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Preoperative evaluation of P53 and bcl-2 over expression in clinical stage 1 endometrial carcinoma and their correlation with surgico-pathological data and prognosis of patients

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ABSTRACT

Introduction: P53 and bcl-2 over expression was reported to affect the biology and prognosis of patients endometrial carcinoma. Methods: This prospective study included 38 patients with histologically confirmed endometrial carcinoma and staged clinically as stage I. Immunohistochemical staining of the tumor specimens obtained by dilatation and curettage (D&C) with P53 and bcl-2 monoclonal antibodies was done. The surgical, pathological and follow up data of all patients were studied. Results: There were 18 cases (47.4%) with positive P53 over expression. P53 over expression was found to be associated with increasing grade of differentiation, advanced stage, and non endometrioid type (significant), and increased depth of myometrial invasion (non-significant). It was associated with more recurrence rate (22% versus 15%) and shorter mean survival time (33.9 versus 29.2 months). Bcl-2 expression was present in24 cases (63.2%) of the studied group. There was significant decrease in bcl-2expression with poorly differentiated, advanced stage, increased depth of myometrial invasion, and non-endometrioid type. It was not significantly correlated with recurrence rate and mean survival time. Conclusion: P53 over expression in the D&C specimens was associated with adverse surgicopathological criteria, increased mortality rate, and shorter survival time in patients with endometrial carcinoma. A significant decrease in bcl-2 expression was associated with adverse surgicopathological criteria, but it was not significantly correlated with prognosis of the patients.

Keywords: Endometrial Carcinoma; Prognosis; P53; Bcl-2

1. INTRODUCTION

Endometrial carcinoma is the most common malignancy of the female genital tract accounting for almost half of all gynecologic cancers in western world. It represents the fourth most common cancer after breast, lungs and bowel cancers, and the seventh leading cause of death from malignancy in women [1].

Mariani *et al.* [2], reported that in the pretreatment curettage specimen, the presence of unfavorable level of P53 and bcl-2 or non endometrioid histological feature or combination of those, can significantly predict lymph node status in patients with endometrial carcinoma.

P53 gene mutation was found to affect the biological features of endometrial carcinoma, as it was found to be associated with more aggressive histologic subtypes than endometrioid carcinoma. Strong expression of p53 correlates with advanced stage and high grade of the tumor and was detected more frequently in endometrial cancer with lympho-vascular space invasion [3].

Bcl-2 belongs to a family of apoptosis-regulatory genes which may either promote cell survival or encourage cell death [4]. Expression of bcl-2 does not only contribute to oncogenesis but also to chemotherapy resistance in variety of tumors by inhibiting apoptosis [5]. Bcl-2 expression was found to be increased in grade 1 and 2 endometrioid adenocarcinoma, while in the serous papillary endometrial cancer showed immuno-negativity to bcl-2 [6]. Bcl-2 may have an importance in the progression of endometrial carcinoma [7].

To the best of our knowledge, there are few reported studies to evaluate the correlation of pre-operative testing of P53 and bcl-2 expression with the surgico-patho-

logical data and prognosis of patients in clinical stage 1 endometrial carcinoma. We tried to evaluate this point in this prospective clinical study.

2. PATIENTS AND METHODS

This prospective clinical study was conducted at the departments of Gynecology and Pathology, Faculty of Medicine, during the period from April 2005 to October 2008. The study included 38 patients with histologically confirmed endometrial carcinoma and staged clinically as stage I.

The routine pre-operative work up of these patients was done. Dilatation and curettage was done, a part of the biopsy was prepared in paraffin sections for histologic examination and another part was used for immunohistochemical staining with P53 and bcl-2 monoclonal antibodies.

*P*53 *immune staining*: Immunohistochemical reaction was carried out for detection of P53 protein over expression in endometrial tumor tissue using monoclonal mouse anti-human P53 protein, clone; DO-7, supplied by DAKO corporation, USA. Positive staining was defined as homogenous pattern of nuclear staining (brownish coloration) that involved more than 5% of the cells.

Bcl-2 *immunohistochemical staining*: The primary antibody used was the second generation monoclonal mouse anti-bcl-2 protein (Biogenex, Cat no. Am 287). Using the high power field, the immunoreactivity (positive cases) for bcl-2 was determined by the percentage of tumor cells showing cytoplasmic (brown) staining that involved more than 5% of the cells.

After that, patients were subjected to surgical treatment, in which peritoneal cytology, extra-fascial hysterectomy, bilateral salpingo-oophrectomy and pelvic lymph node sampling were done.

