

Immunohistochemical Expression of Cytokeratins 7 And 20 as Well as Mucins 1 And 2 in Egyptian Cases of Primary Colorectal Carcinomas

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Abstract: Colorectal cancer (CRC) development is a multi-steps process that spans, at least, 10-15 years and represents a major health world-wide burden. Almost half of the patients undergoing surgery develop recurrent disease; therefore, surveillance is advocated even through various means at different intervals. However, current screening and surveillance efforts have had a limited impact due to suboptimal compliance. So, the expression of both cytokeratins 20 and 7 and mucin core proteins (MUC1 and MUC2) in these primary neoplasms were investigated in this study aiming at detection of their role in early diagnosis and histogenesis in addition to determine their relationship to the clinical features. The study was performed on 120 primary surgically resected CRC divided into two groups; group 1 consisted of 70 cases of non-mucinous adenocarcinoma and group 2 including 50 cases of mucinous carcinoma. In addition, 20 normal colorectal specimens were taken as controls (group 3). Among the clinicopathological characteristics of the malignant groups, group 2 cases histologically were more likely to be of high grade than those of group 1 that conversely were more likely to be of low grade despite of statistical insignificance. Moreover, there were significant differences in CKs expression by the tumor stage of both malignant groups (early stages were more likely to be CK20+/CK7- while late stages were more likely to be CK20+/CK7+) and in CK20 expression between either malignant groups and the control one (either in CK20+/CK7- or in CK20+/CK7+ phenotype). Considering the mucin core proteins, MUC1 was more greatly expressed in each of the carcinoma groups than the control one, however, MUC2 was markedly lower in group 1 than in group 2 or in control one. In addition, most of group 1 cases were of MUC1+/MUC2- phenotype and most of group 2 cases had MUC1+/MUC2+ phenotype. Exclusively, MUC1 was not detected in any control case in contrast to MUC2 that was expressed in all control cases. It was concluded that the relevance of the CKs phenotypic profile in tumor stage as CK7 may be an indicative marker for tumor progression. Also, mucinous carcinomas, through MUCs phenotypic profile, can be grouped together as goblet cell type (MUC2+) carcinomas. In addition, MUC1 expression was strongly associated with colorectal carcinomas. Therefore, reevaluation of the histological classification of colorectal carcinomas is recommended.

Key words: Primary colorectal carcinomas • Cytokeratins 20 and 7 antigens • Mucins 1 and 2 proteins • Immunohistochemistry

INTRODUCTION

Colorectal cancer (CRC) is the third most frequent cancer in the world, with a high incidence rate in North America, Western Europe, Australia, New Zealand and

France. The general survival rate of colorectal carcinoma patients doesn't exceed 40% [1]

Colorectal carcinoma develops either sporadically (85%), as a part of hereditary cancer syndrome (<10%), or on a background of inflammatory bowel disease.

Traditionally, the adenoma-carcinoma sequence has been believed to underlie the development of most CRC cases, however, in the recent years, two distinct pathways have been implicated in that development; the microsatellite instability (MSI) and the chromosomal instability (CIN) ones [2, 3] Detection of these two pathways has led to the paradigm shift in thinking of CRC as a genetically heterogeneous disease [4-6].

The risk of CRC increases after age of 40 years rises sharply at the ages of 50-55 years and reaches its peak by age 75 years. Cancer as such is due to an accumulation of genetic alterations that allows growth of neoplastic cells with phenotypic hallmark characteristics including insensitivity to anti-growth signals, evasion of apoptosis, limitless replicative potential, sustained angiogenesis and the invasiveness ability with metastasis [7-9].

The effective therapy of cancer patients essentially depends upon the primary anatomical site in the digestive system as well as the histologic type of tumor. Thus, the classification of primary cancers into several groups is important for this point [10].

The use of antibodies immunohistochemically for detecting specific tumor antigens has led to significant improvement in their diagnosis. Since the immunohistochemical profiles of specific antibodies in metastatic cancers corresponded to those of the primary sites, thus, the patterns of their expression in primary tumors may help to identify the origin of tumors [11-13].

Cytokeratins (CKs) are the principal intermediate filamentous proteins of normal epithelia and epithelial tumors. Twenty different subtypes of CKs have been numbered depending upon their molecular weight and isoelectric Ph [10]. The expression pattern of CKs depends upon the embryologic development and the degree of cellular differentiation [14]. Cytokeratin 7 is found in many ductal and glandular epithelia, like those of the lung, breast, ovary and endometrium, while CK20 is expressed in gastro-intestinal epithelium, urothelium and Merkel cells[10]. In the last years, the combined patterns of CK7and CK20 have been extensively expressed in various primary and metastatic carcinomas [13].

