International Journal of Academic Health and Medical Research (IJAHMR)
ISSN: 2643-9824
Vol. 5 Issue 6, June - 2021, Pages: 17-20

Immunohistochemical Detection of Bcl2 in Cervical Tumors among Sudanese Women

1Abu Elgasim Abass Awad Elkareem and 2Mai Omer Elfadni Suliman

1Sudan University of Science and Technology, College of medical laboratory science, Khartoum, Sudan
Sudan International University, College of medical laboratory science, Khartoum, Sudan
gassomy2@gmail.com
2Sudan University of Science and Technology, College of medical laboratory science, Khartoum, Sudan

Abstract*: This is a descriptive retrospective hospital based study conducted in Khartoum state hospitals. The study aimed to detect the expression of Bcl-2 proteins in cervical tumors using immunohistochemical method.Forty paraffin embedded blocks previously diagnosed as cervical tumors were selected. Samples include 30 (75%) malignant tumors (28/30) samples were squamous cell carcinoma and (2/30) samples were adenocarcinoma and 10 (25%) samples were benign tumors.The patient's age ranged between 30 and 85 years with mean age of 55 years, most patients 26 (65%) were less than 70 years and the remaining 14 (35%) patients were more than 70 years.One section of 3 µm thickness was cut from each paraffin block by rotary microtome and stained by immunohistochemical method (modified new indirect method) for detection of Bcl-2. Data collected from patient's files and results obtained were analyzed using SPSS computer program.Immunohistochemical expression of Bcl-2 was revealed positive result in 12/30 samples and negative result in 18/30 in malignant samples while all benign tumors showed negative result for Bcl2, with significant statistical association between Bcl-2 expression and histopathology diagnosis (P=0.02).Out of 12 Bcl2 positive samples, 11 samples were squamous cell carcinoma and only one sample was adenocarcinoma with no statistical association between Bcl-2 expression and type of cancer (P=0.78).Comparison between the expression of Bcl-2 and grade of the cancer the positive result was detected in 2/4 samples of well differentiated tumors, 5/12 samples of moderately differentiated tumors and 4/12 samples of poorly differentiated tumors with no relation between histological grade of tumor and Bcl-2 expression (P=0.55).This study concludes that there is association between Bcl-2 expression and malignant tumors of cervix, with no association with both type of cancer and the histological grade of tumor.*

Keywords— Bcl2; cervical tumors; Immunohistochemistry.

1. INTRODUCTION

Cervical cancer is a malignant neoplasm arising from cells originating in the cervix uteri. One of the most common symptoms of cervical cancer is abnormal vaginal bleeding, but in some cases there may be no obvious symptoms until the cancer has progressed to an advanced stage [(1)].

Cervical cancer is the third most commonly diagnosed cancer word wide and the fourth leading cause of cancer death in women [(2)]. It affects about 16 per 100,000 women per year and kills about 9 per 100,000 per year [(3)].

Approximately 80% of cervical cancers occur in developing countries [(4)]. Cervical cancer death rates have been decreasing, but the disease still accounted for 200,000 deaths in 2010; in developing countries, 46,000 of these women were aged 15-49 years, and 109,000 were aged 50 years or older [(5)].

Infection with some types of human papilloma virus (HPV) is the greatest risk factor for cervical cancer, followed by smoking. Other risk factors include human immunodeficiency virus, oral contraceptive use, hormone replacement therapy use and previous cancer [(6)].

Not all of the causes of cervical cancer are known, however, several other contributing factors have been implicated [(7)].

Papa Nicola smear can be used as a screening test for pre cancers and cancer, but do not make a final diagnosis of cervical cancer. Confirmation of the diagnosis of cervical cancer or pre-cancer requires a biopsy of the cervix; this is often done through colposcopy [(8)].

Bcl-2 is a proto-oncogene situated in the inner mitochondrial membrane. It is a 25 KD protein with 239 amino acids which protects the cells from apoptosis and is localized on the long arm of the 18thchromosome [(9)].

Bcl2 over-expression is present in premalignant and malignant lesions of cervix. It has been suggested that bcl2 may play a vital role in a relatively early stage of cervical cancer. Bcl2 positivity has also been shown to confer a better 5 year survival rate and prognosis [(10)].

Bcl2 is responsible for the prevention of apoptotic cell death in several situations. Inappropriate expression of bcl-2 may prolong survival of defective and harmful cells, including those involved in HPV infection, thus increasing the probability of malignant conversion [(11)].

