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RESEARCH ARTICLE

IMMUNOHISTOCHEMICAL EXPRESSION OF CYTOKERATIN 20 (Ck20) IN COLORECTAL TUMORS AMONG SUDANESE PATIENTS.

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Abstract

Background and Objective: Cytokeratin 20 (CK20) is a widely expressed epithelial protein used as immunohistochemical marker for routine diagnosis. The aim of the current study was to detect the immunohistochemical expression of Ck20 tumor markerin colorectal tumors among Sudanese patients.

Methods: Retrospective analytical case control study was conducted at Khartoum state. One hundred and fifty previously diagnosed colorectal tumor blocks were enrolled including 100 malignant tumors and 50benign colorectal tumors. The paraffin sections were tested by immunohistochemical method for CK20 expression.

Results: Females were significantly at greater risk of getting colorectal malignant tumors than males (p. value 0.003). Malignant tumors were significantly more common in the rectal site than the colon site (p 0.000). Significant positive immunohistochemical expression of Ck20 was noticed in colorectal malignant tissues (58%) when compared to benign tumors (22%) (p 0.000).

Conclusion: The study concluded that colorectal cancer was more distributed in females and the rectal was the most affected site. Immunohistochemical expression of Ck20 in malignant tissues was twice that of benign colorectal tumors confirming the importance of CK20 staining colorectal carcinogenesis regardless of cancer grade.

Introduction: -

Colorectal cancer is a major cause of morbidity and mortality throughout the world. It accounts for over 9% of all cancer incidence[(1)]. It is the third most common cancer worldwide and the fourth most common cause of death[(2)]. Cytokeratin 20 (CK20) is a polypeptide with molecular weight of 48.5 kDa encoded by a gene located on chromosome 17q21.2[(3, 4)]. The immunohistochemical expression of CK20 marker was considered suitable for the localization and therapy checks. The levels of Ck20 reflect the success of surgery, radiotherapy and chemotherapy on the patients[(5)]. Immunohistochemical studies with highly specific antibodies showed that CK20 expression in normal tissues is limited to epithelial cells of the gastrointestinal (GI) tract, the urothelium and Merkel cells, and that

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this profile is maintained in malignant tumors of these cells. Both the specificity of CK20 antibodies and the restricted CK20 expression make CK20 a valuable diagnostic marker. Accordingly, CK20 IHC is a useful diagnostic tool for the classification of tumors, especially in the case of distant metastasis where the tissue of origin is unknown, as well as for the detection of disseminated tumor cells[6].The objectives of this study were to detect the immunohistochemical expression of Ck20 tumor marker in colorectal tumors among Sudanese patients and to correlate immunohistochemical expression of Ck20 tumor marker with cancer grades and demographical data of patients.

Materials and Methods: -

Study subjects: -

A retrospective analytical case control study aimed to detect the immunohistochemical expression of Ck20tumor in colorectal tumors among Sudanese patients. The study was conducted at Khartoum state in IbnSina and Soba Teaching Hospitals during the period April 2014 – December 2015. One hundred and fifty blocks of colorectal tumors were enrolled in the study including one hundred blocks previously diagnosed as colorectal cancer and fifty benign colorectal tumor blocks.

Immunohistochemical staining: -

Sections of 5µm in thickness were obtained from each formalin-fixed paraffin embedded tissue block using rotary microtome. Sections were immune stained using monoclonal antibodies by new indirect techniques. Briefly, sections placed on coated slides were dewaxed in hot plate oven and cleared in two changes of xylene for two minutes. Sections were then hydrated through a series of ethanol concentrations (100%, 90%, 70%, 50%) and a final wash in water for two minutes for each. Slides were retrieved by water bath heat retrieval technique and treated with hydrogen peroxide for fifteen minutes. After that, sections were washed in phosphate buffer saline (PBS, pH 7.4) for five minutes and treated with protein blocker solution for fifteen minutes. Sections were treated with anti- Ck20 primary antibodies for thirty minutes, and then rinsed in PBS before being treated with secondary polymer conjugate for thirty minutes and rinsed in PBS. Slides were treated with DAB for seven minutes then washed in PBS for five minutes. For the staining step, sections were counter stained in Mayer's hematoxylin for one minute washed and blued in running tap water before they were dehydrated through ascending concentrations of ethanol (50%, 70%, 90% &100%). Sections were finally cleared in xylene and mounted in DPX [7].

Statistical analysis: -

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

Ethical consideration: -

Before the study, was conducted the proposal of the study were ethically approved by ethical committee of the Sudan University of Science and Technology and Ministry of Health research committee. An official written permission to conduct the study was obtained by the investigator from responsible authorities.