Mucins are large, abundant, filamentous glycoproteins that are located at the interface between various epithelia and their extracellular environments [15, 16]. Several mucins are known to form mucus layers while others form the glycocalyx on the intestinal enterocytes [16]. Mucin1 (MUC1), located in 7q22, is a membrane-bound mucin that is expressed in the mammary glands and the pancreatic ducts as well as the gallbladder and colorectal adenocarcinomas [15-17], however, MUC2

is a large secretory mucin located within the 11q15 locus and expressed in goblet cell and observed in intestinal-type tumors, mucinous carcinomas as well as in early colorectal cancer [10].

In order to determine the usefulness of markers for classification of colorectal carcinomas in addition to explain their role in early diagnosis and their relationship to clinical profiles, the expression of CK7 andCK20 as well as MUC1and MUC2 were examined in these primary neoplasms.

MATERIAL AND METHODS

The material of this study consisted of surgically resected 70 primary sporadic colorectal non-mucinous carcinomas (Group 1) as well as 50 mucinous carcinomas irrespective to their mucus component (MC; Group 2), selected from Pathology Departments, Al-Azhar and Kasr El-Aini University Hospitals during the period from July 2005 to December 2007. Thirty seven (52.9%) of group 1 patients were males, while thirty three were females (47.1%) with ages ranged from 47 to 62 years (mean; 59.7 ± 2.1). Twenty eight (56%) of group 2 patients were males, while the remaining (44%) were females. The ages ranged from 49 to 67 years (mean; 58 ± 1.9). The clinicopathological data were taken from patient files. Tumors associated with inflammatory bowel disease, post-irradiation, or chemotherapy were excluded from this study. In addition, twenty normal colonic (Fig.1) and rectal biopsies were taken 5 cm far from the tumor-free margins as control tissues (Group 3). Ten control biopsies were taken from males (50%) and similar number from females (50%). The ages ranged from 48 to 67 years (mean; 50.2 ± 2.3 ; Table1). Formalin-Fixed and paraffin-embedded blocks were sectioned at $5\mu\text{m}$ thickness and examined microscopically after H. andE. staining to confirm the diagnosis. Tumor staging was done according to American Joint Committee on Cancer [18]. In addition, immunohistochemical study was performed as the sections were deparaffinized, dehydrated and incubated with 3% H_2O_2 for 5 minutes to block the endogenous peroxidase activity. Immunohisto-chemical staining for CK7(OV-TL 12/30,1:50,Dako Cytomation), CK20 (Ks 20.8,1:50,Dako Cytomation), MUC1(C 595,1:100, Santa Cruz Biotechnology, Inc.) and MUC2 (B306.1,1:100, Santa Cruz Biotechnology, Inc.) monoclonal antibodies using an avidin-biotin peroxidase complex (ABC) technique was carried out after antigen retrieval by microwave heating in citrate solution. Immunohistochemical evaluations were performed for areas with and without mucus component.

Table 1: Histopathological diagnosis of the studied cases (n=140)

| Variables | Group1 (non mucinous adenocarcinoma n=70) | Group2 (mucinous adenocarcinoma n=50) | Group 3; control (n=20) |
|----------------|---|---------------------------------------|-------------------------|
| Age (Years): | | | |
| Range | 47-62 | 49-67 | 48-67 |
| Mean | 59.7±2.1 | 58±1.9 | 50.2±2.3 |
| Sex: | | | |
| Male | 37(52.9)* | 28(56) | 10(50) |
| Female | 33(47.1) | 22(44) | 10(50) |
| Anatomic site: | | | |
| Rt. colon | 16(22.9) | 15(30) | 8(40) |
| Lt colon | 25(35.7) | 16(32) | 6(30) |
| Rectum | 29(41.4) | 19(38) | 6(30) |

* Values are expressed in n(%), total percent is 100%

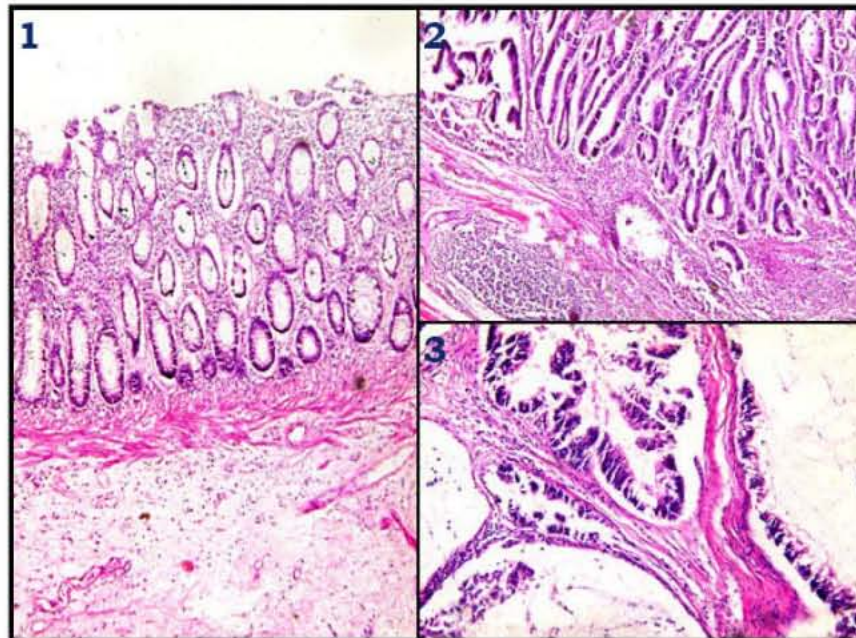


Fig. 1: Normal colonic mucosa (H andE X 200)

Fig2: Low grade non-mucinous adenocarcinoma (H andE X200).

