



Investigation of JC Polyomavirus (JCV) Genome in Colorectal Cancer Patients from Iran

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Abstract

Background: JC polyomavirus (JCV) is an epitheliotropic and neurotropic virus that identified in relationship with some devastating complications such as progressive multifocal leukoencephalopathy (PML) and linked to colorectal cancer. The aim of current study was to identify the prevalence of JCV in colorectal cancer for the first time in Iran.

Methods: This retrospective case-control study was conducted by the hospitals affiliated to Iran University of Medical Sciences, Tehran, Iran from 2011 to 2016. Formalin-fixed paraffin-embedded (FFPE) blocks were used for DNA extraction by QIAamp® DNA FFPE Tissue Kit. The SYBER Green Real-time PCR assay performed by specific primers for JCV T-Large Ag. Melting curve analysis used for evaluation of amplification specificity. Positive control cloned in pTZ57R/T plasmid by Generey Biotechnology system.

Results: Of 157 specimens 66 were colorectal cancer by the mean age (y) ± std. deviation 59.35±14.48 and 91 healthy control by the mean age (y) ± std. deviation 57.21±14.66. All 157 specimens tested for JCV T-Large Ag gene by Real-time PCR method and we found that there was not any positive result although the melting analysis showed specificity of positive control amplification.

Conclusion: Low prevalence of JCV infection in Iranian CRC population confirmed by the current study results; there was not any JCV positive result in CRC and healthy control groups. Further studies by broader and different populations are recommended.

Keywords: JC virus; Colorectal cancer; Real-time polymerase chain reaction

Introduction

John Cunningham virus (JCV) is a common polyomavirus that infected about 80% of human population (1). JCV has some genetic similarity to the BK and SV40 polyomaviruses (2). These viruses are classified as *Polyomaviridae* family. They

have a circular DNA molecule that packaged in the mini-chromosome like structure (3, 4). JC virus first was described by the electron microscopy in 1965 (5).

JCV transmission route is still undetermined but its primary infection occurred in childhood (4). Generally, majority of infected patients have no symptoms. JCV initial infection may occur in the tonsils or gastrointestinal tract and then it could establish a latent infection there. Also, it could become latent in kidneys, where its replication causes virus shedding in the urine (2, 4). The virus could penetrate into central nervous system (CNS), probably by 5-HT_{2A} serotonin receptor and infects oligodendrocytes and astrocytes (1, 3). Reactivation of the virus could be triggered by serious immunosuppression, transplantation, and advanced HIV disease. Progressive multifocal leukoencephalopathy (PML) is one of the serious demyelinating diseases associated with JCV (6, 7).

Recently, the JCV genome was detected in colorectal cancer (CRC) patients which may indicate the probable role of this virus in the development of cancer (8, 9). Globally, CRC is the third most common cancer in humans, and accounts for 10% of all cases of cancer (10). In 2012, approximately 1.4 million new cases of CRC and 694,000 deaths occurred, and the five-year survival rate of these patients in the United States is about 65% (10). Early diagnosis of colonic adenoma infected with JCV could be helpful for using suppressive procedures in treatment strategies and to prevent its progression to CRC (11, 12).

The purpose of this study was to evaluate the prevalence of JCV in CRC cases against a control group.

Materials and Methods

Research Population

The current study was designed as a retrospective case-control study conducted by the hospitals affiliated to Iran University of Medical Sciences, Tehran, Iran from 2011 to 2016.

All participants were diagnosed by an expert pathologist and written consent was obtained from each one. Ethics were approved by the ethical committee of Iran University of Medical Sciences, Tehran, Iran via record number IR.IUMS.REC 1394.26669.

