**Original Article**
Middle East Journal of Cancer; October 2015; 6(4): 211-218

# Analysis of Colorectal Cancer and Polyps for the Presence of Herpes Simplex Virus and Epstein - Barr virus DNA Sequences by Polymerase Chain Reaction

**Sahar Mehrabani-Khasraghi[*,♦], Farzad Khalily[**], Mitra Ameli[***]**

**Department of Microbiology, Tonekabon Branch, Islamic Azad University, Mazandaran, Iran*
***Gastroenterology and Hepatology Research Center, Karaj University of Medical Sciences and Health Services, Alborz, Iran*
****Department of Medicine, Tonekabon Branch, Islamic Azad University, Mazandaran, Iran*

## Abstract

**Background:** Colorectal cancer is one of the most common malignancies worldwide with more than one million new cases diagnosed each year. The aim of this study is to investigate the prevalence of herpes simplex virus and Epstein-Barr virus in patients with colorectal carcinomas and polyps in comparison with healthy subjects by using the polymerase chain reaction technique.

**Methods:** In this analytical case-control study, we selected 15 patients with colorectal cancer, 20 patients with colorectal polyps and 35 patients without malignancy as controls. Biopsy specimens were frozen under sterile conditions at -20ºC. After DNA extraction, analysis of polymerase chain reaction to detect herpes simplex virus and Epstein-Barr virus DNA in tissue samples was performed. Statistical analysis was performed with the $\chi 2$ test.

**Results:** We observed herpes simplex DNA in 33.3% of tumor samples (5 of 15) and 20% from the non-malignant control group (7 of 35). There was no herpes simplex DNA in the polyp tissues (0 of 20). Epstein-Barr DNA was found in 60% of tumor samples (9 of 15), 35% of polyp samples (7 of 20), and 40% of the non-malignant control group (14 of 35). Statistical analysis showed no significant association between the prevalence of herpes simplex and Epstein-Barr viruses and the incidence of colorectal cancer and polyps compared with the control group.

**Conclusion:** The results demonstrate a lack of direct molecular evidence to support an association between herpes simplex and Epstein-Barr viruses with human colorectal malignancies. These results do not exclude a possible oncogenic role of these viruses to infect different colon cells.

***Keywords:*** Colorectal cancer, Polyp, Herpes simplex virus, Epstein-Barr virus, Polymerase chain reaction

♦Corresponding Author:
Sahar Mehrabani Khasraghi, MSc
Department of Microbiology, Tonekabon Branch, Islamic Azad University, Mazandaran, Iran
Tel: +98-939-0671927
Fax: +98-115-4274415
Email: saharmehrabani1@gmail.com

