Mucins in the colorectal neoplastic spectrum with reference to conventional and serrated adenomas

Konvansiyonel ve "serrated" adenomların referans alındığı kolorektal neoplazi spektrumunda müsinler

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Background/aims: *Alterations in expression of mucins and aberrant expression of various types of mucin genes were observed in colorectal adenomas and carcinomas, though their significance in neoplastic transformation of colorectal epithelium is yet to be determined. The aim of this study was to determine expression of MUC1, MUC2, MUC5AC, and MUC6 through conventional adenoma-carcinoma sequence and polyps involved in the "serrated" pathway of the colorectum using tissue array technique.* ***Methods:*** *In this study, a total of 172 cases including 100 colorectal polyps [8 hyperplastic polyps, 10 sessile serrated adenomas, 19 tubular, 37 tubulovillous, and 26 villous adenomas], 16 adenomas with intramucosal carcinoma, 28 conventional colorectal cancers, and 28 normal mucosae were examined. Tissue array blocks were prepared and sections were stained immunohistochemically for MUC1, MUC2, MUC5AC, and MUC6.* ***Results:*** *Expression of MUC1 significantly increased in close correlation with the neoplastic process and reached its highest values in intramucosal carcinomas and conventional colorectal cancers (p<0.001). In contrast, MUC2 expression showed a significant decrease in intramucosal carcinoma and conventional colorectal cancer groups (p<0.001). Sessile serrated adenomas exhibited the highest MUC5AC expression while adenomatous polyps showed an increase in MUC5AC expression in parallel with neoplastic progression (p<0.001). Hyperplastic polyps seemed to lie between normal mucosa and sessile serrated adenomas in terms of mucin expression, suggesting that they are morphologically and histogenetically linked.* ***Conclusions:*** *Upregulation of MUC1 and MUC6 through the adenoma-carcinoma sequence together with downregulation of MUC2 and MUC5AC at the neoplastic end of the spectrum seem to follow the steps of malignant transformation.*

Key words: Polyps, colorectum, mucin genes, immunohistochemistry, tissue array

Amaç: *Kolorektal adenom ve karsinomlarda müsin ekspresyonunda değişiklikler ve farklı müsin gen tiplerinin aberan ekspresyonu gözlenmesine karşın bunların kolorektal epitelin neoplastik transformasyonundaki öneminin belirlenmesi gerekmektedir. Bu çalışmada doku array tekniği kullanılarak, konvansiyonel adenom-karsinom sekansı ve kolorektumun "serrated" arayolu boyunca MUC1, MUC2, MUC5AC ve MUC6 ekspresyonlarının belirlenmesi amaçlanmıştır.* ***Yöntem:*** *Bu çalışmada 100 kolorektal polip [8 hiperplastik polip, 10 sesil "serrated" adenom, 19 tubüler, 37 tubülovillöz ve 26 villöz adenom], 16 intramukozal karsinom gösteren adenom, 28 konvansiyonel kolorektal kanser ve 28 normal mukoza içeren toplam 172 olgu incelendi. Doku array blokları hazırlandı ve kesitler immünhistokimyasal olarak MUC1, MUC2, MUC5AC ve MUC6 ile boyandı.* ***Bulgular:*** *MUC1 ekspresyonu neoplastik süreçle ilişkili olarak anlamlı artış gösterdi ve intramukozal karsinom ve konvansiyonel kolorektal kanserlerde en yüksek seviyelere ulaştı (p<0.001). Bu durumun aksine MUC2 ekspresyonu, intramukozal karsinom ve konvansiyonel kolorektal kanser gruplarında anlamlı bir azalma gösterdi (p<0.001). Sesil "serrated" adenomlar en yüksek MUC5AC ekspresyonu sergilerken, adenomatöz polipler neoplastik progresyone paralel olarak MUC5AC ekspresyonunda artış gösterdiler (p<0.001). Hiperplastik poliplerin müsin ekspresyonu açısından normal mukoza ve sesil "serrated" adenomlar arasında yer aldıkları ve bu polipler ile morfolojik ve histogenetik olarak ilişkili oldukları düşünüldü.* ***Sonuç:*** *MUC1 ve MUC6 ekspresyonunun adenom-karsinom sekansı boyunca artışı ile MUC2 ve MUC5AC ekspresyonunun karsinogenezin neoplastik döneminde azalma göstermesi, bu proteinlerin malign transformasyon basamaklarını izlediklerini düşündürmektedir.*

Anahtar kelimeler: Polipler, kolorektum, müsin genleri, immünhistokimya, doku array

INTRODUCTION

Mucins are the major components of the mucous viscous gel covering the surface of epithelial tissues. They are high molecular weight glycoproteins having oligosaccharides attached to their serine or threonine residues of the apomucin protein backbone by O-glycosidic linkages (1-3). To date, 14 distinct epithelial mucin genes (MUCs 1, 2, 3A, 3B, 4, 5AC, 5B, 6, 7, 8, 11, 13, 14, 16), which can be broadly classified into two groups as secreted mucins and membrane bound mucins, have been identified (3-5).

