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Original Article

Analysis of colorectal cancer and polyp for presence herpes simplex virus and cytomegalovirus DNA sequences by polymerase chain reaction

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Abstract

Introduction: In recent years, it was demonstrated that there is a clear association between the complicated course of colorectal cancer (CRC) and the presence of herpes viruses. Despite a great number of published reports, the exact pathogenic role of herpes viruses remains unclear in these patients. The purpose of this study is to explore the prevalence of herpes simplex virus (HSV) and cytomegalovirus (CMV) in patients with CRC and polyp in comparison with healthy subjects using the polymerase chain reaction (PCR) method.

Methods: In this case-control study, 15 biopsies of patients with CRC and 20 colorectal polyp sample were selected. From each patient, two tissue samples were obtained: one sample from malignant tissue, and the other from normal colorectal tissue in an area located 15 cm away from the malignant tissue. Furthermore, 35 samples from healthy people as controls were selected. After DNA extraction, PCR was used to determine HSV and CMV genomes by specific primers. A statistical analysis was performed using the chi-square test.

Results: Five CRC patients (33.3%) had HSV DNA detected in both the malignant and the matched normal tissue. Five CRC patients (33.3%) and seven polyp patients (35.0%) had CMV DNA detected in both the malignant and the matched normal tissue. HSV DNA was found in 20% and CMV DNA in 37.1% of samples from healthy people as a control group. Thus, no significant association was observed between the prevalence of HSV and CMV, and an incidence of CRC and polyps according to the location of the samples as compared with the control group.

Conclusion: The findings demonstrated that there is no direct molecular evidence to support the association between HSV and CMV and human colorectal malignancies. However, the results from this study do not exclude a possible oncogenic role of these viruses in the neoplastic development of colon cells.

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Introduction

Colorectal cancer (CRC) is the most common gastrointestinal cancer and the leading cause of cancer deaths in Iran.[1] According to the World Health Organization (WHO), approximately, 875000 new cases of CRC are diagnosed annually worldwide.[2] In general, the most CRCs are immunologically silent tumors, grow slowly and often do not produce symptoms until they reach a large size. The incidence of CRC varies worldwide, with higher frequencies in America, North-Western Europe, Australia, Japan, China, Singapore and Canada, and lower

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frequencies in African and Asian countries, including Iran.[3] Regardless of the etiology, the majority of CRC are demonstrated to arise from adenoma polyps. There are three forms of adenomatous polyps including tubular histology, villus, and tubular-villus. Although a variety of risk factors, such as viral infections, have been found to involve in the development of CRC, inherited genetic predisposition and molecular mechanisms related to CRC remain under investigation.[4,5]

Viral etiologies of human malignancies are an intriguing subject for basic researchers and clinicians. With the exception of hepatitis C virus, all known human tumor viruses contain DNA as their genetic material.[6] Herpes simplex virus (HSV) and cytomegalovirus (CMV) are ubiquitous herpes viruses that infect and establish persistent infections in the host. A potential role of HSV and CMV in human carcinogenesis has also been investigated in a variety of studies.[7-11]

Available data from clinical studies have so far provided contradictory results, some of which were able to detect the DNA of these viruses in colorectal adenocarcinomas by different laboratory techniques such as in-situ hybridization and polymerase chain reaction (PCR). In contrast, others failed to demonstrate the presence of these viruses in tissue samples of CRC, even using the same detection methods. Considering the importance of CRC as the most common gastrointestinal cancer, and the possible role of oncogenic viruses in tumorigenesis, the present study aimed to investigate the prevalence of HSV and CMV in patients with CRC and polyps by the PCR technique, in comparison with healthy subjects.

Methods

This case-control study was conducted on a total of 35 subjects including 15 patients with CRC and 20 ones with colorectal polyp. Written and 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. Two tissue samples were obtained from each patient, one from malignant tissue and the other from normal colorectal tissue in an area located 15 cm away from the malignant tissue. In addition, 35 samples from patients without malignancy were used as a negative control. Tissue fragments were sampled by endoscopic biopsy, and an average tissue size of 25 mg was calculated for each patient. All collected tissues were kept frozen at −20 °C until analysis.

DNA extraction

DNA was extracted using the KiaSpin® Tissue Kit (Kiagen CA, Iran) according to the manufacture's instructions. DNA concentrations were determined from absorbance values at a wavelength to 260 nm using a Biophotometer system (Eppendrof, Germany). The ratio of absorbance at 280/260 nm and 230/260 nm was used to assess the purity of DNA.