The use of adjuvant radiotherapy was individualized in the tumor board meetings .Candidates of postoperative radiotherapy were suggested by use of prognostic factors such as surgical staging, depth of myometrial invasion, histologic type and tumor grading.

Follow up: Patients were followed up every three months, by history, physical examinations, and ultrasound. MRI was done every 6 months. The surgico- pathological data of all patients were studied. Overall survival, recurrence rate and duration were estimated.

2.1. Statistical Analysis

Statistical analysis was done by using SPSS (Statistical package of social science) program version 10, 1999. The data were parametric by using Kolmogrov-Smirnov test. The qualitative data were presented in the form of means, standard deviation and range. Student test was used for comparison of the two groups. One way ANOVA test was used to compare more than two groups. Man-Whitney test was used to compare the non-parametric data. Sensitivity, specificity and accuracy were calculated. Kaplan-Meier survival analysis was done to calculate the cumulative survival. Significance was considered when P value is less than 0.05.

2.2. Ethical Considerations

The research was approved by The Ethical Review Committee of our Faculty of Medicine. Formal and written consents were taken from all patients.

3. RESULTS

This study included 38 cases of histologically confirmed endometrial carcinoma, clinical stage I, during the period from April 2005 to October 2008. The mean follow up duration from the treatment was 21.5 month, (range 6 - 40 months). After surgical staging (according to FIGO staging 1988), there were 30 cases with stage I, 4 cases with stage II and 4 cases with stage III.

Pretreatment study of P53 over expression by "Immuno-histochemical stain" was done for the studied group; there were 18 cases (47.4%) with positive P53 over expression.

P53 over expression was found to be associated with increasing grade of differentiation, advanced stage, and non endometrioid type (significant), and increased depth of myometrial invasion (non-significant) as can be concluded from **Table 1**.

The relation between P53 over expression and recurrence of the disease in the study group: Although the recurrence of the disease in patients with negative P53 over expression was 15% (3 of 20 cases), in the positive group it was 22% (4 of 18 cases) but this difference was not statistically significant (P value 0.576).

The survival time was prolonged in P53 negative patients, as the mean survival time in p53 negative group was 33.9 months while it was 29.21 months in p53 positive group (**Table 2**).

Figure 1: Kaplan-Meier estimates the influence of P53 over expression in the recurrence of the studied group. There was difference in the mean recurrence duration in both groups.

The relation between P53 over expression and mortality of the studied group is shown in **Table 3**, the mortality between P53 positive patients was higher 16.6% (3 of 18 cases) in comparison to the mortality between negative patients, which was 5% (1 of 20 cases) the difference is significant (P value 0.026).

Figure 2: Kaplan-Meier estimates the influence of P53 over expression in the mortality duration of the studied group: There was difference in the mean mortality duration in both groups. It was shorter in positive group.

Bcl-2 expression was studied also, bcl-2 expression

Table 1. Relationship between P53 over expression and degree of differentiation of the tumor, surgical staging, depth of myometrial invasion, and histological type in the study group.

Grade	P53		Total	P value
	Negative	Positive		
G1	11 (78.6%)	3 (21.4%)	14 (100%)	
G2	6 (37.5%)	10 (62.5%)	16 (100%)	**0. 037**
G3	3 (37.5%)	5 (62.5%)	8 (100%)	
Total	20 (52.6%)	18 (47.4%)	38 (100%)	
Stage				
Stage I	19 (63.3%)	11 (36.7%)	30 (100%)	
Stage II	1 (25.0%)	3 (75.0%)	4 (100%)	**0. 013**
Stage III	0 (0%)	4 (100%)	4 (100%)	
Total	20 (52.6%)	18 (47.4%)	38 (100%)	
Myometrial invasion				
No invasion	4 (66.7%)	2 (33.3%)	6 (33.3%)	
Invasion < 50%	9 (52.9%)	8 (47.1%)	17 (100%)	**0.709**
Invasion > 50%	7 (46.7%)	8 (46.7%)	15 (100%)	
Total	20 (52.6%)	18 (47.4%)	38 (100%)	
Histopathologic type				
Endometrioid	18 (62.1%)	11 (37.9%)	29 (100%)	
Adenosquamous	1 (25.0%)	3 (75.0%)	4 (100%)	**0. 047**
Papillary serous	1 (20.0%)	4 (80.0%)	5 (100%)	
Total	20 (52.6%)	18 (47.4%)	38 (100%)	

Table 2. The relation between P53 over expression and the mean survival time in the study group.

