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Clinicopathologic Aspects of Squamous Cell Carcinoma of the Uterine Cervix: Role of PTEN, BCL2 and P53

Ali Yousif Babiker [1,2], Ahmad Almatroudi [1], Khaled S. Allemailem [1], Nazik Elmalaika O. S. Husain [3], Mohamed A. Alsammani [4], Mohammed A. Alsahli [1] and Arshad H. Rahmani [1,*]

1 Department of Medical Laboratories, College of Applied Medical Sciences, Qassim University, Buraidah 51452, Saudi Arabia; alibabkr99@gmail.com (A.Y.B.); aamtrody@qu.edu.sa (A.A.); k.allemailem@qu.edu.sa (K.S.A.); shly@qu.edu.sa (M.A.A.)
2 Department of Histopathology and Cytology, College of Medical Laboratories Science, University of Sciences and Technology, Omdurman P.O. Box 407, Sudan
3 Department of Pathology, Faculty of Medicine, Omdurman Islamic University, Omdurman P.O. Box 382, Sudan; nazikhusain@gmail.com
4 Department of Obstetrics and Gynecology, College of Medicine, Qassim University, Buraidah 51452, Saudi Arabia; m_sammani@yahoo.com
* Correspondence: ah.rahmani@qu.edu.sa

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Abstract: Background: Squamous cell carcinoma (SCC) of the uterine cervix is a leading cause of morbidity and mortality among women. The alterations of Phosphatase and tensin homolog (*PTEN*), B-cell lymphoma 2 (*Bcl2*) and p53 expression seem to be significant in the development of various types of cancers. The altered expressions of PTEN, Bcl2 and p53 and their involvement in cancer of the uterine cervix are not well recognized. **Aim**: This study aimed at examining the expression patterns of PTEN, Bcl2 and p53 proteins and comparing them with the grade and stage of cervical cancer. **Materials and Methods**: Tissue blocks of SCC and ten cases of inflammatory lesions of the uterine cervix were examined immunohistochemically for the expression of PTEN, Bcl2 and p53 proteins. **Results**: Loss of PTEN expression was identified in 45.33% of cervical SCC and high expression was found in inflammatory lesions ($p \leq 0.05$). PTEN expression was significantly associated with the clinical stage of SCC (61.36% and 45.16% in stages I–II and III–IV, respectively) ($p < 0.05$), but not with the degree of differentiation of the SCC. The expression of *Bcl2* was significantly high (60%) in cancer cases than in control cases ($p < 0.05$). Bcl2 did not show any significant association with the histologic type and clinical stage of the SCC of the uterine cervix. The expression of p53 protein was significantly high (57.33%)) in cancer tissue, and no expression was noted in control cases ($p < 0.05$). Moreover, the expression pattern of p53 protein in cervical cancer tissue samples was not linked with the patient age, grade and stage of the cervical SCC ($p > 0.05$). **Conclusion**: The reduced expression of PETN and overexpressions of Bcl2 and p53 might play an indispensable role in carcinogenesis of cervical SCC. Moreover, a relationship was detected between PTEN expression and clinical stage of the cervical SCC.

Keywords: PTEN; Bcl2; p53; gene; immunohistochemistry; prognostic role; pathogenesis; carcinogenesis; SCC; cervical cancer

1. Introduction

Cervical cancer is a cancer rising from the cervix and is a common malignancy of female gynecologic systems and contributes approximately 8% of altogether cancers [1]. The exact mechanisms behind development and progression have not been completely understood. Numerous factors are involved in the pathogenesis of cervical cancer, including human papillomavirus and alteration of cell signaling pathways. Previous findings have reported that the human papillomavirus infection is commonly detected in invasive cervical squamous cell carcinoma (SCC) [2]. However, with the introduction of screening programs based on population, the incidence and mortality rate of cervical cancer has been decreased [3]. However, still, it is vital to recognize potential biomarkers that could be used in the screening and inhibition of cervical cancer.

Tumor suppressor genes, as well as apoptotic genes, are vital for keeping genome integrity and the cell cycle. *PTEN* is a tumor-suppressor gene that encodes a phosphatase upstream of Akt in the phosphatidylinositol-3-kinase (PI3K) pathway [4], and its down-regulated activity has been noticed in many types of cancer [5–7]. AKT/protein kinase B is an essential downstream target of growth factor receptor tyrosine kinases that signal via PI3K [8–11]. Precisely, loss of PTEN causes activation of Akt, which in turn promotes anti-apoptotic as well as pro-cell cycle entry pathways supposed to be important in tumorigenesis [12]. Moreover, The PI3K/AKT cascade has been concerned in promoting cell survival downstream of extracellular stimuli [13,14].

