# Study of Epstein - Barr virus Infection in Relation to the Immunohistochemical Expression of Bcl-2 gene in Tissues of Patients with Adenocarcinoma of the Colon

**Sarmad M. Zeiny[1], Saad Hasan Mohammed Ali[2], Jabbar R. Zangoor[2], Shakir H .Mohammed Al-Alwany[3]**

*[1]Assist. [2]Prof. Sarmad M. Zeiny, College of Medicine / Microbiology department / University of Baghdad, [3]Assist. Prof., College of Science / University of Babylon*

## Abstract

**Background:** EBV infection in tissue micro-environment is challenged by the precisely regulated survival and apoptosis mechanisms. Abnormal bcl-2 proto-oncogene expression in colonic carcinomas allows accumulation and propagation of these genetically altered cells.

**Objective:** To analyze the relevant concordance of BCL-2 gene , EBNA1 s and LMP-1-EBV expression in issues from a group of Iraqi patients with colonic adenocarcinomas.

**Patients and Methods**: One hundred (100) tissue biopsies, belonged to (40) patients with colorectal cancers, (40) patients with benign colon tumors, and (20) apparently normal colorectal control tissues, were enrolled in this study. The detection of EBNA1 s and LMP-1-EBV as well as BCL-2 was done by immunohistochemistry (IHC).

**Results:** The positive-IHC results of EBNA1 detection in 40 tissues with malignant colonic tumors were 57.5%, while in 40 colonic benign tumors tissues were 32.5 %. In those apparently healthy colonic control tissues, EBNA1 detection was 20%. The positive results of LMP-1 –EBV -IHC detection where 45 % from colonic carcinoma group, while the percentage of benign colonic tumors was 30%(12 out of 40). Lastly, in AHC was15% showed positive signals. Malignant colonic tumors showed 47.5% positive results of Bcl-2-IHC detection while benign colonic tumor tissues showed 40% positive bcl-2-IHC reactions and in the colonic control tissues was 20%.

**Conclusion**: It concluded from the present high rates of EBV infection in colonic adenocarcinoma along with the concordance bcl-2 expression that they could played an important role in the development and progression of both malignant and benign colonic tumors in our group of Iraqi patients.

***Keywords:*** *malignant and benign colonic tumors, Colonic carcinogenesis; EBNA1; LMP-1-EBV; Bcl-2; Immunohistochemistry.*

## Introduction

Colonic cancers are one of the major cancer problems worldwide that ranking the third most common cancers and third leading cause of cancer-related deaths in both men and women. Worldwide, the annual newly diagnosed cases are approximately 1.36 million, constituting about 9.7% of all cancer cases. WHO stated that approximately 55% of colonic cancers were reported in more developed regions [(1)].

Previous studies found that environmental conditions, lifestyles, sequential genetic changes and viral agents are the major risk factors for colorectal cancers [(2)]. Among others, microbial-epithelial interactions have proposed as a potential oncogenic trigger for the development of colonic cancers [(3)].

(In contrast to gastric carcinoma and MALT-type lymphoma where both were strongly associated with *Helicobacter Pylori* infection , a direct causal

link between various microbial infections (as, *Helicobacter pylori, Streptococcus bovis, Escherichia Coli, Bacteroides*, JC virus, cytomegalovirus, human papillomavirus, Epstein-Barr virus (EBV)) and CRC has not been established yet [4].

Previous data regarding the role of EBV expression in colorectal cancer are sparse as well as contradictory and without a clear evidence of the active role of EBV in colorectal carcinogenesis (4). In addition, the EBV detection assays vary significantly across these studies, having a significant impact on the obtained results [4,5].

The expression patterns of the 6 EBV nuclear antigens (EBNA1, -2, -3A, -3B, -3C and -LP), 3 latent membrane proteins (LMP1, -2A, and -2B), and multiple non-coding RNAs (EBERs and miRNAs) define the distinct latency programs linked to the types of cancers associated with EBV infection

Earlier studies revealed that LMP1-mediated upregulation of Fascin was depending on the NF-κB, where NF-κB and Fascin have both contributed to an invasive migration of LMP1-expressing lymphocytes [6,7]. It is evident, and based on these studies, that EBV infection and Fascin expression have a strong correlation with malignancy progression in several human carcinomas especially moderately- and poorly-differentiated grades [7].

The deregulation of Bcl-2 family proteins can cause various pathological consequences including the development of cancer. The anti-apoptotic protein Bcl-2 (B-cell lymphoma-2) is an important member in that family that controls the release of pro-apoptotic factors which are responsible for the activation of caspases (by stabilizing the mitochondrial outer membrane) [8,9]. An aberrant expression of Bcl-2 has been implicated in several cancers, including CRC[5]. However, the data obtained from different researches were often in disagreement [9].

