Investigate the Relationship Between the Presence of JCV DNA and the Immunohistochemical Expression of CK2 0, CK7, and CDX2 in Colorectal Cancer

Hind Jaber Hassoon, Jasim Mohammed Muhsin

Department of Medical Laboratory Techniques, College of Health and Medical Techniques, Middle Technical University (MTU), Baghdad, Iraq

Abstract

Background: Globally, colorectal cancer (CRC) is the most common malignancy and has a high fatality rate. Early childhood the John Cunningham virus or JC virus (JCV) infection persists throughout life and has been linked through multiple routes to colorectal cancer. The expression of cytokeratins 7 (CK7), 20 (CK20), and CDX2 have been investigated in a variety of primary and metastatic carcinomas, and their patterns of expression may be used to determine the site of origin of metastatic carcinomas. **Objectives:** The aim is to assess the relationship between JCV DNA and tumor markers (CK7, CK20, and CDX2) in patients with colorectal cancer, this study was carried out. **Materials and Methods:** Ninety CRCs (45 of which were squamous cell carcinomas and 45 of which were adenocarcinomas) had their paraffin sections randomly chosen, extracted, and immunostained for CK7, CK20, CDX2, and for the detection of JCV DNA by real-time (PCR). **Results:** JCV DNA was detected in 22 (24.4%) of AD-CRC and 24 (26.7%) of SCC-CRC ($P = 0.004$). The presence of JCV was significantly correlated with tumor stages ($P = 0.04$) and age categories ($P = 0.05$). Moreover, JCV presence was significantly correlated with all studied tumor markers ($P < 0.05$). **Conclusion:** JCV might play a role in the development of colorectal cancer, and CDX2, which is highly specific and sensitive as markers of colorectal origin should be helpful in the detection of intestinal adenocarcinomas.

Keywords: CDX2, CK20, CK7, Colorectal cancer, JC polyomavirus

Introduction

The cells of the colon or rectum, which are components of the large intestine, are where colorectal carcinoma develops. It is the third most prevalent cancer in the world after lung and breast cancer in women and lung and prostate cancer in men. It is a common and potentially fatal condition that can afflict both men and women. Additionally, it ranks third in both sexes for deaths from cancer. Age, family history, lifestyle choices, and genetic susceptibility are just a few of the variables that can affect the development of colorectal cancer.[1,2]

Progressive multifocal leukoencephalopathy (PML), a form of opportunistic infection, can be brought on by the JC virus, a human polyomavirus. While JCV is seen in 40% of normal colon mucosa, a higher prevalence of JCV (90%) has been seen in cases of colorectal cancer, which is thought to occur in people with compromised immune systems. The influence of viral agents in CRC has not been thoroughly researched. The connection between polyomaviruses and CRC has received a lot of attention recently, however, research findings are conflicting.[2]

The normal gastrointestinal (GI) system contains JCV, which has been connected to colorectal cancer in humans. JCV encodes the three structural capsid proteins VP1,

Address for correspondence: Dr. Hind Jaber Hassoon, Department of Medical Laboratories Techniques, College of Health and Medical Techniques, Middle Technical University (MTU), Baghdad, Iraq.
E-mail: hindjaber@mtu.edu.iq

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VP2, and VP3. The T antigen proteins known as large T and various small t splice variants, which are required for the creation of viral particles, are also encoded by JCV. The tumor suppressor proteins p53 and members of the pRB family can be damaged by T-Ag,[3] which may cause carcinogenesis in animal models. T-Ag plays a role in regulating viral transmission and cell growth. There have been reports of the presence of a wide range of human tumors, including esophageal carcinoma, colorectal cancer, anal carcinoma, and stomach cancers have been reported to contain the nucleic acid and T-Ag of JCV.[4]