P53 is a tumor suppressor gene and guardian of genome and it shows a pivotal role in determining the fate of cells exposed to DNA damage stimuli [15]. Wild-type p53 is a tumor suppressor protein that shows an important task in managing genomic stability through the cell cycle control and also shows a role in the induction of apoptosis when cell damage is beyond repair [16–18]. Studies advocate that *p53* gene is the most frequently mutated tumor suppressor gene in human malignancy [19].

The B cell lymphoma-2 (Bcl2) family of proteins are vital controllers of apoptosis [20] and they are expressed in solid tumors, including breast, prostate, colorectal, lung, stomach, and ovarian cancers [21,22]. There is increasing proof to recommend that Bcl2-targeting therapy may be an effective treatment for cancers [23,24].

Exploration of the relationship between the pattern of immunohistochemical expression of PTEN, Bcl2 and p53 and pathological grading and clinical staging of uterine cervix cancer was the aim of this study.

2. Materials and Methods

In this case-control study, 75 cases of squamous cell carcinoma of the uterine cervix were retrieved randomly from different histopathology departments in Khartoum State, Sudan. In this study, control cases (n = 10) as inflammatory lesions of uterine cervix cases were included, and the mean age of the patients was 46.0 $\pm$ 12.0 years old. The incidence of cervicitis was high in patients in the 4th decade. Tissue samples were fixed in 10% formalin; tissue was embedded in paraffin, and stained with hematoxylin and eosin for histopathological evaluation. Clinicopathologic characteristics, such as tumor size, differentiation grade, and stage, were recorded.

2.1. Expressional Evaluation of PTEN, Bcl2, and P53 through Immunohistochemical Staining

Concisely, formalin-fixed paraffin-embedded tissue sections were deparaffinized, rehydrated and rinsed in phosphate-buffered saline (PBS) (pH 7.0) and the rest of the procedure was performed as the previously described method [25]. Blocking of endogenous peroxidase activity was done through 0.3% hydrogen peroxide in methanol for 20 min. Antigen retrieval was done in citrate buffer (pH 6.0) in a pressure cooker for 25 min, and then sections were kept at room temperature for 10–15 min. Then, the blocking step was performed with protein block for 10 min, and slices were washed. Expressional evaluation of PTEN, p53, and Bcl2 was examined through the streptavidin–biotin method. The monoclonal antibody of PTEN (PM 278AA, Biocare Medical, CA, USA), p53 (9D3DE3,

Abcam, Cambridge, U.K.) and Bcl2 (PM003AA. Biocare Medical, USA) used as primary antibodies and sections were incubated for overnight at 4 °C. Following incubation with secondary antibody (Abcam, Cambridge, U.K., Biotynylated Goat anti-mouse, ab 64259 lot GR 234312-2 lot GR 234312-2) for 60 min, followed by incubation with streptavidin–biotin enzyme (Streptavidin Peroxidase ab 64259) complex was applied for 45 min as the manufacturer instructions. Then, diaminobenzidine (DAB) (Abcam, Cambridge, U.K., ab 64259) chromogen was used, and sections were counterstained with hematoxylin and results were observed under a light microscope.

2.2. Consideration of Positive and Negative Cases for Each Marker

A total of five fields from each section were selected, and 100 cells from each area were counted and the mean percentage positivity was calculated. Expressions of *PTEN*, *p53*, and *Bcl2* were considered as the positive case if more than 5% of cells showed positivity and less than 5% positivity was taken as the negative case [26]. PTEN, Bcl2, p53 protein staining among the cervix tumor cases was either negative, weakly positive or strong positive.

2.3. Statistical Analysis

Markers' expression and its association with a histologic grade or clinical stage were analyzed by Chi-square $(\lambda)^2$. The *p*-Value of $p < 0.05$ was considered statistically significant.