Neither the function nor the prognostic value of Bcl-2 expression in patients with CRC is clear. This was evidenced from the contradictories in data from many studies that have found expression of Bcl-2 to correlate with better prognosis [9,10], while many other investigators demonstrated that Bcl-2 was a poor prognostic marker for cancer patients [11] or Bcl-2 expression in CRC has no prognostic significance [12].

Under physiological conditions, the pro-apoptotic members (like Bax and Bak) are sequestered and inhibited by anti-apoptotic relatives (like Bcl-xL, Bcl-2 or Mcl-1). It was found that anti-apoptotic proteins are overexpressed in different cancers, supporting the avoidance of cell death. In CRC, high expression of these anti-apoptotic members has been shown to correlate with lower tumor differentiation [13].

## Materials and Methods

This research was designed as a retrospective one. The study groups collectively comprised of (100) colonic tissue which have processed as formalin-fixed and paraffin embedded blocks and retrieved from the archives of the period from 2014 till 2020 from many hospitals and private histopathological laboratories in Baghdad, Babylon, Kerbela, and Al-Najaf provinces and enrolled in this study as both patients and control samples from patients whom their age ranged from 17 to 75 years.

These blocks included 40 biopsies from patients with colonic cancers, 40 biopsies from patients with benign colonic tumors and 20 biopsies from patients with apparently normal colonic tissues and used as an age- and sex- matched control group.

Trimming process and tissue sectioning of the tissue blocks were conducted in the histopathological department of Teaching laboratories at the Baghdad Medical City Complex where one paraffin embedded (4 mm) thick-tissue section was stained with hematoxyline and eosin, while other tissue sections were stuck on positive- charge slides for EBV-EBNA 1 and LMP 1 antigens as well as Bcl-2 detection by using Mouse and Rabbit Specific HRP/DAB immunohistochemistry detection kit (purchased from Abcam, UK). The details of techniques for performing IHC reaction as well as these antibodies conducted according the instructions of that manufacturing company and done in the Research Laboratories of the Clinical Communicable Diseases Research Unit, at College of Medicine, University of Baghdad as well as in the Advanced Microbiology Research Laboratory at College of Science, University of Babylon.

The specification of this IHC kit after proper use of the detection system gives an intense brown signals at the specific sites of the expressed proteins in tissues with a positive test. These signals evaluated under light microscopy using × 100 lens for counting the positive cells, where given intensity and percentage scores based on the counted in 10 different fields of 100 cells in each sample where their averaged percentage within 10 fields was determined and given a scale of 0-3 for relative intensity where 0 corresponding to no detectable IHC reaction, and 1, 2, 3 equivalents to low, moderate, and high intensity, respectively. Reactions assigned to one of the following percentage scores where 1%–25% = score 1, 26%–50% = score 2 and r > 50% = score 3.

## Statistical Analysis

T test, ANOVA test, and Chi square applied by using the SPSS program (version-22) and Excel application.

## Results

### I. Study Group Characteristics

The archival specimens collected in this study were related to patients with colonic tumor whom ages were ranged from seven years to eighty five years. The mean age of patients in colonic carcinoma (CC) (56.7 years) was higher than the mean age of the benign colonic tumors (BCT) (52. 3 years), whereas the mean age of apparently healthy control(AHC) was (49.8 years). The distribution of gender frequency in this study where 52.5%, 55% & 60% were males in CC, BCT & AHC, respectively; while, 47.5% ,45%& 40% were females in CC, BCT & AHC, respectively. This study has revealed that well differentiated carcinomas were seen in 47.5% of CC group, while 37.5% and 15% of CC have moderately and poorly differentiated grade carcinomas, respectively. Anatomically, 6 cases of colonic carcinoma were in the cecum (15%), 11 cases in the transverse colon (27.5%), 2cases in the ascending colon (5%), 2 cases in the descending colon (5%), 11 cases in the sigmoid (27.5%), and 10 cases in the rectum (25%) (Table 1).

**Table 1. The Study Group Characteristics**

| | | **Malignant colonic tissues (CC), N= 40 (%)** | **Benign colonic tissues(BCT), N = 40(%)** | **Healthy colonic tissues (AHC) N=20(%)** |
|---|---|---|---|---|
| Age | Mean of Age (Years) | 56.7 | 52.3 | 49.8 |
| | Range of Age | 17 – 85 | 24 -74 | 38 – 75 |
| Gender | Male | 21 (52.5) | 22 (55) | 12 (60) |
| | Female | 19 (47.5) | 18 (45) | 8 (40) |
| Grade | Poorly | 6 (15 ) | | |
| | Moderately | 14 (37.5) | | |
| | Well | 18(47.5) | | |