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MUC1 gene located on chromosome 1q21-24 is fully sequenced and encodes a transmembrane glycoprotein. It is a non-secretory glycoprotein expressed along the apical membrane of columnar cells (6) and is upregulated in various adenocarcinomas (1,5,7-10). Gel-forming (secreted) mucins (MUC2, MUC5AC, MUC5B, and MUC6) are found in a cluster on chromosome 11p15.5. MUC2 gene codes for typical secretory mucin that predominates in intestinal goblet cells while MUC5AC gene is mainly expressed in gastric and tracheobronchial mucosae (4,7,11-14). MUC6 is expressed by mucous neck cells of the corpus and deep glands of the antrum (3,12,14).

Over the past decade, mucins and mucin-associated carbohydrate structures have been found to undergo drastic changes in malignant tumors (1,5,7-9,15). However, it is not clear at which step of malignant transformation these alterations occur and/or become detectable and how they are related to the morphogenesis of carcinoma (7). It is hypothesized that mucins are involved in the regulation of differentiation and proliferation of tumor cells through ligand-receptor interactions. They also seem to take part in morphogenetic signal transduction and tumor invasion by disrupting the adhesions between opposing cells and establishing new ligands for the invading cells (5,7,10,16,17).

Colorectal cancer constitutes a suitable model for studying the mechanisms of carcinogenesis and tumor progression through the well-established adenoma-carcinoma sequence (18) in which one can observe progression from a benign adenomatous polyp to adenoma with varying degrees of dysplasia, to intramucosal and invasive carcinoma (5). In recent years, however, an alternative "serrated" pathway involving a hyperplastic polyp-serrated adenoma-carcinoma sequence has been introduced (19-22). Alterations in expression of mucins and aberrant expression of various types of mucin genes were observed in colorectal adenomas and carcinomas (1,8,15,16,23-27), though their significance in neoplastic transformation of colorectal epithelium is yet to be determined.

The aim of the present study was, therefore, to determine differential expression of MUC1, MUC2, MUC5AC, and MUC6 through conventional adenoma-carcinoma sequence and also through polyps involved in the "serrated" pathway of the colorectum in a large case series using the advantage of tissue array technique.

MATERIALS AND METHODS

A total of 172 cases including 100 colorectal polyps [8 hyperplastic polyps (HP), 10 sessile serrated (SSA), 19 tubular (TA), 37 tubulovillous (TVA), and 26 villous adenomas (VA)], 16 adenomas with intramucosal carcinoma (ACA), 28 conventional colorectal cancers (CCA), and 28 normal mucosae (NM) derived from specimens resected for purposes other than cancer were retrieved from the archives of the Department of Pathology, Ankara University Medical School. Adenomas were further classified according to the degree of glandular intraepithelial neoplasia (dysplasia) as low and high grades while carcinomas were graded as well, moderately, and poorly differentiated according to World Health Organization (WHO) classification (28). After reviewing the hematoxylin and eosin (H&E)-stained slides of each case, for polyps with a diameter above 4 mm, representative fields of the lesions were marked on the glass slides and core tissue samples were prepared manually from the paraffin blocks using a 4 mm diameter dermatologic biopsy needle while smaller polyps were totally represented in the cores. A total of 13 array blocks, each containing 14 cases represented by one core per case, were prepared. Sections of 4 micron thickness were cut, mounted on poly-L-lysine coated slides and were stained with monoclonal antibodies raised against MUC1 (Ma695, Novacastra, 1:100), MUC2 (CCP58, Novacastra, 1:100), MUC5AC (CLH2, Novacastra, 1:50), and MUC6 (CLH5, Novacastra, 1:30) using Ventana NexEs automated immunostainer for secondary visualization. Antibody detection was performed by using a biotinylated secondary antibody of Ventana (Ventana Medical Systems, Tucson, AZ, USA) and 3,3'-diaminobenzidine. Positive control tissues were used as recommended by the suppliers whereas exclusion of the primary antibody served as negative control.