Results: -

A total of 150 paraffin blocks previously diagnosis as colorectal tumors were collected in the study. According to the histopathological diagnosis, 100 tumors were classified as malignant cases and 50 as benign controls.

According to gender, 44% were males and 56% were females among patients with malignant tumors, while the majority of benign tumors were among males (70%). Females were found to be at significant greater risk of getting malignant tumors (p. value= 0.003, OR= 2.9, CI= 1.25-3.5) as malignant tumors constituted 79% of all tumors among females. According to age group, patients with malignant tumors were slightly older at presentation (41% were less than 50 years old and 59% were more than 50 years), while the opposite was among patients with benign tumors in which 58% were less than 50 years and 42% were more than 50 years, with significant relation between the elder age group and malignant histopathological diagnosis (p 0.049). Concerning the site of the tumor, colon site was less presented among malignant tumors (41%) when compared to rectal site tumors (59%), while the majority of benign tumors were at the colon site (72%), with significant relation between the rectal site of tumor and malignant histopathological diagnosis (p 0.000) (Table 1).

Positive immunohistochemical expression of Ck20 was represented in 58% of malignant tissues. While only 22%of benign tissues were positive for this marker with significant relation between immunohistochemical expression of Ck20 and histopathological diagnosis (Table 2) (Fig. 1).

The immunohistochemical expression of Ck20 was mostly positive in moderately differentiated and well differentiated tumors (65% and 54% respectively),with no significant relation between the immunohistochemical expression of Ck20 and cancer grade (p 0.123) (Table 2).

Table 1:-Relation between demographical data and histopathological diagnosis among study population.

Demographical Data		**Histopathological diagnosis**		
		Malignant	**Benign**	***P. value***
		NO. (%)	**NO. (%)**	
Gender	Male	44 (44%)	35 (70%)	0.003
	Female	56 (56%)	15 (30%)	
	Total	100 (100.0%)	50 (100.0%)	
Age	< 50	41(41%)	29 (58%)	0.049
	≥ 50	59 (59%)	21 (42%)	
	Total	100 (100.0%)	50 (100.0%)	
Site of tumor	Colon	41(41%)	36 (72%)	0.000
	Rectal	59 (59%)	14 (28%)	
	Total	100 (100.0%)	50 (100.0%)	

Table 2: -Relation between Ck20 immunohistochemicalexpression, histopathological diagnosis and cancer grade.

Ck20 Immunohistochemical expression	**Histopathological Diagnosis**		**Cancer Grade**		
	Malignant	**Benign**	**Well Differentiated**	**Moderately Differentiated**	**Poorly Differentiated**
	Number (%)	**Number(%)**	**NO. (%)**	**NO. (%)**	**NO. (%)**
Positive	58 (58.0%)	11 (22.0%)	22 (54.0%)	33 (65.0%)	3 (37.5%)
Negative	42 (42.0%)	39 (78.0%)	19 (46.0%)	18 (35.0%)	5 (62.5%)
Total	100 (100%)	50 (100%)	41 (100%)	51 (100%)	8 (100%)
P. Value	0.000		0.123		

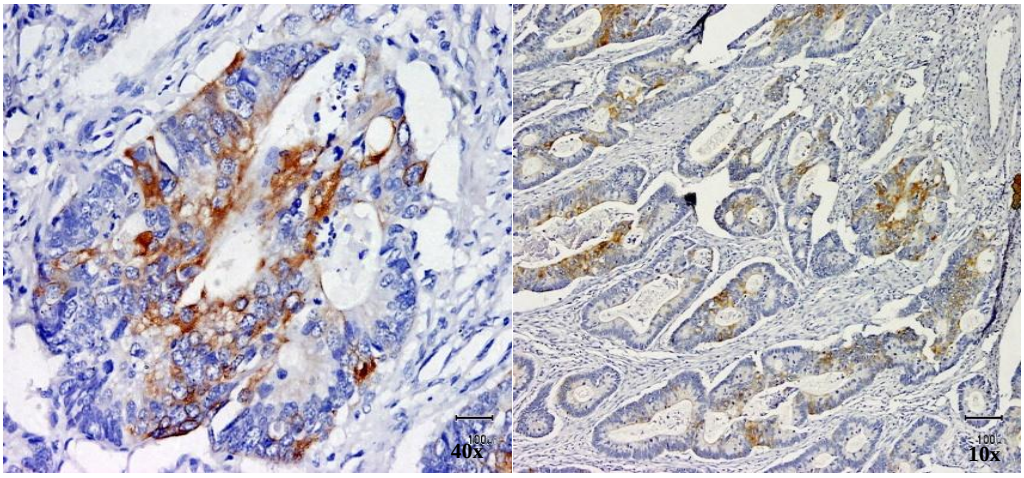

Figure 1: -Moderately differentiated colorectal cancer showing positive CK20 expression (10X and 40X).