Middle East Journal of Cancer

Received: March 3, 2015; Accepted: June 15, 2015

## Introduction

Colorectal cancer (CRC) is the most common gastrointestinal cancer and the leading cause of cancer deaths in Iran.[1] Colorectal cancer is third in incidence among women after lung and breast cancers, and in men after lung and prostate cancers. According to the World Health Organization, each year 875,000 new cases of CRC are recorded.[2] The incidence of CRC varies throughout the world. America, North-Western Europe, Australia, Japan, China, Singapore and Canada have the highest rates whereas African and Asian countries such as Iran have the lowest rate.[3] The majority of CRC (regardless of the etiology) originate from adenomatous polyps. Adenomatous polyps may be pedunculated or sessile. Cancer is more common in the sessile types. Adenomatous polyps may have a tubular histology, villus and tubular-villus. Although many risk factors for development of CRC have been identified, such as viral infections, the inherited genetic predisposition and molecular mechanisms related to CRC remain under investigation.[4, 5] Viral etiologies of human malignancies are an intriguing subject. With the exception of hepatitis C virus (HCV), all known human tumor viruses contain DNA as their genetic material.[6] The oncogenic role of a few viral agents is recognized in human tumors, such as HTLV-1 (a T-cell leukemia virus), Epstein-Barr virus (EBV), human herpes virus type 8 (HHV8), HCV, hepatitis B virus (HBV), and human papilloma virus (HPV). Herpes simplex virus (HSV) and EBV are member of the Herpesviridae family, which includes cytomegalovirus, varicella-zoster, and herpes viruses 6, 7 and 8.[7] Herpes simplex virus and EBV are ubiquitous herpes viruses that infect and establish persistent infections in the host. Epstein-Barr virus infection can occur in immunocompetent individuals, but it most frequently occurs in immunocompromised patients such as organ transplant recipients, patients undergoing hemodialysis, those receiving immunosuppressive drugs, and in patients with acquired immune deficiency syndrome. A potential role of HSV and EBV in human carcinogenesis has also been investigated. Yang et al. have reported that HSV is associated with cervical and oral tumors. Their results suggest that HSV is a risk factor in cervical and oral cancers.[8] Recent studies report that EBV-transformed lymphoblastoid cell lines have demonstrated alterations in methylation patterns when compared to peripheral blood leukocytes.[9] These findings raise the question of whether persistent HSV and EBV infections can include oncogenic pathways that result in colorectal carcinomas. Detection of an infectious agent in human cancers might have important implications in cancer treatment and prevention. Give the importance of CRC as the most common gastrointestinal cancer and the possible role of oncogenic viruses in tumorigenesis, the present study intends to investigate the prevalence of HSV and EBV in patients with CRC and polyps in comparison with healthy subjects by the polymerase chain reaction (PCR) technique.

## Materials and Methods

### *Patients*

In this analytical case-control study, informed consent was received from all patients admitted to the Endoscopy Clinic of Toos and Firoozgar Hospitals in Tehran, Iran, between January 2013 and June 2013. We

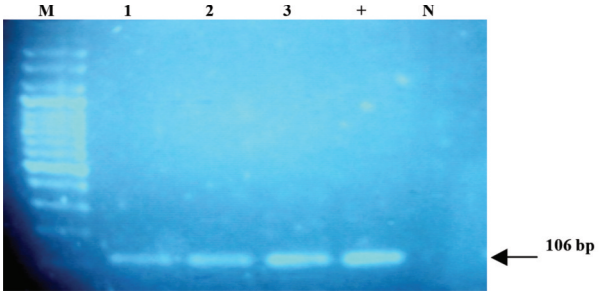


**Figure 1.** PCR analysis of β-globulin. DNA extracted from tissue samples was amplified for β-globulin gene using primers described in Materials and Methods. Amplification yielded a band of 106 bp. As positive control (+), we used human DNA from fresh tissue; the negative control (N) was PCR master mix without DNA. Clinical samples, lanes 1-3. DNA molecular weight marker, M.

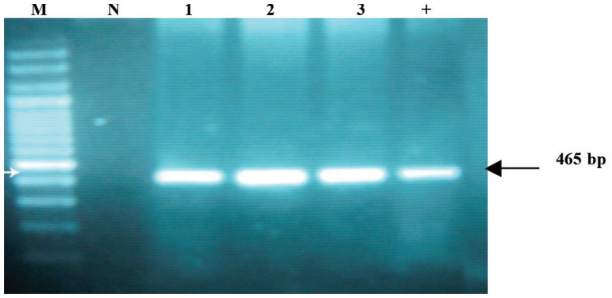


**Figure 2.** PCR analysis for the detection of herpes simplex virus (HSV) from tissue samples. DNA extracted from tissues was amplified with specific primers. Amplification of fragment yielded a band of 465 bp. Positive control (+); negative control (N); clinical samples, lanes 1, 2 and 3; DNA molecular weight marker, M.

enrolled 15 patients with CRC, 20 with colorectal polyps and 35 without malignancy as the controls. Sampling was performed by endoscopic biopsy and a tissue sample size of 25-50 mg was calculated for each patient. All collected tissues were kept frozen at -20ºC until analysis.