The extent of staining was assessed as percentage of positively stained areas within the tissue core while staining intensity was graded as weak (+), moderate (++), and strong (+++) using an arbitrary scale. An expression score was calculated after multiplying percentage expression by the intensity of staining. All microscopic evaluations were performed by one observer (AE) who has experience in gastrointestinal pathology.

Age and gender of the patients, location, size, number and gross features of the polyps, and grade and stage of the carcinoma (28) cases were ret-

rieved from patients' files. Location of the polyps was grouped as rectum, left colon including splenic flexura, descending colon and sigmoid, and right colon including cecum, ascending colon and transverse colon.

Statistical analysis was performed using Kruskal Wallis variance analysis for the comparison of the groups while Multiple Comparison Tests were used to determine the differences between the groups. Chi-square test was used to evaluate the difference between the groups for nominal variables. A p value d 0.05 was considered as significant.

RESULTS

A total of 172 cases including 100 colorectal polyps (8 HP, 10 SSA, 19 TA, 37 TVA, and 26 VA), 16 ACA, 28 CCA (of which 4 were well-differentiated, 21 were intermediate and 3 were poorly differentiated adenocarcinomas), and 28 NM were studied. There were 117 males and 55 females with an age range of 21-84 years. Though mean age was lower in the HP group compared to all other groups, no significant difference was observed between the polyp groups. There was no significant difference between the male and female patients with regard to the prevalence of different types of polyps. A great majority of polyps and carcinomas were located in the rectum and/or left colon while only 18% of polyps and 7.1% of carcinomas were located in the right colon. Adenomas with carcinomatous foci were significantly larger (mean diameter=20.25 mm) when compared to other polyps (HP=9.38 mm, SSA=7.6 mm, TA=8.0 mm, TVA=11.06 mm, VA=11.73 mm) ($p<0.001$). Clinicopathological features of the polyps and tumors are presented in Table 1. Low grade dysplasia was observed in 27.8% (n=30) of adenomas, including 10 SSA, whereas 57.4% (n=62) of adenomas had high grade dysplasia. No significant association was observed between location, size, and dysplasia in the study groups.

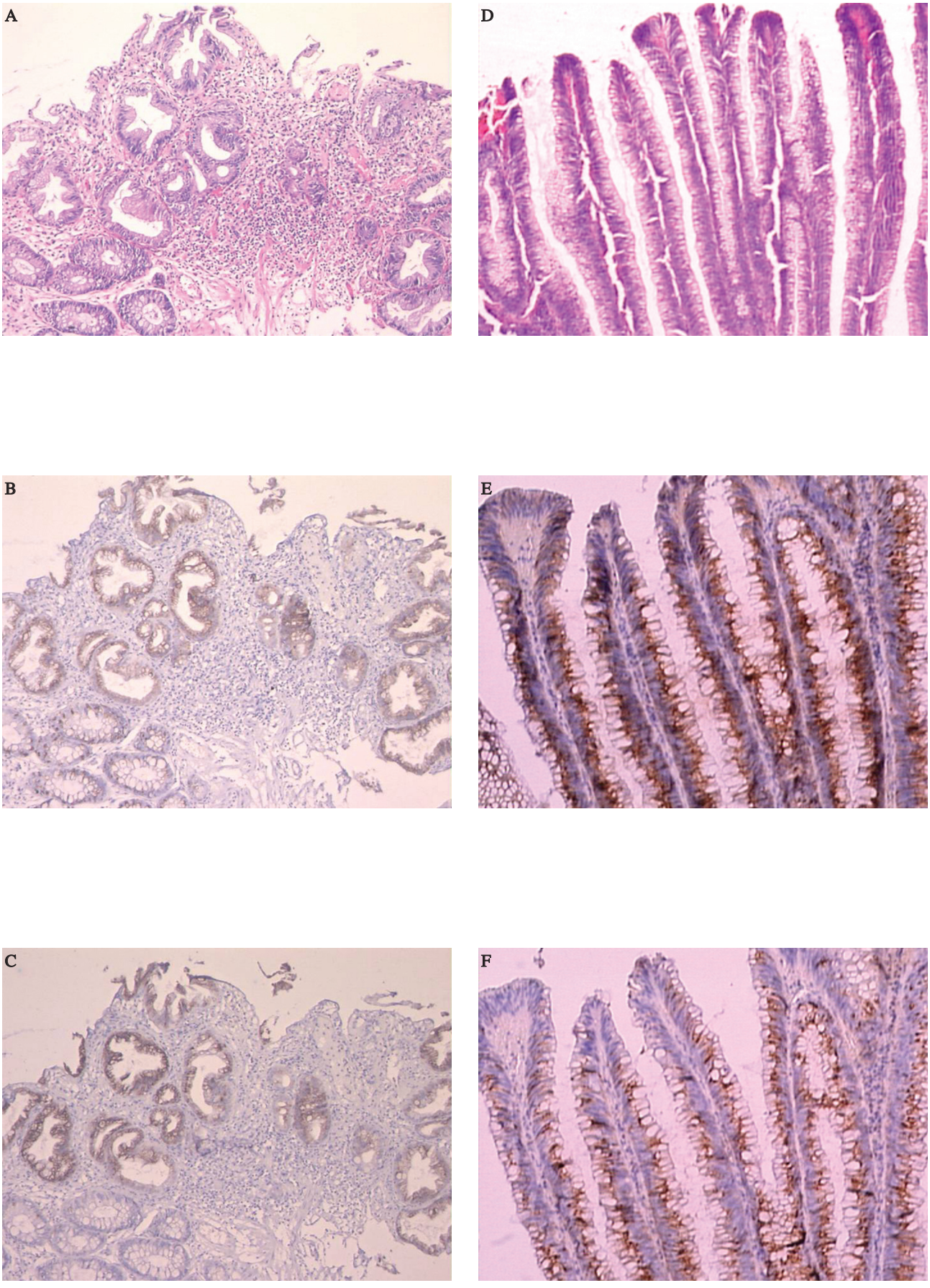
MUC antigens were differentially expressed in normal colorectal mucosae, polyps, and carcinomas. Predominant expression patterns were defined as supranuclear, apical, basolateral, and diffuse cytoplasmic staining. No significant association was observed between the expression patterns and polyp types or carcinomas, and all four antibodies showed a random mixture of expression patterns (Figure 1).