Discussion: -

Colorectal adenocarcinoma is a heterogeneous disease that involves multiple tumorigenic pathways. The disease is one of the most commonly diagnosed cancers, and a leading cause of cancer deaths, around the world[(8)].

According to the literature, colorectal cancer has higher prevalence amongelder individuals[9]. In the current study, patients with malignant tumors were slightly older at presentation (59% were more than 50 years) with a mean age of 52±15 years while the opposite was observed among patients with benign tumors (58% were less than 50 years) with a mean age of 50±15 years. Marginal significant relation between the elder age group and malignant histopathological diagnosis (p 0.049) was noticed in the study.

In contrast to most of the reported data[9, 10], females were found at greater risk of developing malignant tumors constituting 79% (56/71) of all tumors, while the majority of benign tumors were among males (70%). Colorectal cancer is broadly considered to be an environmental disease with a wide range of cultural, social, and lifestyle factors[11]. Thus, the higher presentation of females might be due to some differences in the predisposing factors among Sudanese or might be due to sampling error that lowered the presentation of females among benign tumors. However, and according to data in hand, being a female increases the risk of getting malignant colorectaltumors by three times among Sudanese.

Concerning the site of the tumor, colon site was less presented among malignant tumors (41%) when compared to rectal site tumors (59%), while the majority of benign tumors were at the colon site (72%), with significant relation between the rectal site and malignant histopathological diagnosis. Interestingly, when gender was included in the analysis, malignant tumors were equally distributed between the two sites among females while 70% of males had tumors at the rectal site supporting other reports of sexual differences in the site of the disease. Male: female ratio of rectal was reported to be higher in most high incidence populations in contrast to low incidence populations where the sex ratio between these two sites is comparable. Therefore, it's reasonable to correlate such changes with adopting modernized life style among Sudanese population. The results of this study also supports that the differences under the influence of environmental factors are site-specific[12].

Cytokeratin 20 (CK20) is a low molecular weight member of the Cytokeratin family of proteins that is expressed in primary colorectal tumors and their metastastetic cells. It was considered a relevant marker for colorectal diagnosis, since its expression is restricted to gastric and intestinal epithelium, urothelial, and Merkel cells, as well as cancers originating from these tissues [13]. Thus, CK20 levels can provide clinically valuable information on the postoperative prognosis of patients with colorectal cancer [14]. The accumulative effects of such gene may play an important role during carcinogenesis [15]. Moreover, Cytokeratin-based tumor marker assays were considered as a simple and cheap tool for efficient management before conventional methods [16]. In concordance with other studies, Ck20 expression in the current study presented a good indicator for malignancy even though it was expressed in both malignant and benign tumors (22% and 58% respectively). Thus, our data agrees with several other studies in considering cytokeratin 20 (CK20) a well-established marker for colon cancer. The detected expression in the current study was lower than most of the reported data[9, 14].For instance reported higher expression values of CK20 in colorectal cancer (80%)[9]. Moreover, other studies showed higher frequencies of CK20 in colorectal adenocarcinomas reaching 81% and 84%[17, 18].It's also important to highlight the significance of the reported negative immunohistochemical expression of CK20 in this study (42%) which was associated with higher microsatellite instability in previous studies and thus bad prognosis[19].

In general, the analyzed subset of samples showed differential expression of CK20 throughout the different histological grades representing 53.6% of well differentiated tumors, 64.7% of moderately differentiated samples and 37.5% of poorly differentiated tumors.However,immunohistochemical expression of CK20 among malignant tumors was not significantly correlated with cancer histological grade ($p > 0.05$). Several studies reported no relevance between cancer grade and CK20 expression[5, 20].Accordingly, CK20 can be considered an indicator to primary colorectal cancer tumors or malignancy in general but that elevated expression can be disingenuous afterwards in the tumor.

Conclusion: -

The study concludes that colorectal cancer was more distributed in female and the rectal was the most affected site. Ck20 expression was higher in the colorectal malignant tumors in general regardless of the histological grade of tumors. Our results sustain the data published in literature concerning the importance of immunohistochemical expression of CK20.