Fig 3: Low grade mucinous adenocarcinoma showing wide areas of mucin secretion (H andE X200).

The results for cytokeratins were considered positive if 10% or more of the tumor cells were stained [10], while staining of mucin core proteins (MUC1 and MUC2) was scored semiquantitatively as 0 (negative), 1+ (<5% positive cells), 2+ (5-30% positive cells), 3+(30-60% positive cells) and 4+ (>60% positive cells). MUC phenotypes were defined such that a score of, at least, 3 was regarded as positive [19]. Immunopositivity for CK7 and MUC2 appeared as a brown cytoplasmic staining while positive CK20 and MUC1 reactions displayed a brownish membranous epithelial staining.

Statistical Analysis: Chi-square test, Fisher exact test, t-test (unpaired) and ANOVA test (Analysis of variance) was performed. The results were considered to be

statistically significant when the P value was < 0.05. All statistical analyses were conducted using the SPSS version 12 statistical package for social science.

RESULTS

Thirty nine (55.7%) of group1 (non-mucinous adenocarcinoma; Fig.2) cases were low and 31 (44.3%) were high graded while 19 (38%) of group 2(mucinous adenocarcinoma) cases were low (Fig.3) and 31 (54%) were high graded. Regarding the tumor staging according to American Joint Committee on Cancer, eight (11.4%) of group1 cases were in stage I, twenty one (30%) were in stage II, fourteen (20%) were in stage III and twenty seven (38.6%) were in stage IV. However, six (12%) of

Table 2: Clinicopathological features of malignant groups (n=120)

| Characteristics | Group 1; non-mucinous carcinoma (n=70) | Group 2; mucinous carcinoma (n=50) |
|-----------------|--|------------------------------------|
| Age: | | |
| Range | 47-62 | 48-67 |
| Mean | 59.7 ±2.1 | 58± 1.9 |
| Sex: | | |
| Male | 37(52.9) * | 28(56) |
| Female | 33(47.1) | 22(44) |
| Anatomic site: | | |
| Rt. colon | 16(22.9) | 15(30) |
| Lt. colon | 25(35.7) | 16(32) |
| Rectum | 29(41.4) | 19(38) |
| Grade: † | | |
| Low | 39(55.7) | 19(38) |
| High | 31(44.3) | 31(62) |
| Stage: | | |
| I | 8 (11.4) | 6 (12) |
| II | 21(30) | 14(28) |
| III | 14(20) | 12(24) |
| IV | 27(38.6) | 18(36) |

*Values are expressed in n (%), total percent is 100%

† Insignificant difference (P>0.05) between the grade (whether low or high) of both groups

Table 3: Expression of cytokeratins in group1 (non mucinous adenocarcinoma) (n=70)

| Characteristics | CK20-/ CK7-(n=9) | CK20-/ CK7+ (n=1) | CK20+/ CK7-(n=31) | CK20+/ CK7+ (n=29) | Total* | P value |
|-------------------|------------------|-------------------|-------------------|--------------------|---------|---------|
| Age group(Years): | | | | | | |
| <50 | 3(14.3) | 0 (0) | 10(47.6) | 8 (38.1) | 21(100) | 0.39 |
| 50-59 | 3(12) | 0 (0) | 11(44) | 11(44) | 25(100) | |
| ≥60 | 3(12.5) | 1(4.2) | 10(41.6) | 10(41.6) | 24(100) | |
| Sex: | | | | | | |
| Male | 4(10.8) | 1(2.7) | 17(45.9) | 15(40.5) | 37(100) | 0.87 |
| Female | 5(15.2) | 0 (0) | 14(42.4) | 14(42.4) | 33(100) | |
| Anatomic site: | | | | | | |
| Rt. colon | 1(6.3) | 0 (0) | 9 (56.2) | 6(37.5) | 16(100) | 0.69 |
| Lt. colon | 4(16) | 0 (0) | 10 (40) | 11(44) | 25(100) | |
| Rectum | 4(13.8) | 1(3.4) | 12 (41.4) | 12 (41.4) | 29(100) | |
| Grade: | | | | | | |
| Low | 5(12.8) | 0 (0) | 16 (41) | 18 (46.2) | 39(100) | 0.99 |
| High | 4(12.9) | 1(3.2) | 15(48.4) | 11 (35.5) | 31(100) | |
| stage:† | | | | | | |
| I | 0 (0) | 1(12.5) | 6 (75) | 1 (12.5) | 8 (100) | <0.05† |
| II | 1 (4.8) | 0 (0) | 19 (90.5) | 1 (4.8) | 21(100) | |
| III | 2 (14.3) | 0 (0) | 3 (21.4) | 9 (64.3) | 14(100) | |
| IV | 6 (22.2) | 0 (0) | 3 (11.1) | 18 (66.7) | 27(100) | |

* Values are given in n(%). Total percent may be slightly more or less than 100 because of rounding off.

