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Original Article

Association between HBV Infection and Colorectal Cancer in Iranian Population

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ABSTRACT

Background:
Colorectal cancer (CRC) as a major health problem has increased globally. The etiology of CRC is among the critical issues. Smoking, obesity, and infectious diseases are probable risk factors of CRC. Meanwhile, chronic infections, such as chronic hepatitis B (CHB) is under investigation. Objectives: The aim of the present study was to determine the rate of HBV genome infection in patients with CRC compared with healthy subjects' colon tissues.

Materials and Methods:
Archived formalin-fixed paraffin-embedded (FFPE) blocks of 157 patients who underwent total colonoscopy that were referred to hospitals affiliated to Iran University of Medical Sciences, Tehran, Iran, were enrolled. They were categorized into 66 CRC cases and healthy colon tissues as the control group. After DNA extraction from FFPE specimens a Syber Green Real-time polymerase chain reaction (PCR) method was carried out. SPSS software version 16 was used for statistical analysis.

Results:
Of a total of 157 specimens, the mean age ± std. deviation of 66 patients with CRC was 59.3 ± 14.4, and 57.6% (38.66) of them were males. The mean age ± std. deviation of 91 healthy controls was 57.2 ± 14.6, and 57.1% (52) of them were males. By using real-time PCR we found that there were 6.4% (10.157) HBV positive, of them 9% (6.66) had CRC, and 4.4% (4.91) were healthy controls. Different variables did no t have any significant differences.

Conclusion:
Although some studies reported the association between HBV infection and CRC outcome, we could not prove it. It suggests the role of other risk factors in colorectal cancer incidence. Further studies with larger sample size and different study populations are recommended.

Keywords: Colorectal cancer (CRC), Chronic hepatitis B (CHB), Hepatitis B virus (HBV)

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INTRODUCTION

Colorectal cancer (CRC) is the fourth leading cause of death in men and third in women (1). It is the first leading cause of malignancy by estimated annual new cases of 135.430 and death of 50.260 in the United States (1). CRC incidence in males is 30-40% more than females that probably depends on their exposure to certain risk factors and various screening tests for men and women. CRC is prevalent (58%) in ages more than 65 years (2). Geographical differences could influence on colorectal cancer burden as in industrialized

countries; it seems to occur more. Recently, some regions such as Eastern Europe, Latin America, and Asia showed more incidence and mortality rates of CRC, although other regions such as certain Western European countries and the United States had steadily decreased rates (1,3). The increasing rate of CRC in different populations is challenging, and the reason is multifactorial. Some of them included the Western lifestyle, such as an unhealthy diet, obesity, tobacco and alcohol consumption, and past medical history, such as chronic diseases (3,4).

Hepatitis B is a major health problem that accounts for about 1 million death annually (5,6). It is estimated that in 90% of adults, hepatitis B virus (HBV) infection is resolved spontaneously, and only 5-10% develop chronic hepatitis B (CHB) worldwide (5,6). HBV is a small enveloped DNA virus from hepadnaviridae family that can integrate its genome into the host genome (7,8). Its carcinogenesis is based on the integration of viral genome and high production of certain viral proteins such as X and pre-S. CHB is related to some malignancies, including pancreatic cancer, non-Hodgkin's lymphoma, and chronic lymphocytic leukemia. Furthermore, it seems that the early detection of colon adenoma in patients with CHB could prevent its progression to CRC by treatment strategies (9,10). Also, chronic hepatitis C (CHC) has been related to a higher incidence of colorectal adenoma (10).

To our knowledge, there is limited data about HBV infection in CRC and benign colon lesions and its relation between different pathologic data. So, we aimed to investigate HBV genome infection in CRC and benign colon lesions.

MATERIALS AND METHODS

Study population

This study was conducted in hospitals affiliated to Iran University of Medical Sciences, Tehran, Iran, from 2011 to 2017. All samples were collected after the diagnosis by an experienced pathologist. Consent was obtained from each patient. Ethics was approved by the ethics committee of Iran University of Medical Sciences, Tehran, Iran (IR.IUMS.REC 1394.26663).

The data of 157 subjects were used for analysis. Archived formalin-fixed paraffin-embedded (FFPE) blocks were collected from each patient for HBV investigation. Fujinon machine (Fujinon, Japan) was used for total endoscopy and colonoscopy for each patient. 66 cases were CRC patients, and 91 were healthy subjects. Patients' data were obtained from the medical record repository. CRC patients were sporadic, and non-familial cancerous and healthy subjects underwent total colonoscopy and did not have any malignant lesions. Healthy subjects were collected based on pathology data which have not diagnosed by any malignancy. Stained slides with Hematoxylin and Eosin (H&E) that were reviewed by experts were used for further analysis.

DNA isolation

Specimens were dissected by vibrating microtome (Leica VT 1000S, Leica Microsystems) into a 20 micron dissection. After deparaffinization, DNA extraction was done by QIAamp® DNA FFPE Tissue Kit (QIAGEN, Hilden, Germany), according to the protocol. Isolated products were evaluated by NanoDrop ND-1000® (Thermo Fisher Scientific Inc., Waltham, MA, USA) spectrophotometry. Purified DNA was kept at-20˚C until use.