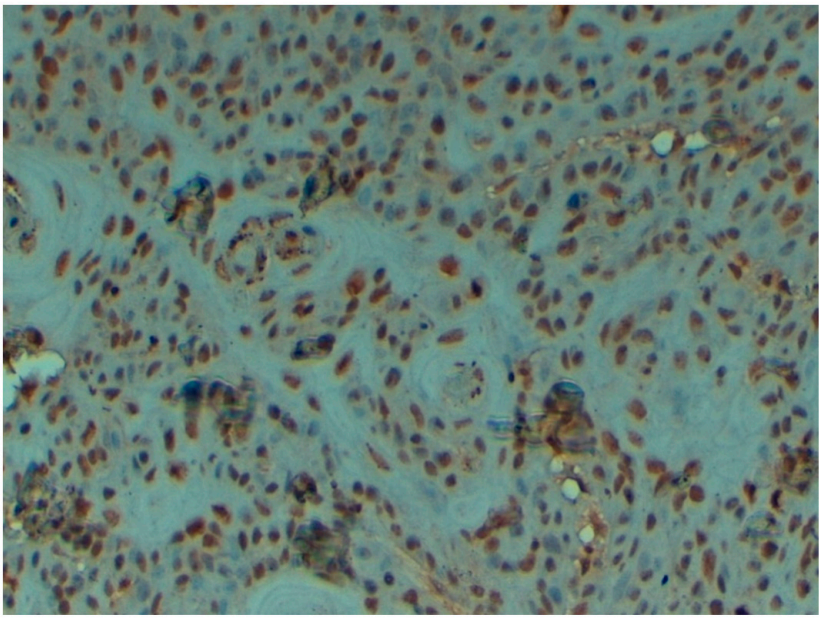
3. Results

3.1. PTEN Protein Expression

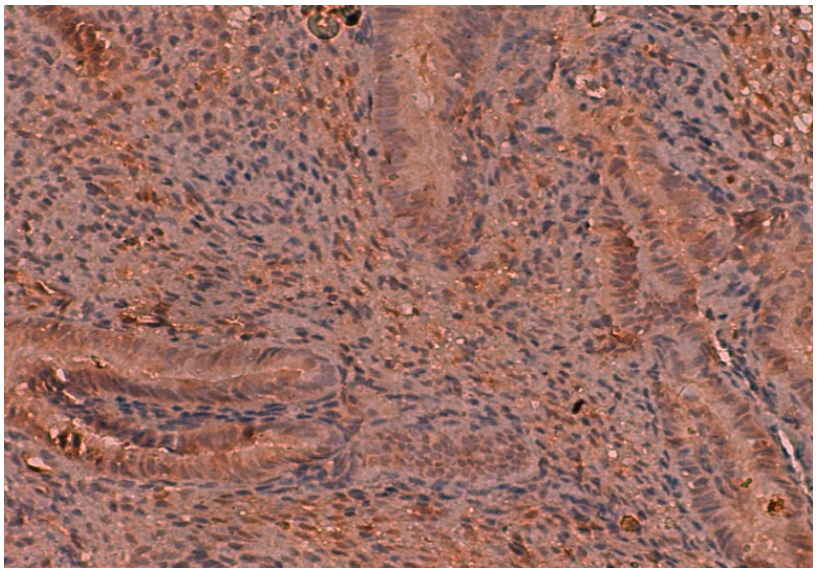
Expression of PTEN protein was noted in cancerous cases and inflammatory lesion of cervix (Figure 1a). Loss of PTEN expression was identified as 45.33% (34/75) in cervix cancer and a high expression was noted in control cases (Figure 1b) with $p < 0.05$ (Table 1). PTEN expression was significantly associated with the clinical stage of the SCC of the uterine cervix (61.36% and 45.16% in stages I–II and III–IV, respectively) ($p < 0.05$) (Table 1). No association was detected between the PTEN expression and grade of differentiation (58.33% in the well differentiated, 55.88% in the moderately differentiated, 47.05% in the poorly differentiated) and age of the patients ($p > 0.05$) (Table 1).

Table 1. Correlation between Phosphatase and tensin homolog (PTEN) and clinico-pathological features in cervical Squamous Cell Carcinoma (SCC).

Variables	Total Cases	PTEN Positive	PTEN Negative	*p*-Value
Cervical carcinoma	75	41 (54.66%)	34 (45.33%)	<0.05
Inflammatory lesion	10	10 (100%)	00 (100%)	
Age (In years)				
≤58 years	31	18 (58.06%)	13 (41.9%)	>0.05
>58 years	44	23 (52.27%)	21 (47.72%)	
Histological grades				
Well differentiated	24	14 (58.33%)	10 (41.66%)	>0.05
Moderately differentiated	34	19 (55.88%)	15 (44.11%)	
Poorly differentiated	17	8 (47.05%)	09 (52.94%)	
Clinical stage				
I and II	44	27 (61.36%)	17 (38.63%)	<0.05
III and IV	31	14 (45.16%)	17 (54.83%)	