***Cont...*** **Table 1. The Study Group Characteristics**

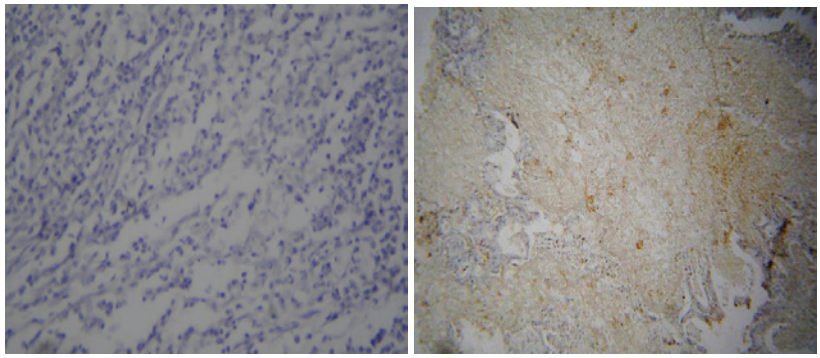
| | | | | |
|---|---|---|---|---|
| Site of tumors | Cecum | 6 (15) | | |
| | Transverse colon | 11 (27.5) | | |
| | Ascending colon | 2 (5) | | |
| | Descending colon | 2 (5) | | |
| | Sigmoid | 11 (27,5) | | |
| | Rectum | 10 (25) | | |

**II. EBV-EBNA1 - Associated Colonic Tumors:**

Twenty- three out of forty (57.5%) tissues with **colonic** carcinoma showed positive immunohistochemical (IHC) reactions for EBV-EBNA1 in this study. The highly signal scores were found in moderate grade score (Score II) (25%; 10/40). While the highly intensity signals were found in weak (Intensity I) 22.5% (9/40) (Table 2 and Figure 1). The benign **colonic** tumors group revealed 32.5% positive signals which represented 13 out of 40 tissues in this group. The highly signal scores was found in low grade score (Score I) (17.5% ; 7/40).While the highly intensity signals was found in moderate (Intensity II) 17.5% (7/40) Lastly, 20% (4 out of 20 cases )of **colonic** control tissues group presented positive signals for EBV-EBNA1 –IHC test. However, in comparison to the percentage of EBNA1 –IHC in healthy **colonic** control group as well as in the group of benign **colonic** tumors, the differences between the percentages of EBNA1 –IHC in tissues of patients with **colonic** carcinoma and each of these groups are statistically significant (P value = < 0.004).

**Table 2: The percentage of EBNA1 –IHC score and intensity signaling in colonic tumors**

| **EBNA1 –IHC** | | **Colonic carcinoma (n=40)** | | **Benign colonic tumors (n=40)** | | **A.H control (n=20)** | | **P-value ($\chi^2$test)** |
|---|---|---|---|---|---|---|---|---|
| | | **No.** | % | **No.** | % | **No.** | % | |
| Negative | | 17 | 42.5 | 27 | 67.5 | 16 | 80 | 0.004 |
| Positive | | 23 | 57.5 | 13 | 32.5 | 4 | 20 | |
| Signal Score | I | 6 | 15 | 7 | 17.5 | 2 | 10 | 0.007 |
| | II | 10 | 25 | 4 | 10 | 1 | 5 | |
| | III | 7 | 17.5 | 2 | 5 | 1 | 5 | |
| Signal Intensity | Weak | 9 | 22.5 | 5 | 12.5 | 3 | 15 | 0.009 |
| | Moderate | 8 | 20 | 7 | 17.5 | 1 | 5 | |
| | Strong | 6 | 15 | 1 | 2.5 | 0 | 0.00 | |



**A** **B**

**Figure (1): Colonic Carcinoma Showing The Results of Immunohistochemistry Staining Protein Over Expression Using Biotinylated Anti –EBNA1 Protein Antibody; Stained By DAB-Chromogen (Brown) and Counter Stained By Mayer's Hematoxylin (Blue).A- Colonic cancers with negative EBNA1-IHC reactions (40X).**

**B- Positive EBNA1-IHC reaction with low score and high signal intensity (40X).**

III. The Results of LMP-1 –EBV -IHC Signal score and intensity signaling in colonic tumors.

Table (3) shows the positive results of LMP-1 –EBV -IHC detection where 45 % (18 out of 40 tissues) from colonic carcinoma group showed positive signals including 27.5 % (11 out of 40 tissues) in the low score (score I), while the percentage of benign colonic tumors was 30%(12 out of 40 ) showed positive signals including 12.5% (5 out of 40 tissues) in the moderate score (score II) .Lastly, in AHC was15% (3 out of 20 cases) showed positive signals including 10 % (2 out of 20 tissues) in the low score (score I) ( Fig. 2).

The percentage of LMP-1 –EBV expression in cells that were evaluated for the intensity of LMP-1 –EBV -IHC reactions showed 30 % (12 out of 40 tissues) in the weak intensity(I), while the percentage of benign colonic tumors was (30%) showed positive signals intensity including 17.5 % (7 out of 40 tissues) in the moderate score (score II). Lastly, in AHC was15% (3 out of 20 cases) showed positive signals including 10 % (2 out of 20 tissues) in the weak score (score I) Fig. (2). Statistically, significant differences were noticed between scoring and intensities of tissues at ($P<0.05$) in colonic tumors group.

