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Immunohistochemical Detection of Cytokeratin 20 in Sudanese Patients with Colorectal Carcinoma

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Abstract: Background: *Colon cancer is cancer of the large intestine at the lower part of digestive system. Rectal cancer is cancer of the last several inches of the colon. Together, they are often referred to as colorectal cancers .The most common cancers of the large intestine (the type called adenocarcinoma) arise from the mucosa, the inner layer of cells. Mistakes (usually a series of mistakes involving genes within the replacement cells) lead to abnormal cells and uncontrolled proliferation of the abnormal cells that give rise to cancer.* ***Objective:*** *To detect the expression of cytokeratin 20 among colorectal carcinoma patients using immunohistochemical method, to correlate between CK20 expression and grade of cancer and to detect the association between colorectal cancer and age, sex of patients.* ***Materials and methods:*** *This is a hospital based cross-sectional retrospective descriptive study was conducted in Khartoum state during the period from May 2018 to July 2021. Thirty paraffin block samples were collected from patients previously diagnosed as colorectal carcinoma in National public health laboratory using simple random collection method. The paraffin blocks were cut by rotary microtome, and then stained by immunohistochemical method for detection of CK20.* ***Results:*** *The study revealed that the most patients were older than 50 years representing 56.7%. Out of thirty patients the study showed that the majority of patients were males representing 70%. CK20 among study population showed strong expression in 76.7% of patients, and weak expression in 23.3% of patients. The grade of cancer of study population revealed that 53.3% were well differentiated tumor.* ***Conclusion:*** *The study concludes that the CK20 expression is positive in all colorectal carcinoma tissue and the majority of expression is strong with no association with grade of cancer.*

Keywords: Colorectal Carcinoma, Cytokeratin 20, Sudanese, Immunohistochemical

Introduction:

Colorectal cancer, commonly known as colon cancer or bowel cancer, the term colorectal cancer covers cancers in both the colon (colon cancer) and the rectum (rectal cancer). Genetic analysis shows that colon and rectal tumors are essentially genetically the same cancer (Karaptis, et al. 2008). In colorectal cancer, cells in the colon or in the rectum start to grow in an uncontrolled way, forming a lump called the primary cancer or primary tumor. Colorectal cancer is the third most commonly diagnosed cancer in the world, there are 1.23 million new cases of colorectal cancer were clinically diagnosed, and that it killed 608,000 people suffered from the disease worldwide, It is the second most common cause of cancer in women and the third most common in men with it being the fourth most common cause of cancer death after lung, stomach, and liver cancer (Ferlay, et al. 2010). Risk factors for colon cancer include hereditary conditions like familial adenomatous polypsis and hereditary non polypsis colorectal cancer, also commonly occurs in people over the age of 50, a diet high in fat especially fat from animal sources and low fiber diets, smoking and alcohols, obesity, it is more common in men than women (Pischon, et al. 2006). The diagnosis of colorectal cancer includes digital rectal examination, followed by a colonoscopy, X-ray and CT-scans. If a colon cancer is suspected, laboratory tests including blood tests and urine analysis will be run. A biopsy may be needed to confirm the diagnosis, also use the tumor markers like CK20 to diagnosis and to monitoring treatment of colorectal cancer (Procsoc, 1997).The curative treatment consists of surgically removing tumors and surrounding tissue. If the lymph nodes are hit, chemotherapy will follow the surgery and radiation may also be used (panzer, et al. 1999).Cytokeratin 20 (CK20) is a newly described polypeptide with molecular weight 48.5 kDa and an isoelectric point at pH 5.66 .This proteins is encoded by the gene located on chromosome 17q21.2 (Bragulla and Homberger, 2009, Schweizer, et al. 2006). CK20 expression is restricted to a few organ systems. Almost cases of colon carcinoma (95-100%) were positive for CK20 (Chu and Duval, 2000, Stopyra, et al. 2001, Tot, 2002). This Immunohistochemical expression of CK20 marker is suitable for the localization, and therapy checks. The levels of Ck20 reflect the success of surgery, radiotherapy and chemotherapy on the patients (Procsoc, 1997). Increase cases of colorectal cancer in the world, the large number of death from this cancer and it consider as a health problem.

Materials and Methods:

Study design: this is a crossectional study

Study population: Thirty colorectal tissue blokes were cut from patients diagnosed with colorectal cancer at National public health laboratory in Khartoum state during the period from May to July 2018. Patient identification data and other information were obtained from patients file.

