[原著] A Histochemical Comparative Study on Four Different Types of Lectins and Carcinoembryonic Antigen (CEA) on Human Colorectal Tissues

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A Histochemical Comparative Study on Four Different Types of Lectins and Carcinoembryonic Antigen (CEA) on Human Colorectal Tissues

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Key words : colon and rectum, neoplastic lesion, adjacent non-neoplastic mucosa, UEA-1 and CEA.

Abstract

To evaluate the specificity and efficacy of lectin histochemical staining characteristics on human colorectal neoplastic and adjacent non-neoplastic tissue, a thorough comparative study has been performed to that of a recognized colorectal tumor marker, carcinoembryonic antigen (CEA). Neoplastic tissue and adjacent non-neoplastic mucosa of 26 surgically extirpated specimens were examined lectin histochemically by four different lectins (Ulex europaeus-I, Arachis hypogaea, Triticum vulgare and Canavalia ensiformis) and immunohistochemically by anti CEA antibody. According to their degree of staining all the four lectins (UEA-I moderate 57.69%, extensive 39.42%; PNA moderate 60.57% extensive 5.76%; WGA moderate 76.92% extensive 1.92% and Con-A moderate 48.07%, extensive 51.29%) and CEA (moderate 66.34%, extensive 22.11%) showed very high percentages of positive staining for neoplastic tissues but almost reciprocal results were observed by UEA-I (moderate 7.69%, extensive 0%) and partially by PNA lectin (moderate 15.38%,extensive 0%) for non-neoplastic mucosa ($P < 0.01$). While CEA (moderate 50%, extensive 19.23%) and the other two lectins did not show much differences in their staining percentages for non-neoplastic mucosa ($P > 0.05$). Further study regarding their staining pattern revealed that for neoplastic tissues UEA-I mainly stained the cytoplasmic (without polarity) (60.58%) or the stromal (28.85%) types but in case of non-neoplastic mucosa the cytoplasmic (with polarity) (57.15%) or the apical (35.71%) types predominated. No such distinct staining patterens were observed for CEA and the other lectins. From our study it is strongly suggestive that UEA-I lectin is by far more specific and localized for the malignant transformation of colorectal tissues with compared to that of carcinoembryonic antigen, CEA.

Introduction

In recent years, the detection of histocemical alterations in gastrointestinal mucins has aroused considerable interest, these changes may be potentially useful tools in the recognition of early neoplastic changes. The transitional mucosa adjacent to colorectal carcinomata

has shown an abnormal mucin pattern that was characterized by a significant increase in sialomucins, accompanied by a marked decrease in sulfomucins. These findings are in contrast to normal colorectal mucosa, in which sulfomucins predominate.

More recently, numerous studies using different lectins have been done on colorectal carcinomata, adenomata and hyperplastic polyps[1,15]. These studies have shown that gorse seed agglutinin (UEA-1) and peanut agglutinin (PNA) mostly bind to carcinomatous and transitional mucosa, but not to normal colonic glands. Other lectins have not shown a similar ability to distinguish between malignant and normal colorectal mucosa. They have reported that UEA-1 and PNA may be a reliable indicator of colorectal malignant transformation[2,4,5,7,9,15]. But no comparative studies have been done to evaluate their efficacy and specificity with respect to a standard colorectal tumor marker[16]. With this aim we selected four most common lectins of different carbohydrate specificity[17] and carcinoembryonic antigen (CEA) as a common denominator to compare the lectin histochemical findings on both human colorectal neoplastic and adjacent non-neoplastic tissues. Both qualitative and quantitative differentiations were satistically evaluated. Also literature concerning lectin histochemistry were discussed explaining the obtained results.

Materials and Methods

Twenty-six surgically extirpated colorectal carcinomata tissues were involved in this study. Their regional distribution, degree of differentiation[18], Duke's[19] and histological staging[20] were summerized in table 1. Five sections were taken from each case, four from tumor site (O, oral side of the tumor; T, topmost part of the tumor; B, bottomost part of the tumor and A, anal side of the tumor) and one (N) from the normal mucosa 10cms from the oral side of the tumor (figure 1).

For lectin histochemical staining a little modified avidinbiotin-complex (ABC) method and for CEA immunohistochemical staining peroxidase-antiperoxidase (PAP) method were performed[21,22].