**Table 3: The percentage of LMP-1 –EBV-IHC score and intensity signaling in Colorectal Tumors**

| LMP-1 –EBV -IHC | | Colonic carcinoma (n=40) | | Benign colonic tumors (n=40) | | A.H control (n=20) | | P-value ($\chi$2test) |
|---|---|---|---|---|---|---|---|---|
| | | No. | % | No. | % | No. | % | |
| Negative | | 22 | 55 | 28 | 70 | 17 | 85 | 0.007 |
| Positive | | 18 | 45 | 12 | 30 | 3 | 15 | |
| Signal Score | I | 11 | 27.5 | 4 | 10 | 2 | 10 | 0.04 |
| | II | 5 | 12.5 | 5 | 12.5 | 1 | 5 | |
| | III | 2 | 5 | 3 | 7.5 | 0 | 0.00 | |
| Signal Intensity | Weak | 12 | 30 | 4 | 10 | 2 | 10 | 0.03 |
| | Moderate | 5 | 12.5 | 7 | 17.5 | 1 | 5 | |
| | Strong | 1 | 2.5 | 1 | 2.5 | 0 | 0.00 | |

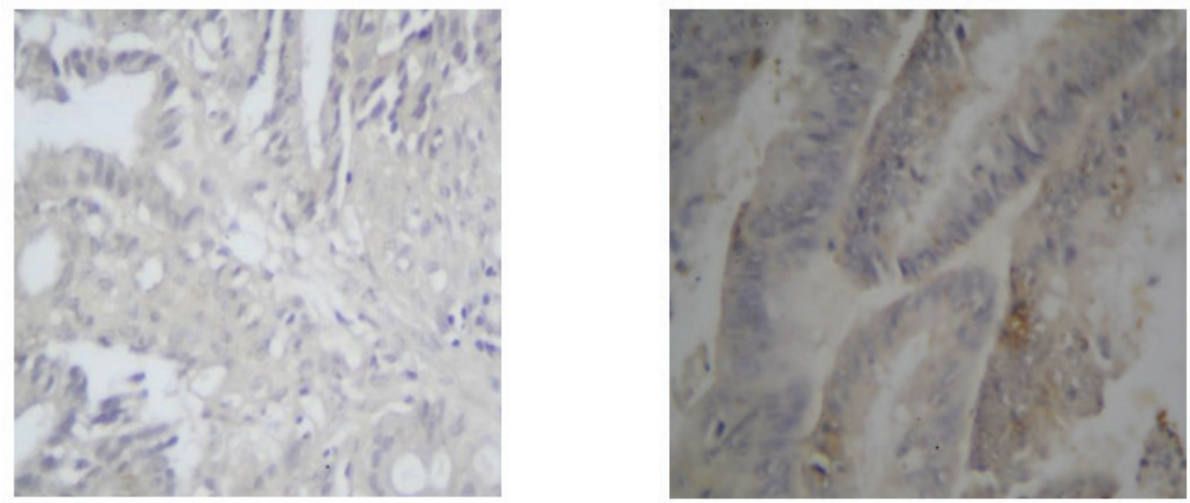



**Figure (2) : Infiltrative Colonic Carcinoma Showing Results of Immunohistochemistry Staining Protein Over Expression Using Biotinylated Anti – LMP-1 –EBV Protein Antibody; Stained By DAB-Chromogen (Brown) and Counter Stained By Mayer's Heamatoxylin (Blue).**
**A- Colonic Cancer with negative staining for LMP-1 –EBV (40x). .**
**B- LMP-1 –EBV -IHC-reaction with high signal score and strong signal intensity (40x).**

**IV. The Results of BCL2 -IHC Signal score and intensity signaling in colonic tumors.**

Table (4) shows the positive results of bcl-2 -IHC detection where 47.5 % (19 out of 40 tissues) from colonic carcinoma group showed positive signals including 20 % (8 out of 40 tissues) in the low score (score I), while the percentage of benign colonic tumor was 40%(16 out of 40) showed positive signals including 17.5% (7 out of 40 tissues) in the moderate score (score II). Lastly, in AHC was 20% (4 out of 20 cases) showed positive signals including 10 % (2 out of 20 tissues) in the low score (score I) ( Fig. 2).