(**a**)

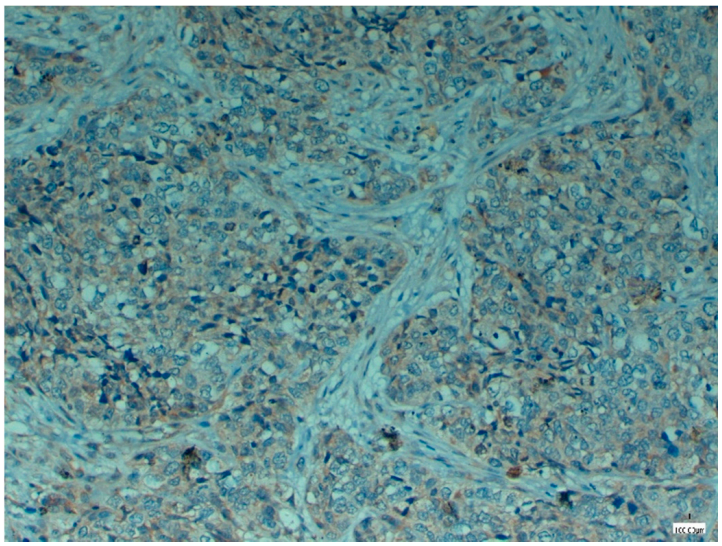


(**b**)

Figure 1. (**a**) Expression of PTEN protein in cervical squamous cell carcinoma (original magnification: 40×). (**b**) Expression of PTEN protein in control cases (original magnification: 40×).

3.2. Bcl2 Expression

The cytoplasmic expressions of Bcl2 protein were noted in 60% (45/75) cases of cervical cancer tissue (Figure 2a). Whereas all control cases (inflammatory lesions) were negative or did not show any expression with $p < 0.05$ (Figure 2b). Bcl2 did not show any significant association with histologic type ($p > 0.05$), as well as clinical stage of the tumor ($p > 0.05$) (Table 2). Additionally, Bcl2 expression did not show any significant association with age of the patients ($p > 0.05$).



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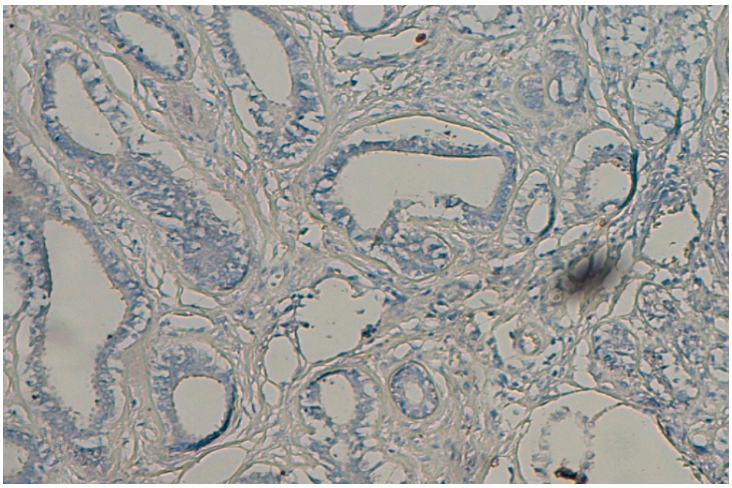


(**b**)

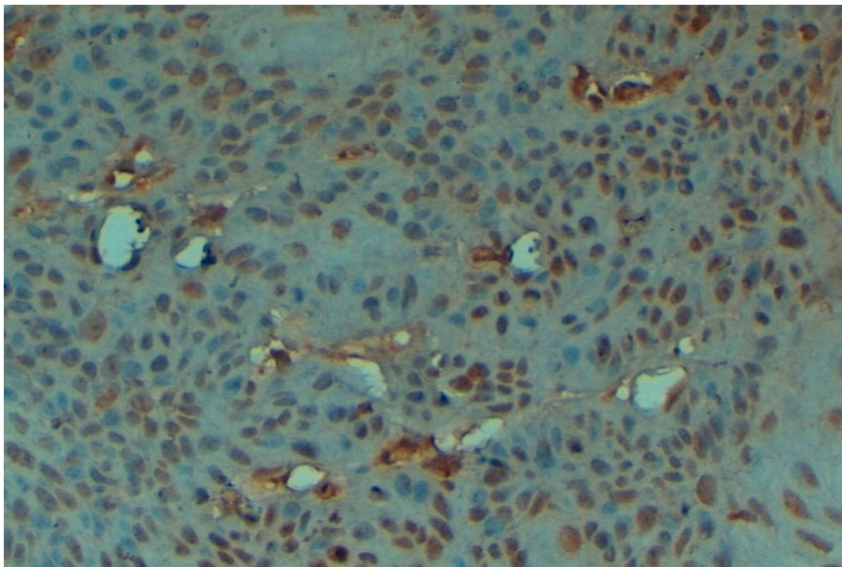
Figure 2. (**a**) Bcl2 protein is showing expression (cytoplasmic) in cervical SCC (original magnification: 40×). (**b**) Bcl2 protein is not showing expression in control cases (original magnification: 40×).