In addition, cytokeratins (CKs) are a class of intermediate-sized cytoskeleton filaments seen in epithelia. There are 20 subgroups in the CK intermediate filaments. These have various molecular weights and display variable expression in various cell types and malignancies. A couple of the most useful cytokeratins include CK7 and CK20. The endometrium, ovary, breast, and lung are among the many ductal and glandular epithelia that contain the protein CK7. Merkel cells, urothelium, and the GI epithelium all express CK20.[5] In a few rare cases, colorectal carcinomas may express CK7 prominently, whereas a variety of non-colorectal adenocarcinomas may do the opposite. As a result, research into developing new, more accurate markers of intestinal differentiation is continuing, and CDX2 appears to be one of them a transcriptional component it is expressed by the caudal-type homeobox gene CDX2 and is essential for the development and differentiation of intestinal epithelial cells.[5] Previous studies showed that CDX2, which is expressed in both healthy and malignant intestinal epithelial cells with a reasonable level of sensitivity and specificity, can be used as an immunohistochemical marker for neoplasms of intestinal origin. However, CDX2 was also expressed by intestinal-shaped carcinomas and other cancers, including gastric cancer.[6,7] Due to the paucity of research on the relationship between JCV and colorectal adenocarcinoma in Iraq, this study was carried out to assess the prevalence of JCV in patients with CRC in relation to the expression of CK7, CK20, and CDX2.

Materials and Methods

Case selection and tissue samples

Archival material from 90 patients who had resection for primary colorectal cancer between February 2022 and July 2023 was used to obtain paraffin-embedded tissue slices. The Department of Pathology, Gastroenterology, and Hepatology Teaching Hospital's patient files were used to select all cases. Pathological characteristics such as the histological type, histological differentiation, depth of invasion, and lymph node status were determined using hematoxylin- and eosin-stained sections. Every patient was evaluated to confirm the diagnosis. Using WHO guidelines, the histological type was carried out. For postoperative pathological staging, the American Joint Committee on Cancer (AJCC) TNM staging method was applied.[8] For immunohistochemistry (IHC) studies, one paraffin block from each patient was used because it had the most tumors and the greatest fixing. According to the American Joint Committee on Cancer staging method, out of the 90 cases of colorectal carcinoma, 45 were categorized as adenocarcinoma and 45 as squamous cell carcinoma, 16 (17.8%) cases were categorized as T1, 34 (37.8%) as T2, 28 (31.1%) as T3, and 12 (13.3%) as T4. According to histological grading, the carcinomas were divided into 32 (35.6%) high-grade carcinomas (poorly differentiated and undifferentiated carcinomas) and 58 (64.4%) low-grade carcinomas (well-moderately differentiated). Tumors were divided into three categories based on where they were found: rectal carcinomas (48, or 53.3%), sigmoid carcinomas (22, or 24.4%), and carcinomas close to the recto-sigmoid region (20, or 22.2%). There were lymph node metastases in 20 (22.2%) of the patients. To detect the presence of JC polyomavirus infection, cut up to 25 mg sections of FFPE for DNA extraction, which is then submitted to the real-time PCR procedure.

Immunohistochemistry

Blocks of paraffin-embedded tissue were sliced into 4-μm-thick sections, which were then deparaffinized and rehydrated as usual. Slides were treated in Hydrogen Peroxide Block for 15 min to lessen endogenous peroxidase-induced non-specific background staining. Prior to immunostaining, the slides were treated with pepsin (Lab-Vision; catalog no. AP-9007) at a dosage of 1 mg/mL for CK20 for 15 min. For CK7, slides were heated in a microwave for 20 min in 10 mM citric acid at pH 6.0. Primary antibodies to CK7 (clone OV-TL 12/30, Lab-Vision; 1:50), CK20 (clone Ks 20.8, Dako; 1:50), and CDX2 (clone AMT 28, Novo-Castra; Leica Biosystems, USA, 1:50) were incubated on the slides for 60 min at room temperature. The ultra-vision detection system anti-polyvalent, HRP, Lab-Vision Secondary Detection Kit (Thermo Fisher Scientific, Fremont, CA, USA) was used to carry out the traditional avidin-biotin-peroxidase complex (ABC) method. As chromogens, AEC and DAB were employed. Mayer's hematoxylin was used as a counterstain on all of the slides. The cytoplasm, cell membrane, or both tumor cell components were positively immunostained for CK7 and CK20, as evidenced by the brown color of DAB. Negative sections were those in which epithelial cell immunostaining was completely absent. The quality of the immunostaining result was evaluated

DNA extraction and JCV DNA detection

Real-time polymerase chain reaction was used to qualitatively examine clinical samples for the presence of JCV. Following the manufacturer's instructions, a commercial kit called the gSYNCTM DNA Extraction Kit (Geneaid Biotech Ltd.) was used to extract DNA from 25 mg slices of paraffin-embedded tissue blocks. DNA was

kept until processing at −20°C. Gel electrophoresis was utilized to gauge the DNA's quality and quantity using a Nanodrop. About 5 µL of extracted DNA was placed in the instrument sample cell to perform them. An extract with a purity of (1.8–2) at an absorption wavelength 260/280 was accepted; otherwise, DNA extraction from the sample would be carried out. On the Roche Diagnostic LightCycler 2.0 Instrument, a Creative Biogene Biotechnology kit for the polyomaviruses JC (Cat. No. PDPS-AR165) was utilized to detect JCV. Initial denaturation at 95°C for 2 min (1 cycle), then denaturation for 15 s (40 cycles) at 95°C, then annealing and extension at 60°C.