P53 expression	Mean	S D*	Minimum	Maximum	P value
Negative	**33.9**	**2.77**	**28.46**	**39.34**	
Positive	**29.21**	**2.79**	**23.73**	**34.68**	
Overall	**32.78**	**2.25**	**28.36**	**37.2**	**0.461**

P value: 0.461 (non significant); S D*: Standard deviation.

Table 3. The relation between P53 over expression and mortality of the study group.

P53 expression	Number of cases	Number of died cases	P value
Negative	20	1 (5%)	
Positive	18	3 (16.6%)	
Total	38	4 (100%)	0.026

P value: 0.026 (significant).

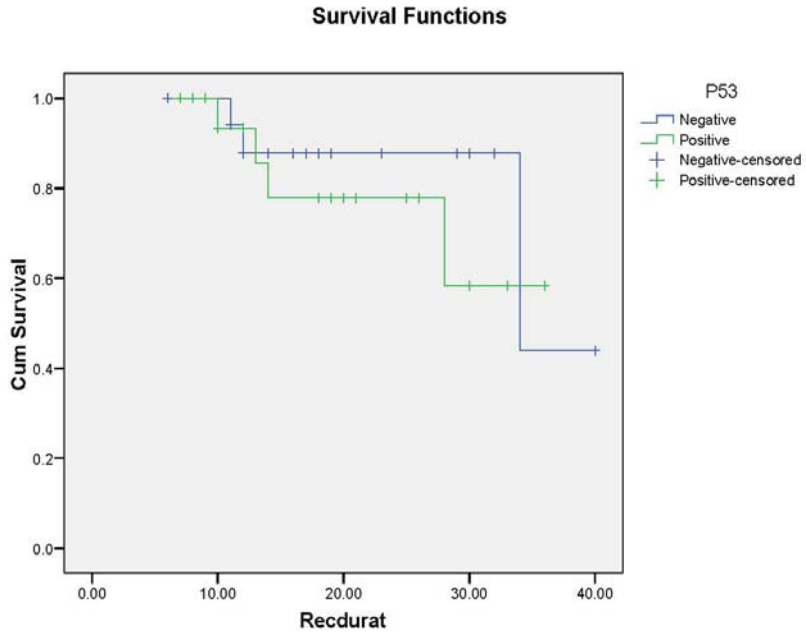

Figure 1. Kaplan-Meier estimates of the influence of P53 over expression in the recurrence of the studied group: As seen in this figure, the faint line represents the positive group while the dark line represents the negative group.

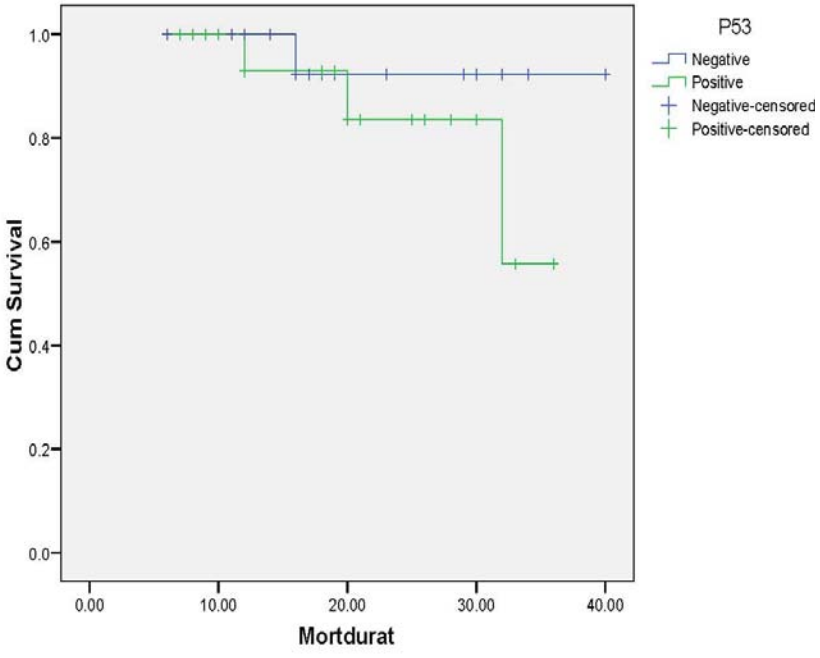

Figure 2. Kaplan-Meier estimates of the influence of P53 over expression in the mortality duration of the studied group: As seen from this figure, the faint line represents the positive group while the dark line represents the negative group. There was difference in the mean mortality duration in both groups. It was shorter in positive group.

