and a very challenging problem which gynaecologist face, regardless of the age of the suffering women.[3] The International Federation of Gynaecology and Obstetrics working group on menstrual disorders developed a FIGO classification system to determine the causes of AUB in non-gravid women in the reproductive age group.[4] It describes the main causes as: polyp, adenomyosis, leiomyoma, hyperplasia and malignancy, coagulopathy, ovulation, endometrial, iatrogenic and not yet classified.

The neoplastic lesions causing AUB are endometrial hyperplasia and endometrial malignancy. Endometrial hyperplasia is described as an increased proliferation of endometrial glands, relative to the stroma resulting in an overall increased gland to stromal ratio as compared to the normal endometrium. These hyperplastic endometrial gland varies in shape and size and may also show cytologic atypia. These atypical hyperplastic glands may progress or even co-exist with carcinoma of the endometrium. Endometrial hyperplasia is most commonly associated with a hyperesterogenic state. The continued and prolonged estrogenic stimulation of the endometrium may be due to anovulatory periods, obesity, polycystic ovarian disease, functional granulosa cell tumors of ovary and estrogen replacement therapy.[5]

The 2014 classification of the WHO has divided endometrial hyperplasias into 2 types: hyperplasia without atypia and atypical hyperplasia/endometrioid intraepithelial neoplasia. This simple 2 tier classification reflects some new understanding of the underlying molecular genetic changes. The hyperplasias without atypia are benign, shows no relevant genetic changes and will regress with time after the endocrine balance of the body has normalized. Whereas, atypical endometrial hyperplasias are shown to exhibit many mutations which are typical for invasive endometrioid endometrial carcinoma. In approximately 60.0% of atypical endometrial hyperplasias, patients have either a coexisting invasive cancer or are at increased risk of developing invasive cancer later.[6,7]

One of the possible mechanisms of endometrial carcinogenesis is loss of growth

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suppression. Apoptosis plays an important role in the death of normal and neoplastic cells. Member of the gene family Bcl-2 is involved in the control of apoptosis, in addition to Bcl-2, BAX is also involved in the control of apoptosis.[8] The Bcl-2 gene was cloned initially in a follicular B-cell lymphoma.[9] Apoptosis is inhibited by expression of Bcl-2 due to formation of hetero- and homodimers between members of the Bcl-2 family. Higher level of Bcl-2 expression is seen both in stroma and glands in endometrial polyps. Decreased Bcl-2 expression is seen in atypical hyperplasia and endometrial carcinoma.[10]

II. MATERIALS AND METHODS

This study was done on 278 women attending the Outpatient and Inpatient Departments of Obstetrics and Gynecology and Pathology with complaints of abnormal uterine bleeding (AUB), after due approval from the hospital ethics committee. A thorough clinical history of patients was enquired and necessary physical examination findings and other relevant investigations were recorded. All the endometrial biopsies/curettage and hysterectomy specimen sent for histopathological evaluation with history of abnormal uterine bleeding were included in the study while all other malignancies of female genital tract and patients not giving informed consent were excluded. Specimens were processed in 10% neutral buffered formalin (10 ml of 40% formaldehyde diluted in 90 ml of water). Tissues were fixed for 24 hours with two changes of formalin and embedded in paraffin which was further cut into 4-5micron thick sections. The sections were routinely stained with haematoxylin and eosin (H&E) stain. Immunohistochemical staining using the Bcl-2 monoclonal antibodies was performed on diagnosed cases of endometrial lesions. The various histomorphological and immunohistochemical patterns were identified and classified according to percentage of cell stained and intensity of staining on immunostained slides.

Bcl-2 staining intensity was calculated as Yigit et al 2012. [11]

- Upto 5% of glands Negative
- 5-25% of glands 1+(weak)
- 26-50% of glands 2+(moderate)
- >50% of glands 3+(Intense)

Statistical analysis was performed using Fisher's exact test. Data was statistically analyzed using SPSS software version (20.0). Chi-square tests were used in the analysis of dichotomous or categorical variables. When expected cell frequencies were <5, the fisher exact test was used. p-value of 0.05 or less was considered to be statistically significant.

