Original Article

CK 20 & CK 7 in Colorectal Adenocarcinoma

Immunoexpression of Cytokeratin 20 and Cytokeratin 7 in Colorectal Adenocarcinoma in Association to Histological Grade

Muhammad Tariq[1], Pervez Mohammad[1] and Muhammad Ashraf Salam[2]

ABSTRACT

Objective: To observe the immunoexpression of CK20/CK7 as a tumor marker and its association with histological grade of colorectal adenocarcinoma.
Study Design: Observational / cross sectional study.
Place and Duration of Study: This study was conducted at the Department of Pathology and Surgery, Post Graduate Medical Institute Lahore and Jinnah Medical College Peshawar from July, 2013 to February, 2015
Materials and Methods: Surgical specimens / paraffin blocks of 60 histopathologically diagnosed cases of colorectal adenocarcinoma were included in this study. Three sections, 4µm- thick from each blocks were cut and were stained with H&E and for CK20 and CK7 immunostain respectively. All the slides were evaluated for colorectal adenocarcinoma along with expression of CK20 and CK7.
Results: CK2-+/CK7 – immune-phenotype was seen in 38 out of60 (63.33%) cases of colorectal adenocarcinomas. While the CK20+/CK7+ immunophenotype was detected in 9 out 60 (15%) cases of colorectal adenocarcinomas. Similarly the CK20-/CK7- immunophenotype expression pattern was observed in 11/60 (18.33%) cases of colorectal adenocarcinomas. We also observed the CK20 expression in 47 out of 60 (78/33%) and CK7 expression in 11 out of 60 (18.33%) in colorectal adenocarcinomas.
Conclusion: There is an association of histological grade and pattern of expression of CK20/CK7.
Key Words: Cytokeratin 20 (CK20), cytokeratin 7 (CK7), colorectal adenocarcinoma, immunohistochemistry (IHC).

Citation of articles: Tariq M, Mohammad P, Salam MA. Immunoexpression of Cytokeratin 20 and Cytokeratin 7 in Colorectal Adenocarcinoma in Association to Histological Grade. Med Forum 2018;29(1):27-31.

INTRODUCTION

The use of tumor biomarkers is two thousand year old which has been published in old Egyptian papyrus, in which inflammation of breast was differentiated from breast carcinoma(Waxman, 1985)[1]. According to the history the 1st cancer indicator in modern medication was recognized by Bence-Jones in 1846 (Waxman, 1985)[1]. The glycoprotein molecule was collected from specimens of carcinoma colon, exposed the initial cancer antigen far ahead labeled as carcinoembryonic antigen (Gold and Freeman, 1965)[2].

Tumor markers consists of different types of materials like the cell surface antigens, hormones, enzymes, proteins, oncofetal antigens, oncogenes and receptors (Diamandis, 2002)[3]. The major role of these markers is screening and early detection, to confirm the diagnosis and prognosis of treatment (Lindblom and Liljiegren, 2000; Sokoll and Chan, 2004; Goedegebuure et al., 2004, Cooper, 2004).[4,5,6,7]

[1.] Department of Pathology / Surgery[2], Jinnah Medical College, Peshawar.

Correspondence: Dr. Muhammad Tariq, Assistant Professor of Pathology, Jinnah Medical College, Peshawar.
Contact No: 0300-5772028
Email: sohaib765@gmail.com

Received: August, 2017; Accepted: October, 2017

The main use of biomarkers in medical treatment is to confirm the diagnosis. It can also be used for the follow up of patient who is taking treatment of cancer (Cooper, 2004).[7]

The Cytokeratins (CKs) are the major complex collection of intermediate filaments proteins. Their role is very important in the growth and differentiation of the epithelial cells and are crucial for the normal tissue morphology and physiology. These intermediate filaments proteins show specificity in both normal tissue and in their tumors. Due to this unique property immunohistochemistry of intermediate filaments is extensively used in histopathology (Moll et al., 1982)[8].

Cytokeratins up to some extent are resistant to degradation and are thus providing resistance to surrounding stress. Barrier defects, inflammation, hyperprolifration and dedifferentiation are keratin related diseases, showing their essential contribution to epithelial functions (Schwarz et al., 2015)[9].

So far, twenty distinct cytokeratins have been discovered. On the basis of molecular weight and isoelectric facts cytokeratins were categorized and classified into 2 groups:

Type one (1) and type two (2) cytokeratins.