†Significant difference was found in CKs expression by tumor stage (P<0.05).

group 2 cases were in stage I, fourteen (28%) were in stage II, twelve (24%) were in stage III and eighteen (36%) were in stage IV. The locations of the group 1 cases were as follows: 16 in the right colon (16/70;22.9%), 25 in the left colon (25/70;35.7%) and the remaining were in rectum (29/70;41.4%), however, 15 of the group 2 cases were located in the right (15/50; 30%), 16 in the left colon (16/50;32%) and the remaining were in the rectum (19/50;38%). Eight of the control group biopsies were located in the right colon (8/20;40%) and twelve in both left colon and rectum (6;30% in either site). The clinicopathological features of carcinoma groups are shown in Table 2. No significant differences were found as regard the age, sex, location and staging between

groups 1 and 2. Histologically, mucinous carcinomas were more likely to be of high grade than non-mucinous group which conversely was more likely to be of low grade although there is a statistical insignificance (>0.05). Signet ring cell malignant components were seen in 9 out of the high grade group 2 cases (29%). Pure signet ring cell carcinoma was not found in this study.

Expression and Locations of Cytokeratins (Tables 3-6):

Cytokeratin 7 was found in 42.8% (Fig.4) and 52% (Fig.5) while CK20 was expressed in 85.7% (Fig.6) and 94% (Fig.7) of group 1 and 2 cases respectively (Table 6). CK20-/ CK7+ phenotype was observed in one (rectal) of group 1 cases (1/70; 1.4%) while CK20+/ CK7-was found

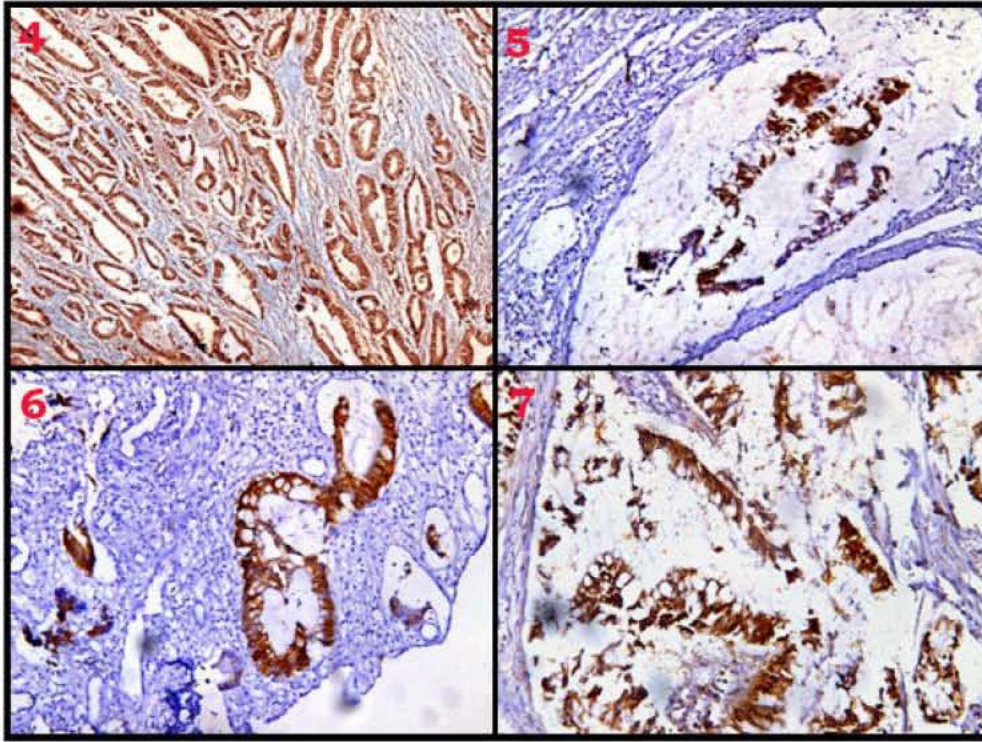


Fig. 4: Diffuse brown cytoplasmic CK7 positive immunostaining in a case of non-mucinous adenocarcinoma (DAB chromogen and hematoxylin counter stain x 200).

Fig. 5: Brown cytoplasmic CK7 positive immunostaining in a case of mucinous adenocarcinoma (DAB chromogen and hematoxylin counter stain x 200).