Overall, 157 archived formalin-fixed paraffin-embedded (FFPE) blocks were collected from patients with CRC (66 cases) and healthy subjects (91 cases). Age and sex matching between cases and control group was done. Endoscopy and colonoscopy for each participant underwent by Fujinon machine (Fujinon, Japan). Patient's data were recruited from the medical record repository. Inclusion criteria for CRC patients were being sporadic cases, non-familial cancerous cases and written consent. Matched age and sex cases that underwent total colonoscopy organized as healthy control group and they had not any malignant lesions. Pathologic slides were stained by Hematoxylin and Eosin (H&E) method and reviewed by a sophisticated pathologist.

DNA isolation

Samples were dissected by Vibrating microtome (Leica VT 1000S, Leica Microsystems). The 20 micron dissection was used for deparaffinization and then genomic DNA extraction by QIAamp® DNA FFPE Tissue Kit (QIAGEN, Hilden, Germany), for each sample according to manufacturer's protocol. Evaluation of isolated DNA purity was quantified by NanoDrop ND-1000® (Thermo Fisher Scientific Inc., Waltham, MA, USA) spectrophotometry and then well-purified ones kept at -20 °C before further use.

Real Time PCR assay

A SYBR Green Real-time PCR assay was performed for detection of JCV genome presence in each sample. The Rotor-Gene-Q 6000 thermocycler (Corbett, Australia) was used for the Real-time PCR assay. Specific forward 5'-TGA GGA ATG CAT GCA GAT CTA C-3' and reverse 5'-TTT GCA GGG CAT TTT GTT TTT TAC-3' primers were used from previous work (6). In addition, reaction specificity was achieved by melting curve analysis based on previous study. To improve the accuracy of the analysis, samples were duplicated. About 15 µl reaction mix was composed of 7.5 µl SYBR RT-PCR Master Mix (Odense M, Denmark) which corresponded to 1X concentration in each tube, 0.5 µl of each forward and reverse primers (10 pmol/µl), 0.2–0.5

µM concentration of each samples or controls, and distilled water added for the rest of the total volume. Cycling program and temperature were as following: 1 cycle of 95 °C for 5 min, 40 cycle of 95 °C for 30 sec and 60 °C for 30 sec (fluorescent acquisition was performed at the end of this step). Melting curve was adjusted for 50 to 99 °C by 5 sec Intervals. Positive control was cloned at pTZ57R/T plasmid by Generay Biotechnology (Generay Biotechnology Co, Shanghai, China).

Statistics

SPSS 16 software (Chicago, IL, USA) used for data analysis. Qualitative variables analyzed by

Chi-square or Fisher test. P-value <0.05 was considered statistically significant.

Results

Study population

All samples were tested for JCV by a Real-time PCR assay. Of 157 specimens 66 were CRC cases by the mean age (y) ± std. deviation 59.35±14.48 and 91 healthy control by the mean age (y) ± std. deviation 57.21±14.66. Table 1 summarized demographic and pathologic characteristics of each groups.

Table 1: Demographic and pathologic characteristics of our study population (n=157)

