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Immunohistochemical Detection of Cytokeratin 20 in Colorectal Carcinoma

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1. Abstract

1.1. Background: Colon cancer is cancer of the large intestine (colon), 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. These cells are exposed to toxins from food and bacteria as well as mechanical wear and tear and are constantly dying off and being replaced. 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

1.2. 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.

1.3. Materials and Methods: This is a hospital based longitudinal retrospective descriptive study was conducted in Khartoum state during the period from May to July 2018. 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.

1.4. Results: The age of study population ranged between 27 and 90 years with mean age of 54. The study revealed that the most patients were older than 50 years representing 17(56.7%) and the remaining 13(43.3%) were younger than 50 years. Out of thirty patients the study showed that the majority of patients were males representing 21(70%) and the remaining 9 (30%) were females.CK20 among study population showed strong expression in 23(76.7%) patients, and weak expression in 7 (23.3%) patients. The grade of cancer of study population revealed that 16 (53.3%) were well differentiated tumor.

1.5 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.

2. 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 [1]. 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. Like other cancers, colorectal cancer starts in a small area but can spread to other parts of the body to form metastatic tumors [1]. 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 [2].

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 [3]. 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 [4]. 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 [5]. Cytokeratin 20 (CK20) is a newly described polypeptide with molecular weight 48.5 kDa and an isoelectric point at pH 5.66. This protein is encoded by the gene located on chromosome 17q21.2 [6, 7]. CK20 expression is restricted to a few organ systems. Almost all cases of colon carcinoma (95-100%) were positive for CK20. 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 [4]. Increase cases of colorectal cancer in the world, the large number of death from this cancer and it consider as a health problem [2].

3. Materials and Methods

3.1. 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.

3.2. Data Analysis

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

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

3.3. Information Takes Form Patients File

Name, Age, Sex, Diagnosis, Grade of cancer.

4. 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 old with mean age of 54 years. Most patients were older than the age of 50 years representing 17 (56.7%) and the remaining 13 (43.3%) were younger than 50 years as indicated in (Table 1) The description of sex as show in (Table 2). Most patients were male representing 21 (70%) and the remaining 9 (30%) were female. Out of 30 patients, the tumor grade revealed well differentiated tumor in 16 (53.3%) and moderately differentiated tumor in 6 (20%) while poor differentiation was seen in 8 (26.7%) as indicated in (Table 3). The intensity of stain as show in (Table 4) most result was strong Ck20 expression 23 (76.7%) and remaining 7 (23.3%) were weak Ck20 expression. The number of patient with well differentiated tumor in strong expression of Ck20 was 12 (40%) and weak expressions of Ck20 were 4 (13.3%). Moderately differentiated in strong expression of Ck20 were 5 (16.7%) and weak expression of Ck20 were 1 (3.3%). Poor differentiated tumor in strong expression of Ck20 were 6 (20%) and weak expression of Ck20 were 2 (6.7%), this result show insignificant statistical association (P value.911), as indicated in (Table 5).

Table 1: Distribution of age among the study population.

Age group (year)	Frequency	Percent (%)
Less than 50 years	13	43.3
50-70 years	14	46.7
70-90 years	3	10
Total	30	100%

Table 2: Distribution of sex among the study population.

Sex	Frequency	Percent (%)
Male	21	70
female	9	30
Total	30	100%

Table 3: Distribution of cancer grade among the study population

Tumor grade	Frequency	Percent (%)
Well differentiation	16	53.3
Moderate differentiation	6	20
poor differentiation	8	26.7
Total	30	100%