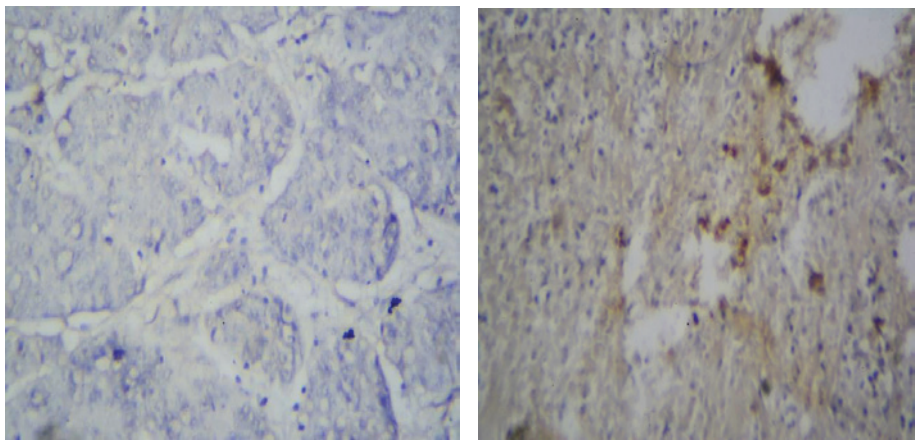
The percentage of bcl-2 expression in cells that were evaluated for the intensity of bcl-2 -IHC reactions showed 25 % (10 out of 40 tissues) in the weak intensity(I), while the percentage of benign colonic tumors was (40%) showed positive signals intensity including 20 % (8 out of 40 tissues) in the moderate score (score II). Lastly, in AHC was 20% (4 out of 20 cases) showed positive signals including 10 % (2 out of 20 tissues) in the weak score (score I) (Figure 2). Statistically, significant differences were noticed between scoring & intensities of tissues at ($P<0.05$) in colonic tumors group.

**Table 4: The percentage of BCL-2-IHC score and intensity signaling in colonic tumors**

| BCL-2 -IHC | Colonic carcinoma (n=40) | | Benign colonic tumors (n=40) | | A.H control (n=20) | | P-value ($\chi$2test) |
|---|---|---|---|---|---|---|---|
| | No. | % | No. | % | No. | % | |
| Negative | 21 | 52.5 | 24 | 60 | 16 | 80 | 0.008 |
| Positive | 19 | 47.5 | 16 | 40 | 4 | 20 | |

***Cont...*** **Table 4: The percentage of BCL-2-IHC score and intensity signaling in colonic tumors**

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Signal Score | I | 8 | 20 | 6 | 15 | 2 | 10 | 0.009 |
| | II | 7 | 17.5 | 7 | 17.5 | 1 | 5 | |
| | III | 4 | 10 | 3 | 7.5 | 1 | 5 | |
| Signal Intensity | Weak | 10 | 25 | 3 | 7.5 | 2 | 10 | 0.007 |
| | Moderate | 6 | 15 | 8 | 20 | 1 | 5 | |
| | Strong | 3 | 7.5 | 5 | 12.5 | 1 | 5 | |



**A** **B**

**Figure 2: Infiltrative Colonic Carcinoma Showing The Results of Immunohistochemistry Staining Protein Over Expression Using Biotinylated Anti – bcl-2 Protein Antibody; Stained By DAB-Chromogen (Brown) and Counter Stained By Mayer's Hematoxylin (Blue).**
**A- Colonic Cancer with negative staining for bcl-2 (40x).**
**B- bcl2 -IHC-reaction with high signal score and strong signal intensity (40x).**

**V. Spearman's Rho Statistical Testing of Age, Grade, EBNA1 –EBV; LMP1-EBV and BCL-2- IHC to Evaluate the Studied Markers in Colonic Tissues.**

A strong positive relationship (with highly significant correlation) was found between **EBNA1 –EBV; LMP1-EBVand BCL-2-** markers in **colonic** tissues (r = 0.648, P = 0.008) and (r = 0.564, P = 0.006), respectively. Similarly, there is a strong positive relationship (with highly significant correlation) between **BCL2** and grade of **colonic** tissues (r = 0. 683; p= 0.009). However, there are no significant correlations among EBV and other markers (as illustrated in table 5).

**Table 5: Spearman's Rho Statistical Testing of Age, Grade, EBNA1 EBV; LMP1--EBV and *BCL-2* -IHC to Evaluate the Studied Markers in Colonic Tissues.**

| Spearman's rho | | Age groups (years) | Grade | EBV-EBNA1 | EBV-LMP-1 |
|---|---|---|---|---|---|
| Grade | r | -0.149 | | | |
| | P | 0.377 | | | |
| EBV-EBNA-1 | r | 0.070 | 0.123 | | |
| | P | 0.862 | 0.512 | | |
| EBV-LMP-1 | r | 0.080 | 0.153 | | |
| | P | 0.794 | 0.449 | | |
| BCL-2 | r | 0.165 | 0.683 | 0.648 | 0.564 |
| | P | 0.371 | 0.009* | 0.008* | 0.006* |

*Correlation is highly significant (P<0.01).

## Discussion

CRC, like other human carcinomas, is a complex multistep process that involves early steps of tumor cell invasion within the microenvironment and then an eventual entering the bloodstream. Consequently, specific genetic and epigenetic changes are required to fulfill these metastasis steps [14]. Several studies had reported that the Fascin gene (produces an actin-binding protein that is upregulated in cancer cells specially during epithelial to mesenchymal transition) plays an important role in the progression of human carcinomas, including CRC and has correlated with poor prognosis in human CRC [6, 15,16].