The type 1 "cytokeratins comprising of acidic, low molecular weights (40-56.6 kDa) proteins contains e.g. CK10, CK11, CK12, CK13, CK14, CK15, CK16, CK17, CK18, CK19, and CK20.

The type 2 "cytokeratins include basic, high molecular weight (52-67 kDa) proteins including CK1, CK2, CK3, CK4, CK5, CK6, CK7, CK8, and CK9 Bragulla & Homberger, 2009, (Iwatsuki & Suda, 2010.

As defined by Bayrak et al. (2011)[10] cytokeratin 7 (CK7) is basic (type II) and cytokeratin 20 (CK20) is acidic (type I) cytokeratain. Their expression was deliberated in different primary and metastatic malignancies and this appearance pattern was found to be altered in coloreactal carcinomas regarding the histological grade. CK7 was identified in 17.3% and CK20 in 81.1% cases of colorectal adenocarcinomas. CK7-/CK20+ had the highest proportion (65.8%) in colorectal carcinomas. The CK7-/CK20+ immunophenotype was detected in 15.3%, CK7-/CK20- in 16.9% and CK7-/CK20- in 2% colon adenocarcinomas. CK20 positively was more common in low grade carcinomas than in high grade carcinomas (85.1% versus 47.6%) (Bayrak et al; 2011)[8]. The CK7-/CK2-+ phenotype is highly sensitive and specific markers founded in colorectal carcinoma (Bayrak et al; 2012). The objective of this study was to observe the immunoexpression of CK20 & CK7 in colorectal adenocarcinoma as a Tumor markers and see the association.

MATERIALS AND METHODS

This study was performed at the Department of Pathology and Surgery. Post Graduate medical Institute Lahore & Jinnah Medical College Peshawar. Sample collection was done from July 2013 to February 2015, keeping in view the inclusion and exclusion criteria. Written informed consent was taken and recorded. The study was approved by the ethical committee. The patients having colorectal adenocarcinoma were selected according to the following recruitment criteria:-

Both male and female patients of all ages diagnosed histopathologically as primary colorectal adenocarcinoma were included in this study.

The patients already on chemotherapy and radiotherapy were excluded.

60 colorectal Adenocarcinoma Biopsy were included in this study. After recording history and findings of physical examination each biopsy was placed in a plastic jar and immersed in tenfold volume of 10% buffered formaline solution. The jar was labeled with the patient name, age, sex and registration number. A proforma was attached to each case. All relevant clinical information like presenting complaints, duration, report of investigations etc were recorded. Detailed gross examination of the specimen 1.Color 2.Weight 3.Size 4. Cute surface was carried out and findings were recorded.

Tissue processing was done according to Spencer and Bancroft, 2008. All the specimens were fixed in 10% buffered formaline solution. After twenty four hours of fixation, representative tissue sections were administered in automated processor in ascending alcohol concentrations and numerous change of xyline. Afterward dispensation, paraffin blocks were prepared in L-shaped metallic moulds. A rotatory microtome was used to obtain 3 to 4 micron thick sections. These sections were taken on the albumenized slides and were processed for staining with Haematoxylin and Eosin staining was done as per standard protocol. (Kiernan, 2008; Gamble.M, 2008)[11].

Microscopy was performed by using Binocular Microscope (Olympus) for the histopathological diagnosis and categorization of colorectal adenocarcinomas (2000; Fleming, 2012.)[12].

Staining Cytokeratin 20 and Cytokeratin 7 Immunohistochemical (IHC) was performed by using commercially available (Dako, Denmark) and reagents according to manufacturer`s instructions. Press et al., 2005).[13]

Data Analysis: Data was analyzed by the method of Levesque, 2007 using SPSS version 20. Qualitative data, like expression of CK20 and Ck7 was presented in the form of frequency and percentages. The association among the qualitative variables was calculated and analyzed statistically using chi square test. Quantitative data like age was given in the form of mean ± S.D. A P-value ≤ 0.05 was measured as statistically significant.

RESULTS

The study was consisted of sixty histopathologically diagnosed cases of colorectal adenocarcinomas. A detailed history of each case was recorded age, sex, complaints and site of colorectal biopsy was recorded. The immunostaining was carried out on all cases for CK20 and CK7 and expression was observed.