MUC1 was totally absent in NM, HP, and TA, while 24.4% of TVA, 19.3% of VA, 20.0% of SSA, 37.5% of ACA, and 89.3% of CCA showed MUC1 positivity. MUC2 was the most widely expressed antigen in every study group, though CCA (82.2%) had fewer MUC2 positive cases compared to other groups. MUC5AC was negative in the majority of NM (89.2%), while all HP, SSA, and TA expressed MUC5AC. MUC5AC expression, though widespread among other groups, showed a dramatic decrease at the neoplastic end of the spectrum with 32.2% positivity in CCA. MUC6 was absent or near absent in NM and HP. The number of MUC6 positive cases increased in parallel with the neoplastic spectrum including SSA. Percentages of positively stained cases with MUCs in all study groups are presented in Figure 2.

Expression of MUC1 significantly increased in close correlation with the neoplastic process and reached its highest values in ACA and CCA ($p<0.001$). In contrast, MUC2 expression showed a significant decrease in ACA and CCA groups ($p<0.001$), although it did not vary significantly between the polyp types. While staining intensity of MUC1 increased, that of MUC2 decreased significantly in ACA and CCA groups when compared to other study groups ($p<0.001$). SSA showed the highest MUC5AC expression in comparison to all

Table 1. Clinicopathological features of the polyps and tumors

Groups (n)	Age (years)	Site						Size (mm)
		Rectum		Left		Right		
	Mean ± SD	n	%	n	%	n	%	Mean ± SD
TA (n: 19)	59.83 ± 9.9	4	21.1	12	63.2	3	15.8	8 ± 3.6
TVA (n: 37)	56.46 ± 11.8	7	18.9	22	59.5	8	21.6	11.06 ± 5.5
VA (n: 26)	56.88 ± 16.4	10	38.5	12	46.2	4	15.4	11.73 ± 5.5
HP (n: 8)	39.62 ± 12.14	6	75	2	25	-	-	9.38 ± 5.4