Ethical approval

The study received permission from the ethical committees of the Middle Technical University (MTU) College of Health and Medical Techniques. The analysis used anonymized clinical data that were acquired after each patient gave written consent to treatment because all of the specimens were recorded, and patient identities were replaced with codes. Patients did not need to give informed consent for the trial as a result.

Statistical analysis

The chi-square test was used to assess the outcomes. *P* value of 0.05 or less were regarded as significant. All statistical evaluations were performed using SPSS 25.0 for Windows.

Results

Clinicopathological features of the study population

Figure 1 provides a summary of the cohort's clinicopathological traits. Samples from 59 (65.6%) male and 31 (34.4%) female patients were included in the study. The patients' ages ranged from 25 to 83 years, with a mean of 48.33 ± 19.52 years. The bulk of the samples were collected from the rectum in 48 (53.3%) cases, while 22 (24.4%) samples came from the sigmoid colon and 20 (22.2%) samples came from other areas of the large bowel. Histologically, 56 (62.2%) of the tumors were adenocarcinomas and 34 (37.8%) were SCCs, 58 (64.4%) of the malignancies were low grade, compared to 32 (35.6%) that were high grade. Distribution based on the size of the tumor T2 instances made up 34 (37.8%), T3 cases made up 28, T4 cases made up 12, and T1 cases made up 16.7%. Finally, according to lymph nodes status (N of TNM), 70 (77.8%) cases showed no nodal involvement, while 20 (22.2%) cases presented with nodal involvement [Table 1].

The association of clinicopathological features with JCV infection

In this study, JCV DNA was detected in 22 (24.4%) samples of AD-CRC and 24 (26.7%) samples of SCC-CRC ($P = 0.004$). The prevalence of JCV was 20 (22.2%) in the age group of 25–35 years compared to 18 (20%) in the age group of 36–45 years ($P = 0.05$). JCV DNA was positively detected in 32 (35.6%) of the CRC patients who were male, but only 14 (15.6%) of the CRC patients who were female ($P = 0.4$). The rate of JCV was 18 (20%) at the pathogenic stage, stage II, while it was 12 (13.3%) in stage I. These numbers differed significantly from the rates in stages III and IV, which were 10 (11.1%) and 6 (6.7%), respectively ($P = 0.04$). JCV DNA was found in the tumors in 26 (28.9%) rectum, 12 (13.3%) sigmoid colon, and 8 (8.9%) other locations ($P = 0.5$). JCV DNA levels in metastatic tumors were 10 (11.1%), while they were 36 (40%) in non-metastatic tumors ($P = 0.9$). The profile of patients with CRC who tested positive and negative for JCV DNA is shown in Table 1.

Interferences of IHC staining of studied markers with the study population

The CK7 expression was detected in 45 (50%) of 90 colorectal carcinomas as 26 (28.9%) in ADCRC and