Table 4: 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%

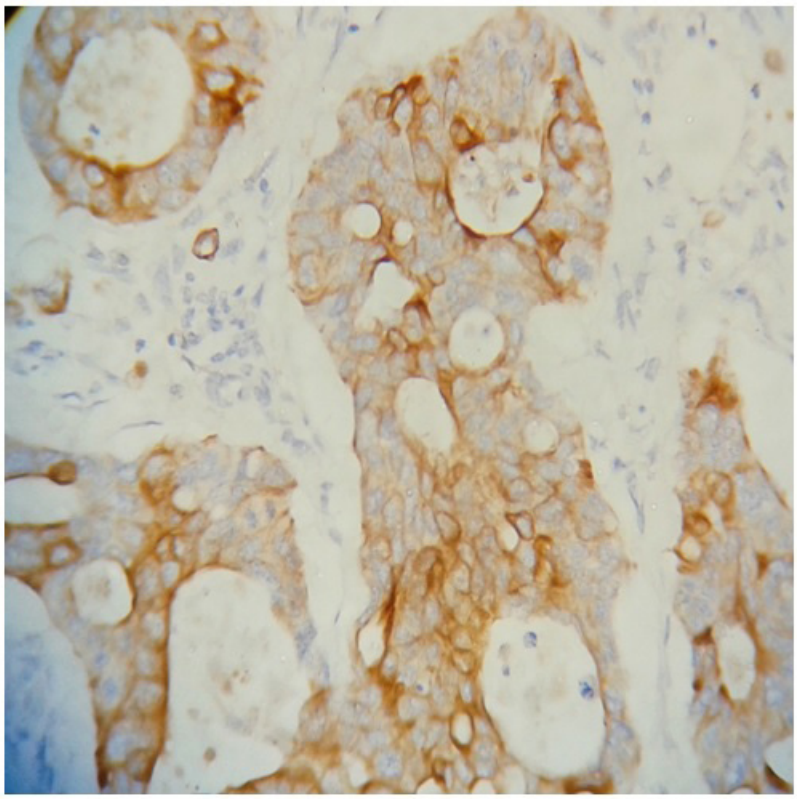
Table 5: Correlation of Ck20 expression with cancer grade

Grade	Intensity				Total		P Value
	Strong expression		Weak expression				
	N	%	N	%	N	%	
Well differentiation	12	40	4	13.3	16	53.3	0.911
Moderate differentiation	5	16.7	1	3.3	6	20	
Poor differentiation	6	20	2	6.7	8	26.7	
Total	23	76.7	7	23.3	30	100	

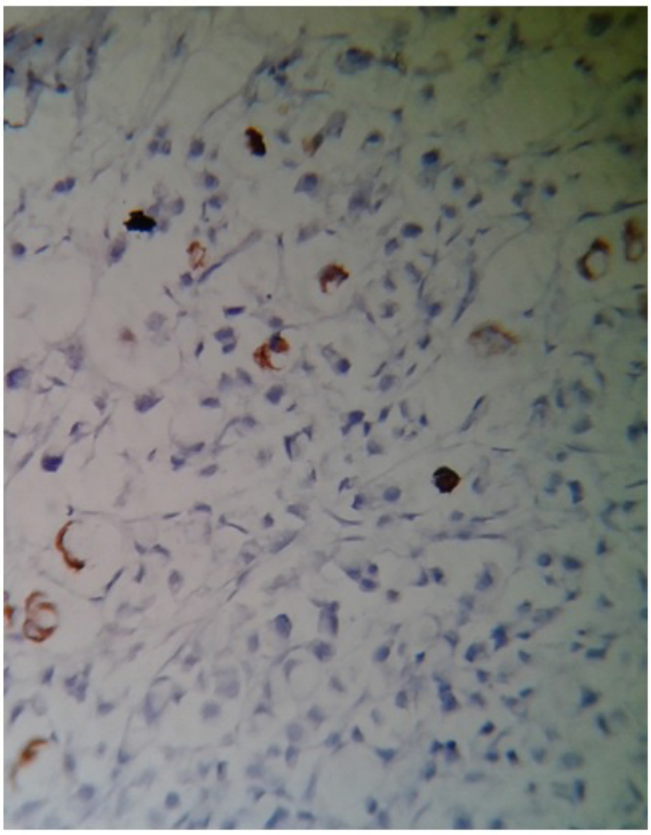
5. Discussion

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 [11]. 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 (Figure 1). 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 years.

Similar finding was described by Pischon, et al (2006) [3], 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 How lader N 2016 [8] 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. Siegel RL, 2009 [9] reported that the colorectal cancer appears 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) [3], they reported that the men are more susceptible to colorectal carcinoma than women. Similar finding was reported by Murphy et al 2015 [10] reported that the incidence of colorectal cancer appears 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 (Figure 2). These result supported by Edwards BK 2013 [11] 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) [12], 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 was described by Moll, et al (1993) [14], 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 (1993) [15], 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 [12] who recorded that the expression of Ck20 not affected by tumor grade but the positivity is seen even in undifferentiated tumor. Tatkraumi 2014 [13] reported the expression of Ck20 in colon cancer appear positive regarding to the histological grade of cancer.



Figures 1: Will differentiated colorectal cancer with strong CK20 expression.



Figures 2: Will differentiated colorectal cancer with weak CK20 expression.

6. Conclusion

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

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