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Immunohistochemical Expression of p53, bcl2 and CD34 in Cervical Intraepithelial Neoplasias and Carcinomas

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Abstract

Background Cervical cancer is the fourth most common cancer affecting women worldwide. Immunohistochemical expression of several biomarkers; including those regulating apoptosis and angiogenesis; may help to distinguish reactive conditions from precancerous and cancerous lesions of the uterine cervix.

Objective To assess the IHC expressions of p53, bcl2 and CD34 in cervical intraepithelial neoplasias and carcinomas.

Methods A cross sectional study included a total of 127 formalin-fixed paraffin-embedded cervical tissue blocks; of which 22 cases were chronic cervicitis, 24 cases were low grade squamous intraepithelial lesion (LSIL), 28 cases were high grade squamous intraepithelial lesion (HSIL) and 53) cases were invasive cervical carcinomas. Sections from each block were immunohistochemically stained for p53, bcl2 and CD34.

Results p53 was not expressed in chronic cervicitis, with significant increase in its expression from LSIL through HSIL to carcinomas had been identified. A significantly higher IHC expression of p53 was observed in adenocarcinomas and adenosquamous carcinomas compared to squamous cell carcinomas. Bcl2 was expressed in all cases with non-significant differences. Regarding CD34 IHC expression; there was a significant increase in microvessel density (MVD) from chronic cervicitis through LSIL and HSIL to carcinomas. A significantly higher MVD was detected in adenosquamous carcinomas and adenocarcinomas, in poorly differentiated carcinomas and was significantly increasing with stage.

Conclusions p53 plays an important role in the progression of the severity of intraepithelial cervical lesions. MVD can be utilized as ancillary marker for the risk of malignant transformation of cervical intraepithelial lesion.

Key words LSIL, HSIL cervical carcinoma, p53, bcl2, CD34, MVD

List of abbreviation: CIN = cervical intraepithelial neoplasia, LSIL = low grade squamous intraepithelial lesion, HSIL = high grade squamous intraepithelial lesion, IHC = Immunohistochemical, TAH = total abdominal hysterectomies, SI = staining index, MVD = microvessel density

Introduction

Cervical cancer is the fourth mostly common cancer affecting women worldwide, after breast, colorectal, and lung cancers; it is most found in the lower resource countries of sub-Saharan Africa. It is also the fourth most common cause of cancer death (266,000 deaths in 2012) in women worldwide [(1)]. In Iraq, and according to the latest Iraqi cancer registry 2011, cervical cancer is out of the commonest ten cancers in Iraqi females [(2)].

Cervical intraepithelial neoplasia (CIN) is a premalignant (dysplastic) lesion that is characterized by abnormal cell proliferation, maturation and nuclear atypia. CIN may return to normal or progress to invasive cancer if left

untreated. Approximately one-third to one-half of cases of CIN-I and CIN-II regress without treatment, even cases of CIN-III have been noted to regress spontaneously. The more severe the abnormality of the lesion, the less likely it is to regress [3]. The average time for progression of CIN to invasive cancer has been expected to be 10 to 15 years, allowing long time windows for early detection and possible preventive therapy of cancer or precancerous lesions [4]. Because the decision with regard to patient management is two-tiered (observation versus surgical treatment), the three-tier classification system has been recently made easy to a two-tiered system, with CIN-I renamed low grade squamous intraepithelial lesion (LSIL) and CIN-II and CIN-III combined into one class referred to as high grade squamous intraepithelial lesion (HSIL) [5].

P53 is a tumor suppressor gene, which inhibit cellular proliferation by blocking entry into the S phase of the cell cycle and is also a principal regulator of apoptosis. Immunohistochemical (IHC) expression of p53 may be a useful marker which can provide information complementary to morphology, prognosis and survival outcome of the patients [6].

bcl2 is a protooncogene, which defends the cell from apoptosis. Inappropriate expression of bcl2 may prolong survival of defective and harmful cells, including those involved in human papilloma virus (HPV) infection, thus increasing the probability of malignant change [7].

Angiogenesis plays an important role in tumerogenesis and metastasis in most human solid tumors [8]. Study of angiogenesis can offer good information about the role of angiogenesis in pre-invasive and invasive cervical tumor progression and can assess the relationship with epidemiological and prognostic pathological parameters, which may develop evaluation models that constitute the basis of investigative trials for possible treatment targets [9].

The aim of the present study is to assess the IHC expression p53, bcl2 and CD34 in chronic cervicitis, LSIL, HSIL and cervical carcinomas and to study the relation of these expressions to certain clinicopathological parameters including patient's age, grade of cervical intraepithelial neoplasia, grade and stage of cervical carcinoma.