### *DNA extraction*

DNA was extracted using the KiaSpin® Tissue Kit (Kiagen CA, Iran) according to the manufacturer's instructions. The concentration of the sample absorbance at a wavelength of 260 nm was determined by the Biophotometer System (Eppendorf, Germany). Absorbances at 280/260 nm and 230/260 nm were used to determine sample purity.

### *Polymerase chain reaction (PCR)*

We performed PCR amplification of the human β-globulin gene (Table 1) to determine the quality of extracted DNA.[10] The PCR amplification was performed in a 20 µL reaction volume contained 10 µL prime Taq premix (2x; Kiagen CA, Iran), 3 µL of sterile distilled water, 1 µL of forward and reverse primers (TAG Copenhagen, Denmark), and 5 µL of DNA template. The PCR reaction was carried out as follows: an initial denaturation at 95ºC for 5 min, followed by 35 cycles at 95ºC for 50 sec, 55ºC for 45 sec, 72ºC for 40 sec and a final elongation at 72ºC for 5 min.

Specific primers listed in Table 1 were used to reproduce the HSV genome of the samples.[10] The PCR amplification was performed in a 20 µL reaction volume contained 10 µL prime Taq premix (2x; Kiagen CA, Iran), 3 µL of sterile distilled water, 1 µL of forward and reverse primers (TAG Copenhagen, Denmark), and 5 µL of DNA template. The PCR reaction was carried out as follows: an initial denaturation at 95ºC for 5 min, followed by 35 cycles at 95ºC for 50 sec, 64ºC for 45 sec, 72ºC for 40 sec and a final elongation at 72ºC for 5 min.

We used specific primers to reproduce the EBV genome from the samples (Table 1).[10] The PCR amplification was performed in a 20 µL reaction volume contained 10 µL prime Taq premix (2x; Kiagen CA, Iran), 3 µL of sterile distilled water, 1 µL of forward and reverse primers (TAG Copenhagen, Denmark), and 5 µL of DNA template. The PCR reaction was carried out as follows: initial denaturation at 95ºC for 5 min, followed by 35 cycles at 95ºC for 40 sec, 65ºC for 40 sec, 72ºC for 40 sec and a final elongation at 72ºC for 5 min. Then, 5 µL of the PCR product was analyzed on a 1.5% agarose gel.

### *Statistical analysis*

Statistical analysis was performed using the SPSS-20 (SPSS, Inc., Chicago, IL, USA) software package. We used the t- and $\chi 2$ tests to analyze the relationship between the prevalence of HSV and EBV and occurrence of CRC and polyps in addition to a comparison with control group tissue samples. Statistical significance was accepted at the 0.05 level.

## Results

Herpes simplex virus DNA was found in 33.3% (5 of 15) of CRC tumor samples. There was no HSV DNA observed in any of the colorectal polyp tissues (0 of 20). Herpes simplex virus DNA was found in 20% (7 of 35) of patients in the control group. Statistical analysis showed no significant association between the prevalence of HSV and the incidence of CRC and polyps compared to the control group ($P$=0.254).

Epstein-Barr virus DNA was detected in 60% (9 of 15) of CRC tumor cases. In patients with colorectal polyps, EBV DNA was found in 35% (7 of 20) of polyp tissues. Epstein-Barr virus DNA was found in 40% (14 of 35) of patients in the control group. Statistical analysis showed no significant association between the prevalence of EBV and incidence of CRC and polyps compared to the control group ($P$=0.161).

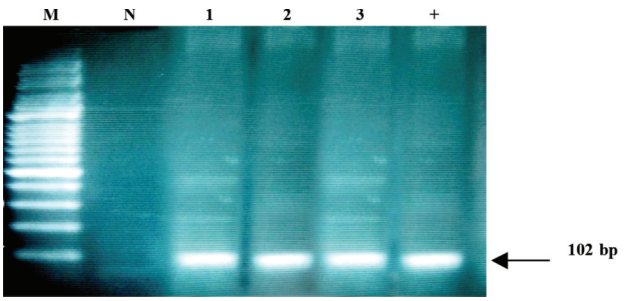


**Figure 3.** PCR analysis for the detection of Epstein-Barr virus (EBV) from tissue samples. DNA extracted from tissues was amplified with specific primers. Amplification of fragment yielded a band of 102 bp. Positive control (+); negative control (N); clinical samples, lanes 1, 2 and 3; DNA molecular weight marker, M.