Table 2. Correlation between Bcl2 expression and clinico-pathological features of cervical SCC.

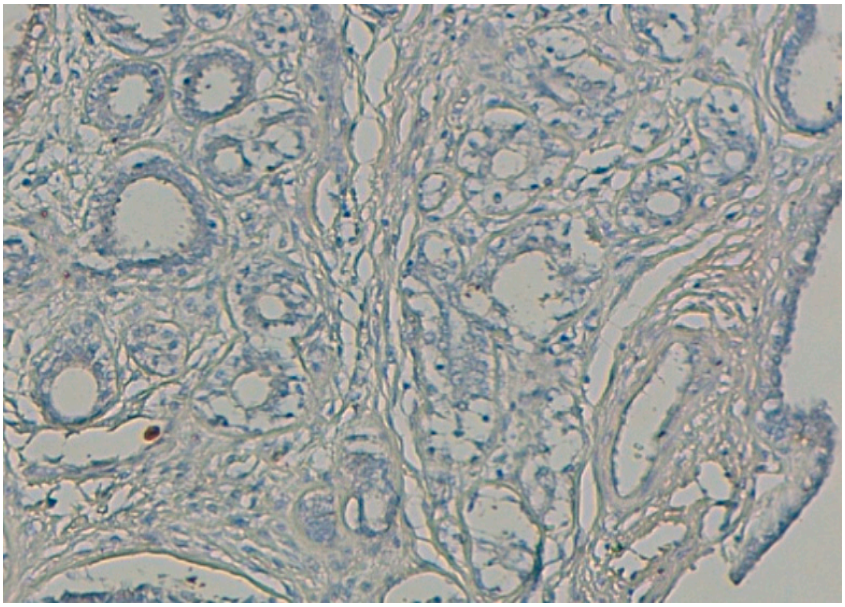
Variables	Total Cases	Bcl2 Positive	Bcl2 Negative	*p*-Value
Cervical carcinoma	75	45 (60%)	30 (40%)	<0.05
Inflammatory lesion	10	00 (00%)	10 (100%)	
Age (In years)				
≤58 years	31	20 (64.51%)	11 (35.38%)	>0.05
>58 years	44	25 (56.81%)	19 (43.18%)	
Histological grades				
Well differentiated	24	15 (62.5%)	09 (37.5%)	>0.05
Moderately differentiated	34	21 (61.76%)	13 (38.2%)	
Poorly differentiated	17	9 (52.9%)	08 (47.05%)	
Clinical stage				
I and II	44	28 (63.63%)	16 (36.36%)	>0.05
III and IV	31	17 (54.83)	14 (45.16%)	

3.3. P53 Expression

The expression of p53 protein, which was mainly located in the nucleus (Figure 3a), was noted in 57.3% (43/75) cases (Table 3). The expression was high in cancer tissue, and no expression was observed in control cases (Figure 3b), and such difference of expression was statically significant ($p < 0.05$). The expression pattern of p53 protein in cervical cancer tissue samples was not associated with patient age, grade and stage of tumors ($p > 0.05$) (Table 3).



(**a**)



(**b**)

Figure 3. (**a**) p53 protein is showing expression (nuclear) in cervical SCC (original magnification: 40×). (**b**) p53 protein is not showing expression in control cases (original magnification: 40×).

Table 3. Correlation between p53 expression and clinico-pathological features of cervical SCC.