Methods

This cross sectional study was approved by Institute Review Board of the Collage of Medicine, Al-Nahrain University. During the period from March 2014 to December 2014; a total of one hundred twenty seven formalin fixed paraffin embedded cervical biopsies were collected (punch, cone and total abdominal hysterectomy). The histological diagnosis of the specimens included chronic cervicitis in 22 cases (17.3%), LSIL in 24 (18.8%) case, HSIL in 28 (22%) and invasive cervical carcinoma in 53 (41.7%) case. The carcinoma cases included 38 (29.9%) cases of squamous cell carcinoma, 12(9.4%) cases of adenocarcinoma and 3 cases (2.4%) of adenosquamous carcinoma types. Cases were retrieved from the archival materials of Teaching Laboratories and Oncology Teaching Hospital in Medical City, and Al-Imamain Al-Kadhimain Medical City for the period from January 2012 to October 2014.

All the cases of chronic cervicitis were punch biopsies; whereas 20 cases of LSIL were punch biopsies and 4 cases were cone biopsies. Regarding HSIL, 20 cases were punch biopsies, 6 cases were cone biopsies and 2 cases were total abdominal hysterectomies (TAH); for invasive cervical carcinomas 45 cases were TAH, 6 cases were punch biopsies and 2 cases were cone biopsies.

All the clinicopathological parameters such as (age; grade of cervical neoplasia; histopathological type, grade and FIGO (International Federation of Gynecology and Obstetrics) pathological stage of cervical carcinomas) were obtained from patients' admission case sheets and pathology reports.

Any sample lacking the clinicopathological information was excluded from this study.
For each case, one representative (4 µ) section was stained with Hematoxylin and Eosin and the histopathological diagnosis was revised. Three (4 µ) sections were placed on positively charged slides and stained immune-histochemically using three steps- indirect streptavidin method for monoclonal mouse antibodies including anti-p53 antibody, clone (BP53-12), anti-bcl2 antibody, clone (Bcl2/100) and anti-CD34 antibody, clone (QBEND-10); all manufactured by Abcam (United States).

Interpretation of the results of IHC staining
IHC reaction is considered positive when brown staining is nuclear for p53 protein, cytoplasmic bcl2 and cytoplasmic (of endothelial cells) for CD34 protein. The positive control for both p53 and bcl2 IHC reaction was taken from the lymphoid tissue in non-Hodgkin lymphoma. The positive control for CD34 was obtained from normal lymph node tissue. Technical negative control for all was obtained by omission of the primary antibody.
The results of IHC expressions of the above molecular markers were analyzed in a semi-quantitative fashion as follow:

p53
The IHC expression of p53 was scored semi-quantitatively by assessing both staining intensity (0 = no staining, 1 = weak, 2 = moderate and 3 = strong) and percentage of stained cells (staining ratio), (1 = 1-5%, 2 = 6-25%, 3 = 26-50%, 4 = 51-75% and 5 = 76-100%). The staining index (SI) was calculated for each case as the product of staining intensity and staining ratio (SI = staining intensity + staining ratio), with final SI range from 0, 2-8 [10, 11].

bcl2
The IHC expression of bcl2 was scored semi-quantitatively by assessing both staining intensity (0 = no staining, 1 = weak, 2 = moderate and 3 = strong) and percentage of stained cells (staining ratio), (1 = 1-5%, 2 = 6-25%, 3 = 26-50%, 4 = 51-75% and 5 = 76-100%). The SI was calculated for each case as the product of staining intensity and staining ratio (SI = staining intensity + staining ratio), with final SI range from 0, 2-8 [10].

CD34
For CD34 IHC expression in carcinoma cases, intratumoral microvessel density (MVD) was calculated based on Weidner method. According to this method, sections were scanned at the low power (x10) for the blood vessels stained with CD34, and three hot spots area were chosen. The hot spot area is an area with the most dense vessel growth. Only hot spots in tumor cell cluster in viable (non-necrotic and non-sclerotic areas) have been taken into account in this study [12]. Regarding SILs cases, the microvessels were counted in the stroma along the basement membrane subtending dysplastic epithelium [13].
Once the region of interest (the vascular hot spot) was defined, a higher magnification was selected in order to be able to count the individual stained blood microvessels. The count of microvessels was done at power (x20) which represent a field size of 0.74 mm^2 which provide microvessel count. Three hot spots were selected for counting MVD. The mean of the examined fields was divided on the high power field area which is 0.74 mm^2 and this represented MVD [12].
Any single brown stained endothelial cell or endothelial cell clusters clearly separated from adjacent microvessels, tumor cells and connective tissue elements were considered as a single countable microvessel [12].