Category of variables	Variables	Male (%)		Female (%)		Total (%)	
		Case	Control	Case	Control	Case	Control
Descriptive	No.	38 (57.6)	52 (57.1)	28 (42.4)	39 (42.9)	66 (100)	91 (100)
	Mean age	59.13	56.50	59.64	58.15	59.35	57.21
	Std. Deviation	14.601	15.624	14.589	13.412	14.486	14.661
	Std. Error of Mean	2.369	2.167	2.757	2.148	1.783	1.537
	Range	27-81	15-89	28-85	26-81	27-85	15-89
Sample location	Colon	15 (39.5)	15 (28.8)	14 (50.0)	14 (35.9)	29 (43.9)	29 (31.9)
	Rectum	10 (26.3)	16 (30.8)	3 (10.7)	9 (23.1)	13 (19.7)	25 (27.5)
	Cecum	7 (18.4)	5 (9.6)	4 (14.3)	11 (28.2)	11 (16.7)	16 (17.6)
	Ileum	1 (2.6)	4 (7.7)	1 (3.6)	1 (2.6)	2 (3.0)	5 (5.5)
	Sigmoid	5 (13.2)	12 (23.1)	6 (21.4)	4 (10.3)	11 (16.7)	16 (17.6)
Differentiation	Well	21 (55.3)	-	15 (55.6)	-	36 (55.4)	-
	Moderate	13 (34.2)	-	8 (29.6)	-	21 (32.3)	-
	Poorly	1 (2.6)	-	-	-	1 (1.5)	-
	Undifferentiated	3 (7.9)	-	4 (14.8)	-	7 (10.8)	-
Lymph node involvement	Involved	10 (26.3)	-	9 (32.1)	-	19 (28.8)	-
	Not involved	28 (73.7)	-	19 (67.9)	-	47 (71.2)	-
Tumor stage	T1	4 (10.5)	-	2 (7.1)	-	6 (9.1)	-
	T2	6 (15.8)	-	3 (10.7)	-	9 (13.6)	-
	T3	18 (47.4)	-	18 (64.3)	-	36 (54.5)	-
	T4	10 (26.3)	-	5 (17.9)	-	15 (22.7)	-
Mucinous	Mucinous	14 (36.8)	-	6 (9.1)	-	20 (30.3)	-
	Non-mucinous	24 (63.2)	-	22 (33.3)	-	46 (69.7)	-
Tumor grade	High grade	16 (42.1)	-	11 (16.7)	-	27 (40.9)	-
	Low grade	22 (57.9)	-	17 (25.8)	-	39 (59.1)	-
Total		90(57.3)		67(42.7)		157 (100)	

Real-Time PCR assay

Real-time PCR assay performed for all specimens for detection of JCV T-Large Ag gene by specific

primers. Although melting curve analysis showed reasonable curve without non-specific products (Fig. 1), from all of 157 specimens there was not

any positive result and we found that all 157 specimens were negative for JCV genome infec-

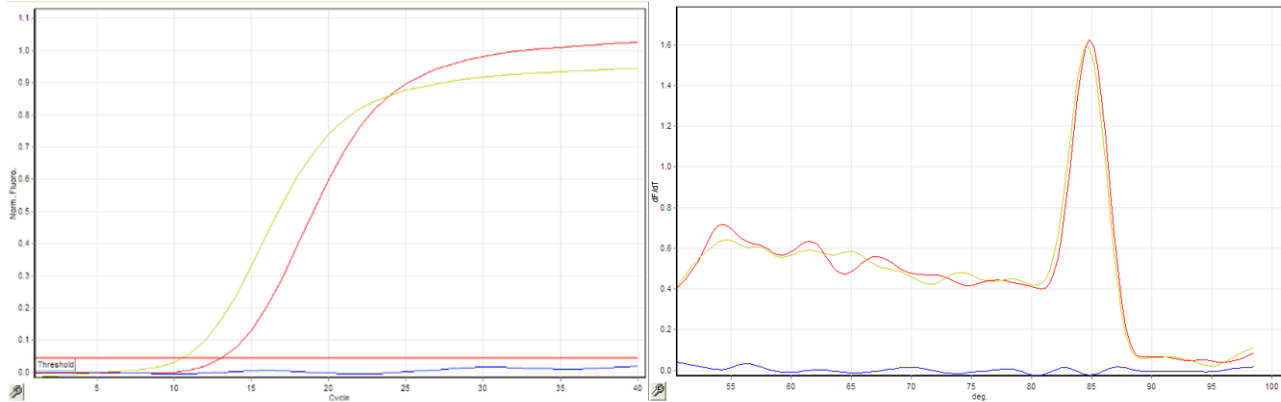


Fig. 1: Melting curve analysis and cycling curve for duplicate positive controls

Statistics

Analysis of different demographic and pathologic variables showed majority of them were not any significant result, although CRC more occurred in males. However, rectum location and mucinous type of CRC in males were statistically significant ($P < 0.05$).