SSA (n: 10)	55.3 ± 10.1	2	20	7	70	1	10	7.6 ± 3.5
ACA (n: 16)	59.35 ± 17.5	4	25	12	75	-	-	20.2 ± 8.5
CCA (n: 28)	58.28 ± 14.86	14	50	12	42.9	2	7.1	42.5 ± 14.8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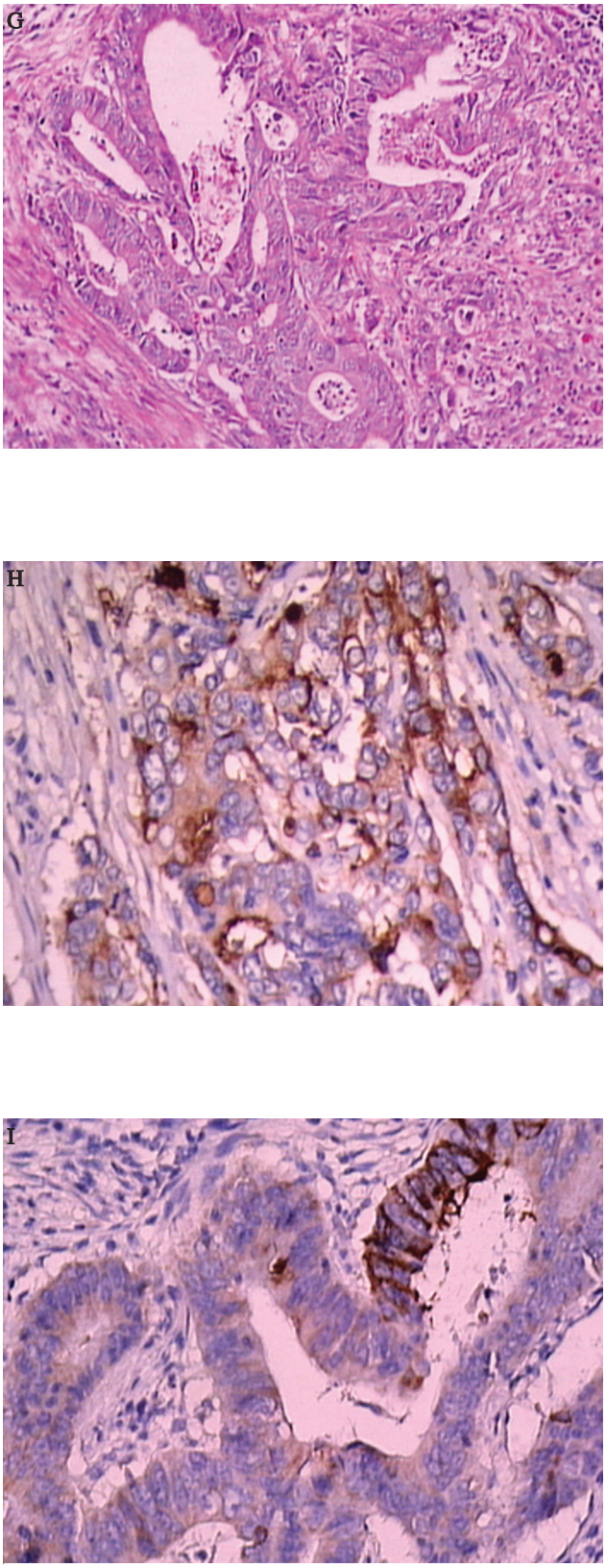

Figure 1. Expression of MUCs in the study groups:
A-C: sessile serrated adenoma (x200; H&E, MUC2, and MUC5AC, respectively), **D-F:** villous adenoma (H&E, x100, x200; MUC2, and MUC5AC, respectively), **G-I:** cancer (H&E, x200, x400; MUC1, and MUC2, respectively)

other groups, while adenomatous polyps showed an increase in MUC5AC expression in parallel with neoplastic progression ($p<0.001$). However, both percentage expression and staining intensity of MUC5AC decreased significantly in the CCA group compared to all other groups ($p<0.001$) except NM, which showed the weakest expression. Expression of MUC6 showed a non-significant increase through the sequence reaching the highest values in the ACA group, while its staining intensity increased significantly in the latter group when compared to NM ($p<0.05$). The expression scores showed similar results to their percentage expressions. Expression of MUCs in all study groups is presented in Table 2.

MUC1 and MUC6 expressions increased in parallel with the degree of dysplasia in adenomatous polyps, while MUC2 expression decreased as the grade of dysplasia increased. MUC5AC showed varied staining in different dysplasia grades, whereas carcinomas showed high MUC5AC expression. Expression of MUCs in adenomas with dysplasia/neoplasia is shown in Figure 3. However, no correlation was observed between the grade of adenocarcinomas and MUC expressions.

