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Molecular detection of john cunningham virus (JCV) in patients with colorectal cancer in khartoum, Sudan

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Abstract

Background: Colorectal cancer (CRC) is the most repetitious malignancies with high mortality worldwide. JC virus (JCV) is ubiquitous Polyomavirus, with seroprevalence rates ranging from 70% to 90% in adult population. Recently the roles of JCV have been reported in many malignant tumors worldwide. The association of JCV was reported in patients with colon and rectum cancers

Objective: the study was conducted to evaluate the association of JCV DNA in patients with colorectal cancer in Khartoum State.

Materials and methods: A total of 70 formalin-fixed paraffin-embedded tissue block samples were collected including ***33*** (47.1%) male ***37***(52.9%) female patients with CRC. DNA was extracted from all the samples. Nested PCR was carried out for detection of Vp1/T Ag junction genome in JCV genome by Nested-PCR assay.

Results: 3/70(4.2%) samples of patients were positive for JCV DNA (P 0.522). Out of 3 samples positive for JC DNA, 2(2.8%) were males and 1(1.4%) was female.

Conclusion: prevalence of JCV DNA was 4.2% among patients with CRC. Including 2.8% male and 1.4% female (p= 0.522). The subsequent T-Ag protein expression might explain the increased risk of colorectal cancer and requires further investigation

Keywords: JC virus- colorectal cancer- polymerase chine reaction

Introduction

Colorectal cancer (CRC) is the most recurrent malignancy, and third leading cancer with high mortality among men and women worldwide [1]. The pathogenesis of this tumor is not well known. Predisposing factors include Crohn's disease, celiac disease, hereditary genetic syndromes and dietary factors [2]. JCV is associated with disease primarily in immune compromised individuals and its replication in glial cells could lead to progressive multifocal leukoencephalopathy (PML), an often-fatal disease of central nervous system [3]. More recently, JCV was also found in non-neural cancers, such as gastric [4] and lung cancers [5]. JCV infection was first reported as a potential risk factor for colorectal cancer (CRC) in a work by Laghi *et al*. [6], which found that 96% of CRC tissues were positive for JCV DNA sequences. Subsequently, other studies have been published showing that JCV sequences were found in 26%–88.9% of CRC tissues [7, 10].

The John Cunningham virus (JCV) is a ubiquitous, small, non-enveloped polyomavirus with a closed, circular, double-stranded DNA genome that frequently resides in the kidneys of healthy individuals and is excreted in the urine of a large portion of the adult population. JCV infection is subclinical and leads to lifelong latency, but may be reactivated when the immune system is impaired [11, 13]. About 90% of the adult population carries antibodies to the virus, and it seems that in most people, the virus remains latent [14]. High concentrations of JCV have been observed in urban sewage worldwide; therefore, it is suspected that the contaminated water is a typical route of JCV infection [15]. JCV encodes three proteins that are the structural capsid proteins: VP1, VP2, and VP3 [16]. JCV also encodes the T antigen proteins (i.e., Large T and multiple small t splice variants) and the agnoprotein which is involved in the assembly of viral particles. T-Ag has several splice sites producing truncated proteins that are involved in regulating cell proliferation and viral transmission [17]. Like other polyomaviruses, JCV encodes a version of a large T-antigen that can bind to and inactivate tumor suppressor proteins p53 and pRB and interfere with several cell-signaling pathways [18, 20]. Moreover, JCV genomic DNA sequences and T-antigen expression have been detected in a broad range of tumor cell types including oligodendrocytes, astrocytomas, glioblastomas, ependymomas, and most other types of brain tumors, indicating that JCV infection may be associated with human carcinogenesis [21]

Studies on association of JCV and colorectal in Sudan is limited, therefore, this study was conducted to evaluate the rate of JCV in patients with CRC in Khartoum city.

Material and Method

Sample preparation

A total of 70 formalin-fixed paraffin-embedded tissue blocks samples were collected from70 patients including 33(51.4%) males and 37(48.6%) female patients (mean age –55) with CRC during May 2018 to May 2019.

Deparaffinization

Deparaffinization was done using xylene and ethanol (Germany, Merk). Initially, all the specimens were placed in microtubes then xylene was added and kept at 45oC for 15 min followed by centrifugation at 14,000 rpm. This step was repeated once more and the supernatant was discarded and 1ml absolute ethanol was added to precipitate and stored at the room temperature for 10 min and centrifuged again at 14,000 rpm for 1 minute. The supernatant was discarded. This process was repeated by adding 70% ethanol, followed the same condition. Finally, supernatant was discarded and all microtubes were placed at 65oC for 5 min to vaporize the ethanol residue and the pellet was used in DNA extraction (Bouzari M, The 4th national Biotechnology Congress Islamic Republic of Iran; 2005)