Tissues were fixed in 10% formalin and processed routinely for paraffin embedding. Serial 4 micro meter sections were cut and one section from each block was stained with hematoxylin and eosin for routine histopathological studies.

a) Lectin histochemical staining (ABC method)

Adjacent serial sections were deparaffinized in zylol, hydrated through graded alcohols and washed in phosphate buffered saline (PBS) pH 7. 2. Endogenous peroxidases were inhibited by treating with 0. 3% H_2O_2 for 5 minutes. Sections were incubated in biotiated lectin (1 : 100) (E. Y. Labs. INC, SanMateo, Cal) for 12 hours at 4°C in a moist chamber, which was diluted in 1% bovine serum albumin (PBS- 1%BSA-Nacl). The sections were then washed three times in PBS (five minutes each wash) and further incubated with avidin (10micro gm/ml)-peroxidase for 30 minutes at room temperature in a moist chamber. Again three times after (five minutes each wash) washing with PBS peroxidase activity was shown by treating with 3.3 'diaminobenzidine tetrahydrochloride (DBA) for about 2 minutes. The sections were then washed, the nuclei were counter stained lightly with haematoxylin, dehydrated and mounted. Four lectins were used in this study : Ulex europaeus-I (UEA-I), Arachis hypogaea (PNA), Triticum vulgare (WGA) and Canavalia ensiformis (Con-A) from E. Y. Labs. INC. (san Mateo, Cal). Parallel experiments, in which lectin binding was inhibited by pre-incubation of each lectin with its appropriate binding sugar, confirmed the specificity of lectin binding (Table 2).

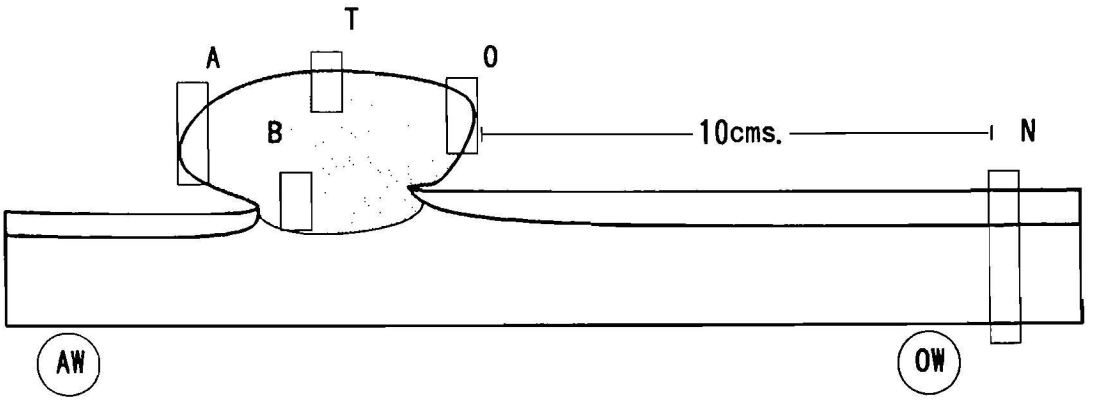

Figure 1. Schemetic diagram of resected colon tissue showing the different sites of tissue sampling. N= adjacent non-neoplastic colonic mucosa (10 cms from tumor margin), O =oral side of the tumor, T = topmost part of the tumor, B = bottomost part of the tumor and A = anal side of the tumor.

Teble 1. Summary of cases studied according to their regional distribution, Dukes and histological staging.

		No. of Cases
A. Site of Lesion:		
Proximal Colon (n=13)	Caecum	5
	Ascending Colon	6
	Transverse Colon	2
Distal Colon (n=13)	Desending Colon	1
	Sigmoid Colon	5
	Rectum	7
B. Tumor Differentiation: (n=26)	Well-differentiated	5
	Moderately-differentiated	18
	Poorly-differentiated	3
C. Dukes Staging: (n=26)	Duke A	3
	Duke B	5
	Duke C	10
	Duke D	8
D. Histological Staging: (n=26)	Stage I	2
	Stage II	6
	Stage III	4
	Stage IV	6
	Stage V	8

b) CEA immunohistochemical staining (PAP method)