The descriptive statistics of age (in years) of 60 cases of colorectal adenocarcinomas, the minimum age was 16 years and maximum 85 years, the mean age of the patients was 54.40 ± 19.66 years. There were 6 out of 60 (10%) patients 16-29 years, 9 out of 60 (15%) 30-49 years, 34 out of 60 (5.67%) patients were 50-69 years and 11 out of 60 (18.33%) were alone 70 years of age. In this study there were 33 out of 60 (55%) males and 27 out of 60 (45%) females patients.

The histological grades of tumors was also assessed as shown in Figure 1, Grade-` was seen in 26/60 (43.33%) Grade 2 15/60 (25%) and Grade 3 was seen in 19/60 (31.67%).

The CK20/CK7 immunophenotype stain results and frequencies of different immunophenotype positively is given in Table 1.

The CK20 and CK 7 expression were imposed with tumors grade Table 2 showing the different vesicles showing P value and its significances.

The CK20-/CK7- immunophenotype was detected in 10/19 cases (52.63%) in high grade carcinoma and in

1/41 cases (2.44%) in low grade carcinoma (X^2 = 23.201; p-value = 0.001) highly significant

Similarly CK20+/Ck7+ immunophentype was observed in 8/19 cases (42.1%) in high grade carcinoma and in 1/41 cases (2.44%) in low grade carcinoma (X^2 = 19.986; p-value = 0.031) significant.

While CK20+/Ck7+ immunophentype was expressed in 2/19 cases (10.52%) in high grade carcinoma and in 0/41 cases (0%) in low grade carcinoma (X^2 = 0.599; p-value = 0.435) insignificant.

The results of this study indicate that the association exists among Ck20/Ck7 expression and the grade of colorectal adenocarcinoma.

Table No. 1: Ck20/Ck7 Stain Results

Results	Frequency	Percentage (%)
$CK20^+/CK7^-$	38	63.33%
$CK20^-/CK7^-$	11	18.33%
$CK20^+/CK7^+$	9	15.00%
$CK20^-/CK7^+$	2	3.33%
Total	60	100%

Table No. 2: Different Variables Showing P-Value and its Significance

Variables	**P-value**	**Significance***
CK20 Positivity (low grade vs high grade)**	0.035	Significant*
CK7 Positivity (high grade vs low grade)	0.039	Significant*
CK20+/CK7- Immunophenotype (low grade vs high grade)	0.000	Highly Significant*
CK20-/CK7- Immunophenotype (high grade vs low grade)	0.001	Highly Significant*
CK20+/CK7+ Immunophenotype (high grade vs low grade)	0.031	Significant*
CK2-+/CK7- Immunophenotype (high grade vs low grade)	0.435	Insignificant

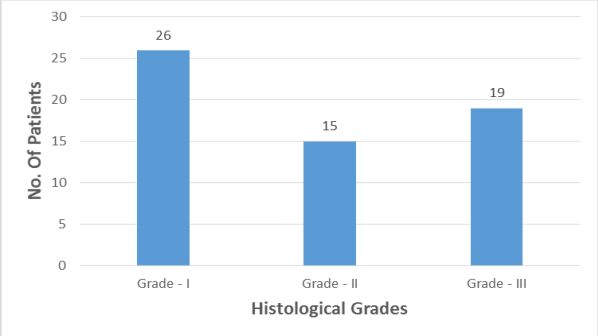

Fig- 1: Histological Grades **Grade I (well differentiated), Grade II (moderately differentiated) and Grade III (Poorly differentiated). Grade I and Grade II is low grade and Grade III is high grade.

Note: - Grade I (well differentiated), Grade II (moderately differentiated) and Grade III (poorly differentiated) Grade I and Grade II is low grade and Grade III is high grade. Grade II (moderately differentiate adenocarcinoma immunostain CK20 shown in Fig 3 and immunostain CK7 (Fig 4).