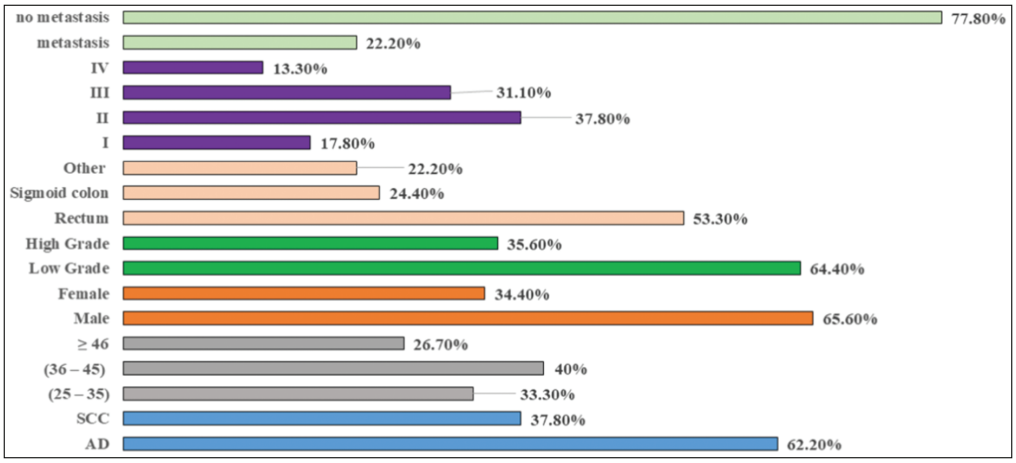

Figure 1: Clinicopathological characteristics of colorectal study populations

Table 1: The association of clinico-pathological features with JCV (PCR) results

Variables		Total *N* (%)	JCV (PCR)		*P* value
			Positive 46 (51.1%)	Negative 44 (48.9%)	
Tumor histology	AD-CRC	56 (62.2%)	22 (24.4%)	34 (37.8%)	0.004
	SCC-CRC	34 (37.8%)	24 (26.7%)	10 (11.1%)	
Age groups (Years)	25–35	30 (33.3%)	20 (22.2%)	10 (11.1%)	0.05
	36–45	36 (40%)	18 (20%)	18 (20%)	
	≥ 46	24 (26.7%)	8 (8.9%)	16 (17.8%)	
Genders	Male	59 (65.6%)	32 (35.6%)	27 (30%)	0.4
	Female	31 (34.4%)	14 (15.6%)	17 (18.9%)	
Tumor grades	Low grade	58 (64.4%)	28 (31.1%)	30 (33.3%)	0.4
	High grade	32 (35.6%)	18 (20%)	14 (15.6%)	
Tumor location	Rectum	48 (53.3%)	26 (28.9%)	22 (24.4%)	0.5
	Sigmoid colon	22 (24.4%)	12 (13.3%)	10 (11.1%)	
	Other	20 (22.2%)	8 (8.9%)	12 (13.3%)	
Tumor (TNM) stages	I	16 (17.8%)	12 (13.3%)	4 (4.4%)	0.04
	II	34 (37.8%)	18 (20%)	16 (17.8%)	
	III	28 (31.1%)	10 (11.1%)	18 (20%)	
	IV	12 (13.3%)	6 (6.7%)	6 (6.7%)	
Lymph node metastasis	Present	20 (22.2%)	10 (11.1%)	10 (11.1%)	0.9
	Absent	70 (77.8%)	36 (40%)	34 (37.8%)	

Table 2: Results of IHC of tumor markers in CRC patients

Tumor markers		Total *N* (%)	AD-CRC N (%)	SCC-CRC N (%)	95% CI			*P* value
					Value	Lower	Upper	
CK7	Positive	45 (50%)	26 (28.9%)	19 (21.1%)	0.68	0.29	1.61	0.3
	Negative	45 (50%)	30 (33.3%)	15 (16.7%)				
CK20	Positive	67 (74.4%)	40 (44.4%)	27 (30%)	0.64	0.23	1.78	0.4
	Negative	23 (25.6%)	16 (17.8%)	7 (7.8%)				
CDX2	Positive	21 (23.3%)	6 (6.7%)	15 (16.7%)	0.15	0.05	0.44	0.000
	Negative	69 (76.7%)	50 (55.6%)	19 (21.1%)				

19 (21.1%) in SCC-CRC, and cytoplasmic CK20 immunoreactivity was more prominent in AD-CRC as 40 (44.4%) with no significant differences ($P < 0.05$). High significant difference ($P < 0.001$) was found in CDX2 positive signals as 6 (6.7%) and 15 (16.7%) for AD-CRC and SCCCRC, respectively, as represented in Table 2.

Interferences of IHC staining of studied markers with JCV infection

As represented in Table 3, a significant association has been detected in the three studied tumor markers with respect to JCV infection in CRC patients, 45 (50%) of the positive CK7 expression was revealed 18 (20%) of JCV DNA ($P = 0.03$), while the positive CK20 signals of 67 (74.4%) showed as 30 (33.3%) of viral DNA ($P = 0.04$). Finally, in 21 (23.3%) of CDX2 positive signals, 15 (16.7%) showed JC polyomavirus infection and some showed positive signals [Figure 2].