DNA extraction

DNA extracted from deparaffinizd tissue by guanidine chloride (Black-well laboratory Cambridge, UK) as fallow: samples were subject to lyses solution containing 400mMNaCl, 6M guanidine chloride and 300 µl of 7.5% ammonium acetate and heat treated at 98°C for 20 minutes in water bath. After cooling10µl (20 mg/ml stock) proteinase K was added and incubated for overnight at 56°C. On day two, second heat treatment was applied by incubating samples at 98 °C for 5 minutes in a water bath. After cooling 10µl proteinase k was added, briefly vortexed and incubated at 56 °C for overnight. During the whole incubation period samples were put on a shaker at interval for about 30 minutes. Chloroform was then added, the supernatant was collected, and DNA was precipitated by ethanol, dissolved in 100 µl TE storage buffer. The purity and quality of the extracted DNA was analyzed based on absorbance of the extracted DNA at 260 and 280 nm wavelengths using a spectrophotometer (NanoDrop-1000, Thermo Fisher Scientific, and Wilmington, USA)

Semi-nested PCR assay to detect JCV DNA

The semi nested PCR was carried out as described in [22] using three primers to amplify JCVT-antigen sequences as follows: T-antigen: T1 (TGGCCTGTAAAGTTCTAGGCA) and T2 (GCAGAGTCAAGGGATTTACCTTC) and Second PCR amplification was conducted using sets of primers for T1 and T3 (AGCAACCTTGATTGCTTAAGAGA) [23]. Three primers display sequences that avoid annealing with Polyomaviruses other than JCV nucleotides. Each reaction was performed in 25 µl volume containing 5 µl master mix, 1 µl of each primer (1 and 2) for the first round and (1 and 3) for the second round, 7 µl of DNA, and 11 µl of distilled water. Reactions were performed using PCR machine under the following cycling conditions: 8 min at 95ºC followed by 35 cycles of 30 s at 95ºC, 30 s at 55ºC and 30 s at 72ºC. A final elongation period of 7 min at 72ºC was applied. 5 µl of the PCR product was analyzed by gel electrophoresis in 2% Agarose, and stained with 0.15% Ethidium bromide and the product was visualized by using UV gel documentation system INGeNius Germany. The expected size of JCV DNA amplicon is 145 bp.

Statistical analysis

Collected data were analyzed using statistical package for social science (SPSS version 16.0). A p value of ≤ 0.05 was considered significant.

Result

In this investigation 3/70(4.2%) samples of CRC were positive for JCV. 2/36 (5.5%) were from male patients while 1/34 (1.4%) was from a female patient with (p=0.589) and of the age groups 41-60 yrs. (Tables 1 and 2). There was no significant association between gender and prevalence and JCV positivity

Table 1: Frequency of positive result according age group

		Age							Total
		10 - 20	21- 30	31 - 40	41- 50	51- 60	61- 70	71- 80	
result	positive	0	0	0	2	1	0	0	3
	negative	5	7	10	14	12	12	7	67
Total		5	7	10	16	13	12	7	70

Table 2: Frequency of positive result according to gender

		Sex		Total
		Male	Female	
Result	Positive	2	1	3(4.2%)
	Negative	34	33	67(95.8%)

Discussion

CRC is one of dominant cause of death among man and women worldwide. Several viruses were suggested to be the risk factors for CRC. Among them, JC virus, BK virus, Human Cytomegalovirus (CMV), Human papilloma viruses (particularly type 16 and 18) have the largest number of report [24]. High prevalence of 82% and 90% of JC virus associated colorectal carcinoma have been reported in Portugal and USA respectively [25, 26]. In the present study 4.2% of our CRC patients were positive for JCV DNA in agreement with results reported by Mou *et al*., (2012) in China [27]. Sarvari *et al*., (2018) have reported low prevalence of 1.42% JCV DNA in patients with CRC, in Shiraz city, Iran, which was lower than our finding [28]. In the present study the rate of JCV DNA was among the male (2.8%) and female (1.4%) patients in contrast to Shalaka *et al*., (2014), who reported that the rate of JCV in male patients with CRC is higher than in females [26]. Recent investigations revealed that, infected patients with JC virus in immunodeficiency status may results in progress to PML, thus, treatment of patients infected with JC virus by rituximab natalizumab and efalizumab lead to progressive multifocal leukoencephalopathy. Therefore, it is suggested that the urine of patients with CRC cancer or patients with autoimmune diseases should be screened for JC virus DNA before chemotherapy treatment or by immunomodulatory drugs (rituximab and natalizumab) therapy [29, 30].

Conclusion

In the present study 4.2% of our study population was found positive for JCV using nested PCR, and no significant differences according to age and sex were discernable. Our present study also represents the first report on JCV infection in colorectal patients in Sudan.

Ethical review

The study was approved by the Ethical Review Committee (ERC) of Alneelain University, the Ministry of Higher Education & Scientific Research, Khartoum State, Sudan.

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