In the present study, twenty- three out of forty (57.5%)and 45 % (18 out of 40 tissues) tissues with colonic carcinoma showed positive immunohistochemical (IHC) reactions for EBV-EBNA1and EBV-LMP-1, respectively.

Epstein-Barr virus by alternating its envelop proteins has exhibited a dual tropism to both B and epithelial cells. As such, this viral infection commonly associated with Burkitt and Hodgkin lymphoma (as B-cell lymphomas) as well as nasopharyngeal, gastric and probably rectal carcinomas (as an epithelial malignancy [17]). An activation of clearly blown colorectal cancers by Epstein-Barr virus can result by the viral wide- ranged effects that result in cell damage by various processes such as metabolism, cell cycle regulation, apoptosis, protein synthesis, angiogenesis and cellular connections [18].

Boguszakova and associates study on patients with colonic adenocarcinoma and adenoma failed to show EBV DNA in the investigated biopsies [19]. Similar no positive findings were reported by Yuen et al. and Cho et al. who tested the presence of EBV in colorectal carcinomas from Chinese patients using ISH for EBERs [20, 21].

In Karpinsky et al. study on 186 colorectal cancer specimens by PCR, 19% positive samples for EBV-DNA found. (16). In addition, Samaha et al. and Kon et al. (10, 12) concluded that rectal lymphoepithelioma-like carcinoma probably related to EBV. However, Yanai and co-workers by using ISH for EBER-1 found that

EBV infection may be related to 60% of IBD diseases such as Crohn's disease and ulcerative colitis cases [22].

During EBV latent infection enhances the oncogenic properties via compromising immune system and enhancing chronic inflammatory microenvironment. This virus encodes 6 viral nuclear antigens (EBNA-1, -2, -3A, -3B, -3C and LP), 3 viral latent membrane proteins (LMP-1, -2A and -2B), 2 small viral non-coding Ribonucleic acids (EBER-1 and -2) and rightward transcripts of BamHI-A, where the main function of these proteins is to evade immune surveillance and to play as "oncogenes" in EBV - infected cells, by silencing the anti-EBV effect of INF-γ and modulating anti-viral cytokines (TNF-α, IL-1β and IL-6 ) and mimicking the IL-10 characteristics [23, 24].

Bcl-2 is well documented as a key inhibitor of apoptosis that play a major role to maintain normal balance between both apoptosis and cellular survival. The defects in the apoptotic pathway in mitochondria are tightly related to carcinogenesis. In addition, abnormal activation of Bcl-2 has been implicated for the CRC evolution, yet, and to date, the exact role of Bcl-2 expression as well as its prognostic value in CRC patients has not been consistently established [25].

Positive results of bcl-2 -IHC detection were detected in 47.5 % of tissues from colonic carcinoma group, including 20 % with low score (score I), while the percentage of bcl-2 -IHC detection in benign colonic tumor was 40% including 17.5% with moderate score (score II) and in AHC was 20% including 10 % with low score (score I).

One eligible meta-analysis that investigated the prognostic value of Bcl-2 in CRC found that high expression of Bcl-2 has significantly increased survival of CRC patients and this is contradictory to the anti-apoptotic function of Bcl-2, and might be related to the interactions of other proteins enrolled in the apoptotic pathways such as p53(9). Highly expressed Bcl-2 was more frequently correlated to highly differentiated and A/B Ducks' staged in tumors. As such, down-regulation of bcl-2 has been associated with higher risk of malignant transformation of colorectal adenoma, and was proved that lacking of Bcl-2 expression correlated with invasion, metastasis and recurrence of CRC [26].

Many previous studies have tested the prognostic significance of the counteracting expression levels of Bcl-2 and Bax with clinicopathological parameters of colorectal cancers (27, 28), and found that Bax/Bcl- 2 ratio determines the cell susceptibility to apoptosis, where lower ratio lead to resistance of cancer cells to apoptosis result in poor prognosis and more infiltrative growth and tumor progression and aggressiveness [29].

Other study [30], and regarding the location of primary tumors from where resected, have demonstrated a critical role in determining tumor characteristics and prognosis of the cancer.

The finding of [31] study indicated that abnormal bcl-2 gene activation is an early event in neoplastic development or progression of colorectal carcinoma, since higher levels of bcl-2 expression in dysplastic and malignant than non-neoplastic cells were found.

However, several other studies [32,33] reported higher rates of bcl-2 expression in adenoma than carcinoma, indicating an important role of bcl-2 to play in the early stage of the adenoma-carcinoma sequence and being followed by other genetic changes, such as p53 accumulation.

**Ethical Clearance:** The study was ethically approved from the College of Medicine / University of Baghdad, College of Medicine / University of Babylon and Ministry of Health in Iraq. The patients were asked for their permission to give samples and information.