Fig. 6,7: Brown epithelial membranous CK20 positive immunostaining in a case of non-mucinous and mucinous adenocarcinoma respectively (DAB chromogen and hematoxylin counter stain x 200).

in 31(31/70;44.3%) distributed as follows: 9 in the right colon,10 in the left colon and 12 in the rectum. CK20+/CK7+ were displayed in 29 (29/ 70; 41.4%) of group 1 cases located as follows: 6 in the right colon, 11 in the left colon and 12 in the rectum. However, the remaining cases (9/70; 12.9%) were immunonegative for both CKs (one in the right, 4 in each of the left colon and rectum; Table 3). Considering the group 2, no cases were of CK20-/CK7+ phenotype while CK20+/CK7-was detected in 21 cases (21/50;42%); 6 of which were in the right colon, 5 in the left and 10 in the rectum. CK20-/CK7-phenotype was found in three cases (3/50;6%); one in each location while both CKs (CK20+/CK7+) were expressed in 26 cases (26/50;52%) distributed as follows: 8 in either right colon or rectum and 10 in the left colon; Table 4). Regarding the control group, neither of the specimens was CK20-/CK7+ nor CK20+/CK7+,however, five were CK20+/CK7-(5/20;25%); 2 of which were in each right and left colon and one in the rectum. The remaining (15/20; 75%) were of CK20-/CK7-phenotype distributed as

follows: 6 in right colon, 4 in left colon and 5 in the rectum (Table 5). The relative expression of CK7 and CK20 in malignant groups was compared by clinicopathological characteristics. There was no significant difference in both CKs expression in either of malignant groups by age, sex, tumor grade or anatomical site ($P > 0.05$) while a statistically significant difference was found in their expression by tumor stage ($P < 0.05$ for group1; Table 3 or for group2; Table 4). Early-stage cancers (stages I and II) were more likely to be CK20+/CK7-and less likely to be CK20+/CK7+. Conversely, advanced colorectal cancers (stages III and IV) were more likely to be CK20+/CK7+. In addition, the expression of CK20 displayed a significant difference ($P < 0.05$) between group 1 or group 2 and control (regarding the expression of CK20 alone or combined with CK7; Table 6).

Expression and Locations of Mucins (Tables 7 and 8): MUC1 expression was seen in 77.1% (Fig.8) while MUC2 was expressed in 10% (Fig.9) of group 1 cases (staining

Table 4: Expression of cytokeratins in group 2 (mucinous adenocarcinoma) (n=50)

| Characteristics | CK20-/CK7-(n=3) | CK20-/CK7+(n=0) | CK20+/CK7-(n=21) | CK7+/CK20+(n=26) | Total* | P value |
|-----------------|-----------------|-----------------|------------------|------------------|----------|---------|
| Age (Years): | | | | | | |
| <50 | 1(4.3) | 0(0) | 8(34.8) | 14 (60.9) | 23(100) | >0.05 |
| 50-59 | 1(5.6) | 0(0) | 8(44.4) | 9 (50) | 18(100) | |
| =60 | 1(11.1) | 0(0) | 5 (55.6) | 3(33.3) | 9(100) | |
| Sex: | | | | | | |
| Male | 2(7.1) | 0(0) | 14(50) | 12(42.9) | 28 (100) | >0.05 |
| Female | 1(4.5) | 0(0) | 7 (31.8) | 14(63.6) | 22 (100) | |
| Anatomic site: | | | | | | |
| Rt. colon | 1(6.7) | 0(0) | 6(40) | 8 (53.3) | 15 (100) | >0.05 |
| Lt. colon | 1(6.3) | 0(0) | 5 (31.2) | 10 (62.5) | 16 (100) | |
| Rectum | 1(5.3) | 0(0) | 10(52.6) | 8 (42.1) | 19 (100) | |
| Grade: | | | | | | |
| Low | 2(10.5) | 0(0) | 8 (42.1) | 9 (47.4) | 19 (100) | >0.05 |
| High | 1(3.2) | 0(0) | 13(41.9) | 17(54.8) | 31(100) | |
| Stage: † | | | | | | |
| I | 0(0) | 0(0) | 5 (83.3) | 1 (16.7) | 6 (100) | <0.05 ‡ |
| II | 0(0) | 0(0) | 12 (85.7) | 2 (14.3) | 14 (100) | |
| III | 1(8.3) | 0(0) | 2(16.7) | 9 (75) | 12 (100) | |
| IV | 2(11.1) | 0(0) | 2(11.1) | 14 (77.8) | 18(100) | |

* Values are expressed in n(%). Total percent may be slightly less than 100 because of rounding off

† TNM stage classification of American Committee of cancer

‡ Significant difference was found in expression of CK20 alone or combined with CK7 by tumor stage (P<0.05)