PCR

PCR amplification of the human β-globulin gene was carried out to monitor the quality of extracted DNA. The identification of HSV and CMV genomes was performed according to Zaravinos et al.[12] using the specific primers shown in table 1. PCR amplification was performed according in a final volume of 20 µl, containing 10 µl of ×2 prime Taq premix (Kiagen CA, Iran), 3 µl of sterile

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

Primer	Sequence (5´-3´)	Size (bp)	Tm
b_2-F	TCCAACATCAACATCTTGGT	106	53.2
b_2-R	TCCCCCAAATTCTAAGCAGA		55.3
HSV-1/2 F	CAGTACGGCCCCGAGTTCGTGA	465	65.8
HSV-1/2 R	TTGTAGTGGGCGTGGTAGATG		59.8
CMV-F	GTCACCAAGGCCACGACGTT	167	59.8
CMV-R	TCTGCCAGGACATCTTTCTC		57.3

HSV: Herpes simplex virus; CMV: Cytomegalovirus; bp: Base pair

distilled water, 1 µl of forward and reverse primers (TAG Copenhagen, Denmark), and 5 µl of DNA template. The human β-globulin gene, as well as HSV and CMV genomes, was amplified under the slightly different conditions; after an initial denaturation at 95 °C for 5 minutes, PCR reactions were followed by 35 cycles of denaturation at 95 °C for 50 seconds, annealing at 55 °C for 45 seconds, extension at 72 °C for 40 seconds, and a final elongation at 72 °C for 5 minutes; 35 cycles of denaturation at 95 °C for 50 seconds, annealing at 64 °C for 45 seconds, extension at 72 °C for 40 seconds, and a final elongation at 72° C for 5 minutes; 35 cycles of denaturation at 95° C for 50 seconds, annealing at 60° C for 45 seconds, extension at 72 °C for 40 seconds, and a final elongation at 72° C for 5 minutes, respectively. Then, 5 µl of the PCR product was analyzed by electrophoresis on a 1.5% agarose gel.

A statistical analysis was performed using the SPSS software (version 20, SPSS Inc., Chicago, USA). The relationship between the prevalence of HSV and CMV and the occurrence of CRC and polyps were investigated according to the location of the samples and compared with the control group. Tissue samples were analyzed using t-test and χ^2 test. The results were considered to be statistically significant at the 5% level.

Results

In all tissue samples, 106 bp band that represents the amplification of human β-globulin gene observed (Figure 1). Due to the quality and reliability of DNA extracted, PCR analysis with HSV and CMV-specific primers was performed; 465 bp bands that represent the replication of HSV (Figure 2), and 167 bp bands that represent the replication of CMV (Figure 3).

In 15 patients with CRC, HSV DNA was found in tumor samples of 5 individuals (33.3%), and the normal tissue surrounding the tumor also displayed HSV DNA in 5 ones (33.3%). In contrast, no HSV DNA was found in polyp samples of patients with colorectal polyps (0 out of 20) while 4 (20.0%) of the patients had HSV DNA only in the normal colorectal tissue surrounding the polyp. HSV DNA was found in 7 (20.0%) of 35 patients with non-malignant conditions. Statistical analysis showed that there is no significant association between the prevalence of HSV and the incidence of CRC and polyps according to the location of the samples, as compared with the control group (P = 0.25).

Out of 15 patients with CRC, 8 ones (53.3%) exhibited detectable CMV DNA in their tumor samples, while the normal tissue surrounding the tumor showed that in 10 ones (66.7%). In 5 patients with CRC (33.3%), CMV DNA was found in tumor tissue and matched normal tissue.