Real-time polymerase chain reaction (PCR)

The Rotor-Gene-Q 6000 thermocycler (Corbett, Australia) was used for real-time PCR analysis. The positive and negative controls and samples were all tested in a duplicate manner. 15µl reaction mixture contains 7.5 µl SYBR RT-PCR Master Mix (Odense M, Denmark) (1X concentration), forward and reverse primers 0.5 µl corresponds to 10 pmol/µl, each sample or controls 0.2–0.5 µM concentration, and the rest of the tube was filled by distilled water. Primers were designed for HBV X gene as following: HBVX-F 5′-GGCCATCAGCGCATGC-3′, and reverse primer HBVX-R, 5′-GCTGCGAGCAAAACA-3′; specificity of primers and amplification was achieved by melting curve analysis. The Thermocycler program was as follows: 1 cycle at 95˚C for 5min, 40 cycle at 95˚C for 30s, and 60˚C for 30s. The fluorescent light acquisition was in annealing/extension. The melting curve was drawn by 5s intervals at 50 to 99˚C. For positive control, we cloned the HBV X gene primers region in pTZ57R/T plasmid by Generay Biotechnology (Generay Biotechnology Co, Shanghai, China).

Table 1: Details of our study participants characteristics (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)	

Carryover contamination was controlled by using AmpErase (PE Applied Biosystems) and dUTP (PE Applied Biosystems) within the master mix.

Real-time PCR standardization

The amplified region of primer sequences was cloned at pTZ57R/T plasmid by Generay Biotechnology (Generay Biotechnology Co, Shanghai, China). A commercially available cloning system (TA Cloning kit of Invitrogen, San Diego, CA) was used in the current study. Synthetized positive control was diluted by distilled water into 20 pmol/µL and used for further analysis.

Statistical analysis

SPSS software version 16 was used for the analysis of different variables. Analysis of classified and qualitative variables was done by Chi-square or Fisher test, and p value ≤ 0.05 was considered statistically significant.

RESULTS

Study population

Of a total of 157 specimens, the mean age ± std. deviation of 66 patients with CRC was 59.3 ± 14.4, and 57.6% (38.66) of them were males. The mean age ± std. deviation of 91 healthy controls was 57.2 ± 14.6, and 57.1% (52) of them were males. The demographic data of each group is shown in table 1.

Table 2: Details of our HBV positive results (n = 10)

Category of variables	Variables	Male (%)		Female (%)		Total (%)	
		Case	Control	Case	Control	Case	Control
Descriptive	No.	4	2	2	2	6	4
	Mean age	55.3	56.5	54.6	57.6	54.9	57.05
	Std. Deviation	6.2	5.2	4.8	3.1	4.6	4.1
Sample location	Colon	2	0	2	1	4	1
	Rectum	1	1	0	0	1	1
	Cecum	0	0	0	1	0	1
	Sigmoid	1	1	0	0	1	1
Differentiation	Well	2	-	1	-	3	-
	Moderate	1	-	1	-	2	-
	Undifferentiated	1	-	0	-	1	-
Lymph node involvement	Involved	1	-	1	-	2	-
	Not involved	3	-	1	-	4	-
Tumor stage	T1	1	-	0	-	1	-
	T3	2	-	2	-	4	-
	T4	1	-	0	-	1	-
Mucinous	Mucinous	1	-	0	-	1	-
	Non-mucinous	3	-	2	-	5	-
Tumor grade	High grade	2	-	0	-	2	-
	Low grade	2	-	2	-	4	-
Total		6		4		10	

Real-Time PCR assay

Overall, real-time PCR for detection of HBV X gene by specific primers was done for 157 specimens. Of them 6.4% (10.157) were HBV positive, which six were from cancerous patients, and four were from non-malignant tissues. Table 2 summarizes the details of 10 positive results, and figure 1 shows graphs of some positive results.

Statistics

Different variables were analyzed by appropriate statistical tests. There was not any association between CRC and HBV infection ($p > 0.05$). Other demographic variables were not significant, although there was higher HBV incidence in colon location, well differentiated, not-involved lymph node, T3 tumor stage, and low-grade tumors.

DISCUSSION

CRC, as a major human malignancy, showed a significant increase in recent decades, especially in developed countries (1). Although studies have been focused on CRC therapeutic procedures, epidemiological, as well as etiological aspects of CRC is more important (4). Meanwhile, HBV, as a major public health problem, affected about 2 billion people, and 248 million of them are chronically infected (CHB). There is some evidence that showed HBV infection was correlated with a higher rate of colorectal malignancy (7,11,12).