Discussion

JCV initial infection occurs in tonsils and digestive tract (6). In some studies, the JCV virus genome was found in sewage and environmental samples. The virus can be transmitted through the fecal-oral routes (13, 14). There were some reports that showed JCV potential roles in carcinogenesis but there are not strong association in JCV infection and human cancers. Furthermore, studies on JCV oncogenesis have been restricted to central nervous system (CNS) and rare studies in CRC patient's tissue and compare them with the disease-free control tissue was done carried on (7). There was no study on the prevalence of JCV infection in gastrointestinal cancer in Iran (3). Indeed, the aim of this study was to evaluate the infection rate of JCV genome in the CRC patients against healthy control subjects. By the present study results, based on the Real-Time PCR data, none of 157 samples was infected with JCV

tion.

genome. JCV prevalence in patients with CRC involvement and healthy individuals is low.

In a study, no association was found between JCV and CRC although the authors used CRC cases and non-cancerous healthy tissue. Overall, 233 CRC cases were used and 233 healthy control tissue but only one from control group was positive for JCV genome infection by PCR method (15). JCV had not linked to CRC, previous studies could get positive results due to the ubiquity of JCV and probable contaminations, and JCV was not causing of genetic instability in CRC. Moreover, a study (16) on 386 CRC males cases and 386 matched controls, that had high seropositivity rate (97%-100%), showed there was not any significant association between JCV genome infection and CRC by PCR method in their tissue samples (OR, 0.9; 95% CI, 0.7-1.3). Probably PCR method probably had not enough specificity to detect JCV in colonic tissue. Our result was same as these findings that showed the prevalence of JCV infection in our population is very low or the probable low specificity of our molecular method that we used.

In a study, 19 tissue samples of patients with CRC lesion were studied and 10 healthy control groups using nested-PCR method (11). The authors identified 21% (4/19) JC virus infection in cases tissue and no positive result in control group tissue. Of 4 JCV positive patients 3 (75%)

had liver metastases. JCV could have broader role in CRC involvement than we thought before and in the end stages of these tumors it has crucial roles. We found the same rate of infection by JCV in the control healthy subjects although there was a great difference in our cases infection rates. This discriminate difference was probably due to the study group of that study cases. They used HCV positive CRC cases that are in higher risk for viral infections due to their immunocompromised situation.

Burnett-Hartman et al (7) reviewed five studies in order to investigate relationship between colorectal cancer and presence of DNA genome of JC virus. They reported presence of genome of this virus in patients with CRC as 26 to 89 percent. Study on 27 well-characterized epithelial malignant tumors of the large intestine showed there were 22 (81%) JCV presence of the viral early genome (17). Study in CRC, adenomas and normal tissue samples by nested PCR for T antigen, VP and agnoprotein, showed T antigen was detected in 26.1% (6/23), 4.8% (1/21) and 0% (0/20), respectively (18). However, they could not find any positive results by VP and agnoprotein nested PCR settings. Their findings showed T antigen integration in colonic tissue without clear and diffuse protein expression could act in oncogenesis process and might use for the hit-and-run mechanism.

Findings of the current study showed that there was no relationship between CRC and infection with JCV genome. Of course, it may be due to low prevalence of the virus in our population or low viral titer in the samples subjected to current study. There were some limitations in the current study that they included lack of using other confirmatory test such as TaqMan qRT-PCR or nested PCR, loss of high quality of genomic DNA by some unexpected thawing the extracted samples, using one section of each blocks that could reduce our chance to find the viral infection and lack of using ELISA method for detection of anti-JCV Ab in patient's sera. Further studies by broader subjects and using sera anti-JCV Ab results with at least on confirmatory test and using several dissections of one patient are recommended.

Conclusion

Although there were some evidence of transformation capacity of JCV and high prevalence of JCV genome in colorectal cancer patients, our finding declares there was not any relationship between colorectal cancer and JCV infection in Iranian subjects and we had not any JCV infection in large intestine tissue of healthy non-malignant lesions. Further studies suggested for more comprehensive results.

Ethical considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

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Conflict of interests

None to declare.

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