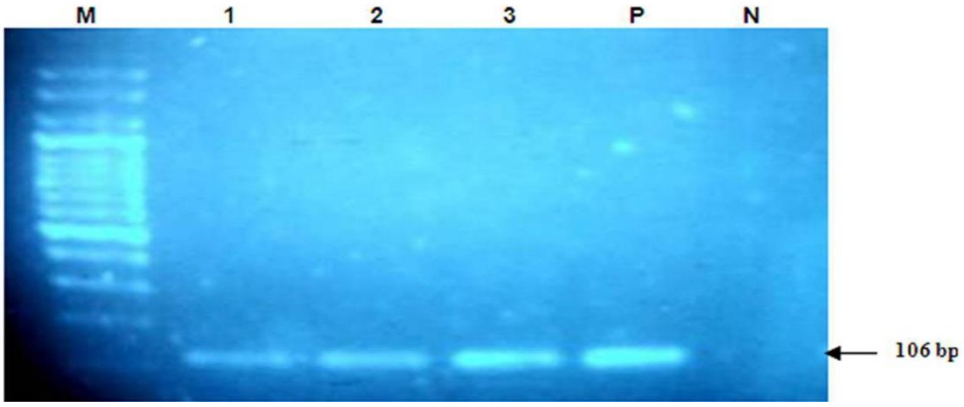

Figure 1. Polymerase chain reaction (PCR) analysis of β-globulin DNA extracted from tissue samples was amplified for β-globulin gene using primers described in methods. Amplification yielded a band of 106 bp. As positive control (P), human DNA from fresh tissue was used; as negative control (N), PCR master mix without DNA was used. Clinical samples, Lanes 1-3. DNA molecular weight marker, M

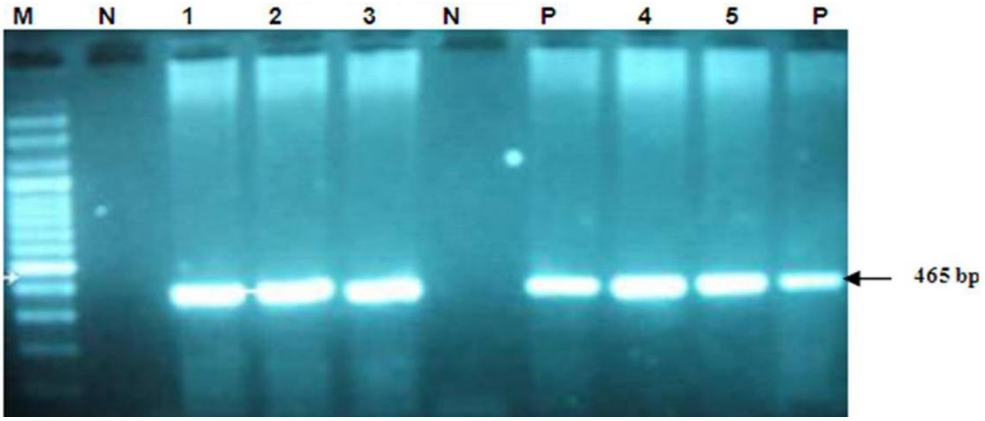

Figure 2. Polymerase chain reaction (PCR) analysis for the detection of herpes simplex virus (HSV) from tissue samples
DNA extracted from tissues was amplified with specific primers. Amplification of fragment yielded a band of 465 bp. Positive controls (P); negative controls (N); clinical samples, Lanes 1-5; DNA molecular weight marker, M

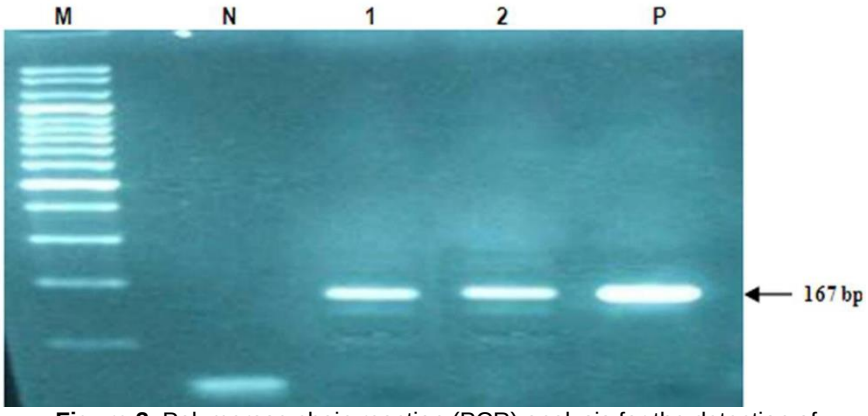

Figure 3. Polymerase chain reaction (PCR) analysis for the detection of cytomegalovirus (CMV) from tissue samples
DNA extracted from tissues was amplified with specific primers. Amplification of fragment yielded a band of 167 bp. Positive control (P); negative control (N); clinical samples, Lanes 1 and 2; DNA molecular weight marker, M

In 20 patients with colorectal polyps, CMV DNA was detected in the polyp samples of 10 patients (50.0%), and in the normal tissue surrounding the polyp of 14 individuals (70.0%). In 7 patients (35.0%) with polyps, CMV DNA was found to be positive in polyp tissue and matched normal tissue. CMV DNA was identified in 13 (37.1%) of 35 patients with non-malignant conditions. Statistical analysis revealed that there is no significant association between the prevalence of CMV and the incidence of CRC and polyps according to the location of the samples in comparison with the control group ($P = 0.28$).