Discussion

One of the most prevalent malignancies in the world, colorectal cancer accounts for 10% of all cancers in both men and women, and its etiology is linked to a number of environmental, genetic, and lifestyle variables. Infection with viral agents accounts for 15% to 20% of cancer causes worldwide, and this percentage is rising as more is understood about the carcinogenic role of viruses in various cancers. Indeed, viruses like the herpes simplex virus (HSV), human papillomavirus (HPV), Epstein-Barr virus (EBV), cytomegalovirus (CMV), and JC polyomavirus (JCPyV) have been linked to the development of CRCs. Recall that these viruses are thought to be possible initiators of a variety of GI tract tumors, including esophageal, stomach, and colorectal cancers or even sexual organs.[9-11]

It has been found that the JC virus has a high incidence of between 82% and 90% in people with colorectal cancer.[12] 46 (51.1%) of the CRC patients in the current

Table 3: The association of IHC results of tumor markers with JCV (PCR) infection

Tumor markers		Total *N* (%)	JCV (PCR)		95% CI			*P* value
			Positive 46 (51.1%)	Negative 44 (48.9%)	Value	Lower	Upper	
CK7	Expressed	45 (50%)	18 (20%)	27 (30%)	0.40	0.17	0.94	0.03
	Not expressed	45 (50%)	28 (31.1%)	17 (18.9%)				
CK20	Expressed	67 (74.4%)	30 (33.3%)	37 (41.1%)	0.35	0.12	0.97	0.04
	Not expressed	23 (25.6%)	16 (17.8%)	7 (7.8%)				
CDX2	Expressed	21 (23.3%)	15 (16.7%)	6 (6.7%)	3.0	1.06	8.83	0.03
	Not expressed	69 (76.7%)	31 (34.4%)	38 (42.2%)				

investigation tested positive for JCV DNA (P = 0.004). The JCV DNA rate was among the male 32 (69.6%) and female 14 (30.4%) patients (P = 0.4), and the frequency of JCV DNA was among the age groups (25-35) years 20 (43.5%) and age group (36–45) years 18 (39.1%) (P = 0.05). Additionally, among the grade II CRC tissues, a high frequency of 18 (39.1%) JCV DNA was discovered. These results were all in line with past studies.[13,14] Immunohistochemical methods are often used to locate the primary site of metastatic cancers with poor differentiation. In our study, we investigated the expression of three immunohistochemical markers, CDX2, CK20, and CK7, in colorectal carcinomas in relation to various clinicopathological variables. In GI tract adenocarcinomas, CDX2, a transcription factor involved in intestinal epithelial cell proliferation and differentiation, is expressed from the esophagus to the rectum. In epithelial cells, there are intermediate filaments in the cytoplasm known as cytokeratins. The distribution of carcinomas varies with the kind of tissue, and the majority of them have cytokeratin profiles that are similar to those of the healthy tissue from which they developed. The two most prevalent cytokeratin indicators are CK20 and CK7.[13,14]The respiratory tract, mammary gland, endometrium, ovaries, biliary tract, apocrine and eccrine glands, urothelium, and mesothelium are only a few examples of the many ductal and glandular epithelia that express CK7. CK20 is exclusive to the urothelium, Merkel cells, and epithelium of the GI tract.[15]

According to earlier research, the percentage of colorectal carcinomas that express CDX2 positively is over 90%, cytokeratin 20 positively expressed in various studies ranges from 62% to 96%, and cytokeratin 7 positively expressed in up to 17% of cases.[16] Our findings concur with these findings. It is apparent that the percentage of CK20 positive varies greatly. This may be brought on by changes in the investigated populations, immunohistochemistry techniques, and interpretation standards.[5,16]

Our finding highlights that CDX2 is the colorectal cancer immunomarker with the highest sensitivity and specificity, and it backs up earlier suggestions that it can be used by itself to confirm the diagnosis of metastatic CRC, while CK20 and CK20 should always be used together in a medical board.

In this study, CDX2 staining was lost in 69 (76.7%) of the colorectal carcinomas. It is yet unknown precisely what caused the expression of this marker to drop. A recent study found a correlation between CRCs' increased T stage, N stage, tumor grade, and proximal location with reduction of CDX2 expression.[17] Loss of CDX2 expression has been linked to lower T stage, N stage, tumor grade, and location on the right side of the body, according to research evaluated CDX2 expression in 713 CRC cases and discovered a connection between low CDX2 expression with proximal location, infiltrative growth, advanced T, N, and M phases, and poor differentiation they also asserted that patients who lost overall survival were decreased for CDX2.[17,18] Currently, it was calculated that there was a highly significant correlation (P = 0.000) between the loss of CDX2 expression and the histologic kinds of CRC.