Statistical Analysis
Statistical analysis was performed with SPSS V. 17 (statistical package for social sciences) and Excel 2007 programs. Continuous variables were expressed as mean±SEM (standard error of the mean), while categorical variables were expressed as numbers and percentages.

Statistical relations between two categorical variables were tested using Chi-square or Fisher exact tests. Relations between categorical and continuous variables were tested using unpaired t-test and ANOVA. Values were considered statistically significant when p-value < 0.05.

Results

Clinicopathological parameters

The clinicopathological parameters of chronic cervicitis, LSIL (Fig. 1), HSIL (Fig. 2) and carcinoma (Fig. 3) cases included in the present study are summarized in Tables 1.

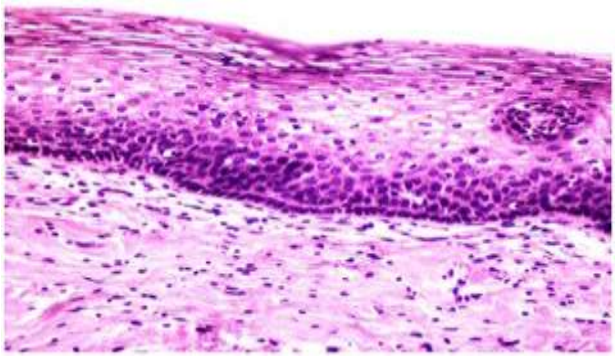

Fig. 1. Low grade squamous intraepithelial lesion shows dysplastic cells (high N/C ratio with hyperchromatic nuclei) limited to the lower one thirds of the epithelium, (H&E), (20x).

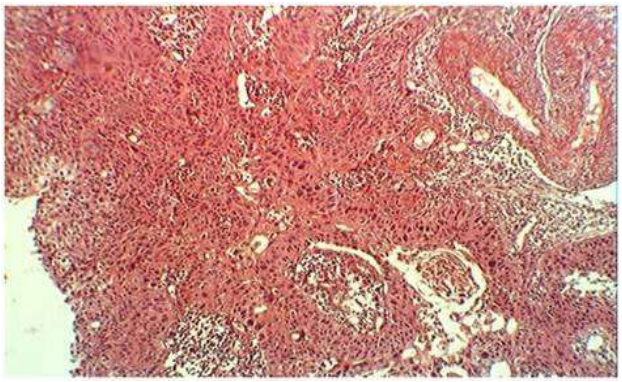

Fig. 3. Moderately differentiated non-keratinizing squamous cell carcinoma composed of irregular islands of tumor cells associated with abundant eosinophilic cytoplasm with individual keratinization and prominent nuclear pleomorphism, (H&E), (10x).

p53 immunohistochemical expression

p53 was not expressed immunohistochemically in all studied chronic cervicitis cases and expressed in 6 (25%) cases of LSIL, 17 (60.7%) cases of HSIL and 43 (81.1%) cases of cervical carcinomas, with significant increase in its expression with increasing severity of the lesions ($p < 0.001$). Regarding p53 staining index (SI), the majority of cases of carcinoma 30 out of 53(56.6%) showed high SI (6-8) (Fig. 4), while only 6 out of 28 cases of HSIL (21.4%) were with high SI (6-8). Regarding LSIL, the majority of cases 18 out of 24 (75%) were negative for p53 and the rest of the cases showed low SI (Fig. 5).

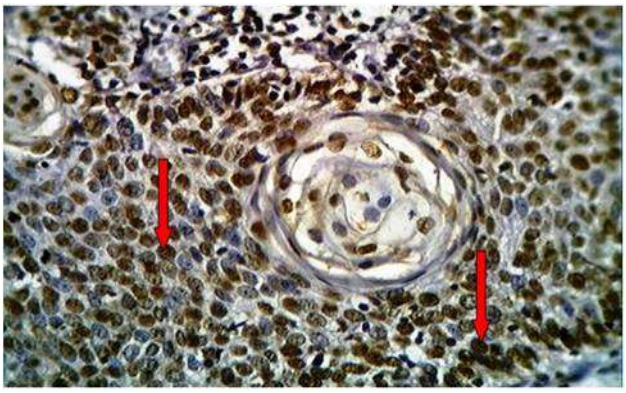

Fig. 4. Well differentiated squamous cell carcinoma of the uterine cervix stained immunohistochemically with anti-p53 monoclonal antibody showing positive brown nuclear staining (arrows) with moderate intensity (2) , high percentage (4) and SI of 6, (40X).