III. RESULTS

The present study included a total of 278 patients between ages of 11-70 years who presented with abnormal uterine bleeding (AUB). The maximum number of patients were in the 5th decade 112 (40.3%) followed by 83 (29.9%) patients in the 4th decade and 38 (13.7 %) patients in the 3rd decade of life. Majority of the patients in our study were parous 261 cases (93.8%) and nulliparous constituted only 17 cases (6.1%). The incidence of abnormal uterine bleeding was high in grandmultipara 109 (39.2%) and multipara 104 (37.4%) as compared with nulliparous 17(6.1%) and primiparous 48 (17.3%) females.

Out of the 278 cases, there were 268 (96.4%) cases of endometrial hyperplasia and 10 (3.6%) cases of endometrial cancer. Table 1 Endometrial hyperplasia was the commonest pathology seen in the perimenopausal age group (41-50 years). This included simple hyperplasia without atypia in 74 (51.7%) cases followed by complex hyperplasia without atypia in 46(60.5%) cases. The other common lesions encountered were endometrial carcinoma in 8 cases and atypical hyperplasia in 4 cases. Table 2

Of the 268 cases of endometrial hyperplasia, the most common subtype was simple hyperplasia without atypia in 143 (51.4%) cases followed by complex hyperplasia without atypia in 76 (28.3%) cases. Complex hyperplasia with atypia was seen in 34 (12.2%) cases and the least common variant was simple hyperplasia with atypia seen in 15 (5.4 %) cases. Table 3 Out of 10 cases of endometrial carcinoma 7(70.0%) cases presented with grade II, 2 (20.0%) cases presented with grade III, 1 (10.0%) case presented with grade I. 4 (40.0%) cases presented with <1/2 myometrial invasion, 3 (30.0%) cases presented with >1/2 myometrial invasion and 3 (30.0%) cases did not show myometrial invasion. 3 (30.0%) cases presented with lymphovascular invasion and 7 (70.0%) cases showed no lympho-vascular involvement. Hence, accordingly 6 (60.0%) cases presented with stage II, 2 (20.0%) cases presented with stage III, 1 (10.0%) case each presented with stage I and stage IV.

Simple Hyperplasia without atypia showed moderate (2+) Bcl-2 positivity in 28/143 (19.5 %) cases and intense (3+) positivity in

115/143 (80.5%) cases (Figures 1). Simple Hyperplasia with atypia showed moderate (2+) Bcl-2 positivity in 12/15 (80.0 %) cases, intense (3+) positivity in 1/15 (6.6%) cases and weak (1+) positivity in 2/15 (13.3%) cases. Complex Hyperplasia without atypia showed moderate (2+) Bcl-2 positivity in 66/76 (86.8 %) cases and weak (1+) positivity in 10/76 (13.2%) cases. Complex Hyperplasia with atypia showed mild (1+) Bcl-2 positivity in 28/34 (82.3%) cases (Figures 2) and absent staining in 6/34 (17.6%) cases. Adenocarcinoma showed weak (1+) Bcl-2 positivity in 1/10 (10%) cases and absent staining in 9/10 (90%) cases (Figures 3). The mean scoring of Bcl-2 staining in Simple Hyperplasia without atypia was found to be 2.8 $\pm$0.41, in Simple Hyperplasia with atypia was 2.0 $\pm$0.41, in Complex Hyperplasia without atypia was1.85$\pm$0.36, in Complex Hyperplasia with atypia was 0.83$\pm$0.41 and in adenocarcinoma was 0.1$\pm$0.32. On applying analysis of variation (ANOVA) test, the pattern was found to be significant ($F=87.61$, $df=4$, $p<0.001$).

IV. DISCUSSION

In gynaecological practice AUB is a very commonly encountered complaint. More than 2/3 of all gynecological consultations in the peri and postmenopausal years are because of AUB.[12] Histopathological diagnosis plays a very important role in identifying the spectrum of diseases underlying the uterine bleeding, more so, in developing countries with limited resources.