**Table 1.** Primers sequences and base pair (bp) length.

| Primer | Sequence (5´-3´) | Size (bp) |
|---|---|---|
| b 2- F | TCCAACATCAACATCTTGGT | 106 |
| b 2- R | TCCCCCAAATTCTAAGCAGA | |
| HSV-1/2 F | CAGTACGGCCCCGAGTTCGTGA | 465 |
| HSV-1/2 R | TTGTAGTGGGCGTGGTAGATG | |
| EBV-F1 | GTGTGCGTCGTGCCGGGGCAGCCAC | 102 |
| EBV-R1 | ACCTGGGAGGGCCATCGCAAGCTCC | |

We observed the highest prevalence of HSV in CRC patients older than 55 years (26.6%) of age and in the non-malignant control group participants who were over 55 years of age (8.6%; Table 2). The highest prevalence of EBV was observed in two groups of CRC patients, those 35-55 years (26.6%) and over 55 years (26.6%) of age. In patients with colorectal polyps, the highest prevalence of EBV was observed in 35-55 years old (20%) participants. In the control group, individuals 35-55 years (14.3%) and those over 55 years (14.3%) had the highest prevalence of EBV (Table 3). Statistical analysis showed no significant association between the prevalence of HSV and EBV in terms of age in patients with CRC and polyps compared to the control group ($P$>0.05; Tables 2, 3).

In terms of gender, the highest prevalence of HSV was observed in male CRC patients (20%) and in non-malignant control group women (11.4%; Table 2). We observed the highest prevalence of EBV in male CRC patients (53.3%), in women patients with colorectal polyps (25%) and in non-malignant control group women (22.9%; Table 3). Statistical analysis showed no significant association between the prevalence of HSV and EBV and gender in CRC patients and those with polyps compared to the control group ($P$>0.05; Tables 2, 3).

The highest prevalence rate according to anatomic location for HSV in CRC patients was the proximal colon (20%) and the distal colon (20%) in the control group (Table 2). The highest prevalence rate according to anatomic location for EBV in CRC patients was the proximal colon (33.3%). In patients with polyps, the highest prevalence was the distal colon (20%). The highest prevalence for the control group was the distal colon (40%; Table 3). Statistical analysis showed no significant association between the prevalence of HSV and EBV and anatomic location in CRC patients and those with polyps in comparison with the control group ($P$>0.05; Tables 2, 3).

In all tissue samples, we observed a 106 bp band that represented amplification of the human β-globulin gene (Figure 1). Due to the quality and reliability of DNA extracted, PCR analysis with HSV specific primers was performed where we observed 465 bp bands that represented the replication (Figure 2). PCR analysis with EBV specific primers was performed. A total of 102 bp bands that represented the replication were observed (Figure 3).

## Discussion

We investigated CRC, polyp and non-malignant tissues for the presence of HSV and EBV DNA by the PCR method. In CRC patients we detected HSV DNA in 33.3% of samples; EBV DNA was detected in 60% of samples. In patients with colorectal polyps, HSV DNA was not observed, whereas EBV DNA was found in 35% of samples. In the control group, 20% had HSV DNA and 40% had EBV DNA. There was no association between viral presence and occurrence of CRC and polyps compared to control group tissue.