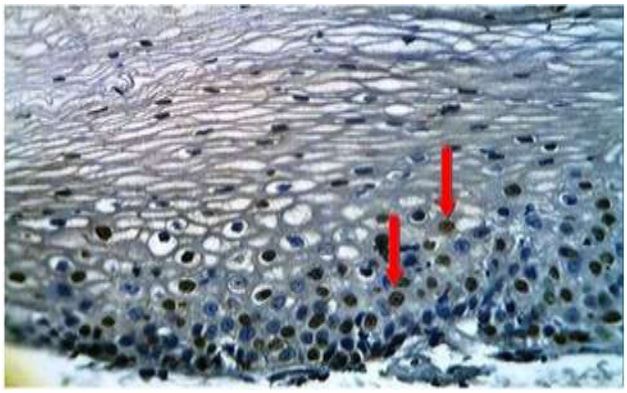

Fig. 5. Low grade squamous intraepithelial lesion of the uterine cervix stained immunohistochemically with anti-p53 monoclonal antibody showing positive brown nuclear staining (arrows) with moderate intensity (2) , moderate percentage (3) with SI of 5, (40X).

Table 1. Clinicopathological parameters of chronic cervicitis, low grade and high grade squamous intraepithelial lesion and invasive cervical carcinoma cases

Parameters		Values
Histopathological diagnosis	Chronic cervicitis	22
	LSIL	24
	HSIL	28
	Invasive carcinoma	53
Age (Mean±SEM) and range (years)	Chronic cervicitis	38.27±2.53 (22-62)
	LSIL	38.46±2.3 (22-62)
	HSIL	43.32±1.92 (27-63)
	Invasive cervical carcinoma	44.13±1.4 (29-70)
Histopathological types of invasive cervical carcinoma	Adenocarcinomas	12 (22.64%)
	Adenosquamous carcinomas	3 (5.66%)
	Squamous cell carcinomas	38 (71.7%)
Grade of invasive cervical carcinoma	Well-differentiated	9 (16.98%)
	Moderately-differentiated	28 (52.83%)
	Poorly-differentiated	16 (30.19%)
Stage of invasive cervical carcinoma(pathological FIGO staging system)*	I	13 (29%)
	II	24 (53%)
	III	8 (18%)

LSIL = low grade squamous intraepithelial lesion, HSIL = high grade squamous intraepithelial lesion, *8 out of 53 carcinoma cases were lacking information about the stage (6 cases were punch biopsies and 2 cases were cone biopsies).

Table 2. Frequency distribution of chronic cervicitis, low grade and high grade squamous intraepithelial lesion and invasive cervical carcinoma cases according to immunohistochemical expression and scoring index of p53.

Frequency of p53 expression and SI (0,2-8)	Chronic cervicitis No. (%)	LSIL No. (%)	HSIL No. (%)	Carcinoma No. (%)
Positive	0 (0%)	6 (25%)	17 (60.7%)	43 (81.1%)
Negative (0)	22 (100%)	18 (75%)	11 (39.3%)	10 (18.9%)
2	0 (0%)	2 (8.3%)	5 (17.9%)	4 (7.5%)
3	0 (0%)	1 (4.2%)	2 (7.1%)	1 (1.9%)
4	0 (0%)	2 (8.3%)	3 (10.7%)	5 (9.4%)
5	0 (0%)	1 (4.2%)	1 (3.6%)	3 (5.7%)
6	0 (0%)	0 (0%)	1 (3.6%)	7 (13.2%)
7	0 (0%)	0 (0%)	3 (10.7%)	10 (18.9%)
8	0 (0%)	0 (0%)	2 (7.1%)	13 (24.5%)
Total	22	24	28	53
p value		<0.001		

LSIL = low grade squamous intraepithelial lesion, HSIL = high grade squamous intraepithelial lesion, SI = scoring index

Table 3. Association of p53 immunohistochemical expression with clinicopathological parameters of squamous intraepithelial lesions, and invasive cervical carcinomas