Out of our 278 patients, the patients complaining of menorrhagia were 39.6% and 12.6% of infertility, the findings are concordant with Kaur et al.[13] We saw a 7.9% incidence of postmenopausal bleeding, which is comparable with Agrawal et al.[14] However, Rifat and Mahmoud had reported a slightly higher percentage (16.3%) of postmenopausal bleeding.[15] Our study also showed that the occurrence of menstrual disorders increases with advancing age. Excessive bleeding was seen in 41–50 years age group in our study. A similar incidence was reported by Mahapatra & Mishra et al. in their study.[16]

The highest incidence of hyperplasia of the endometrium was seen in the age group 40-49 years, which was similar to the study done by Doroiswami et al.[17] Hyperplasias is seen in perimenopausal age group mostly due to failure of ovulation. The persistent unriped follicles exposes the endometrium to excessive estrogenic state. In our study 51.7% cases of simple hyperplasia without atypia were noted and the majority seen in the perimenopausal age group, a finding similar to Jairajpuri et al and Munro, who have reported 64.4% to 66.6% cases respectively.[18,19] Complex hyperplasia without atypia was seen in 27.3% cases which was similar to the reports of Rifat and Mahmoud.[15] Endometrial carcinoma was the least common pathology in our study, found only in 10 cases, same results were reported by Riaz et al and Abdullah et al.[20,21] Making a diagnosis of endometrial hyperplasia gains importance because they are increasingly identified as a precursor to endometrial carcinoma. There is 5-10% chances of progression of hyperplasia to cancer.[22] Simple, complex, simple atypical and complex atypical hyperplasia have progression risks of 1.0%, 3.0%, 8.0% and 29.0% respectively to develop carcinoma.[22]

The mean scoring of Bcl-2 staining decreased from simple hyperplasia without atypia to complex hyperplasia with atypia and was the least in adenocarcinoma. It can be seen that the intensity of Bcl-2 decreased from benign to malignant lesions. Our finding was in concordance with the study by Risberg et al 2002 and Kapucuoglu et al 2007. [23,24]

V. CONCLUSIONS

Loss of Bcl-2 function by mutational or other mechanisms is an early event in endometrial tumrogenesis and offers an informative immune-histochemical biomarker for premalignant and malignant endometrial lesions. Bcl-2 may also serve as a therapeutic target with the advent of anti-Bcl-2 antibodies and drugs.

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Table 1: Histopathological distribution of cases in patients of AUB

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Endometrial Histopathology	No. of patients	Percentage
Endometrial hyperplasia	268	96.4
Endometrial cancer	10	3.6
Total	**278**	**100**

Table 2: Histopathological distribution of endometrial lesions according to age in years

Histopathological diagnosis	Reproductive (15-40 years)	Perimenopausal (41-50 years)	Postmenopausal (>50 years)	Total
	No of cases			
Simple hyperplasia without atypia	60 (42.0)	74 (51.7)	9 (6.3)	143 (100)
Complex hyperplasia without atypia	28 (36.8)	46 (60.5)	2 (2.6)	76 (100)
Atypical hyperplasia	10 (20.4)	35 (71.4)	4 (8.2)	49 (100)
Endometrial carcinoma	-	2 (0.2)	8 (0.8)	10 (100)
Total	**98**	**157**	**23**	**278 (100)**

Table 3- Bcl-2 immuno-staining in endometrial hyperplasia and carcinoma

Category	No of cases	Bcl-2 staining			
		0 Negative (%)	1+ (Weak) (%)	2+ (Moderate) (%)	3+ (Intense) (%)
Simple Hyperplasia without atypia	143	_	_	28 (19.5)	115 (80.5)
Simple Hyperplasia with atypia	15	_	2 (13.3)	12 (80.0)	1 (6.6)
Complex Hyperplasia without atypia	76	_	10 (13.2)	66 (86.8)	_
Complex Hyperplasia with atypia	34	6 (17.6)	28 (82.3)	_	_
Adenocarcinoma	10	9 (90.0)	1 (10.0)	_	_
Total	**278**	**10**	**9**	**24**	**17**

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Figure 1- Simple Hyperplasia without Atypia: Bcl-2 shows intense diffuse (3+) cytoplasmic staining in the lining epithelial cells of endometrial glands. IHC Bcl-2 x 10X