Table 4. The relation between bcl-2 expression and the degree of differentiation, surgical staging, depth of myometrial invasion and histological type in the studied group.

Grade	Bcl-2		Total	P value
	Negative	Positive		
G1	2 (14.2%)	12 (14.2%)	14 (100%)	0.006
G2	6 (37.5%)	10 (62.5%)	16 (100%)	
G3	6 (75.0%)	2 (25.0%)	8 (100%)	
Total	14 (36.8%)	24 (63.2%)	38 (100%)	
Stage				
Stage I	9 (30.0%)	21 (70.0%)	30 (100%)	0.018
Stage II	2 (50%)	2 (50%)	4 (100%)	
Stage III	3 (75%)	1 (25%)	4 (100%)	
Total	14 (36.8%)	14 (36.8%)	38 (100%)	
Myometrial invasion				
No invasion	1 (16.7%)	5 (83.3%)	6 (100%)	0.083
Invasion <50%	5 (29.4%)	12 (70.6%)	17 (100%)	
Invasion >50%	8 (53.3%)	7 (46.7%)	15 (100%)	
Total	14 (46.7%)	7 (46.7%)	38 (100%)	
Histopathological type				
Endometroid	9 (31%)	20 (69%)	29 (100%)	0.070
Adenosquamous	1 (25%)	3 (75%)	4 (100%)	
Papillary	4 (80%)	1 (20%)	5 (100%)	
Total	14 (36.8%)	24 (63.2%)	38 (100%)	

was present in 24 cases (63.2%) of the studied group. **Table 4**, Shows the relation between bcl-2 expression and the degree of differentiation, surgical staging, depth of myometrial invasion and histologic type in the studied group. There was significant decrease in bcl-2 expression with poorly differentiated, advanced stage, increased depth of myometrial invasion, and non-endometrioid type.

Table 5: Show the relation between bcl-2 expression and the recurrence of the disease in studied group. There was no significant relation between bcl-2 over expression and recurrence of the disease in the studied group (P value 0.627).

Figure 3: Kaplan-Meier estimates the influence of bcl-2 expression on the recurrence duration of the studied group: There was no significant difference in the mean recurrence duration in both groups, the mean recurrence duration for the negative group was 34 months and for the positive group about 32 months.

Table 6: shows the relation between bcl-2 gene expression and the mortality in the studied group. As can be seen from the table, there was no significant difference in the mortality rate between bcl-2 positive and negative cases. (P value 0.615).

Figure 4: Kaplan-Meier estimates the influence of bcl-2 expression on the mortality duration of the studied group. There was no significant difference in the mean recurrence duration in both groups, the mean mortality duration for the negative group was 34 months and for the positive group about 33.5 months.

4. DISCUSSION

Endometrial carcinoma is the most common malignancy of the female genital tract accounting for almost half of all gynecologic cancers in western world [1].

The aim of this randomized trial was to evaluate of P53 and bcl-2 expression in clinical stage 1 endometrial carcinoma and their correlation with surgico-pathological data and prognosis of patients.

In this study P53 and bcl-2 expression were investigated immunohisto-chemically in 38 patient with clinical stage-1 endometrial carcinoma. After surgical staging, stage I represented 79% of our studied group. This result was comparable to that obtained by Creasman *et al.* [8].

In this work P53 over expression was detected in 18 cases (47.4%) of our studied group .This finding was

similar to that obtained by Pilka *et al.* [9].

In this study, P53 expression was increased with the grade of malignancy (**Table 1**), which was statistically significant (P 0.03). This was in accordance with the observations reported by other authors [10,11].There was also a positive correlation between P53 gene mutation and stage of endometrial carcinoma (**Table 1**). So, P53 gene expression increased significantly in advanced stages (P value 0.01). This report was in agree with that of Veralucia *et al.* [12], but did not agree with other authors [13,14].

In our study p53 gene over expression increased with increased depth of myometrial invasion but it was not statistically significant as noticed in **Table 1**, this finding was in agree with that of other authors [14,15]. On the other hand, Cerchi *et al.* [16] and Pilka *et al.* [9], found a

Table 5. The relation between bcl-2 expression and recurrence of the disease in the studied patients.