Clinicopathological parameter		Positive p53	Negative p53	p value
Age (years) (mean±SEM)	SIL	40.3±2.22	37.45±2.08	0.335
	Invasive carcinoma	37.7±1.73	42±2.68	0.266
Histopathological type of invasive carcinomas	Adenocarcinoma	12 (100%)	(0%)	
	Adenosquamous	3 (100%)	(0%)	0.046
	Squamous	28 (73.7%)	10 (26.3%)	
Grade of invasive carcinoma	Well-differentiated	8 (88.9%)	1 (11.1%)	
	Moderately-differentiated	23 (82.1%)	5 (17.9%)	0.718
	Poorly-differentiated	12 (75%)	4 (25%)	
Pathological stage of invasive carcinoma	I	10 (76.9%)	3 (23.1%)	
	II	19 (79.2%)	5 (20.8%)	0.686
	III	7 (87.5%)	1 (12.5%)	

SILs: squamous intraepithelial lesion

Table 4. Frequency distribution of chronic cervicitis, low grade and high grade squamous intraepithelial lesion and invasive cervical carcinoma cases according to immunohistochemical expression and scoring index of bcl2.

Frequency of bcl2 expression and SI (0,2-8)	Chronic cervicitis No. (%)	LSIL No. (%)	HSIL No. (%)	Carcinoma No. (%)
Positive	13 (59.1%)	16 (66.7%)	16 (57.1%)	32 (60.4%)
Negative (0)	9 (40.9%)	8 (33.3%)	12 (42.9%)	21 (39.6%)
2	5 (22.7%)	4 (16.7%)	4 (14.3%)	9 (17%)
3	3 (13.6%)	3 (12.5%)	3 (10.7%)	4 (7.5%)
4	2 (9.1%)	3 (12.5%)	2 (7.1%)	5 (9.4%)
5	1 (4.5%)	2 (8.3%)	3 (10.7%)	3 (5.7%)
6	1 (4.5%)	2 (8.3%)	2 (7.1%)	4 (7.5%)
7	0 (0%)	1 (4.2%)	2 (7.1%)	5 (9.4%)
8	1 (4.5%)	1 (4.2%)	0 (0%)	2 (3.8%)
Total	22	24	28	53
p value		0.999		

LSIL = low grade squamous intraepithelial lesion, HSIL = high grade squamous intraepithelial lesion, SI = scoring index

The differences in frequency of distribution of cases with positive and negative expression of p53 of the studied cases and the distribution of cases according to the SI of p53 are highly significant ($p < 0.001$) as shown in table 2.

There was no significant association between age and IHC expression of p53 in studied SILs and carcinomas ($p = 0.335$; $p = 0.226$, respectively). In invasive cervical carcinoma, p53 expression was not statistically different according to grade and FIGO pathological stage, however, the current study revealed a significantly higher IHC expression of p53 in adenocarcinomas and adenosquamous carcinomas cases compared to squamous cell carcinomas ($p = 0.046$) as shown in table 3.

bcl2 immunohistochemical expression

Statistical analysis showed no significant difference in IHC expression and SI of bcl2 and

in different study groups, (p= 0.999) (Fig. 6 and 7 and table 4).

Regarding clinicopathological parameters, the present work revealed non-significant association of bcl2 expression with age of patients in both SILs and invasive carcinomas (p = 0.37) and (p = 0.873), respectively. bcl2 expressions was not statistically different according to histopathological types, grade and FIGO pathological stage of invasive cervical carcinomas (p = 0.696; p = 0.449; P = 0.915, respectively (Table 5).

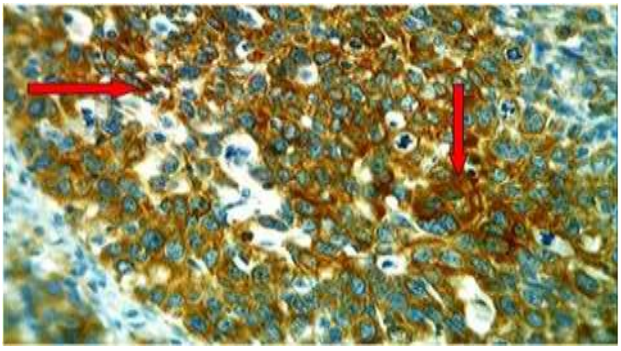

Fig. 6. Moderately differentiated squamous cell carcinoma of the uterine cervix stained IHC with anti-bcl2 monoclonal antibody showing positive brown cytoplasmic staining (arrows) with moderate intensity (2), high percentage (5) and SI of 7, (40X).

CD34 immunohistochemical expression

From statistical point of view there was a highly significant increase in MVD (detected by IHC expression of CD34) with increasing severity of cervical lesion (p < 0.001). The mean MVD in chronic cervicitis, LSIL, HSIL (Fig. 8) and carcinomas (Fig. 9-11) were 3.01±0.32; 9.57±1.59; 16.94±1.13; 55.51±2.15, respectively.