Bcl2 overexpression is present in premalignant and malignant lesions of cervix. It has been suggested that it play a vital role in a relatively early stage of cervical tumorigenesis in association with HPV infection. Bcl2 positivity has also been shown to confer a better 5 year survival rate [(10)].

Two studies of bcl-2 expression and cervical cancer have been found 61 to 63 percent of invasive cervical cancer to have bcl-2 over expression, and in both studies, this correlated with increase overall survival [(12)].

Other studied of bcl-2 expression and cervical cancer showed; there are 12 of the 27 samples stained positively (44%). This positively increase in early stage (grade one) as 50%, this correlated indicated that Bcl-2 is good to using as prognostic marker for cervical cancer [(13)]. Over expression of Bcl-2 protein was activated in the early stage of cervical cancer [(14)].

Bcl-2 expression as immunohistochemistry techniques in 40 cases, Bcl-2 was expressed in 65% of cervix squamous cell carcinoma [15].

2. Materials and methods:

2.1 Materials:

Archived tissue blocks obtained from samples cervical tumors were used in this study.

2.2 Methods:

2. 2.1 Study design:

This is a hospital based descriptive retrospective case study aimed to detect expression of bcl-2 tumor marker in cervical tumor using immunohistochemical method.

2.2.2 Sample processing:

Section to be stained were cut at 3µm thickness by rotary microtome, mounted in positively charged glass slides and put at 60˚C oven for 30 minutes

2.2.2.1 Immunohistochemical staining:

The section of 3µm thickness were obtained from formalin fixed paraffin embedded tissue using a rotary microtome, then immunostained using monoclonal antibodies by new indirect technique as follows:

Sections were dewaxed in hot oven and cleared in two changes of xylene for two minutes, then hydrated through descending concentrations of ethanol (100%, 90%, 70%, 50%) and water two minutes for each, then Ag retrieval by water bath retrieval technique for thirty minutes at 97°c (coplin jar containing citrate buffer PH 6.0), then washed in phosphate buffer saline (PH 7.4) for five minutes, then section use circulated by Dako pen, then treated with hydrogen peroxide solution for fifteen minutes, then washed in phosphate buffered saline (PH 7.4) for five minutes, then treated with anti Bcl-2 (Bcl-2 alpha Ab-1) primary antibody for thirty minutes, then rinsed in phosphate buffered saline (PH 7.4), then treated with secondary polymer conjugated antibody for thirty minutes, then rinsed in phosphate buffer saline (PH 7.4), then treated with DAB for seven minutes, then washed in phosphate buffer saline (PH 7.4) for five minutes, then counter stained in Mayer's haematoxylin for one minutes, then washed and blued in 0.05% ammoniated water for 16 second, then washed in tap water, then dehydrated through ascending concentrations of ethanol (50%, 70%, 90%, 100%), then cleared in xylene and mounted in DPX mountant [16].

2.2.3 Result interpretation:

Detection of more than 5 cells with brown cytoplasm per one field considered as positive result.

All quality control measures were adopted; positive and negative control slides were used during immunohistochemical staining.

2.2.4 Data analysis :

Data analysis was done using SPSS 11.5 computer program. Frequencies mean and chi-square test values were calculated.

2.2.5 Ethical consideration:

Sample collected after taking ethical acceptance from hospital administration .

RESULTS

A total of 40 samples of patients with cervical tumors were investigated, 30 of them were malignant cervical tumors representing 75%, and the remaining 10 (25%) were benign (Table 1).

The age of study population ranged between 30 to 85 years with mean of age 55 years. Subject less than 70 years were 26 (65%), and older than 70 years were 14 (35%) (Table 2).

The description of tumor grade revealed that well differentiated tumor in 4 (13.3%) samples, moderately differentiated tumor in 12 (40%) samples, and poorly differentiated tumor in 12 (40%) samples. And not graded in 2 sample (6.7%) (Table 3).

Malignant cervical tumors revealed positive expression of Bcl-2 in 12 (40%) samples and negative expression in 18 (60%) samples, while all benign tumors showed negative expression of Bcl-2. This result showed significant statistical association (P.value=0.02) (Table 4).

Out of 12 positive samples of Bcl-2, 11 were SCC and one was AC and negative in 18 samples, 17 were SCC and one was AC. With no significant association (P.value=0.78) (Table 5).