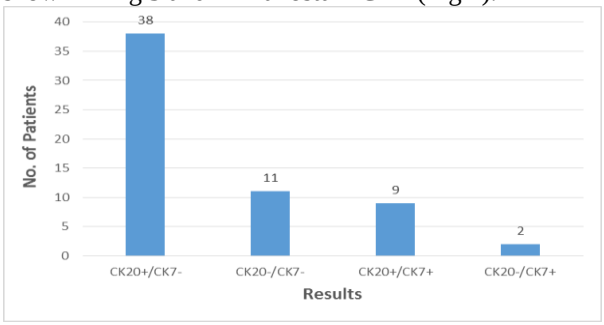

Figure No.2: Frequencies of different immunophenotype positivity *Association is significant at p-value ≥ 0.05 **Grade I (well differentiated), Grade II (moderately differentiated) and Grade III (poorly differentiated). Grade I and Grade II is low grad3e and Grade III is high grade.

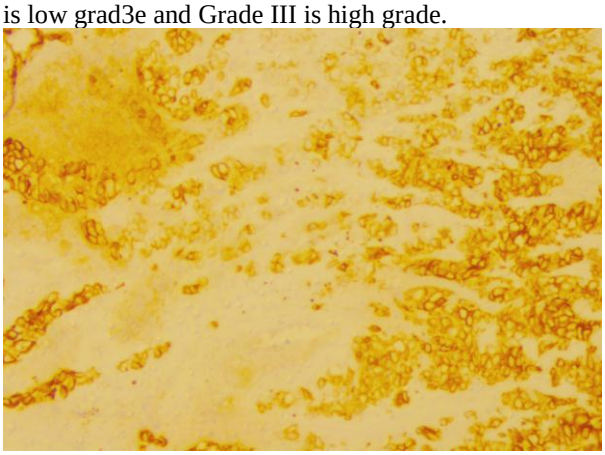

Fig-3: Moderately differentiated (Grade II) adenocarcinoma (Immunostain CK20+ X40)

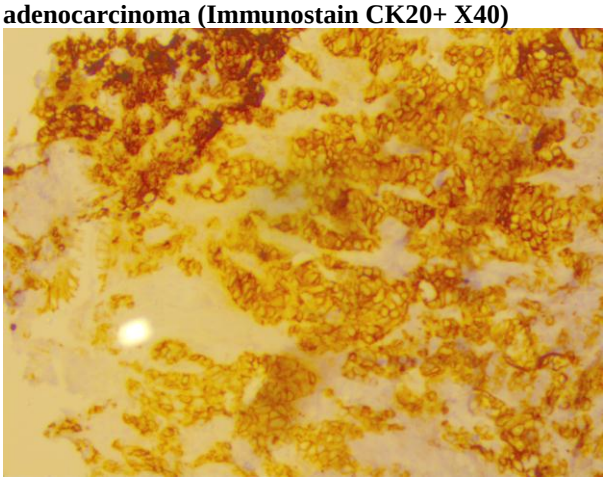

Fig-4: Moderately differentiated (Grade II) adenocarcinoma (Immunostain CK7+ X40)

DISCUSSION

Cytokeratin (CKs) are the major complex collection of intermediate filaments proteins. Their role is very important in the growth and differentiation of the epithelial cells and are crucial for the normal tissue morphology and physiology. These intermediate filaments protein show specificity in both normal tissue and their tumors. Due to this unique property immnuohistochemistry of intermediate filaments is extensively used in histopathology 2010.

A study conducted by Bayrak et al; (2012)[14] claimed that CK20 was detected in 99/118 (83.89%) and CK7 was expressed by 26/118 (2%) of colorectal adenocarcinomas. These observations are in accordance with the present study.

The studies are required to find the role of CK20 and CK7 in high grade colorectal adenocarcinomas, a study was conducted by (Hernandez et al; 2005)[15] in their findings claimed that CK7 expression and CK20 loss are highly associated to high grade colorectal adenocarcinomas. These observations are also seen our study.

A study also observed that in colorectal adenocarcinoma loss of CK20 was associated with high tumor grade, $P \leq 0.001$ (significant) as has also been seen in the present study.

According to our observations the CK20 and CK7 patterns of expression varied according to histological grade in colorectal adenocarcinomas. CK20 was expressed in 81.1% and CK7 in 17.3% cases of colorectal adenocarcinoma. CK20+/CK7- had the highest proportion (65.8%) in colorectal adenocarcinomas. The CK20+/CK7- immunophenotype was identified in 15.3%, CK20-/CK7- in 16.9% and CK20-/CK7+ in 2% colorectal adenocarcinomas. CK20 positivity was more common in low grade adenocarcinomas than in high grade adenocarcinomas (85.1% versus 47.6%), more or less similar results were recorded in this study.