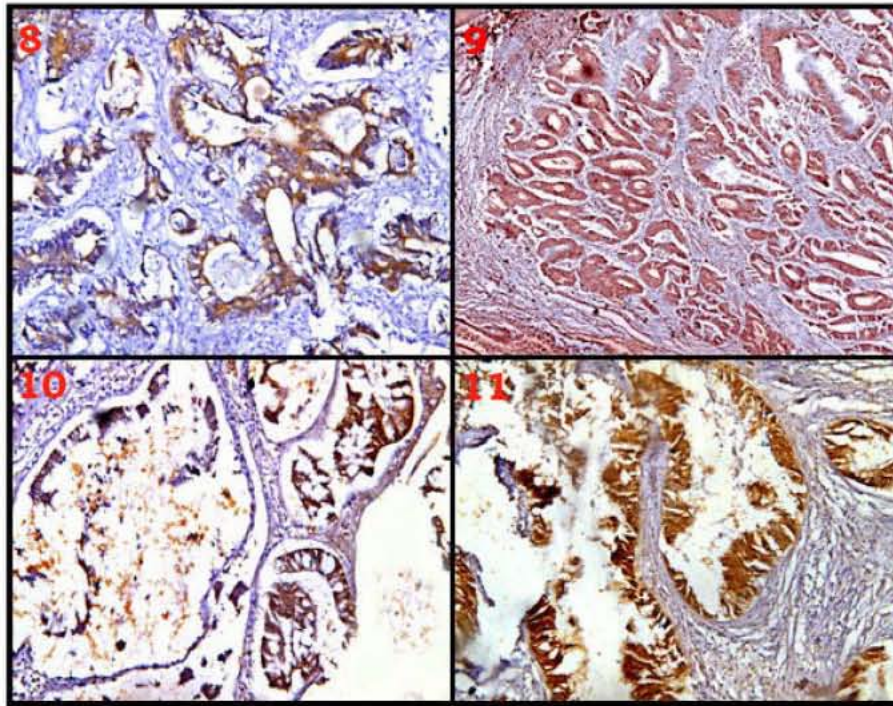
Table 5: Expression of cytokeratins in control group (n=20)

| Characteristics | CK20-/ CK7-(n=15) | CK20-/ CK7+(n=0) | CK20+/ CK7-(n=5) | CK7+/ CK20+ (n=0) | Total | P value |
|--------------------|-------------------|------------------|------------------|-------------------|---------|---------|
| Age group (Years): | | | | | | |
| <50 | 3 (60) | 0 (0) | 2 (40) | 0 (0) | 5 (100) | >0.05 |
| 50-59 | 9 (81.8) | 0 (0) | 2 (18.2) | 0 (0) | 11(100) | |
| =60 | 3 (75) | 0 (0) | 1 (25) | 0 (0) | 4 (100) | |
| Sex: | | | | | | |
| Male | 7 (70) | 0 (0) | 3 (30) | 0 (0) | 10(100) | >0.05 |
| Female | 8 (80) | 0 (0) | 2 (20) | 0 (0) | 10(100) | |
| Anatomic site: | | | | | | |
| Rt. colon | 6 (75) | 0 (0) | 2 (25) | 0 (0) | 8 (100) | >0.05 |
| Lt. colon | 4 (66.7) | 0 (0) | 2 (33.3) | 0 (0) | 6 (100) | |
| Rectum | 5 (83.3) | 0 (0) | 1 (16.7) | 0 (0) | 6 (100) | |

Table 6: Cytokeratins expression in the three groups (n=140):

| CK20/CK7 Phenotypes | Group 1 (Non mucinous adenocarcinoma n=70)* | Group2; (Mucinous adenocarcinoma n=50) | Group3; (Control n=20) |
|---------------------|---|--|------------------------|
| +/+ | 29 (41.4) | 26 (52) | 0(0) |
| +/- | 31(44.3) | 21(42) | 5(25) |
| -/+ | 1(1.4) | 0(0) | 0(0) |
| -/- | 9(12.9) | 3(6) | 15(75) |

* Significant differences (P < 0.05) in CK20 expression between group 1 or group 2 and control one regarding CK20+/CK7+ & CK20+/CK7- phenotypes.



Figs. 8 and 10: Brown epithelial membranous MUC1-positive immunostaining in cases of non-mucinous and mucinous adenocarcinoma respectively (DAB chromogen and hematoxylin counter stain x 200).

Figs. 9 and 11: Diffuse brown cytoplasmic MUC2-positive immunostaining in cases of non-mucinous and mucinous adenocarcinoma respectively (DAB chromogen and hematoxylin counter stain x 200).

Table 7: Mucin core protein expression in the three studied groups (n= 140)

| Group | Mucin core proteins | |
|------------------------------|---------------------|----------|
| | MUC-1* | MUC-2* |
| Non-mucinous (group1) (n=70) | 54 (77.1)† | 7 (10)† |
| Mucinous (group 2) (n=50) | 41(82) | 46 (92) |
| Control (group 3) (n=20) | 0 (0) | 20 (100) |

*Values are expressed in n(%).

† MUC2 expression is significantly lower in group1 than in group 2 or in control one (P < 0.05).