Ethical consideration: All samples were taken ethically after leader permission and according to ethics from National public health laboratory.

Data collection: Information takes form patients files: Name, Age, Sex, Diagnosis, and Grade of cancer.

Data analysis: The data were analyzed using SPSS computer program. Frequencies, means and chi-square test values were calculated.

Methodology:

Sample processing:

Histopathology tissue processing:

Two sections of 5µm in thickness were obtained from each formalin fixed paraffin wax embedded tissue using rotary microtome.

Haematoxylin and eosin stain for histopathology diagnosis:

Sections required for histopathology were stained using haematoxylin and eosin stain (Mayer's technique) through the following steps:

Sections were dewaxed in hot plate oven and cleared in two changes of xylene for two minutes, then hydrated through descending concentrations of ethanol (100%, 90%, 70%, 50%) and water two minutes for each, then stained in Meyer's haematoxylin for 7 minutes, then washed in water, then blued in running tap water for ten minutes, then stained in eosin for three minutes , then washed in distilled water, then dehydrated through ascending concentrations of ethanol (50%, 70%, 90%, 100%), then cleared in xylene and mounted in DPX mountant (Bancroft and Marilyn, 2002).

Immunohistochemical staining procedure:

Sections of 5µm thickness were obtained from formalin fixed paraffin embedded tissue using a rotary microtome. Sections required for immunohistochemistry were retrieved using water bath retrieval technique, then immunostained using monoclonal antibodies by indirect decxtraine polymer technique as follows:

Sections were dewaxed in hot plat oven and cleared in two changes of xylene for two minutes, then hydrated through descending concentrations of ethanol (100%, 90%, 70%, 50%) and water two minutes for each, then retrieved by water bath retrieval technique(citrate buffer) for fourteen minutes, then endogenous peroxaidase blocker for thirty minutes, then washed in phosphate buffer saline (PH 7.4) for five minutes, then treated with Ck20 for thirty minutes, then rinsed in phosphate buffer saline (PH 7.4), then treated with secondary antibody for thirty minutes, then rinsed in phosphate buffer saline (PH 7.4), then treated with DAB for ten minutes, then washed in phosphate buffer saline (PH 7.4) for five minutes, then counterstained in Mayer's haematoxylin for one minutes, then washed and blued in running tap water for ten minutes, then dehydrated through ascending concentration of ethanol (50%, 70%, 90%, 100%), then cleared in xylene and mounted in DPX mountant (Bancroft and Marilyn 2002).

Result interpretation:

Immunohistochemical results were detected by research, and confirmed with experian pathologist group.

Results:

A total of 30 patients with colorectal cancer were investigated by conventional histopathology and immunohistochemistry methods. Their ages ranged between 27 to 90 years. The descriptions of patients according to sex 70%were male and the 30% were female. Out of 30 patients, the tumor grade revealed well differentiated tumor in 53.3% and moderately differentiated tumor in 20% while poor differentiation was seen in 26.7%. The intensity of stain as show in table (2) most results were strong Ck20 expression 76.7% and remaining 23.3% were weak Ck20 expression. The number of patient with well differentiated tumor in strong expression of Ck20 was 12 (75%) and weak expressions of Ck20 were 4 (25%). Moderately differentiated in strong expression of Ck20 were 5 (83.3%) and weak expression of Ck20 were 1 (16.7%). Poor differentiated tumor in strong expression of Ck20 were 6 (75%) and weak expression of Ck20 were 2 (25%), this result show insignificant statistical association (P value 0.911), as indicated in Table (3).

Table (1) The demographic data of colorectal carcinoma patients:

Parameters	Frequency	Percent (%)
Age group (year)		
Less than 50 years	13	43.3
50-70 years	14	46.7
70-90 years	3	10.0
Total	30	100%
Sex		
Male	21	70.0
Female	9	30.0
Total	30	100%
Tumor grade		
Well differentiation	16	53.3