The present series showed no significant correlation between age and MVD (detected by IHC expression of CD34) in both studied SILs and carcinoma cases (r = -0.228, p = 0.103; r = -0.061, p = 0.665, respectively).

Adenosquamous carcinomas and adeno-carcinomas (Fig. 10) revealed significantly higher MVD compared to squamous cell carcinomas (Fig. 9) (p = 0.01). Regarding grade of carcinoma, there was a significant increment in MVD with decreasing the degree of differentiation (p < 0.001) as shown in fig. 9-11. MVD was increasing significantly according to FIGO pathological stage of carcinomas (p = 0.012) as seen in table 6.

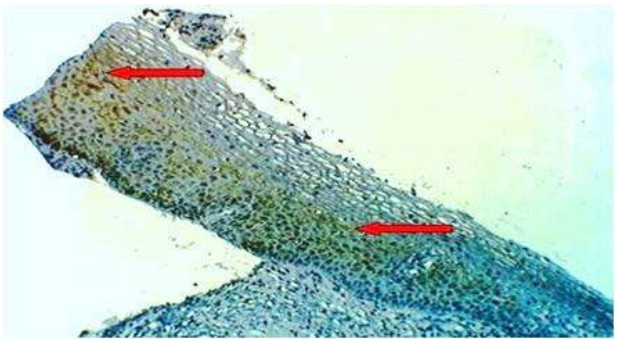

Fig. 7. Low grade squamous intraepithelial lesion of the uterine cervix stained IHC with anti-bcl2 monoclonal antibody showing positive brown cytoplasmic staining (arrows) with weak intensity (1), moderate percentage (3) and SI of 4, (4X).

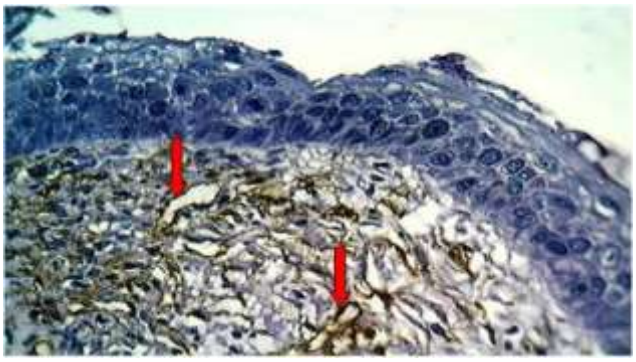

Fig. 8. Low grade squamous intraepithelial lesion of the uterine cervix stained IHC with anti-CD34 monoclonal antibody showing positive brown cytoplasmic staining of endothelial cells of microvessels (arrows) with moderate increase in microvessl density in the connective tissue underneath basement membrane of the dysplastic epithelium (40X).

Discussion

The present study shows lack of p53 IHC expression in all studied chronic cervicitis cases, with significant increase in its expression from LSIL through HSIL to cervical carcinomas. This result is comparable to the literatures which stated that the p53 was not expressed in non-neoplastic cervical lesions with increase in

its expression with increasing severity of the lesion from LSIL through HSIL to carcinoma [10,11,14,15]. In the current work, actually it could not be confirmed which type of p53 protein (mutant or wild) since the antibody used in the study can detect both types.

Taking the age in consideration, this study revealed non-significant association between age and IHC expression of p53 in SILs and carcinomas .This result agrees with that obtained by other Iraqi study done by Baythoon *et al* [16] and with a study done by Koyamatsu *et al* [17].

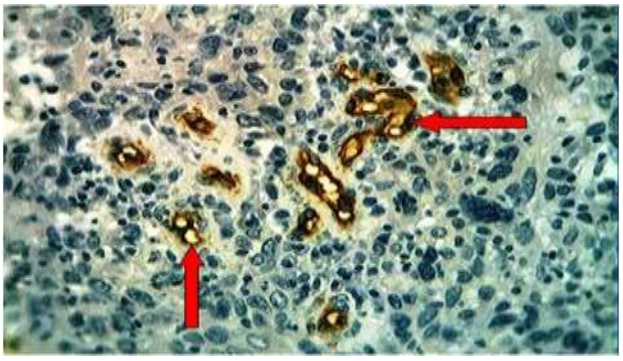

Fig. 9. Well differentiated squamous cell carcinoma of the uterine cervix stained IHC with anti-CD34 monoclonal antibody showing positive brown cytoplasmic staining of endothelial cells of microvessels (arrows) with moderate increase in the intratumoralmicrovessl density (MVD) (40 X).