DISCUSSION

Colorectal mucosa normally expresses MUC1, MUC2, MUC3, and MUC4, of which MUC2 is the predominantly secreted mucin glycoprotein. In normal colorectal epithelium, MUC1 is rarely expressed while MUC2 is abundantly expressed in the cytoplasm of goblet cells and columnar cells (3,4,7,11). It has also been reported that overexpression of MUC1 and downregulation of MUC2 are associated with progression and metastasis in colorectal adenomas and carcinomas (10,16,17).

Similar to the previous observations, in the present study, MUC1 was absent in normal mucosae in contrast to MUC2, which was widely expressed by normal mucosae. While the expression of MUC1 increased throughout the sequence, MUC2 expression remained high, except for a slight decrease in carcinoma cases. Sylvester et al. (27) found that MUC2 was expressed in normal mucosae while the expression ratio decreased in the carcinoma group as confirmed by immunohistochemistry and in situ hybridization. In another study performed on a small series of colorectal carcinomas, MUC1 was detected in only 40% of the cases, whereas MUC2 was present in all cases (8). Li et al. (17), on the other hand, studied a large group of

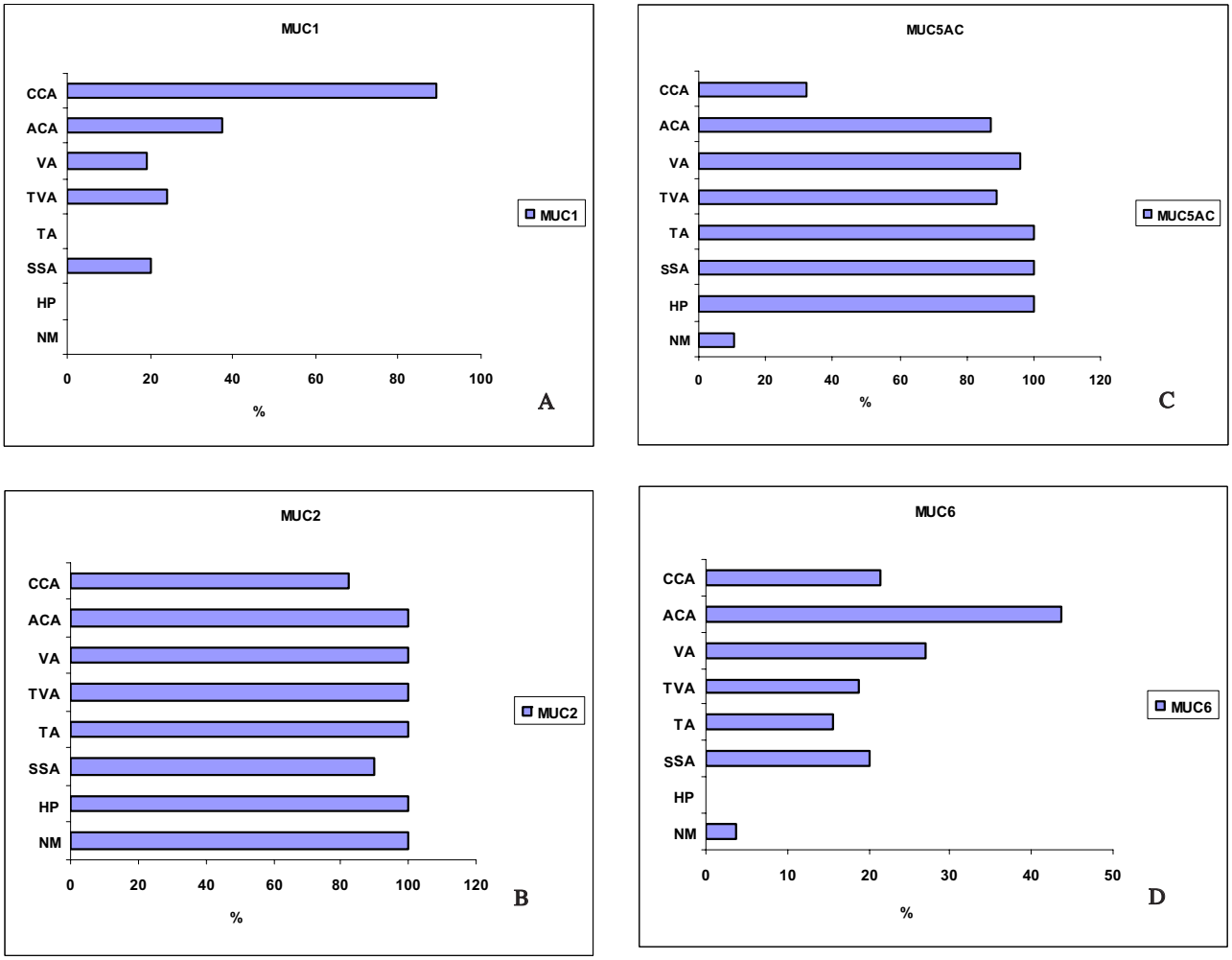

Figure 2 A-D: Percentages of positively stained cases with MUC1, 2, 5AC, and 6, respectively