Moderate and differentiation	14	46.7
Total	30	100%

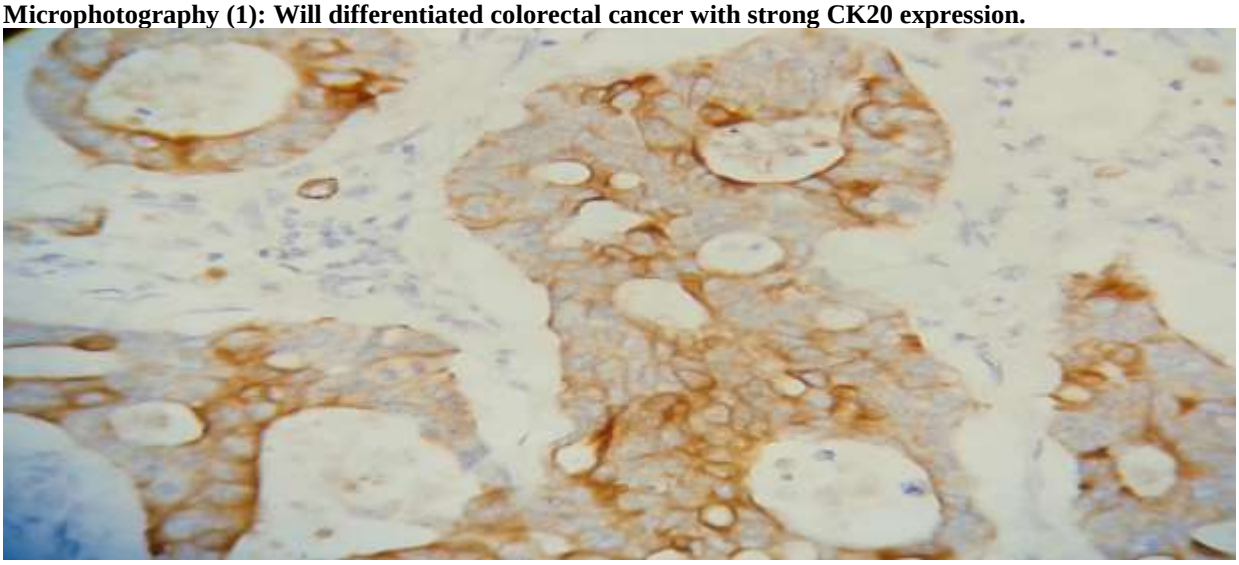
Table (2): Immunohistochemical expression of Ck20 among the study samples

Intensity of stain	Frequency	Percent (%)
Strong expression	23	76.7
Weak expression	7	23.3
Total	30	100%

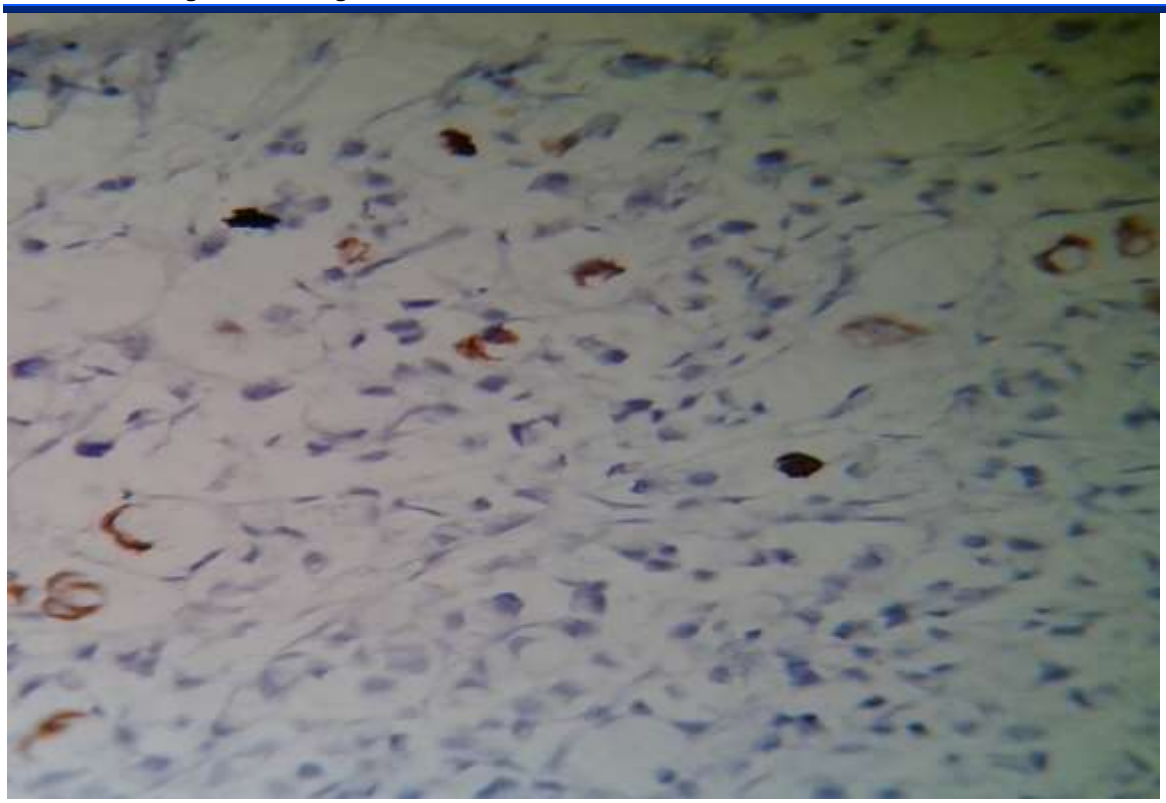
Table (3) Correlation of Ck20 expression with cancer grade