Bcl-2	Total number	Recurrence	No recurrence	Percent
Negative	**14**	**2**	**12**	**85.7%**
Positive	**24**	**5**	**19**	**79.2%**
Overall	**38**	**7**	**31**	**81.6%**

P value: 0.627 (Non significant). Chi square: 0.076

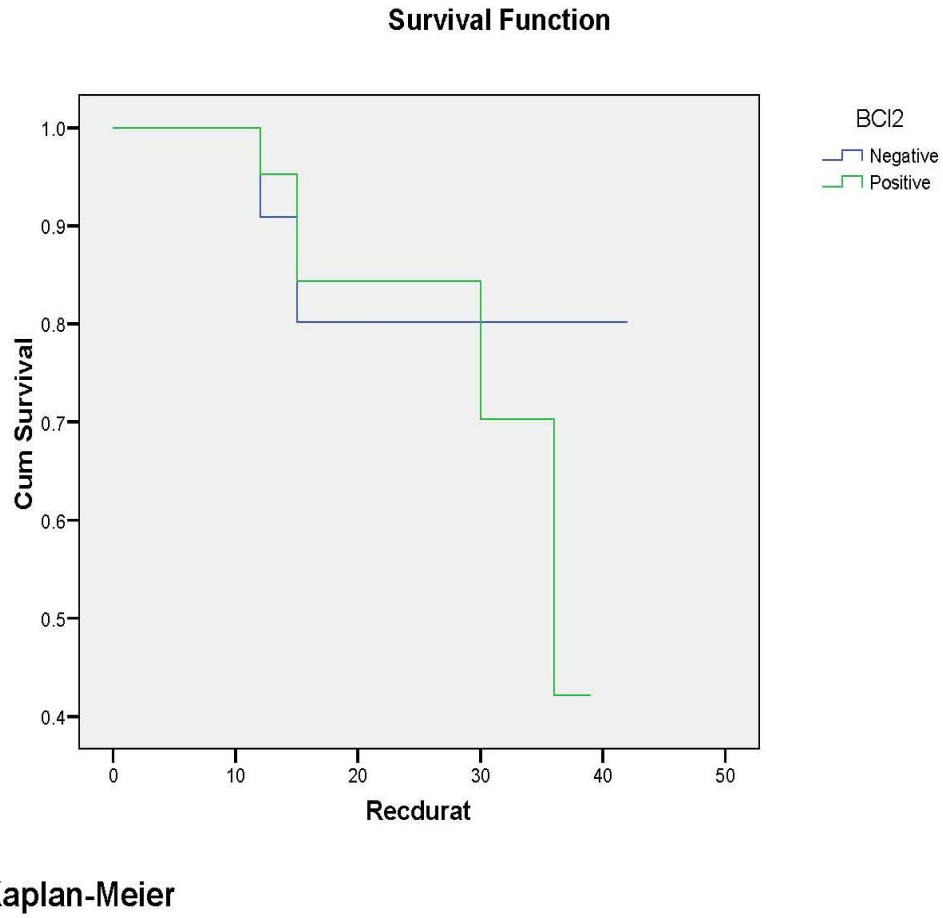

Figure 3. Kaplan-Meier estimates of the influence of bcl-2 expression on the recurrence duration of the studied group: As seen from this figure, the faint line represents the positive group while the dark line represents the negative group. There was no significant difference in the mean recurrence duration in both groups, the mean recurrence duration for the negative group was 34 months and for the positive group about 32 months.

Table 6. The relation between bcl-2 expression and mortality.

Bcl-2 expression	Number	Number of died cases	P value
Negative	14	2 (14.2%)	
Positive	24	2 (8.3%)	**0.541**
Over all	38	4(10.5%)	

P value: 0.615 (Non significant).