colorectal carcinomas and adenomas and showed that MUC1 was expressed in 24% and MUC2 in 38% of the carcinomas. However, they did not find any MUC1 expression in adenomas with low and intermediate grades of dysplasia and showed that only 4% of adenomas with high grade dysplasia expressed MUC1. MUC2 decreased as the grade of dysplasia increased. Though NM and adenomas with low grade dysplasia gave similar results, we observed a gradual increase in MUC1 expression in parallel with the degree of dysplasia as well as an increase through the adenoma-carcinoma sequ-

Table 2. Percentage expression and staining intensity of MUCs in the study groups

Study	MUC1		MUC2		MUC5AC		MUC6	
Groups	%Exp	Intensity	% Exp	Intensity	% Exp	Intensity	% Exp	Intensity
(n)	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
NM (n: 28)	.	.	67.5±17.83	2.8±0.39	0.92±2.78	0.18±0.55	0.18±0.96	0.07±0.3
HP (n: 8)	.	.	61.87±26.31	2.5±0.53	18.75±24.89	2.12±0.35	.	.
SSA (n: 10)	16±34	0.2±0.4	79±33.97	2.3±0.94	48.5±24.83	2.6±0.69	12±30	0.2±0.4
TA (n: 19)	.	.	73.15±21.74	2.89±0.3	10.27±17.54	2.27±0.57	5.05±15.41	0.16±0.38
TVA (n: 37)	10.3±26.5	0.31±0.5	62.48±31.96	2.56±0.5	19.78±24.39	2.16±0.95	6.27±16.7	0.24±0.54
VA (n: 26)	12.4±27.12	0.4±0.8	63.68±30.42	2.56±0.5	25.96±28.96	2.5±0.7	8.9±21	0.38±0.69
ACA (n: 16)	19.12±32.7	0.93±1.2	49.5±33.7	2.37±0.5	32.43±36.14	1.93±0.99	14.31±30.27	0.75±0.93
CCA (n: 28)	37.8±33.1	2.32±1.02	16.78±23.26	1.92±1.05	4.1±8.05	0.6±0.99	5.35±11.77	0.25±0.51

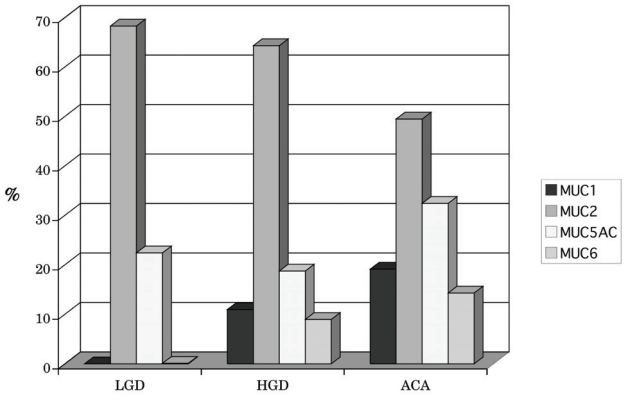

Figure 3. Expression of MUCs in adenomas with dysplasia/neoplasia

ence, while MUC2 expression decreased through the stages of dysplasia. In a study comprised of flat and polypoid tubular adenomas with varying degrees of dysplasia, Ajioka et al. (23) found that MUC2 expression was higher in low grade dysplasia in contrast to MUC1, which increased in high grade dysplasia.

MUC1 functions as an anti-adhesion molecule that inhibits cell-cell adhesion inducing the release of cells from the tumor. This is supported by the dramatic increase in MUC1 expression in carcinomas which have discohesive cells with invasive properties. MUC2 downregulation observed in carcinomas, however, seems to be related to carcinomatous transformation of the intestinal epithelium, which loses its ability to express the native mucin type due to defective glycosylation observed in the late stages (1,10,16,17). Hence, our results support the fact that upregulated MUC1 together with downregulated MUC2 at the neoplastic end of the sequence could serve as a marker to detect intraepithelial/intramucosal carcinomatous foci, as suggested previously (23).