Grade	Intensity				Total		P.Value
	Strong expression		Weak expression				0.911
	N	%	N	%	N	%	
Well differentiation	12	75	4	25	16	100	
Moderate and Poor differentiation	11	78.6	3	21.4	14	100	

Microphotography (1): Will differentiated colorectal cancer with strong CK20 expression.



Microphotography (2): Will differentiated colorectal cancer with weak CK20 expression.



Discussion:

Colorectal cancer, commonly known as colon cancer or bowel cancer is the third most commonly diagnosed cancer in the world. Like other cancers, colorectal cancer starts in a small area but can spread to other parts of the body to form metastatic tumors (Karaptis, et al. 2008). In this study out of thirty patients diagnosed with colorectal carcinoma investigated by histopathology and immunohistochemistry, the age of patients ranged between 27 to 90 years. The majority of patients were older than 50 years representing 17 (56.7 %). This attributed to decrease immunity and activity of digestive system after 50 year. Similar finding were described by Pischon, et al (2006), they reported that the 60 – 80% of people diagnosed with colon cancer are older than 50 years. These result not far away to the finding of Howlader N (2016), they reported that the condition is rare in people under 40 years and the majority of cases are diagnosed in age over 55 year old. Also Siegel RL, (2009), reported that the colorectal cancer appear mainly after the age of 50 years. Based on this study the colorectal cancer is more common in males than females, the males representing 21 (70%) and the remaining 9 (30%) were females. This is attributed to increase smoking and consumption of alcohol in males than females. This result supported by Pischon, et al (2006), who reported that the men are more susceptible to colorectal carcinoma than women. Similar finding were reported by Murphy et al (2015) reported that the incidence of colorectal cancer appear in males higher than females for all racial and ethnic groups. Histopathological analysis of tumor grade in the 30 cases of colorectal carcinoma revealed high percent of well differentiated tumor in 16 (53.3%) patients, in compared with moderately differentiated tumor in 6 (20%) patients and poor differentiation tumor seen in 8 (26.7%) patients. This results attributed to painful of symptoms of colorectal cancer that make the patients reach the medical care early for investigation. These result supported by Edwards BK (2013) they reported that the symptoms of colorectal cancer responsible for increase cases of well differentiated tumor and decrease cases of poor differentiated tumor. But these results differ from the study of Lynch and Chappele, (2003), they reported that the moderately differentiated tumors are more than the well and poor differentiated tumor in case of colorectal carcinoma. The analysis of the quality of Ck20 immunohistochemical in 30 cases of colorectal carcinoma revealed that most staining results were strong Ck20 expression representing 23 (76.7%) and the remaining 7 (23.3%) were weak Ck20 expression. Similar finding were described by Moll, et al (1993), who reported that the CK-20 strong positively was seen in the majority of adenocarcinoma of the colon. Based on this study the statistical association between Ck20 expression and tumor grade is insignificant (P value > 0.05), this attributed to efficiency of Ck20 to react strongly even in poor or undifferentiated tumor. These result supported by Moll, et al (1992), they reported that the strong immunostaining of Ck20 was seen not only in well differentiated tumor but also in tumor with less morphological differentiated. These result similar to the finding of Agnieszka Jasik (2012) who recorded that the expression of Ck20 not affected by tumor grade but the positivity is seen even in undifferentiated tumor. Also Tatkraumi (2014) reported the expression of Ck20 in colon cancer appear positive regarding to the histological grade of cancer. These result unlike the Varmus (2010), who report that the positivity of CK20 is increase in poor differentiated tumors.

Conclusion:

Most cases of colorectal carcinoma in this study appear after 50 years old. The male were affected by colorectal carcinoma more than female. Ck20 expression did not affected by histological grade of tumors.

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