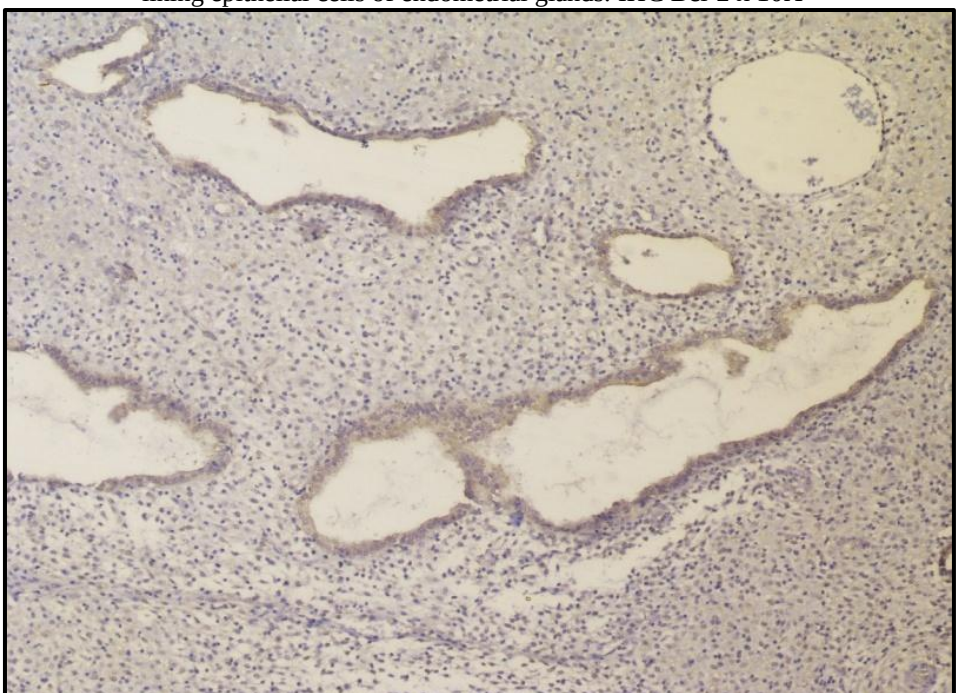

Figure 2- Complex Hyperplasia with Atypia: Photomicrograph shows weak focal (1+) Bcl-2 cytoplasmic positivity. IHC Bcl-2 x 10X

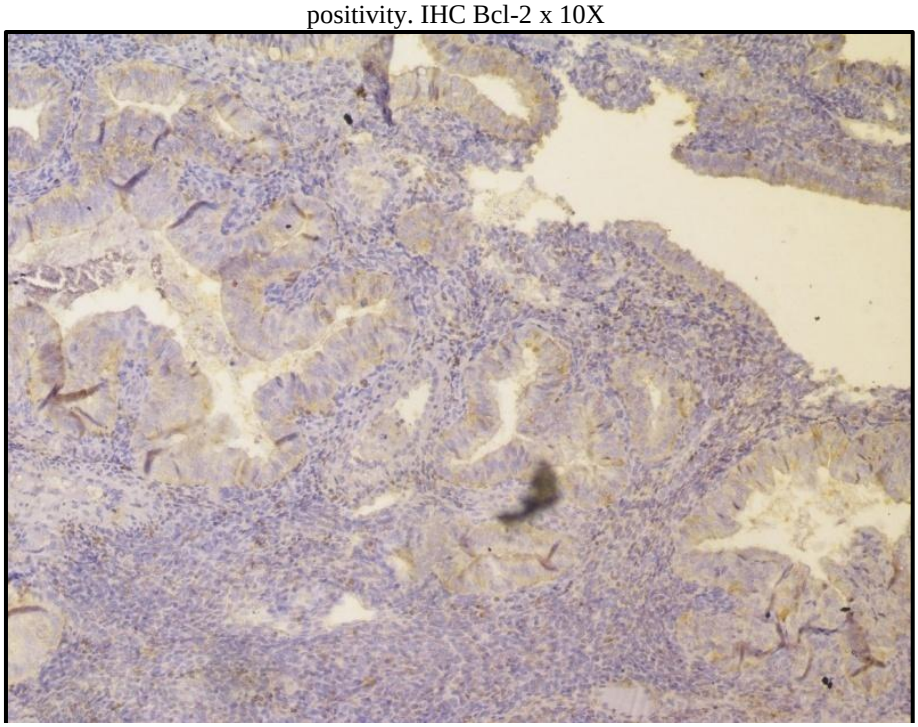

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Figure 3- Photomicrograph shows negative Bcl-2 cytoplasmic positivity (0).IHC Bcl-2 x 10X