A significance difference in the relative expression of CK20 and CK7 between malignant and normal colorectal tissues and by the tumor differentiation was detected in this study. Specifically, it was demonstrated that compared with normal tissues, colorectal adenocarcinomas were likely to be highly positive for CK20 (both in CK20+/CK7- and CK20/CK7+ immunoprofiles). Furthermore, compared with low grade colorectal adenocarcinomas, high grade colorectal adenocarcinomas showed a higher proportional of CK20+/CK7+, CK20-/CK7- and CK20-/CK7+ immunoprofile.

CONCLUSION

This study concludes that there is an association of histological grade and pattern of expression of CK20/CK7.

Author's Contribution:

Concept & Design of Study:	Muhammad Tariq
Drafting:	Pervez Mohammad
Data Analysis:	Muhammad Ashraf Salam
Revisiting Critically:	Pervez Mohammad, Muhammad Ashraf Salam
Final Approval of version:	Muhammad Tariq

Conflict of Interest: The study has no conflict of interest to declare by any author.

REFERENCES

1. Waxman J Tumor markers. Quart J Med 1985; 88:233-41.
2. Gold P, Freeman SO.. Detection of tumor specific antigens in human colonic carcinoma by immunological tolerance and absorptions techniques. J Exp Med 1965;121:439-62.
3. Diamandis EP, Chan D, Fritsche EP, Lilja H, Schwartz M. Tumor markers: Physiology, Pathology, technology, and clinical applications. Press. Washington DC; AACC: 2002.p.3-8.
4. Lindblom A, Liljegren. A Tumor marker in malignancies. Br Med J 2000;320:424-27.
5. Sokoll LJ, Chan DW. Clinical chemistry: Tumor markers. In: Abeloff MD, Armitage JO, Niederhuber JE, Kastan MB, McKenna WG, editors. In Abeloff: Clinical Oncology. 3rd ed. Pennsylvania: Elsevier Churchill Livingstone; 2004.
6. Goedegebuure PS, Eberlein TJ, Liyanange U. Tumor biology and tumor markers. In: Townsed CM, Beauchamp RD, Evers BM, Mattox KL, editors. Townsed: Sabiston textbook of surgery. 17th ed. Philadelphia: Elsevier Saunders; 2004.p. 4-12.
7. Cooper K, Booth A, Squires H, Carroll C, Papaiannou D, Logan RF, et al. Chemoprevention of colorectal cancer: systematic review and economic evolution. Health technology assessment (Winchester, England) 2010;14:1-206.
8. Moll R, Schiller DL, Franke WW. The catalog of human cytokeratins: patterns of expression is normal epithelium, Tumors And Cultured Cells 1982;31:11-24.
9. Schwarz N, Leube RE, Windoffer R, Magin TM. Dissection of keratin network formation, turnover and reorganization in living murine embryos, Published online 2015, March, 11. Doi: 10.1038/ srep 09007.
10. Bayrak R, Hatlas H, Yenidunya S. Cytokeratin 7 cytokeratin 20 expressions in colorectal adenocarcinomas. Pathol Res Pract 2011;207: 156-60.

11. Keirnan JA. Histochemical Methods: Theory and Practice. 4th ed. Blocham UK: Scion; 2008.
12. Fleming M, Tatishchev FS, Ravula S, Wang LH. Colorectal carcinomas: Pathologic aspects. J Gastrointest Oncol 2012;3:153-73.
13. Press MF, Mirlacher M, Sauter G, Bernstein L, Villalobos IE. Diagnostic evaluation of HER-2 as a molecular target: an assessment of accuracy and reproducibility of laboratory testing in large, prospective randomized clinical trials. Clinical Cancer Research 2005;15:6598-07.
14. Bayrak R, Yenidunya Hatlas H. The value of CKX2 and cytokeratins 7 and 20 expression in differentiating colorectal adenocarcinomas from extra intestinal gastrointestinal adenocarcinomas: cytokeratin 7-/20+ phenotype is more specific than CDX2 antibody. Diagnostic Pathol 2012;7:9-10.
15. Hernandez BY, Clegg L, Frierson HF, Moskaluk CA, Li YJ, Cote TR, et al. CK20 and CK7 protein expression in colorectal cancer: demonstration of utility of a population based tissue microarray. Hum Pathol 2005;36:275-81.