While MUCs 1, 2, and 3 are normally synthesized by normal and hyperplastic epithelial cells, no MUC5AC or MUC6 expression was shown in normal colorectal epithelium (24,25). Therefore, it can be suggested that de novo aberrant expression of MUC5AC and MUC6 in adenomatous polyps may provide a more specific marker for neoplastic transformation of colonic epithelium. In accordance with this view, aberrant expressions of MUC5AC and MUC6 genes have been observed in colorectal adenomas in various studies (2,24-26). Though the molecular mechanism responsible for their aberrant expression remains unclear, it may be due to changes in their methylation status, transcriptional regulation, or aberrant differentiation (24,25). Similarly, in our study, despite the absent or near absent expression of MUC5AC and MUC6 in normal mucosae, both showed increased expression throughout the adenoma-carcinoma sequence. Previous studies demonstrated that adenomas exhibited marked upregulation of MUC5AC and to a lesser extent MUC6 (2,24,25). In some of these studies, MUC5AC expression decreased as the grade of dysplasia increased (2,25). Carcinomas, on the other hand, showed decreased expression of both MUC5AC and MUC6 in many studies (8,27), including our study, suggesting that these molecules may be involved in the early stages of neoplastic change.

In recent years, a group of colorectal cancers, mucinous type in particular, were believed to follow a different pathway that involves hyperplastic polyps, sessile serrated adenomas (SSA), and traditional serrated adenomas (19-22). Traditional serrated adenoma is an entity that shares histopathological features with both hyperplastic polyps and conventional adenomas (22), while SSA can be simply defined as a “dysplastic” hyperplastic polyp variant with characteristic differential features such as dilated, boot-shaped basal crypts and an abnormal proliferation zone (21). In the present study, SSA expressed all four MUC molecules at levels higher than hyperplastic and adenomatous polyps, suggesting that they form a unique group with different morphogenesis. Upregulation of MUC2, the intestinal-type mucin, in SSA seems to result from the rich goblet cell content of serrated epithelium in comparison to conventional adenomas. However, hyperplastic polyps, which stand between normal mucosa and SSA in the morphological spectrum, did not show MUC2 upregulation despite their goblet cell-rich epithelia. Interestingly, hyperplastic polyps seem to show a MUC profile similar to normal mucosa except for the presence of gastric mucin MUC5AC, which also increases in SSA, suggesting that these two lesions represent a histogenetic continuum. In a similar study, Biemer-Huttmann et al. (25) reported that MUC2 and MUC5AC are consistently and markedly expressed in serrated adenomas as well as hyperplastic polyps. Yao et al. (29) demonstrated the frequent expression of human gastric mucin, which is encoded by MUC5AC core protein, in serrated adenomas and hyperplastic polyps and suggested that both lesions may have

cell differentiation of gastric foveolar-type epithelium in common, as well as a histogenetic linkage. Hirono et al. (30) moved on to show that hyperplastic polyps and serrated adenomas expressed not only foveolar type but also pyloric gland type mucin, represented by MUC6 expression. We did not, however, observe any MUC6 expression in hyperplastic polyps, while 20% of SSA showed MUC6 expression. Our findings, in parallel with previous observations (25,29,30), are consistent with the view that hyperplastic epithelium may give rise to adenomatous change as demonstrated in the form of mixed hyperplastic-adenomatous polyps/traditional serrated adenomas, and that MUC5AC could be involved in the early stages of this transformation, unlike MUC6, which may take part later in the neoplastic continuum.

The present study seems to be unique for the composition of the cases, which form a complete spectrum of the adenoma-carcinoma sequence including normal colorectal mucosae, hyperplastic polyps, sessile serrated and conventional adenomas, as well as adenomas with carcinomatous foci and adenocarcinomas. The results of our study suggest that upregulation of MUC1 and MUC6 together with downregulation of MUC2 and MUC5AC, at the malignant end of the spectrum, in particular, seem to follow the steps of neoplastic transformation of colorectal mucosa through the adenoma-carcinoma sequence. Hyperplastic polyps seem to lie between normal mucosa and sessile serrated adenomas, in terms of mucin expression, suggesting that they are morphologically and histogenetically linked. In terms of MUC1 and MUC6 expressions, on the other hand, sessile serrated adenomas seem to possess features similar to conventional adenomas and thus, lie closer to the neoplastic end of the spectrum.

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