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 CMV was observed in older than 55 years (26.6%) group of CRC patients. In patients with colorectal polyps, the highest prevalence of CMV was observed in two groups, those 35-55 years (25.0%) and over 55 years (25.0%) of age. In the control group, individuals under 35 years (14.3%) had the highest prevalence of CMV (Table 3). Statistical analysis showed no significant association between the prevalence of HSV and CMV in terms of age in patients with CRC and polyps compared to the control group ($P > 0.05$) (Tables 2 and 3).

In terms of gender, the highest prevalence of HSV was observed in male CRC patients (20.0%) and in non-malignant control group women (11.4%) (Table 2).

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		Total [n (%)]	P
	Positive [n (%)]	Negative [n (%)]		
CRC				
Age groups (year)				> 0.05
Under 35	0 (0)	1 (6.7)	1 (6.7)	
35-55	1 (6.7)	4 (26.7)	5 (33.4)	
Over 55	4 (26.6)	5 (33.3)	9 (59.9)	
Gender				> 0.05
Male	3 (20.0)	8 (53.3)	11 (73.3)	
Female	2 (13.3)	2 (13.3)	4 (26.7)	
Location				> 0.05
Proximal colon (C.A.T)*	3 (20.0)	3 (20.0)	6 (40.0)	
Distal colon (D.S)**	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 (year)				> 0.05
Under 35	0 (0)	0 (0)	0 (0)	
35-55	0 (0)	7 (35.0)	7 (35.0)	
Over 55	0 (0)	13 (65.0)	13 (65.0)	
Gender				> 0.05
Male	0 (0)	7 (35.0)	7 (35.0)	
Female	0 (0)	13 (65)	13 (65.0)	
Location				> 0.05
Proximal colon (C.A.T)*	0 (0)	9 (45.0)	9 (45.0)	
Distal colon (D.S)**	0 (0)	8 (40.0)	8 (40.0)	
Rectum	0 (0)	3 (15.0)	3 (15.0)	
Total	0 (0)	20 (100)	20 (100)	
Control group				
Age groups (year)				> 0.05
Under 35	2 (5.7)	7 (20.0)	9 (25.7)	
35-55	2 (5.7)	11 (31.4)	13 (37.1)	
Over 55	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)*	0 (0)	0 (0)	0 (0)	
Distal colon (D.S)**	7 (20.0)	28 (80.0)	35 (100)	
Rectum	0 (0)	0 (0)	0 (0)	
Total	7 (20.0)	28 (80.0)	35 (100)	

*C: Cecum, A: Ascending colon, T: Transverse colon, **D: Descending colon, S: Sigmoid colon
CRC: Colorectal cancer; HSV: Herpes simplex virus

We observed the highest prevalence of CMV in male CRC patients (33.3%), in women patients with colorectal polyps (40.0%), and in non-malignant control group women (20.0%) (Table 3). Statistical analysis showed no significant association between the prevalence of HSV and CMV and gender in CRC patients and those with polyps compared to the control group (P > 0.05) (Tables 2 and 3).

The highest prevalence rate according to anatomic location for HSV in CRC patients was the proximal colon (20.0%) and the distal colon (20.0%) in the control group (Table 2). The highest prevalence rate according to anatomic location for CMV in CRC patients

was the proximal colon (26.7%). In patients with polyps, the highest prevalence was the proximal colon (25.0%). The highest prevalence for the control group was the distal colon (37.1%) (Table 3). Statistical analysis showed no significant association between the prevalence of HSV and CMV and anatomic location in CRC patients and those with polyps in comparison with the control group (P > 0.05) (Tables 2 and 3).

Discussion

In this study, CRC, polyp, and non-malignant tissues were investigated for the presence of HSV and CMV DNA by the PCR method. In patients with CRC, HSV and CMV DNA were found in 33.3 and 53.3% of the samples, respectively.