Variables	Total Cases	p53 Positive	p53 Negative	*p*-Value
Cervical carcinoma	75	43 (57.33%)	22 (29.33%)	<0.05
Inflammatory lesion	10	00 (00%)	10 (100%)	
Age (In years)				
≤58 years	31	17 (54.83%)	14 (45.16%)	>0.05
>58 years	44	26 (59.09%)	18 (40.90%)	
Histological grades				
Well differentiated	24	15 (62.5%)	09 (37.5%)	>0.05
Moderately differentiated	34	20 (58.82%)	13 (38.2%)	
Poorly differentiated	17	08 (47.05%)	08 (47.05%)	
Clinical stage				
I and II	44	25 (56.81%)	16 (36.36%)	>0.05
III and IV	31	18 (58.06)	14 (45.16%)	

4. Discussion

To the best of our knowledge, the prognostic role of PTEN protein expression through immunohistochemistry has not been evaluated in patients with cervix cancer earlier on Sudanese's patients. In the present study, it was perceived that PTEN protein expression was high in inflammatory lesions and loss of PTEN protein expression was noted in cervical cancer tissue samples, and such difference was significantly significant ($p < 0.05$). An earlier finding based on Chinese patients was in accordance with this finding and results showed that PTEN expression progressively decreased with the normal epithelium of the tissue to squamous cell carcinoma of the cervix [27]. Another recent study from other part of world reported that nuclear PTEN expression was detected in all cervicitis cases whereas 63/102 (62%) cases of cervical cancer showed PTEN expression [28]. Moreover, the current study showed that loss of PTEN expression was significantly associated with clinical stage of the SCC of the uterine cervix (61.36 and 45.16% in stages I–II and III–IV, respectively) ($p < 0.05$). No associations were found between the PTEN expression and grade of differentiation and age of the patients ($p > 0.05$). In this regards, a previous finding also reported that PTEN protein expression was significantly correlated with the stage of the cervical SCC and lymph node metastasis [28] and positive PTEN immunostaining was associated with clinical stage and tumor size [27].

The *Bcl2* gene belongs to the anti-apoptotic genes in the *Bcl2* gene family, and its altered expression has been noticed in several types of tumors including carcinoma of the cervix. In the current study, it was reported that cytoplasmic expressions of Bcl2 protein were noted in 60% (45/75) cases of cervical cancer tissue. The expression pattern of Bcl2 was high in cancer cases than in control cases, and such a difference was statically significant ($p < 0.05$). There are scanty of data available on the expression of Bcl2 protein in cancer based on Sudanese patients. Previous study based on the oral cancer sample of Sudan revealed that expression of Bcl2 was found to be restricted to tumor cells in well and moderately differentiated tumors. There is no expression or undetectable expression of Bcl2 in basal cells [29]. The current findings are in keeping with an earlier study on cervical cancer from China, which reported that Bcl2 in the tissue of cervical cancer was significantly higher than that in normal cervical tissue [30]. Moreover, Bcl2 did not show any significant association with the histologic type ($p > 0.05$), the age of the patients and clinical stage of the cervical SCC ($p > 0.05$). These findings are similar to a previous result on cervical cancer, which reported that expression of Bcl2 had no significant association with the histologic type or clinical stage [31]. Another study said that Bcl2 was expressed in 65% of the tumors and there was a statistically significant association between Bcl2 expression and poorer disease-free survival (DFS) and overall survival (OS) in stage IIB cases [32].

The *p53* gene is a tumor suppressor gene that encodes a 393-amino-acid nuclear DNA-binding phosphoprotein [33]. Studies have suggested that *P53* gene is the most commonly mutated tumor suppressor gene in human malignancy and high expression has been noticed in oral cancers [34]. The current study finding reported that p53 protein expression was noted in 57.3% (43/75) cases. The expression was high in cancer tissue, and no expression was observed in control cases, and such difference of expression was statically significant ($p < 0.05$). P53 protein expression in cervical cancer tissue samples was not correlated with patient age, grade and stage of tumors ($p > 0.05$). An earlier report on cervical cancer stated that the protein expression level of p53 in the tumor tissues was significantly higher compared to that of the normal adjacent tissue [35]. Moreover, the same study reported that p53 protein expression in cervical cancer tissue samples was not correlated with the patient age, tumor size, or family history [35]. A study based on Sudanese patients reported that p53 was expressed in 19.3% of head and neck cancers whereas expression in benign tumors was low and only 4.0% benign cases showed expression [36].

In conclusion, our findings demonstrate that the loss of PTEN protein and overexpressions of Bcl2 and p53 protein play a vital role in the development and progression of cervix cancer.

Author Contributions: A.H.R. and N.E.O.S.H. conceived and designed the experiments; A.Y.B. and A.A. performed the experiments; K.S.A. and M.A.A. (Mohamed A. Alsammani) analysed the data. M.A.A. (Mohammed A. Alsahli) Editing & Review.

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Conflicts of Interest: The authors have declared that no competing interests exist.

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