Finally, as a first study of its sort, a substantial correlation between three colonic tumor markers and JCV infection in CRC patients has been found. However, further research is still needed to fully understand this biological relationship.

Conclusion

Our data unmistakably demonstrate the prevalence of JCV in CRC patients and suggest that it may play a co-factory role in tumor growth. Additionally, because CDX2 is so sensitive and specific, it should be useful in the diagnosis of intestinal adenocarcinomas.

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Nil.

Conflicts of interest

The authors have no conflicts of interest to declare.

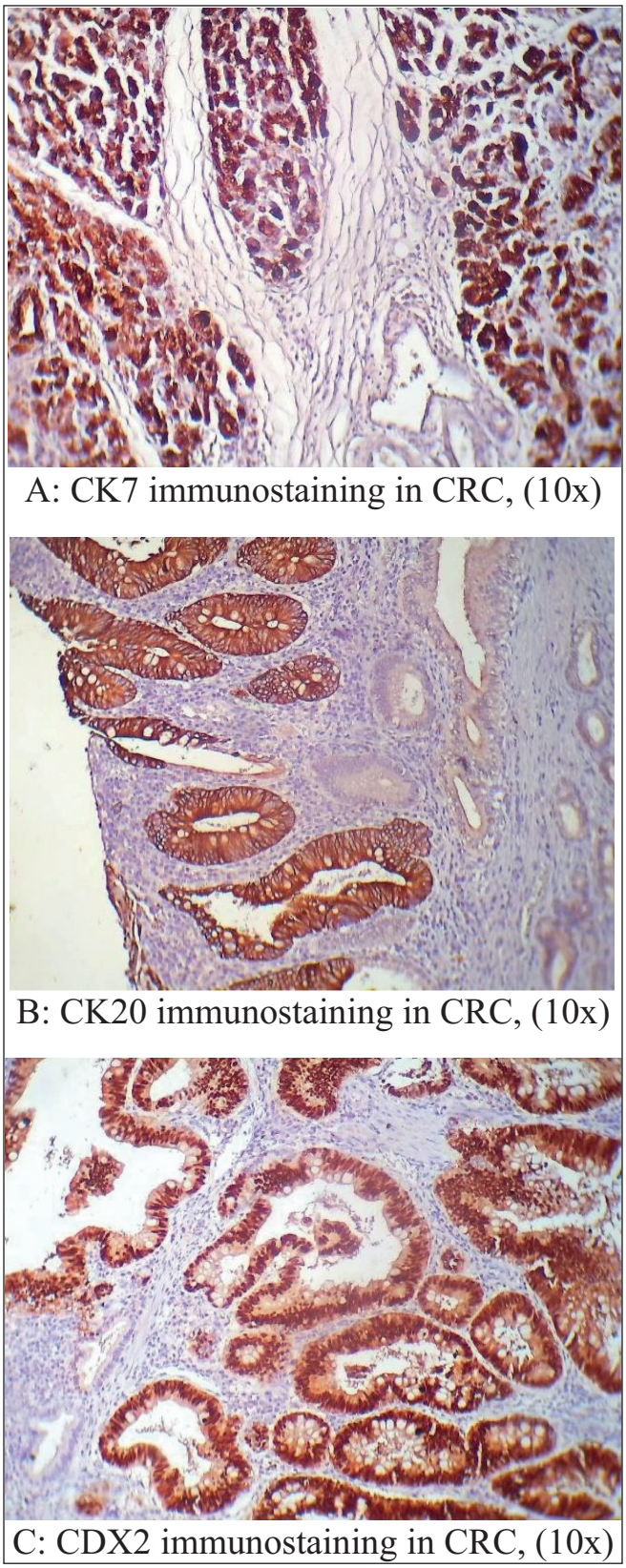

Figure 2: Photomicrographs (A, B, C) of studied colonic markers expression in CRC (10x). A: CK7 immunostaining in CRC (10x). B: CK20 immunostaining in CRC (10x). C: CDX2 immunostaining in CRC (10x)

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