Since the discovery by Gross of a viral cause for murine leukemia, the search for oncogenic viruses in human malignancies has increased rapidly. Based on the current understanding, it is estimated that approximately 15% of the global cancer burden can be linked to oncogenic tumor viruses.[11] Oncogenic viruses may contribute to human carcinogenesis favoring genetic instability and inducing chromosomal aberrations.[12] Genetic instability is a feature common to many human malignancies that seems to play a role in tumor progression, allowing the emergence of cell clones with growth advantages over normal cells.[6]

**Table 2.** Clinical and pathological features of the colorectal cancer (CRC), polyp and control group patients related to the presence of herpes simplex virus (HSV).

| Patients | HSV DNA | | *P*-value | Total |
|---|---|---|---|---|
| | Positive | Negative | | |
| **CRC** | | | | |
| **Age groups** | | | >0.05 | |
| Under 35 years | 0 (0%) | 1 (6.7%) | | 1 (6.7%) |
| 35-55 years | 1 (6.7%) | 4 (26.7%) | | 5 (33.4%) |
| Over 55 years | 4 (26.6%) | 5 (33.3%) | | 9 (59.9%) |
| **Gender** | | | >0.05 | |
| Male | 3 (20%) | 8 (53.3%) | | 11 (73.3%) |
| Female | 2 (13.3%) | 2 (13.3%) | | 4 (26.7%) |
| **Location** | | | >0.05 | |
| Proximal colon (C.A.T)a | 3 (20%) | 3 (20%) | | 6 (40%) |
| Distal colon (D.S)b | 2 (13.3%) | 2 (13.3%) | | 4 (26.7%) |
| Rectum | 0 (0%) | 5 (33.3%) | | 5 (33.3%) |
| **Total** | 5 (33.3%) | 10 (66.7%) | | 15 (100%) |
| **Colon polyp** | | | | |
| **Age groups** | | | >0.05 | |
| Under 35 years | 0 (0%) | 0 (0%) | | 0 (0%) |
| 35-55 years | 0 (0%) | 7 (35%) | | 7 (35%) |
| Over 55 years | 0 (0%) | 13 (65%) | | 13 (65%) |
| **Gender** | | | >0.05 | |
| Male | 0 (0%) | 7 (35%) | | 7 (35%) |
| Female | 0 (0%) | 13 (65%) | | 13 (65%) |
| **Location** | | | >0.05 | |
| Proximal colon (C.A.T)a | 0 (0%) | 9 (45%) | | 9 (45%) |
| Distal colon (D.S)b | 0 (0%) | 8 (40%) | | 8 (40%) |
| Rectum | 0 (0%) | 3 (15%) | | 3 (15%) |
| **Total** | 0 (0%) | 20 (100%) | | 20 (100%) |
| **Control group** | | | | |
| **Age groups** | | | >0.05 | |
| Under 35 years | 2 (5.75%) | 7 (20%) | | 9 (25.75%) |
| 35-55 years | 2 (5.75%) | 11 (31.4%) | | 13 (37.15%) |
| Over 55 years | 3 (8.6%) | 10 (28.5%) | | 13 (37.1%) |
| **Gender** | | | >0.05 | |
| Male | 3 (8.6%) | 12 (34.3%) | | 15 (42.9%) |
| Female | 4 (11.4%) | 16 (45.7%) | | 20 (57.1%) |
| **Location** | | | >0.05 | |
| Proximal colon (C.A.T)a | 0 (0%) | 0 (0%) | | 0 (0%) |
| Distal colon (D.S)b | 7 (20%) | 28 (80%) | | 35 (100%) |
| Rectum | 0 (0%) | 0 (0%) | | 0 (0%) |
| **Total** | 7 (20%) | 28 (80%) | | 35 (100%) |

a C: Cecum; A: Ascending colon; T: Transverse colon; b D: Descending colon; S: Sigmoid colon

The role of EBV in gastric cancer is well known and has been reported to range from 4% to 18% of gastric carcinomas.[13] Although there are many similar features in histology and pathogenesis between gastric and CRC, there are few papers about the relationship of EBV with CRC. However, a tremendous amount of evidence supports an etiologic role for EBV in carcinogenesis in patients with EBV-positive gastric carcinomas.[14-16] Epstein-Barr virus can be expressed in transcripts to activate the proto-oncogene c-Myc, resulting in cell damage in various processes such as metabolism, cell cycle regulation, apoptosis, protein synthesis, angiogenesis and cellular connections. However, the role of HSV in patients with gastrointestinal cancers, particularly CRC, has yet to be reported in the literature. This is the first study that has investigated the prevalence

