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Immunohistochemical Expression of Cytokeratin 20 in Colorectal Cancer

ABU ELGASIM ABASS AWAD ELKAREEM
Assistant Professor of Histopathology and Cytology
College of Medical Laboratory Sciences
Sudan University of Science and Technology, Sudan
SALMA OSMAN MOHAMMED
Lecturer, Histopathology and Cytology Department
Faculty of Medical Laboratory Sciences
Wad Madani Alhlia College, Sudan

Abstract:

This is a hospital based retrospective descriptive case study aimed to evaluate the expression of cytokeratin 20(CK20) in colorectal carcinoma patients using immunohistochemistry. The study was conducted in Khartoum state, Sudan. Thirty paraffin block samples were collected from patients samples previously diagnosed as colorectal carcinoma. The paraffin blocks were cut by rotary microtome, and then stained by immunohistochemical method (New indirect method) for detection of CK20.

The age of study population ranged between 27 and 90 years with mean age of 54 years. 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.

The grade of cancer of study population revealed that 16 (53.3%) samples were well differentiated tumor, 6 (2.0%) samples were moderate differentiated tumor and 8 (26.7%) samples were poorly differentiated tumor. CK20 among study population showed strong

expression in 23(76.7%) samples, and weak expression in 7 (23.3%) samples. The relation between tumor grade and CK 20 expression showed no association (P=0.911).

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 the grade of cancer.
Key words: Colorectal carcinoma, Cytokeratin 20

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 polyps 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)].
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 [(5)(6)]. 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)].

MATERIALS AND METHODS:

Materials:

Thirty issues blokes obtained from patients samples affected with colorectal cancer were used in this study.

Study design:

This is a hospital based retrospective descriptive study aimed at evaluating excretion of CK20 in colorectal cancer by immunohistochemical methods.

Sample processing:

Histopathology tissue processing:

One section of 4μm in thickness was obtained from each formalin fixed paraffin wax embedded tissue using rotary microtome.

Immunohistochemical staining procedure:

Sections were dewaxed in hot plate 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 (citrate buffer) for fourteen minutes, then peroxidase was blocked 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, then treated with secondary antibody for thirty minutes, then rinsed in phosphate buffer saline, then treated with DAB for ten minutes, then washed in phosphate buffer saline 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 [(7)].

Result interpretation:

Immunohistochemical results were detected by research, and confirmed with experience histopathologist.

Statistical analysis:

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

RESULTS:

A total of 30 patients with colorectal cancer were investigated by 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 (Table 1). The description of sex as showed that most patients were male representing 21 (70%) and the remaining 9 (30%) were female (Table 2). Out of 30 patients, the tumor grade revealed that

well differentiated tumor in 16 (53.3%) samples, moderately differentiated tumor in 6 (20%) samples and poorly differentiated tumor was seen in 8 (26.7%) samples (Table 3). The intensity of stain as showed that most result was strong CK20 expression in 23 (76.7%) samples and remaining 7 (23.3%) samples were weak CK20 expression (Table 4). The number of patient with well differentiated tumor showed strong expression of CK20 in 12 (40%) samples and weak expressions in 4 (13.3%) samples. Moderately differentiated showed strong expression of CK20 in 5 (16.7%) samples and weak expression in 1 (3.3%) samples. Poor differentiated tumor showed strong expression of CK20 in 6 (20%) samples and weak expression in 2 (6.7%) samples, this result show insignificant statistical association (P =0.911) (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	10.0%
Total	30	100%

Table (2): Distribution of sex among the study population

Sex	Frequency	Percent (%)
Male	21	70.0%
Female	9	30.0%
Total	30	100%

Table (3) Distribution of cancer grade among the study population

Tumor grade	Frequency	Percent (%)
Well differentiated tumors	16	53.3%
Moderately differentiated tumor	6	20.0%
Poorly differentiated tumor	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 differentiated tumors	12 (40%)	4 (13.3%)	16 (53.3%)	0.911
Moderately differentiated tumor	5 (16.7%)	1 (3.3%)	6 (20.0%)	
Poorly differentiated tumor	6 (20.0%)	2 (6.7%)	8 (26.7%)	
Total	23(76.7%)	7 (23.3%)	30 (100%)	

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 [(1)].