References: -

1. Siegel R, DeSantis C, Virgo K, Stein K, Mariotto A, Smith T, et al. Cancer treatment and survivorship statistics, 2012. CA: a cancer journal for clinicians. 2012;62(4):220-41.
2. Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. International journal of cancer. 2010;127(12):2893-917.
3. Bragulla HH, Homberger DG. Structure and functions of keratin proteins in simple, stratified, keratinized and cornified epithelia. Journal of anatomy. 2009;214(4):516-59.
4. Schweizer J, Bowden PE, Coulombe PA, Langbein L, Lane EB, Magin TM, et al. New consensus nomenclature for mammalian keratins. The Journal of cell biology. 2006;174(2):169-74.
5. Awad-Elkareem A, Mohammed S, Osman. Immunohistochemical Expression of Cytokeratin 20 in Colorectal Cancer. European Academic Research. 2016;III(11):11683-91.
6. Lassmann S, Bauer M, Soong R, Schreglmann J, Tabiti K, Nährig J, et al. Quantification of CK20 gene and protein expression in colorectal cancer by RT-PCR and immunohistochemistry reveals inter-and intratumour heterogeneity. The Journal of pathology. 2002;198(2):198-206.
7. Bancroft JD, Gamble M. Theory and practice of histological techniques: Elsevier Health Sciences; 2008.
8. Ferkolj I, Gangl A, Galle P, Vucelic B. Pathogenesis and clinical practice in gastroenterology: Springer; 2008.
9. Zlatian OM, Comănescu M, Roşu A, Roşu L, Cruce M, Găman A, et al. Histochemical and immunohistochemical evidence of tumor heterogeneity in colorectal cancer. Romanian journal of morphology and embryology= Revue roumaine de morphologie et embryologie. 2014;56(1):175-81.
10. Al-Attraqhchi AA, Abdulhassan BA, Mahmood FM, Hana DB. Molecular and Immunohistochemical Detection of JC Polyomavirus in Human Colorectal Polyps in sample of Iraqi Patients. Int J Curr Microbiol App Sci. 2015;4(2):743-55.
11. Haggar FA, Boushey RP. Colorectal cancer epidemiology: incidence, mortality, survival, and risk factors. Clinics in colon and rectal surgery. 2009;22(04):191-7.
12. Koo JH, Leong RW. Sex differences in epidemiological, clinical and pathological characteristics of colorectal cancer. Journal of gastroenterology and hepatology. 2010;25(1):33-42.
13. Kust D, Šamija I, Kirac I, Radić J, Kovačević D, Kusić Z. Cytokeratin 20 positive cells in blood of colorectal cancer patients as an unfavorable prognostic marker. Acta Clinica Belgica. 2016:1-9.
14. Li W, Xiao H, Hong X, Niu W. Predictive value of CK20 in evaluating the efficacy of treatment and prognosis after surgery for colorectal cancer. Genetics and molecular research: GMR. 2015;14(2):5823.
15. Wang Y, Liu S, Hao D. [Mutations of APC gene MCR region in sporadic colorectal adenomas and carcinomas]. Zhonghua zhong liu za zhi [Chinese journal of oncology]. 1998;20(4):284-6.
16. Barak V, Goike H, Panaretakis KW, Einarsson R. Clinical utility of cytokeratins as tumor markers. Clinical biochemistry. 2004;37(7):529-40.
17. Bayrak R, Haltas H, Yenidunya S. The value of CDX2 and cytokeratins 7 and 20 expression in differentiating colorectal adenocarcinomas from extraintestinal gastrointestinal adenocarcinomas: cytokeratin 7-/20+ phenotype is more specific than CDX2 antibody. Diagnostic pathology. 2012;7(1):1.
18. Wong SCC, Chan CML, Ma BBY, Hui EP, Ng SSM, San Lai PB, et al. Clinical significance of cytokeratin 20-positive circulating tumor cells detected by a refined immunomagnetic enrichment assay in colorectal cancer patients. Clinical Cancer Research. 2009;15(3):1005-12.
19. McGregor DK, Wu T-T, Rashid A, Luthra R, Hamilton SR. Reduced expression of cytokeratin 20 in colorectal carcinomas with high levels of microsatellite instability. The American journal of surgical pathology. 2004;28(6):712-8.
20. Vrabie CD, Ceausu M, Petrescu A, Waller M, Dina I. The usefulness of immunohistochemistry in sporadic colorectal cancer. Romanian Journal of Morphology and Embriology. 2008;49(4):525-36.