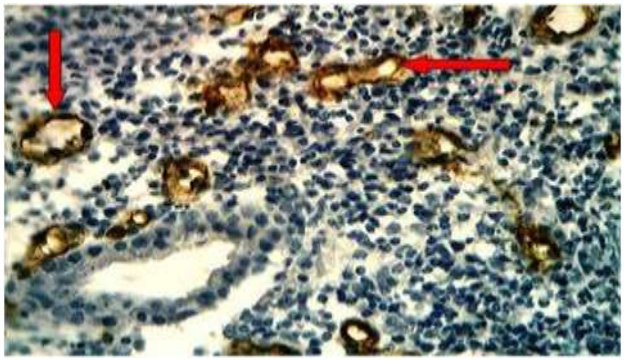

Fig. 10. Moderately differentiated adenocarcinoma of the uterine cervix stained IHC with anti-CD34 monoclonal antibody showing positive brown cytoplasmic staining of endothelial cells of microvessels (arrows) with high intratumoralmicrovessl density (MVD) (40 X).

Concerning the histopathological types of cervical carcinoma, this study shows a statistically significant higher IHC expression of p53 in adenocarcinomas and adenosquamous carcinomas compared to that of squamous cell carcinomas. This result is in accordance with other Iraqi study done by Baythoon *et al* [16] and with other studies done by Cheah and Looi [18] and Abdelall *et al* [11].

Fig. 11. Poorly differentiated squamous cell carcinoma of the uterine cervix stained IHC with anti-CD34 monoclonal antibody showing positive brown cytoplasmic staining of endothelial cells of microvessels with marked increase in the intratumoralmicrovessl density (MVD) (arrows) (10 X).

The higher IHC expression of p53 in adenocarcinoma compared to that of squamous cell carcinoma may be due to higher frequency of mutations in adenocarcinoma [18]. Most mutations induce conformational changes causing over-expression of p53 protein, stabilizing it and rendering it detectable by IHC analysis [19]. It has been suggested that p53 over-expression represents an adverse prognostic factor [20]. Since p53 expression in adenocarcinoma is significantly higher than that of squamous cell carcinoma of the cervix, this would contribute to the less favorable prognosis of the former than the latter [21].

Regarding the histopathological grade of cervical carcinomas, the current study shows non-significant difference in p53 IHC expression among different grades of studied cervical carcinoma. This result is in agreement with other Iraqi study done by Baythoon *et al*

[16] and with other studies worldwide [22-24]. The present study also showed that IHC expression of p53 was not significantly associated with pathological stage of invasive cervical carcinoma. This result is comparable to that obtained by other studies [3,14,22,24].

The current study recorded non-significant difference in IHC expression of bcl2 in studied groups. This result is in agreement with other studies [10,22,25-28]. In a study done by Grace *et al* [15], a significant increase in IHC expression of bcl2 with increasing severity of cervical lesions from mild dysplasia to carcinoma had been observed. This difference is possibly due to different sample size, sensitivity and specificity of different antibodies used, and different modes of scoring systems and interpretations of the results.

This research reported non-significant association between age and IHC expression of bcl2 in both SILs and carcinoma cases. This result agrees with study done by Wootipoom *et al* [24].

Taking the histopathological type of cervical carcinoma in consideration, the current work found non-significant relation between histopathological types of cervical carcinoma and bcl2 immunoreactivity. This finding goes with that obtained by Tjalma *et al* [29].

Regarding cervical carcinoma cases, the present study showed non- significant association of grade and pathological stage to the IHC expression of bcl2. This result is supported by other studies [22,24].

MVD is a commonly used as a measure of angiogenesis. Measuring MVD requires labeling the vessels to be counted using antibodies against any of the antigens naturally expressed by endothelial cells like Factor-VIII, CD31, CD34 and CD105 [31]. In the present study, CD34 was selected based on its superior sensitivity, with detection of a greater number of microvessels in cervical tumors compared to other antibodies [32]. MVD is considered a significant prognostic factor that correlates with increased metastasis and worse prognosis in many tumor types [33].