The comparison between Bcl-2 expression and the grade of tumor showed that Bcl-2 expression was positive in 2 well differentiated tumors, 5 moderately differentiated tumors and 4 poorly differentiated tumors. With insignificant association (P.value=0.55) (Table 6).

Table (1): Distribution of sample among the study population:

Sample	Frequency	Percent
Malignant	30	75%
Benign	10	25%
Total	40	100%

Table (2): Distribution of age group among study population:

Age group	Frequency	Percent
Less than 70 years	26	65%
More than 70 years	14	35%
Total	40	100%

Table (3): Distribution of cancer grade among malignant cervical tumors:

Grade	Frequency	Percent
Well differentiated tumors	4	13.3%
Moderately differentiated tumors	12	40%
Poorly differentiated tumors	12	40%
Not graded	2	6.7%
Total	30	100%

Table (4): Relation between Bcl-2 expression and histopathology diagnosis of cervical tumors:

Sample	Positive	Negative	Total	P.value
Benign	0	10	10	0.02
Malignant	12	18	30	
Total	12	28	40	

Table (5): Relation between Bcl-2 expression and type of cervical cancer:

Sample	Bcl-2 expression		Total	P.value
	Positive	Negative		
SCC	11	17	28	0.78
AC	1	1	2	
Total	12	18	30	

Table (6): Relation between Bcl-2 expression and the grade of cervical cancer:

Grade	Bcl-2 expression		Total	P.value
	Positive	Negative		
Well differentiated tumors	2	2	4	0.55
Moderately differentiated tumors	5	7	12	
Poorly differentiated tumors	4	8	12	
Total	11	17	28	

DISCUSSION

In this study patients age ranging between 30-85 years, with mean age 55 years, indicating that increasing age is associated with high risk of developing cervical tumors probably due to hormonal disturbance that may initiate such type of tumors. A similar result was observed by Tjalma *et al*. [12], their study reported that the mean age of patients ranging between 18-80 years was 51 years .

The samples revealed that 28 (70%) were squamous cell carcinoma, while 2 (5%) were adenocarcioma, our finding revealed that most malignant cervical tumors were squamous cell carcinoma representing 28/30, this finding was consistent with the result of Tewari *et al*. [17], who reported that cervical squamous cell carcinoma is the most frequent cervical cancer subtype internationally.

Bcl-2 is a mitochondrial protein associated with anti-apoptotic function. Over expression of Bcl-2 is found to be in a variety of tumor due to degradation of Bcl-2. In this study over expression of Bcl-2 is observed in malignant cervical tumors 12/30, while benign cervical tumors showed no expression of Bcl-2. This relation showed significant association (P.value=0.02) this finding is compatible with results observed by Sema *et al.* [13], in their study in Bcl-2 expression in carcinoma of the cervix only 12 (44%) out of 27 showed cytoplasmic Bcl-2 expression.

Bcl-2 positive samples were compared with the type of tumor and we found that 11/12 positive samples were diagnosed as cervical squamous cell carcinoma, and 1/12 was adenocarcinoma. With insignificant relationship between each type of malignancy and the Bcl-2 positive results (P.value=0.78) and this was contradicted with results obtained by Shukla *et al.* [18], who reported that a higher percentage of adenocarcinomas were positive for bcl-2 compared with squamous cell carcinomas (66.67% vs. 33.33%).

Bcl-2 expression and grade of cancer showed that positive expression in 2/2 of well differentiated tumors, 5/12 of moderately differentiated tumors and 4/12 of poor differentiated tumors, this relation showed insignificant association (P.value=0.55) indicating that rising of cancer grade is not affected by the Bcl-2 dysfunction. This finding is compatible with result observed by Shukla *et al*. [18], reported that 50% of well differentiated and 33.3% moderately differentiated carcinoma were bcl-2 positive, but the difference was not statistically significant (P.value=0.622). Incompatible result observed by Grace *et al.* [19], showed an increasing expression of bcl-2 with the rising grade of cervical cancer.

References

1. Kumar, V., Abbas, AK., Fausto, N. and Mitchell, RN. (2007). Robbins Basic Pathology 8th Edition Saunders Elsevier. pp. 718–721.

2.Duenas-Gonzalez, A., Serrano-Olvera, A. and Cetina, L. (2014). New molecular targets against cervical cancer. *Int J Womens Health.* **5** (6):1023-31.