As described above, the sections were treated with 0.3% H_2O_2 and nonimmunized normal goat serum (1 : 20) for 30 minutes in room temperature to inhibit nonspecific reactions. After washing with PBS they were incubated with rabbit-antihuman-CEA-antibody (Dako Co.Denmark) at a concentration of 1:250 for 2 hours at 4°C in a moist chamber as the primary antibody. They were then treated with goat-antirabbit immunoglobulin antibody (1:50) for 30 minutes at room temperature in a moist chamber and followed by PAP (rabbit peroxidase-antiperoxidase) solution (1:100) for 30 minutes at room temperature in a moist chamber. Finally, the sections were treated with 0.05% DAB in 0.05 M Tris-HCI buffer, pH 7.2 containing 0.01% H_2O_2 counter stained with hematoxylin and mounted. All the slides were observed at a magnification of 100 times and were analysed i) quantitatively : according to their degree of staining as follows : no cells were positively stained (− or negative); less than 25% cells were positively stained (± or mild); 25-50% cells were positively stained (+ or moderate) and more than 50% cells were positively stained (++ or extensive)(figure 2).ii) qualitatively: according to Hamada's criteria[23] of localization of staining as follows: staining restricted to the apical border of the cell (apical type); staining restricted to the cytoplasmic region with polarity (supraunclear with polarity, SWP type); staining restricted to the cytoplasmic region without polarity (supranuclear without polarity, SWOP type) and stining observed all over the cytoplasmic area including the

Table 2. Lectins used with their source and appropriate carbohydrate specifcity.

Name	Abbreviation	Source	Specificity
1. Ulex europaeus agglutinin	UEA-1	Gorse seed	alpha-L-fucose
2.Arachis hypogaea agglutinin	PNA	Peanut	D-Gal-B (1-3) GalNAc
3.Triticum vulgare agglutinin	WGA	Wheat germ	NeuNAc GluNAc
4.Canavalia ensiformis agglutinin	Con-A	Jack bean	alpha-D-Man alpha-D-Glc

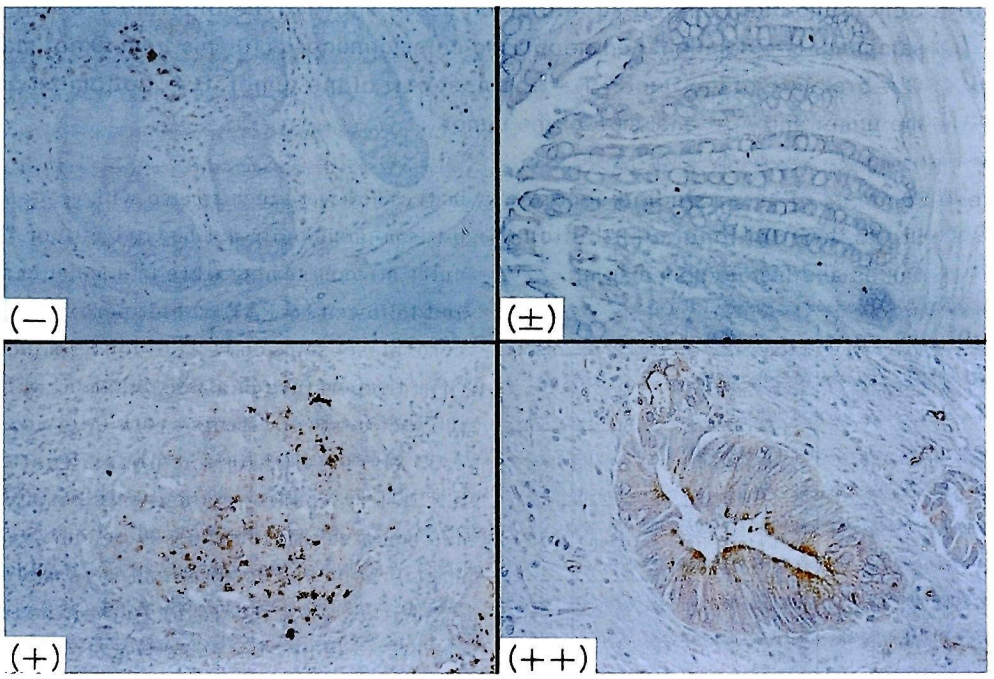

Figure 2. Quantitative analysis of staining according to degree of staining: (-) or negative (Top left), (±) or mild (Top right), (+) or moderate (Bottom left) and (++) or extensive (Bottom right). ABC stain x100.

stromal site (stromal type) (figure 3).

All statistical deductions were performed by paired two tailed t-test.