In the present study, we investigated HBV infection in CRC and healthy colon tissues to identify the correlation between HBV infection and colon malignancy in the Iranian population. We examined 66 CRC cases and 91 colon tissue samples of healthy people. A Syber Green real-time PCR method by specific primers was used for the rest of 157 FFPE extracted viral genomic DNA. Totally, we found 10 (6.4%) positive results that of them 6 (9%) were in the CRC group and 4 (4.4%) were in the healthy group. Statistics couldn't show any associations between different variables and HBV incidence. Our study

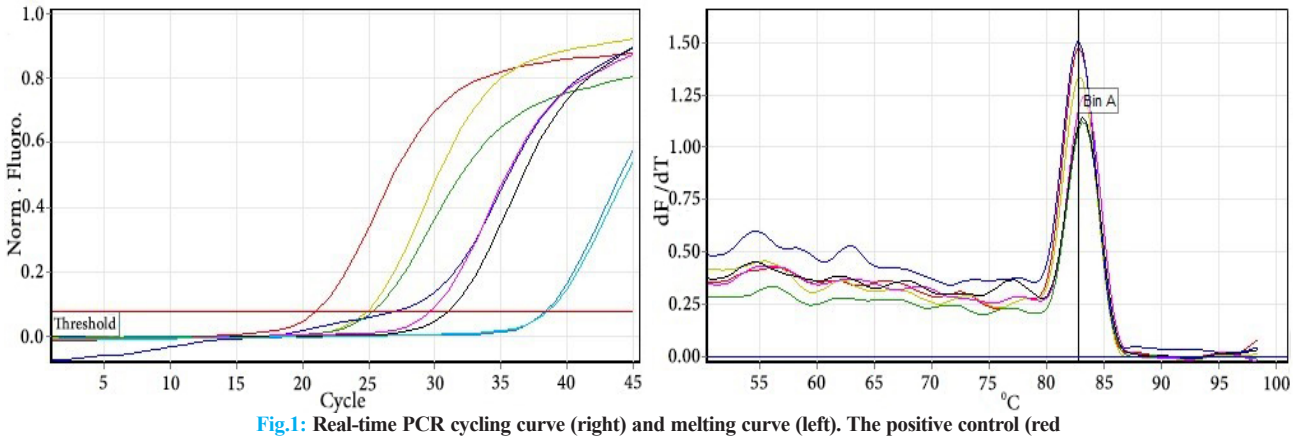

Fig.1: **Real-time PCR cycling curve (right) and melting curve (left). The positive control (red line) Ct was 21 by the threshold lower than 0.1.**

limitation was the lack of HBV antibody tests due to unavailable patients' sera and limited sample size.

HBV carcinogenesis has been investigated, and studies showed the interference of HBV proteins in different pathways P53, PB Wnt/ β-catenin, transforming growth factor β (TGF-β), and Ras signaling (13). HBV X protein (HBx) could interfere with P53 and proceeds malignancy. The integration of HBV DNA is another mechanism for establishing chromosomal instability and cancer (13, 14). Not only liver cells are the main region for HBV replication and its complications, but also extrahepatic malignancies such as polyarteritis nodosa glomerular disease are related to CHB (15, 16). Rare studies assessed the association between colorectal cancer and HBV infection. Based on our findings, we could not find any relationship between HBV infection and CRC. It could be related to the interfering risk factors such as ethnicity. However, studies showed that African American people had a higher rate of CRC compared with whites (17,18).

Patel and colleagues (10) studied the link between HBV infection and colorectal adenoma in 2015. They used 558 consecutive patients that were screened by colonoscopy and had previous hepatitis B history. Of them, 487 patients were in control group and 71 patients were in the hepatitis B group. They found that 23.9% of the hepatitis B group and 15.9% in the non-hepatitis B group had adenoma in colonoscopy, but it was not statistically significant. They reported a significant result in a higher number of adenomas in the distal colon vs. control group. Other demographic and pathologic variables were not significant.

Fahal and others (11) studied the association between different gastrointestinal tract tumors and HBV infection, but they did not find any significant results although they reported HBsAg in primary hepatocellular (25%), gastric (12%), rectal (10%), and colonic carcinoma (8%). Huo and co-workers (12) assessed the effect of chronic hepatitis B infection on the risk of synchronous colorectal liver metastasis (synCRLM) in 4033 consecutive patients with newly diagnosed CRC with hepatitis B and found the prevalence of synCRLM was significantly higher in the HBsAg+ patients than that in the HBsAg– patients and concluded that the concomitant chronic HBV infection significantly increased the risk of CRLM.

Kim and colleagues (19) studied 133 CHB patients who underwent colonoscopy with a matched healthy control group and found that the HBV group had a higher rate of the colorectal polyp, colorectal adenoma, advanced adenoma, and colorectal cancer than the control group. Also, they reported HBV DNA positivity was significantly associated with colorectal adenoma and advanced adenoma.

In conclusion, although there were some associations between HBV infection and colorectal malignancies, others did not find significant results. Also, our findings did not show any associations. Our limitations could affect the findings for which we recommend further studies with larger sample sizes and HBV serologic tests.

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CONFLICT OF INTEREST

The authors declare no conflict of interests related to this work.

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