‡ MUC1 expression was higher in either of malignant groups than in control.

Table 8 : MUC phenotypes and their location in the malignant groups (n=120):

| Group & location | MUC phenotype (MUC1/MUC2) | | | | Total |
|---|---------------------------|---------|------------|------------|---------|
| | -/- | -/+ | +/- | +/+ | |
| Group 1 (Non mucinous adenocarcinoma n=70): † | | | | | |
| Rt. colon | 3 (18.8)* | 0 (0) | 11 (68.7)‡ | 2 (12.5) | 16(100) |
| Lt.colon | 4 (16) | 0 (0) | 18 (72) | 3 (12) | 25(100) |
| Rectum | 9 (31) | 0 (0) | 18 (62.1) | 2 (6.9) | 29(100) |
| Group 2 (Mucinous adenocarcinoma n=50): | | | | | |
| Rt. colon | 1 (6.66) | 1(6.66) | 1 (6.66) | 12 (80)§ | 15(100) |
| Lt.colon | 1 (6.25) | 1(6.25) | 1 (6.25) | 13 (81.25) | 16(100) |
| Rectum | 0 (0) | 5(26.3) | 0 (0) | 14 (73.7) | 19(100) |

*Values are expressed in n(%).

† Insignificant value (P > 0.05) in any malignant group between mucins expression and their location.

‡ MUC1+/MUC2- phenotype was detected in 47 (67.1%) of group 1 cases.

§ MUC1+/MUC2+ phenotype was seen in 39 (78%) of group 2 cases.

score of 3 or more). In addition, MUC1 expression was seen in 82% (Fig.10) while MUC2 was expressed in 92% (Fig.11) of group 2 cases. Malignant groups showed no significant difference in MUC1 expression ($p>0.05$). However, MUC2 expression was significantly lower in group 1 than either in group 2 or in control one ($P<0.05$; Table 7). MUC phenotypes were generated by combining both mucin core proteins expression. MUC1+ / MUC2- was detected in forty seven (47/70; 67.1%) of group 1 cases; 11 of which were located in the right, 18 were in either left colon or rectum. However, MUC1- / MUC2+ were not displayed in any case (0%). MUC1+ / MUC2+ phenotype was detected in 7(10%) cases; 2 of which were in each of the right colon and rectum while 3 in the left colon while sixteen of group1 cases were found to be immunonegative for both mucins (MUC1-/MUC2-); 3 of which were in right, 4 in the left and 9 in the rectum. Considering the group2, MUC1+/MUC2- was detected in two cases; one in the right and the other in left colon while MUC1-/MUC2+ was found in 7; one in either right or left colon and 5 in the rectum. Although MUC1-/MUC2-phenotype was displayed in two cases; one in each of right and left colon, MUC1+ /MUC2+ was found totally in 39 cases(39/50;78%) distributed as follows: 12 in right, 13 in left and 14 in rectum. Regarding the control group, MUC1 was not displayed in any case (0%) in spite of the expression of MUC2 (Focal in the perinuclear cytoplasm of normal goblet cells) in all cases (100%).No significant difference was detected in any malignant group between mucins expression and their location (Table 8).

DISCUSSION

Every year, nearly one million people worldwide develop colorectal cancer (CRC) of whom 50% approximately suffer from recurrence and eventually die of systemic disease within 5 years from diagnosis [20]. Increased understanding of the molecular biology has revealed several promising diagnostic, prognostic and predictive biomarkers [9, 21, 22]. Cancer in general is driven by the time sequence of DNA changes and is phenotypically determined by a limited number of underlying rules [7]. This functional perception has several clinical application for the biological history, detection, prognostication and development of new therapeutic tumor targets for patients with colorectal cancer including: screening to reduce mortality by detection of the mutated genes, incorporating molecular features in staging systems, prediction of chemotherapy

response by microsatellite instability, individualized therapy by pharmacogenomics and target antibodies directed at molecular features of the cancer [23]. This study displayed a male predominance in the malignant groups whether mucinous or non-mucinous. This notice agreed with that observed by *Lee et al.* [10] and *Tozawa et al.*[24] and in contrast to *Yajima et al.* [25] who found a female predominance (1:13) in mucinous carcinoma group. Moreover, insignificant differences among the malignant groups regarding age, sex, anatomic site and staging were found in this study. Although statistically insignificant ($p>0.05$), the cases of mucinous carcinoma were more likely to be of high grade and the non-mucinous cases were more likely to be of low grade. Also, signet ring cell carcinoma components were histologically seen in some of group 2 cases. These data coincided with those detected by *Tozawa et al.* [24] who, in addition, displayed this above mentioned component in poorly differentiated carcinomas.