**Source of Funding**: Self-funded.

**Conflict of Interest:** Nil

## References

1. Prashanth Rawla, Tagore Sunkara, Adam Barsouk. Epidemiology of colorectal cancer: incidence, mortality, survival, and risk factors. Gastroenterology Rev 2019; 14 (2): 89–103.
2. Shivan J. Mehta ; Vikranth Induru,; David Santos,; Catherine Reitz,; Timothy McAuliffe,; Charles Orellana,; Kevin G. Volpp, ,;David A. Asch,; Chyke A. Doubeni, . Effect of Sequential or Active Choice for Colorectal Cancer Screening Outreach A Randomized Clinical Trial. JAMA Network Open. 2019;2(8):e1910305.
3. Collins D, Hogan AM, Winter DC. Microbial and

viral pathogens in colorectal cancer. Lancet Oncol. 2011;12(5):504–512.

4. Shahinaz Bedria, Ali A. Sultanb, Moussa Alkhalafc, Ala-Eddin Al Moustafad,e,f, and Semir Vranic. Epstein-Barr virus (EBV) status in colorectal cancer: a mini review. HUMAN VACCINES & IMMUNOTHERAPEUTICS 2019, VOL. 15, NO. 3, 603–610.
5. de Lima MAP, Neto PJN, Lima LPM, Goncalves Junior J, Teixeira Junior AG, Teodoro IPP, Facundo HT, da Silva CG, Lima MV. Association between Epstein-Barr virus (EBV) and cervical carcinoma: a meta-analysis.Gynecol Oncol. 2018;148(2):317–328.
6. Noor Al-Antarya, Hanan Farghalyb, Tahar Aboulkassimc, Amber Yasmeenc, Nizar Akil d,e, and Ala-Eddin Al Moustafa. Epstein–Barr virus and its association with Fascin expression in colorectal cancers in the Syrian population: A tissue microarray study. HUMAN VACCINES & IMMUNOTHERAPEUTICS 2017, VOL. 13, NO. 7, 1573–1578.
7. Mohr CF, Kalmer M, Gross C, Mann MC, Sterz KR, Kieser A, Fleckenstein B, Kress AK. The tumor marker Fascin is induced by the Epstein-Barr virus-encoded oncoprotein LMP1 via NF-kappaB in lymphocytes and contributes to their invasive migration. Cell Commun Signal 2014; 12:46.
8. Kirsteen J. Campbell, and Stephen W. G. Tait. Targeting BCL-2 regulated apoptosis in cancer. Open Biol. 2018 May; 8(5): 180002.
9. Qi Huang, Shu Li, Pu Cheng, Mei Deng, Xin He, Zhen Wang, Cheng-Hui Yang, Xiao-Ying Zhao, Jian Huang. High expression of anti-apoptotic protein Bcl-2 is a good prognostic factor in colorectal cancer: Result of a metaanalysis. Gastroenterol 2017 July 21; 23(27): 5018-5033.
10. Balzi M, Ringressi MN, Faraoni P, Booth C, Taddei A, Boni L, Bechi P. B-cell lymphoma 2 and β-catenin expression in colorectal cancer and their prognostic role following surgery. Mol Med Rep. 2015;12:553–560.
11. Fucini C, Messerini L, Saieva C, Orzalesi L, Carroni V, Bartolini N. Apoptotic proteins as prognostic markers and indicators of radiochemosensitivity in stage II/III rectal cancers. Colorectal Dis. 2012;14:e64–e71.
12. Melincovici CS, Mihu CM, Mărginean M, Boşca AB, Coneac A, Moldovan I, Crişan M. The prognostic significance of p53, Bax, Bcl-2 and cyclin E protein overexpression in colon cancer - an immunohistochemical study using the tissue microarray technique. Rom J Morphol Embryol. 2016;57:81–89.
13 Anna-Lena Scherr , Georg Gdynia , Mariam Salou, Praveen Radhakrishnan , Katarina Duglova , Anette Heller, Sophia Keim, Nicole Kautz. Bcl-xL is an oncogenic driver in colorectal cancer. Cell Death Dis. 2016 ; 7(8): e2342.
14. Kankana Bardhan and Kebin Liu. Epigenetics and Colorectal Cancer Pathogenesis. Cancers (Basel). 2013 Jun; 5(2): 676–713.
15. Papaspyrou K, Brochhausen C, Schmidtmann I, Fruth K, Gouveris H, Kirckpatrick J, Mann W, Brieger J.Fascin upregulation in primary head and neck squamous cell carcinoma is associated with lymphatic metastasis.Oncol Lett 2014; 7(6):2041-6.
16. Alam H, Bhate AV, Gangadaran P, Sawant SS, Salot S, Sehgal L, Dange PP, Chaukar DA, D'cruz AK, Kannanl S, Gude R, et al. . Fascin overexpression promotes neoplastic progression in oral squamous cell carcinoma. BMC Cancer 2012; 12:32.
17. Karthik Sathiyamoorthy, Yao Xiong Hu, Britta S. Möhl, Jia Chen, Richard Longnecker, andTheodore S. Jardetzky. Structural basis for Epstein–Barr virus host cell tropism mediated by gp42 and gHgL entry glycoproteins. Nat Commun. 2016; 7: 13557.
18. Lorenzo Galluzzi , Federico Pietrocola, José Manuel Bravo-San Pedro1, Ravi K, Amaravadi , Eric H Baehrecke , Francesco Cecconi, Patrice Codogno, et al. Autophagy in malignant transformation and cancer progression. EMBO J. 2015 Apr 1; 34(7): 856–880.
19. Boguszakova L, Hirsch I, Brichacek B, Faltyn J, Fric P, Dvorakova H, Vonka V. Absence of cytomegalovirus, Epstein-Barr virus, and papillomavirus DNA from adenoma and adenocarcinoma of the colon.Acta virologica. 1988;32(4):303–308.
20. Yuen ST, Chung LP, Leung SY, Luk IS, Chan SY, Ho J. *In situ* detection of Epstein-Barr virus in gastric and colorectal adenocarcinomas. Am J Surg Pathol. 1994;18:1158–1163.
21. Cho Y.J., M.S. Chang, S.H. Park, H.S. Kim and W.H. Kim (2001).