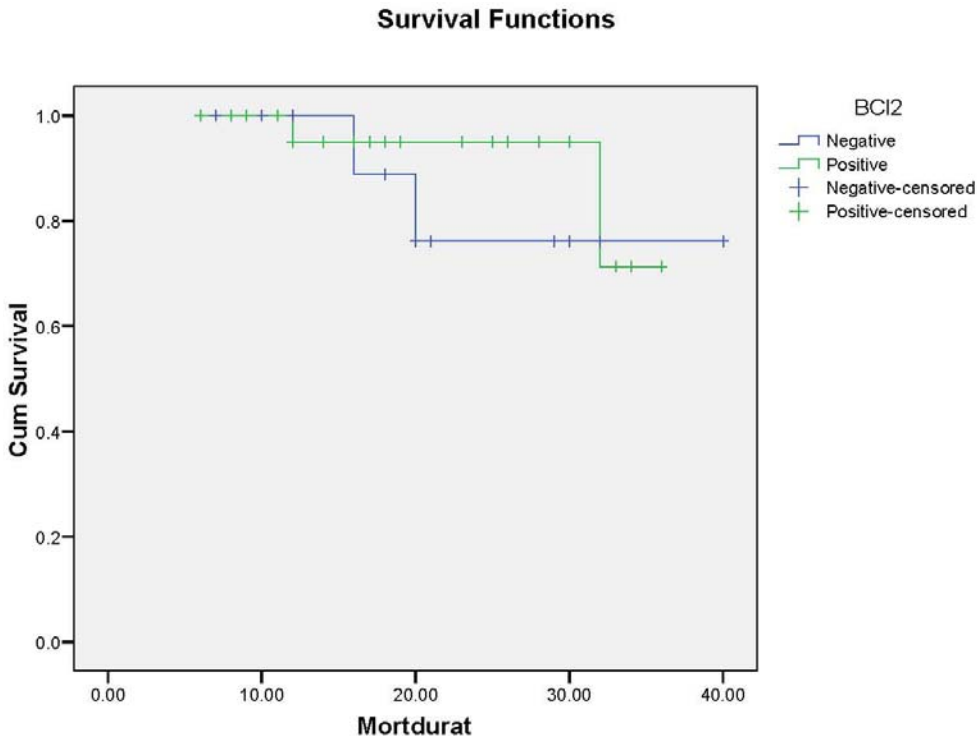

Figure 4. Kaplan-Meier estimates the influence of bcl-2 over expression on the mortality duration of the studied group: As seen from this figure, the faint line represents the positive group while the dark line represents the negative group. There was no significant difference in the mean recurrence duration in both groups, the mean mortality duration for the negative group was 34 months and for the positive group about 33.5 months.

significant positive correlation between P53 over expression and depth of myometrial invasion.

There was significant correlation between P53 gene mutation and histologic type of the tumor (**Table 1**). In endometrioid type, it was 37.9%, while in papillary serous type it was 80% (P value 0.047). These findings were in agree with that of other authors [13,15].

In our study there were 3 cases (3 of 20) of recurrence in the P53 negative group (15%) and 4 cases in P53 positive group (4 of 18) (22.2%) this difference was not statistically significant (P value 0.57) .These results were in agree with Marcia *et al.* [14].On the other hand, Pilka *et al.* [9] and Appel [11], found that a significant correlation between P53 gene mutation and recurrence of the disease. The difference between these studies and our study may be due to shorter follow up time and smaller sample size in our study.

The mean survival time for P53 negative patients was 38.15 months and for the P53 positive patients was 31.68 months. So, P53 over expression in our study was associated with increased mortality rate and shorter survival time in patients with endometrial carcinoma .These results were supported by findings of other authors [7, 11].

Bcl-2 expression in endometrial carcinoma:

In this study bcl-2 expression was investigated in 38 cases of histologically documented endometrial carcinoma. It was found that bcl-2 was expressed in the cyto-

plasm of tumor cells in 24 cases (63.2%). Nearly the same results obtained by Erkanli *et al.* [15] and Appel *et al.* [11].

There were negative correlation between bcl-2 expression and the grade of the tumor. These findings were in agree with Halperin *et al.* [17]. There were also a significant negative correlation between bcl-2 expression and the stage of endometrial carcinoma (**Table 2**). These findings were in agree with other authors [7,9].

Regarding the depth of myometrial invasion in our study (**Table 2**), there were negative correlation between bcl-2 over expression and the depth of myometrial invasion, but the difference was not statistically significant (P value 0.08). These findings were in agree with Marcia *et al.* [14] and Appel *et al.* [11]. On the other hand, other authors [7,9], reported a significant immuno-negativity with increasing the depth of myometrial invasion.

Regarding the correlation between bcl-2 expression and the histologic type of endometrial carcinoma in our study (**Table 2**), there was high expression of bcl-2 in endometrioid type than non-endometrioid types, this was not statistically significant (P value 0.07). These findings were in agree with that of Geisler *et al.* [18].

In the current study there was no significant correlation between bcl-2 expression and recurrence or survival of the patients with endometrial carcinoma (**Tables 6** & **7**). These findings were supported by the findings obtained by other authors [11,14,19].

5. CONCLUSIONS

P53 over expression in the D&C specimens was associated with adverse surgico-pathological criteria, increased mortality rate, and shorter survival time in patients with endometrial carcinoma.

A significant decrease in bcl-2 expression was associated with adverse surgico-pathological criteria, but it was not significantly correlated with prognosis of the patients.

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