**Table 3.** Clinical and pathological features of the colorectal cancer (CRC), polyp and control group patients related to the presence of Epstein-Barr virus (EBV).

| Patients | EBV DNA | | *P*-value | Total |
|---|---|---|---|---|
| | Positive | Negative | | |
| **CRC** | | | | |
| **Age groups** | | | >0.05 | |
| Under 35 years | 1 (6.7%) | 0 (0%) | | 1 (6.7%) |
| 35-55 years | 4 (26.6 %) | 1 (6.7%) | | 5 (33.3%) |
| Over 55 years | 4 (26.6 %) | 5 (33.3%) | | 9 (60%) |
| **Gender** | | | >0.05 | |
| Male | 8 (53.3%) | 3 (20%) | | 11 (73.3%) |
| Female | 1 (6.7%) | 3 (20%) | | 4 (26.7%) |
| **Location** | | | >0.05 | |
| Proximal colon (C.A.T)a | 5 (33.3 %) | 1 (6.7%) | | 6 (40%) |
| Distal colon (D.S)b | 1 (6.7%) | 3 (20%) | | 4 (26.7% |
| Rectum | 3 (20%) | 2 (13.3%) | | 5 (33.3%) |
| **Total** | 9 (60%) | 6 (40%) | | 15 (100%) |
| **Colon polyp** | | | | |
| **Age groups** | | | >0.05 | |
| Under 35 years | 0 (0%) | 0 (0%) | | 0 (0%) |
| 35-55 years | 4 (20%) | 3 (15%) | | 7 (35%) |
| Over 55 years | 3 (15%) | 10 (50%) | | 13 (65%) |
| **Gender** | | | >0.05 | |
| Male | 2 (10%) | 5 (25%) | | 7 (35%) |
| Female | 5 (25%) | 8 (40%) | | 13 (65%) |
| **Location** | | | >0.05 | |
| Proximal colon (C.A.T)a | 2 (10%) | 7 (35%) | | 9 (45%) |
| Distal colon (D.S)b | 4(20%) | 4 (20%) | | 8 (40%) |
| Rectum | 1 (5%) | 2 (10%) | | 3 (15%) |
| **Total** | 7 (35%) | 13 (65%) | | 20 (100%) |
| **Control group** | | | | |
| **Age groups** | | | >0.05 | |
| Under 35 years | 4 (11.4%) | 5 (14.3%) | | 9 (25.7%) |
| 35-55 years | 5 (14.3%) | 8 (22.8%) | | 13 (37.1%) |
| Over 55 years | 5 (14.3%) | 8 (22.8%) | | 13 (37.1%) |
| **Gender** | | | >0.05 | |
| Male | 6 (17.1%) | 9 (25.7%) | | 15 (42.8%) |
| Female | 8 (22.9%) | 12 (34.3%) | | 20 (57.2%) |
| **Location** | | | >0.05 | |
| Proximal colon (C.A.T)a | 0 (0%) | 0 (0%) | | 0 (0%) |
| Distal colon (D.S)b | 14 (40%) | 21 (60%) | | 35 (100%) |
| Rectum | 0 (0%) | 0 (0%) | | 0 (0%) |
| **Total** | 14 (40%) | 21 (60%) | | 35 (100%) |

a C: Cecum; A: Ascending colon; T: Transverse colon; b D: Descending colon; S: Sigmoid colon

of HSV and EBV in CRC and polyps in Iran. Although some studies have detected EBV DNA in colorectal adenocarcinomas, others failed to demonstrate the presence of EBV by the use of various laboratory techniques.[9, 13, 17-25] Boguszakova et al. examined biopsy specimens from 13 patients with adenocarcinoma of the colon and 10 with endoscopic polypectomies for colon adenoma for the presence of the EBV DNA. Their results failed to detect viral DNA in the biopsy specimens tested.[17] Yoen et al. used in situ hybridization (ISH) and an anti-sense EBER probe to investigate the presence of EBV in 74 cases of gastric adenocarcinoma and 36 cases of colorectal adenocarcinoma in Chinese patients. None of the CRC had positive signals.[18] Cho et al. also reported the lack of an association between EBV and colorectal tumors.[19] In a study by Karpinski et al. on