Cytokeratins belong to a group of approximately 20 cytoskeletal structural proteins found in epithelial cells. The expression of individual cytokeratins generally varies by tissue type as well as epithelial type and level of differentiation [25]. The relative expression of CK20 and CK7 has been observed to vary among different epithelial tumors and these markers are currently used as diagnostic tools helping to determine the site of origin of metastatic carcinomas. Although colonic adenocarcinomas typically express CK20 and not CK7. *Zhang et al.* [26] found that rectal cancers could also be positive for CK7. Significant differences in CK20 and CK7 expression in either mucinous or non-mucinous adenocarcinomas by tumor stage ($P<0.05$) were demonstrated here in, so that early stage cancers were more likely to be CK20+/CK7- while a greater percentage of advanced tumors was CK20+/CK7+ indicating that CK7 may be a marker for progression of colorectal cancer. In addition, a higher appearance of CK20+ (CK20+/CK7- and CK20+/CK7+) were detected in the malignant lesions, compared to the normal tissue. Moreover, a significant differences ($P<0.05$) in CKs expression regarding CK20+/CK7- and CK20+/CK7+ phenotypes was found between either malignant group and the control one while insignificant differences ($P>0.05$) were detected between CKs expression and other clinicopathologic features including the age, sex, anatomic site and tumor grade in each group. These results were identical to those given by *Hernandez et al.* [27] who resorted the differences between normal and tumor tissues for CK20 to the fact that its expression in the normal colorectal epithelium is most often highly

expressed in the non proliferative surface epithelium and can be undetectable in the proliferative crypt one. Moreover, they mentioned that since the epithelium in malignant colorectal neoplasms is thought to have global proliferative activity, therefore, CK20 expression is aberrant when compared to its compartmentalization to the non proliferative epithelial population of non neoplastic colorectal mucosa. They also stated that the correlation of CK7 expression (combined to CK20) with higher clinical stage, irrespective to tumor site (colonic or rectal), underlies the growing awareness that CK7 positivity is present in a considerable number of colorectal carcinomas and its presence doesn't exclude a primary tumor from this site.

The present study showed an exclusive difference in mucin core protein expression between mucinous and non-mucinous carcinomas. Mucinous tumors had a tendency to express MUC2+ (its expression was significantly higher than in group1) while colorectal tumors whether mucinous or not were significantly expressed MUC1. The current data agreed with those reported by *Tozawa et al.* [24] and indicated that the colorectal mucinous carcinomas might be grouped into one category regarding MUC1 and MUC2 expression. As well, this study, demonstrated an insignificant difference in any malignant group between mucins expression and the location of the tumor in the large bowel. This result coincided with that mentioned by *Percesepe et al.* [28] and *Losi et al.* [29]. Differences in malignant phenotype based on mucin core protein expression would correspond to differences in the cell lineage that cancer cells belong to [23]. *Ajioka et al.* [1] reached the fact that mucinous carcinomas were most often of the MUC2+ phenotype indicating goblet cell lineage of their predominant cancer cell component. Co-expression with MUC1 which was seen in 78% of group 2 cases may imply up regulation of MUC1 by neoplastic goblet cells or accumulation of MUC1 due to increased longevity of transformed and immortalized goblet cells as stated by *Tozawa et al.* [24] who detected MUC1+/MUC2+ phenotype in a nearby percentage (81.6%) of mucinous carcinomas. The MUC1+/MUC2-phenotype was found in 47 out of 70 (67.1%) group1 cases simulating those of *Ajioka et al.* [1] and *Gum* [30] who displayed this phenotype in most cases of non-mucinous carcinomas denoting that their constituent malignant cells were of columnar cell lineage. Finally, the current study revealed absence of MUC1 and presence of MUC2 expression in all control cases with significant differences in MUC1 detection between either malignant groups and the

control one as well as in MUC2 expression between any of the malignant groups and control one. These data were identical to those of *Yajima et al.* [25] and *Losi et al.* [29] who attributed their results to the aberrant expression of MUC1 (membrane-associated) in only neoplastic epithelium. Moreover, *Lee et al.* [10], *Huttmann et al.* [31] and *Byrd and Bresalier* [32] implicated MUC1 expression in the recurrent malignant neoplasms. *Yajima et al.* [25] also found that most of the MUC1-positive mucinous and non-mucinous cases were associated with p53 mutation. In contrast, *Winterford et al.*, [33] demonstrated that both MUCs are synthesized by goblet cells in normal colon. *Lee et al.* [10], *Chu and Weiss* [34] and *Chen and Wang* [35] stated that CKs and MUCs are widely used for determining histological nature of tumors in alimentary tract and their phenotypic alteration is known during the transformation from benign to malignant lesions.

In conclusion, this study provided the CKs and MUCs immuno-phenotypic profile of mucinous and non-mucinous colorectal carcinomas. The CKs phenotypic profile is relevant in tumor stage and CK7 may be an indicative marker for tumor progression. Mucinous colorectal carcinomas, through MUCs phenotypic profile, can be grouped together as goblet cell type (MUC2+) carcinomas. In addition, MUC1 expression (since being absent in all normal tissue specimens) was strongly associated with colorectal carcinomas.

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