Table 3. Clinical and pathological features of the colorectal cancer (CRC), polyp and control group patients related to the presence of cytomegalovirus (CMV)

Patients	CMV DNA		Total [n (%)]	P
	Positive [n (%)]	Negative [n (%)]		
CRC				
Age groups (year)				> 0.05
Under 35	1 (6.7)	0 (0)	1 (6.7)	
35-55	3 (20.0)	2 (13.4)	5 (33.4)	
Over 55	4 (26.6)	5 (33.3)	9 (59.9)	
Gender				> 0.05
Male	5 (33.3)	6 (40.0)	11 (73.3)	
Female	3 (20.0)	1 (6.7)	4 (26.7)	
Location				> 0.05
Proximal colon (C.A.T)*	4 (26.7)	2 (13.3)	6 (40.0)	
Distal colon (D.S)**	2 (13.3)	2 (13.4)	4 (26.7)	
Rectum	2 (13.3)	3 (20)	5 (33.3)	
Total	8 (53.3)	7 (46.7)	15 (100)	
Colon polyp				
Age groups (year)				> 0.05
Under 35	0 (0)	0 (0)	0 (0)	
35-55	5 (25)	2 (10)	7 (35)	
Over 55	5 (25)	8 (40)	13 (65)	
Gender				> 0.05
Male	2 (10)	5 (25)	7 (35)	
Female	8 (40)	5 (25)	13 (65)	
Location				> 0.05
Proximal colon (C.A.T)*	5 (25)	4 (20)	9 (45)	
Distal colon (D.S)**	3 (15)	5 (25)	8 (40)	
Rectum	2 (10)	1 (5)	3 (15)	
Total	10 (50)	10 (50)	20 (100)	
Control group				
Age groups (year)				> 0.05
Under 35	5 (14.28)	4 (11.41)	9 (25.8)	
35-55	4 (11.41)	9 (25.75)	13 (37.1)	
Over 55	4 (11.41)	9 (25.75)	13 (37.1)	
Gender				> 0.05
Male	6 (17.1)	9 (25.8)	15 (42.9)	
Female	7 (20)	13 (37.1)	20 (57.1)	
Location				> 0.05
Proximal colon (C.A.T)*	0 (0)	0 (0)	0 (0)	
Distal colon (D.S)**	13 (37.1)	22 (62.9)	35 (100)	
Rectum	0 (0)	0 (0)	0 (0)	
Total	13 (37.1)	22 (62.9)	35 (100)	

*C: Cecum, A: Ascending colon, T: Transverse colon, **D: Descending colon, S: Sigmoid colon
CMV: Cytomegalovirus; CRC: Colorectal cancer

In patients with colorectal polyps, CMV DNA, but not HSV DNA, was found in 50.0% samples, respectively. In the control group, it was established that 20.0 and 37.1% of the samples were positive for HSV and CMV DNA, respectively. However, data showed that there is no association between the presence of the virus and the occurrence of CRC and polyps when compared with tissues of the control group.

Since the discovery of a viral cause for murine leukemia by Gross, the search for oncogenic viruses has rapidly increased in human malignancies. Based on the current understanding, it is estimated that approximately 15.0% of the global cancer burden can be linked to oncogenic viruses.[13] Oncogenic viruses may contribute to human carcinogenesis favoring genetic instability and inducing chromosomal aberrations.[14]

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 of HSV in CRC and polyps in Iran. Various in-vitro studies have demonstrated that the gene products of CMV are able to modulate cell cycle progression and apoptosis by regulating the expression of several important host genes. For example, CMV infection has been shown to transcriptionally activate the expression of the proto-oncogenes c-foc, c-jun, and c-myc.[7] Kalejta and Shenk[8] reported that the CMV UL82 gene product pp71 stimulates cell cycle progression by including protein degradation of another important tumor suppressor Rb and its family members p107 and p130. The possible association of CMV with human colorectal adenocarcinomas was first reported by Huang and Roche[10] who detected CMV DNA in 4 out of 7 colonic adenocarcinomas by membrane complementary RNA-DNA hybridization. Interestingly, CMV DNA was also detected in 1 out of 2 cases of familial adenomatous polyposis, but not in normal colonic tissues from the same patients or control cases of Crohn disease. Furthermore, Harkins et al.[15] detected CMV nucleic acids and proteins in a high proportion of CRC but not in non-neoplastic colonic mucosa. However, in other studies, no evidence of a direct association was found between CRC and HCMV infection.[7,9]