the presence of EBV DNA in 186 sporadic CRC cases, after PCR analysis there were 19% of the tumor samples positive for EBV. These results indicated no association between EBV and sporadic CRC.[9] Our results confirmed the results of Boguszakova et al.,[17] Yoen et al.,[18] Cho et al.,[19] and Karpinski et al.[9] where no correlation existed between EBV infection and progression of CRC. However, Yanai et al. found that EBV was detected in 63.3% of Crohn's disease cases and 60% of ulcerative colitis cases using ISH for EBV-encoded small RNA 1 (EBER-1). This indicated a possible relation between EBV and IBD colon diseases.[20] Samaha et al. and Kon et al. reported that lymphoepithelioma-like carcinoma of the rectum was probably related to EBV.[21,22] Ruschoff et al. used PCR to detect EBV DNA in 20 cases of colorectal adenocarcinomas. They identified EBV DNA in 3 cases. These findings suggested that EBV might be associated with colorectal tumors.[23] Kim et al. investigated the presence of EBV in 20 cases of colorectal adenocarcinomas and found 2 EBER-positive cases.[24] Grinstein et al. reported that the EBV might play an oncogenic role in epithelial cancers such as CRC. In addition, EBV could be involved in hyperplasia and dysplasia.[25] In another study, Liu et al. detected EBV in CRC patients from China by PCR. EBV DNA was detected in 26 samples from of 130 CRC cases. In addition, the prevalence of EBV in men with cancer was observed compared to women. These researchers introduced EBV carcinogenic factors in CRC.[13]

The results presented herein have demonstrated a lack of direct molecular evidence to support an association of HSV and EBV with human colorectal malignancies. These results do not exclude the possibility of an oncogenic role for these viruses to infect various colon cells. The carcinogenesis mechanism needs to be clarified further.

## Acknowledgement

This paper was extracted from proposal no. 23796 (Performer: Sahar Mehrabani-Khasraghi). We would like to express our appreciation to Mr. Shabani for his kind assistance with this project and to all patients who participated in this study.

## Conflict of interest

No conflict of interest is declared.