3. Jemal, A., Bray, F., Center, MM., Ferlay, J., Ward, E. and Forman, D. (2011). Global Cancer Statistics. *CA Cancer J Clin.* **61**(2):69-90.

4. Kent, A. (2010). HPV Vaccination and Testing. *Reviews in obstetrics and gynecology*. **3** (1): 33–34.

5. Forouzanfar, MH., Foreman, KJ., Delossantos, AM., Lozano, R., Lopez, AD. and Murray, CJ. (2010). Breast and Cervical Cancer In 187 Countries.a Systematic Analysis. *Lancet.* **378**(9801):1461-84.

6. Gadducci, A., Barsotti, C., Cosio, S., Domenici, L. and Riccardo, GA. (2011). Smoking Habit, Immune Suppression, Oral Contraceptive Use, And Hormone Replacement Therapy Use And Cervical Carcinogenesis: *A review of the literature. Gynecological Endocrinology.* **27** (8): 597–604.

7. Stuart, C. and Ash, M. (2006). Gynaecology by Ten Teachers 18th ed. Hodder Education. pp: 285-310.

8. Noller, KL. (2012). Intraepithelial Neoplasia Of The Lower Genital Tract (Cervix, Vulva): Etiology, Screening, Diagnostic Techniques, Management. In: Katz, VL., Lentz, GM., Lobo, RA., Gershenson, DM. eds. Comprehensive Gynecology. 6th ed. Philadelphia, PA: Elsevier Mosby; pp: 156-175.

9. Hockenbery, D., Nunez, G., Milliman, C., Schreiber, RD. and Korsmeyer, SJ. (1990). Bcl-2 Is An Inner Mitochondrial Membrane Protein That Blocks Programmed Cell Death. *Nature*. **348**:334–336.

10. Dimitrakakis, C., Kymionis, G., Diakomanolis, E., Papaspyrou, I., Rodolakis, A. and Arzimanoglou, I. (2000). The Possible Role Of p53 And bcl-2 Expression In Cervical

Carcinomas And Their Premalignant Lesions. *Gynecol Oncol*.**77**:129–36.
11. Green, DR., Bissonnette, RP. and Cotter, TG. (1994). Apoptosis and cancer.In De Vita VT, Hellman S, Rosenberg SA, eds. Important Advances in Oncology. Philadelphia, Lippincott, 37–52.
12. Tjalma, W., Decuyper, E. and Weyler, J. (1998). Expression of Bcl-2 In Invasive And Insitu Carcinoma Of The Uterine Cervix. *AmjObstet Gynecol*.**178**:113-7.
13. Sema, Z., Tayfun, G., Orhans, S., Emel, E. and Oya, G. (2001).BCL-2 Expression In Carcinoma Of The Uterine Cervix And Its Relationship With Prognostic Variables. *Turk J Med Sci*. **31**: 401-404.
14. Tang, ZH., Cai, QF. and Ye, XA. (2003). Expression of bcl-2, c-er-bB2 and p53 Protein In Cervical Epithelial Carcinogenesis. *Ai-Zheng*. **22**: 1057-61.
15. Rajkumar, T., Rajan, S. and Baruch, Rk. (1998). Prognostic Significant Of Bcl-2 And P53 Protein Expression In Squamous Cell Carcinoma Of The Cervix. *Eur Gynaecoloncol*.**19**(6):556-60.
16. Bancroft, JD. and Marilyn, G. (2008). Theory and practice of histological techniques, 6th ed. Churchill Livingstone, London, pp 125.
17. Tewari, KS., Monk, BJ., Raghavan, E., Brecher, ML. and Johnson, DH. (2006). Tumors of the cervix. 3th ed. Chichester, England: John Wiley & Sons. 45: pp. 501-520.
18. Shukla, S., Dass, J. and Pujani, M. (2014). P53 and Bcl-2 Expression in Malignant And Premalignant Lesions Of Uterine Cervix And Their Correlation With Human Papilloma Virus 16 and 18. *South Asian J Cancer*. **3**:48-53.
19. Grace, VM., Shalini, JV., Lekha, TT., Devaraj, SN. and Devaraj, H. (2003). Co-Overexpression Of p53 and bcl-2 Proteins in HPV-Induced Squamous Cell Carcinoma of the Uterine Cervix. *GynecolOncol*. **91**:51-8.