Akintola-Ogunremi et al.[7] attempted to examine 23, 65 and 51 cases of colorectal hyperplastic polyps, colorectal adenomas and colorectal adenocarcinomas, respectively, by immunohistochemical analysis with two different antibodies. No nuclear HCMV antigen positivity was detected in any of the cases studied. In addition, PCR analysis failed to detect viral DNA in 24 selected cases showing non-specific cytoplasmic immunostaning. In a study by Bender et al.[9] on the presence of HCMV in CRC samples, 6 cases (11.0%), of the 56 tested tissue samples, were found to be positive for HCMV nested PCR amplification. More precisely, 1 (5.0%) of 20 cases and 5 (21.0%) of 24 cases were found to be adenoma and moderately differentiated adenocarcinoma, respectively. Surprisingly, no PCR positivity was obtained in samples from well- and poorly-differentiated adenocarcinomas. Knosel et al.[16] investigated the presence of HCMV DNA and antigens using PCR analysis. 57 primary tumors and 20 metastases of fresh CRC tissue were tested including 13 tumors pairs (primary and metastases) from the same patients. Four (7.0%) of 57 primary tumors were found positive for HCMV DNA by PCR, whereas all metastases were negative.

Conclusion

The results from this study demonstrated that there is no direct molecular evidence to support the association between HSV and CMV with human colorectal malignancies. However, these findings do not exclude the possible oncogenic role for these viruses to infect various colon cells, and their carcinogenesis mechanism needs to be further elucidated.

Conflict of Interests

Authors have no conflict of interest.

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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. Annals of Colorectal Research 2014; 2(1): e17264. DOI: 10.17795/acr-17264
2. Janout V, Kollarova H. Epidemiology of colorectal cancer. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub 2001; 145(1): 5-10. DOI: 10.5507/bp.2001.001
3. Rezaianzadeh A, Safarpour AR, Marzban M, Mohaghegh A. A systematic review over the incidence of colorectal cancer in Iran. Annals of Colorectal Research 2015; 3(1): e25724. DOI: 10.17795/acr-25724
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: 481-92. DOI: 10.1159/000007525
6. Elgui de OD. DNA viruses in human cancer: an integrated overview on fundamental mechanisms of viral carcinogenesis. Cancer Lett 2007; 247(2): 182-96. DOI: 10.1016/j.canlet.2006.05.010
7. Akintola-Ogunremi O, Luo Q, He TC, Wang HL. Is cytomegalovirus associated with human colorectal tumorigenesis? Am J Clin Pathol 2005; 123(2): 244-9. DOI: 10.1309/9QVRHDJUK6H2TURB
8. Kalejta RF, Shenk T. Proteasome-dependent, ubiquitin-independent degradation of the Rb family of tumor suppressors by the human cytomegalovirus pp71 protein. Proc Natl Acad Sci U S A 2003; 100(6): 3263-8. DOI: 10.1073/pnas.0538058100
9. Bender C, Zipeto D, Bidoia C, Costantini S, Zamò A, Menestrina F, et al. Analysis of colorectal cancers for human cytomegalovirus presence. Infect Agent Cancer 2009; 4: 6. DOI: 10.1186/1750-9378-4-6
10. Huang ES, Roche JK. Cytomegalovirus D.N.A. and adenocarcinoma of the colon: Evidence for latent viral infection. Lancet 1978; 311(8071): 957-60. DOI: 10.1016/S0140-6736(78)90248-9
11. 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.
12. Zaravinos A, Bizakis J, Spandidos DA. Prevalence of human papilloma virus and human herpes virus types 1-7 in human nasal polyposis. J Med Virol 2009; 81(9): 1613-9. DOI: 10.1002/jmv.21534
13. zur Hausen H. Oncogenic DNA viruses. Oncogene 2001; 20(54): 7820-3.
14. 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. DOI: 10.1128/JVI.77.22.12331-12335.2003
15. Harkins L, Volk AL, Samanta M, Mikolaenko I, Britt WJ, Bland KI, et al. Specific localisation of human cytomegalovirus nucleic acids and proteins in human colorectal cancer. Lancet 2002; 360(9345): 1557-63. DOI: 10.1016/S0140-6736(02)11524-8
16. Knosel T, Schewe C, Dietel M, Petersen I. Cytomegalovirus is not associated with progression and metastasis of colorectal cancer. Cancer Lett 2004; 211(2): 243-7. DOI: 10.1016/j.canlet.2004.02.011