In this study out of thirty patients diagnosed with colorectal carcinoma investigated by 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*., [(3)], who reported that 60 – 80% of people diagnosed with colon cancer are older than 50 years. Also Marphy *et al*., [(8)], 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. This is attributed to increase smoking and consumption of alcohol in males than females. This result supported by Pischon *et al*., [(3)], who reported that the men are more susceptible to colorectal carcinoma than women. Also Marphy *et al*., [(8)], 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 (53.3%) patients, in compared with moderately differentiated tumor and poor differentiation tumor. This results attributed to painful of symptoms of colorectal cancer that make the patients reach the medical care early for investigation. This result supported by Holyok *et al*., [9], who reported that the symptoms of colorectal cancer responsible for increase cases of well differentiated tumor and decrease cases of poor differentiated tumor. But this results differ from the study of Lynch and Chappele and Lynnch [10], they reported that the moderately differentiated tumors are more than the well and poorly differentiated tumor in case of colorectal carcinoma

The analysis of the quality of CK20 immunohistochemical stain in 30 cases of colorectal carcinoma revealed that most staining results were strong expression (76.7%). Similar finding were described by Moll *et al*., [11], 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 showed insignificant association ($P > 0.05$), this attributed to efficiency of CK20 to react strongly even in poor or undifferentiated tumor. This result supported by Moll *et al*., [11], who 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 unlike the Varmus [12], who report that the positivity of CK20 is increase in poor differentiated tumors.

REFERENCES:

1- Karapetis CS, Khambata A, Ford S, Jonker DJ. Mutations and benefit from Cetuximab in advance colorectal cancer. *New England Journal of Medicine*, 2008; **359**: 1757-65.
2- Ferlay J. Shin HR. Bray F. *Estimates of worldwide burden of cancer. Int J Cance,* 2010; **127**: 2893-2917.
3- Pischon T, Lahmann PH, Boeing H. 2006. Body size and risk of colon rectal cancer. *Journal of national cancer*, 2006; **98**: 920- 931
4- Warren AG, Sheilds PG. Molecular epidemiology. Carcinogen. DNA adults and genetic susceptibility*, Proc soc Expert Biol Med* 1997; **21**6(2): 172-180.
5- Bragulla, HH. Homberger, DG. Structure and functions of keratin proteins in simple, stratified, keratinized and cornfield epithelia. *Journal of Anatomy*, 2009: **214**: 516-559.
6- Schweizer J, Bowden PE, Coulombe PA, Langbein L, Lane EB, Magin TM, Maltais L, Omary MB, Parry DAD, Rogers, MA, Wright MW. 2006. New consensus nomenclature for mammalian keratins. *The Journal of Cell Biology*. 2006; **174**(2):169-74
7- Bancroft JD, Marilyn G. Theory and practice of histological techniques. 5th ed. London: Churchill Livingstone. 2002; 125.
8- Murphy G, Devesa SS, Cross AJ, Inskip PD, McGlynn KA, Cook MB. Sex disparities in colorectal cancer incidence by anatomic subsite, race and age. *Int J Cancer*. 2011;**128** (7:1668–1675
9- . Recalde M, Holyoke ED, Elias EG. Carcinoma of the colon, rectum, and anal canal in young patients. *Surg Gynecol Obstet*. 1974;**139**:909–913
10- Chappele A, Lynnch HT. Herditary colorectal cancer *N Engl J Med*, 2003; **348**(10): 919-32

11- Moll R, Zimbelmann R, Goldschmidt MD, Keith M, Laufer J, Kasper M, Koch PJ, Franke WW. 1993. The human gene encoding cytokeratin 20 and expression during fetal development and in gastrointestinal carcinomas. *Differentiation*, 1999; **53**(2): 75-93.
12- Varmus H. 2010. Stages of colon cancer. *National Cancer Institute,* 2010 ; **101**(4):195-200.