Table 5. Association of bcl2 immunohistochemical expression with clinicopathological parameters of squamous intraepithelial lesions, and invasive cervical carcinomas

Clinicopathological parameter		Positive bcl2	Negative bcl2	p value
Age (years) (mean±SEM)	**SIL**	37.62±1.78	40.45±2.74	0.37
	Invasive carcinoma	38.31±2.05	38.81±2.21	0.873
Histopathological type of invasive carcinomas	**Adenocarcinoma**	7 (58.3%)	5 (41.7%)	
	Adenosquamous	1 (33.3%)	2 (66.7%)	0.696
	Squamous	24 (63.2%)	14 (36.8%)	
Grade of invasive carcinoma	**Well-differentiated**	7 (77.8%)	2 (22.2%)	
	Moderately-differentiated	15 (53.6%)	13 (46.4%)	0.449
	Poorly-differentiated	10 (62.5%)	6 (37.5%)	
Pathological stage of invasive carcinoma	**I**	9 (69.2%)	4 (30.8%)	
	II	14 (58.7%)	10 (41.3%)	0.915
	III	5 (62.5%)	3 (37.5%)	

SILs = squamous intraepithelial lesion

Table 6. Association of MVD (detected by CD34 immunohistochemical expression) with histopathological type, grade and pathological stage of invasive cervical carcinomas

Parameter		MVD Mean ± SEM	p value
Histopathological type	Adenocarcinoma	65.09 ± 3.7	0.01
	Adenosquamous	67.57 ± 7.8	
	Squamous	51.53 ± 2.44	
Grade	Well-differentiated	37.69 ± 3.54	< 0.001
	Moderately-differentiated	52.7 ± 2.22	
	Poorly-differentiated	70.44 ± 2.43	
Pathological stage	I	46.47 ± 4.05	0.012
	II	59.74 ± 3.11	
	III	64.19 ± 3.82	

MVD = microvessel density, SILs = squamous intraepithelial lesion

This study recorded a significant increase in MVD (detected by IHC expression of CD34) with increasing severity of cervical lesion from chronic cervicitis through LSIL and HSIL to carcinomas. This result is concordance with other studies, which also used CD34 for detecting MVD [34-36].

Stepan *et al* [37] also noticed a significant increase in MVD (detected by IHC expression of CD105) with increasing severity of cervical lesion from normal cervix through LSIL and HSIL to carcinoma. In a study done by Dellas *et al* [31], in which CD31 was used for detecting MVD, a significant increased in MVD from benign cervical lesions through preinvasive lesions to invasive cervical carcinomas had been identified.

The loss, or inactivation, of wild type p53 has been reported to indirectly promote tumor angiogenesis by up-regulation of angiogenesis promoting protein, VEGF and down-regulation of a potent angiogenesis inhibitor, thrombospondin-1 (TSP-1), providing rationale for the contribution of angiogenesis to cervical cancer early in carcinogenesis [34,38].

The present series found non-significant correlation between age and MVD (detected by IHC expression of CD34) in studied SILs and carcinoma cases. This finding is in accordance with other studies [39,40].

Concerning cervical carcinoma types, the current study showed a significant higher MVD in adenosquamous carcinomas and adenocarcinomas compared to squamous cell carcinomas. This result is in agreement with other studies [39,41]. In a study done by Vieira *et al* [42], a higher MVD had been identified in squamous cell carcinoma. This difference may be attributable to different sample size, different percentage of each type of carcinoma and different methods used to assess MVD.

The current work showed a significant higher MVD in poorly differentiated carcinomas and lowest value was detected in well differentiated carcinomas. This result agrees with other studies [37,39,41].

Regarding pathological FIGO stage of cervical carcinomas, the present research showed a significant higher MVD with increasing pathological FIGO stage of cervical carcinoma cases. This result is similar to that found by other study done by Landt *et al* [43].

The majority of studies trying to find a correlation between angiogenesis and outcome in cervical cancer report conflicting results, but most have concluded that more extensive tumor angiogenesis is associated with higher rates of tumor recurrence following treatment and poorer survival in cervical cancer [40, 44]. In SILs,

many studies demonstrate a significant increased in MVD with increasing severity of the lesions [31, 34- 36].

In conclusion, P53 plays an important role in the progression of the severity of intraepithelial cervical lesions. Thus, testing this marker in dysplastic cervical lesions might improve the accuracy, precision and sensitivity of cervical lesions diagnosis. bcl2 plays no role in cervical neoplastic transformation and has no utility to differentiate premalignant from malignant lesions of the uterine cervix. MVD is sequentially increased from LSIL through HSIL, and then into invasive carcinoma and can be utilized as ancillary marker for the risk of malignant transformation of cervical intraepithelial lesion. Intratumoral quantification of MVD in cervical carcinoma reflects the grade and pathological stage of the tumor so that it may be a useful additional prognostic factor.

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Author contributions

All authors contributed to this manuscript. They coordinated study subject recruitment, implementation and progress of this study, and helped with data interpretation and manuscript organization and editing.

Conflict of Interest

The authors have no conflicts of interest

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