*In situ* hybridization of Epstein-Barr virus in tumor cells and tumorinfiltrating lymphocytes of the gastrointestinal tract. *Hum. Pathol.* 32(3): 297–301.

22. Yanai H, Murakami T, Yoshiyama H, *et al.* Epstein-Barr virus-associated gastric carcinoma and atrophic gastritis. J Clin Gastroenterol 1999;29:39–43.
23. Rickinson A.B. Co-infections, inflammation and oncogenesis: Future directions for EBV research. Semin. Cancer Biol. 2014;26:99–115. doi: 10.1016/j.semcancer.2014.04.004.
24. Queenie Fernandes, Ishita Gupta, Semir Vranic, and Ala-Eddin Al Moustafa. Human Papillomaviruses and Epstein–Barr Virus Interactions in Colorectal Cancer: A Brief Review. Pathogens. 2020; 9(4): 300.
25. Giuseppa Pistritto, Daniela Trisciuoglio,[2] Claudia Ceci, Alessia Garufi, and Gabriella D'Orazi. Apoptosis as anticancer mechanism: function and dysfunction of its modulators and targeted therapeutic strategies. Aging (Albany NY). 2016 Apr; 8(4): 603–619.
26. Eman AE. Badr, Mohamed FA. Assar, Abdel Monem A. Eltorgoman, Azza Zaghlol Labeeb, Gehad A. Breaka, and Enas A. Elkhouly. A correlation between BCL-2 modifying factor, p53 and livin gene expressions in cancer colon patients. Biochem Biophys Rep. 2020 Jul; 22: 100747.
27. Sun N, Meng Q, Tian A. Expressions of the anti-apoptotic genes Bag-1 and Bcl-2 in colon cancer and their relationship. Am J Surg. 2010 Sep;200(3):341–5.
28. Nehls O, Okech T, Hsieh CJ, Enzinger T, Sarbia M, Borchard F, Gruenagel HH, Gaco V, Hass HG, Arkenau HT, et al. Studies on p53, BAX and Bcl-2 protein expression and microsatellite instability in stage III (UICC) colon cancer treated by adjuvant chemotherapy: major prognostic impact of proapoptotic BAX. Br J Cancer. 2007 May;96(9):1409–18.
29. Hemminki K, Santi I, Weires M, Thomsen H, Sundquist J, Bermejo JL. Tumor location and patient characteristics of colon and rectal adenocarcinomas in relation to survival and TNM classes. BMC Cancer.2010;10.
30. Wray CM, Ziogas A, Hinojosa MW, Le H, Stamos MJ, Zell JA. Tumor subsite location within the colon is prognostic for survival after colon cancer diagnosis. Dis Colon Rectum. 2009 Aug;52(8):1359–66.
31. Mohd. Anwar Miya, G. Vandana, S. Swarnalatha, S. Sandhya. A study of expression of BCL-2 in colorectal carcinoma with histopathological correlation. IAIM, 2018; 5(10): 21-36.
32. Menezes HL, Jucá MJ, Gomes EG, Nunes BL, Costa HO, Matos D. Analysis of the immunohistochemical expressions of p53, bcl-2 and Ki-67 in colorectal adenocarcinoma and their correlations with the prognostic factors. Arq Gastroenterol 2010;47(2):141-7.
33. Yan-fang A, Yong M, Jing-hua L. The expressions of PCNA and Bcl-2 in colorectal adenoma and carcinoma and their clinicopathological and prognostic significance. Acta Academiae Medicinae Xuzhou 2006;6:11-7.