## References

1. Hoseini S, Moaddabshoar L, Hemati S, Mohammadianpanah M. An overview of clinical and pathological characteristics and survival rate of colorectal cancer in Iran. *Ann Colorectal Res*. 2014; 2(1): e17264.
2. Janaut V, Kollarova H. Epidemiology of colorectal cancer. *Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub*. 2001; 145(1): 5-10.
3. Rezaianzadeh A, Safarpour AR, Marzban M, Mohaghegh A. A systematic review over the incidence of colorectal cancer in Iran. *Ann Colorectal Res.* 2015; 3(1): e25724.
4. Allen JI. Molecular biology of colorectal cancer: A clinician's view. *Perspect Colon Rectal Surg*. 1995;8: 181-202.
5. Lynch HT, Lynch JF. Genetics of colonic cancer. *Digestion*. 1998; 59(5): 481-492.
6. Elgui de Oliveira D. DNA viruses in human cancer: An integrated overview of fundamental mechanisms of viral carcinogenesis. *Cancer Lett.* 2007; 247(2): 182-96.
7. Hommes DW, Sterringa G, Van Deventer SJH, Tytgat GNJ, Well J. The pathogenicity of cytomegalovirus in inflammatory bowel disease. *Inflamm Bowel Dis.* 2004; 10(3): 245-50.
8. Yang YY, Koh LW, Tsai JH, Tsai CH, Wong EFC, Lin SJ, et al. Correlation of viral factors with cervical cancer in Taiwan. *J Microbiol Immunol Infect.* 2004; 37: 282-7.
9. Karpinski P, Myszka A, Ramsey D, Kielan W, Sasiadek MM. Detection of viral DNA sequences in sporadic colorectal cancers in relation to CpG island methylation and methylator phenotype. *Tumour Biol.* 2011; 32(4): 653-9.
10. Zaravinos A, Bizakis J, Spandidos DA. Prevalence of human papilloma virus and human herpes virus type 1-7 in human nasal polyposis. *J Med Virol.* 2009; 81(9): 1613-9.
11. Harald-Zur H. Oncogenic DNA viruses. *Oncogene.* 2001; 20(54): 7820-3.
12. Duensing S, Munger K. Human papillomavirus type 16 E7 oncoprotein can induce abnormal centrosome duplication through a mechanism independent of inactivation of retinoblastoma protein family members. *J Virol.* 2003; 77(22): 12331-5.
13. Liu HX, Ding YQ, Li X, Yao KT. Investigation of Epstein-Barr virus in Chinese colorectal tumors. *World J Gastroenterol.* 2003; 9(11): 2464-8.
14. Tokunaga M, Land CE, Uemura Y, Tokudome T, Tanaka S, Sato E. Epstein-Barr virus in gastriccarcinoma. *Am J Pathol.* 1993; 143(5): 1250-5.
15. Nakamura S, Ueki T, Ueyama T, Tsuneyoshi M. Epstein-Barr virus in gastric carcinoma with lymphoid stroma. Special reference to its detection by the polymerase chain reaction and in situ hybridization in 99 tumors, including a morphologic analysis. *Cancer.* 1994; 73(9): 2239-49.
16. Takano Y, Kato Y, Saegusa M, Mori S, Shiota M, Masuda M, et al. The role of the Epstein-Barr- virus in the oncogenesis of EBV (+) gastric carcinomas. *Virchows Arch.* 1999; 434(1): 17-22.

17. Boguszakova L, Hirsch I, Brichacek B, Faltyn J, Fric P, Dvorakova H, et al. Absence of cytomegalovirus, Epstein-Barr virus, and papillomavirus DNA from adenoma and adenocarcinoma of the colon. *Acta Virol.* 1988; 32(4): 303-8.
18. Yoen 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(11): 1158-63.
19. Cho YJ, Chang MS, Park SH, Kim HS, Kim WH. In situ hybridization of Epstein-Barr virus in tumor cells and tumor infiltrating lymphocytes of the gastrointestinal tract. *Hum Pathol.* 2001; 32(3): 297-301.
20. Yanai ST, Chung LP, Luk IS, Chan SY, Ho J. In situ detection of Epstein-Barr virus in gastric and colorectal adenocarcinomas. *Am J Surg Pathol.* 1994; 18(11): 1158-63.
21. Samaha S, Tawfik O, Horvat R, Bhatia P. Lymphoepithelioma like carcinoma of the colon: report of a case with histologic, immunohistochemical, and molecular studies for Epstein-Barr virus. *Dis Colon Rectum.* 1998; 41(7): 925-8.
22. Kon S, Kasai K, Tsuzuki N, Nishibe M, Kitagawa T, Nishibe T, et al. Lymphoe pithelioma-like carcinoma of rectum: possible relation with EBV. *Pathol Res Pract.* 2001; 197: 557-582.
23. Ruschoff J, Dietmaier W, Luttges J, Seitz G, Bocker T, Zirniqibl H, et al. Poorly differentiated colonic adenocarcinoma, medullary type: clinical Phenotypic, and molecular characteristics. *Am J Pathol. 1*997; 150(5): 1815-25.
24. Kim YS, Paik SR, Kim HK, Yeom BW, Kim I, Lee D. Epstein-Barr virus and CD21 expression in gastrointestinal tumors. *Pathol Res Pract.* 1998; 194(10): 705-11.
25. Grinstein S, Preciado MV, Gattuso P, Chabay PA, Warren WH, De Matteo E, et al. Demonstration of Epstein-Barr virus in carcinomas of various sites. *Cancer Res.* 2002; 62(17): 4876-8.