Results

The results of degree of staining for both neoplastic and adjacent non-neoplastic tissues are summerized in table 3. The results for four different tumor sites did not show any recomendable differences in their staining charecteristics for all the lectins and CEA, therefore a combined mean result was made for better understanding. UEA-1 (moderate 57.69%,extensive 39.42%), Con-A (moderate 48.07%, extensive 51.92%) and CEA (moderate 66.34%, extensive 22.11%) showed higher percentages of positive staining for neoplastic tissues but PNA

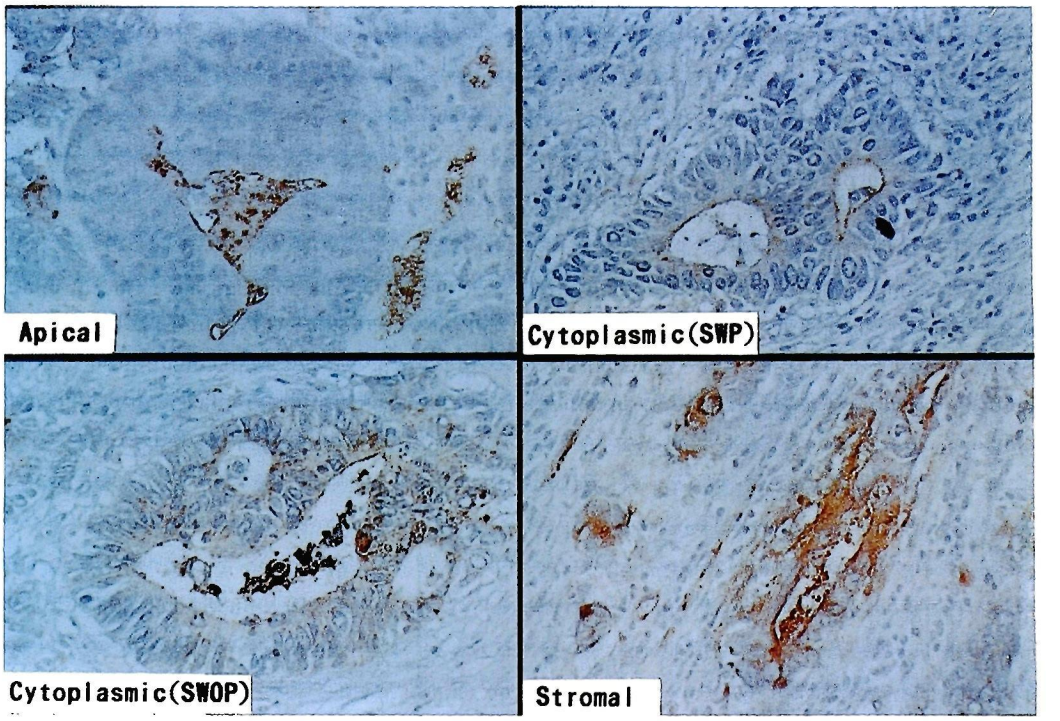

Figure 3. Qualitative analysis of staining according to localization of staining: Apical (Top left), Cytoplasmic with polarity (SWP) (Top right), Cytoplasmic with out polarity (SWOP) (Bottom left) and Stromal (Bottom right). ABC stain x100.

Tabl 3. Data of non-neoplastic mucosa (n= 26) and neoplastic lesion (n=104) for the degree of staining

		− Negetive	± Mild	+ Moderate	++ Extensive
UEA I	Non-neoplastic mucosa	12 (46.15)	12 (46.15)	2 (7.69)	0
	Neoplastic lesion	0	3 (2.88)	60 (57.69)	41 (39.42)
PNA	Non-neoplastic mucosa	14 (53.84)	7 (26.92)	4 (15.38)	0
	Neoplastic lesion	9 (8.65)	26 (25.00)	63 (60.57)	6 (5.76)
WGA	Non-neoplastic mucosa	4 (15.38)	7 (26.92)	13 (50.00)	2 (7.69)
	Neoplastic lesion	0	22 (21.15)	80 (76.92)	2 (1.92)
Con A	Non-neoplastic mucosa	0	3 (11.53)	17 (65.38)	6 (23.07)
	Neoplastic lesion	0	0	50 (48.07)	54 (51.92)
CEA	Non-neoplastic mucosa	0	8 (30.76)	13 (50.00)	5 (19.23)
	Neoplastic lesion	0	12 (11.53)	69 (66.34)	23 (22.11)

Percentages are given in parenthesis.

(mild25%, moderate 60.57%) and WGA (mild 21.15%,moderate 76.92%) did not show much extensive positive staining. For adjacent non-neoplastic mucosa only UEA-1 (46.15%) and PNA (53.84%) showed higher percentage of negative staining. WGA (mild 26.92%,moderate 50%), Con-A (moderate 65.38%,extensive 23.07%) and CEA (mild 30.76%,moderate 50%) showed higher percentage of positive staining for non-neoplastic mucosa also. Figure 4. shows the comparative graphic representation of UEA-1 ($P < 0.01$, $t = 13.9358$), PNA ($P < 0.01$, $t = 5.04767$) and CEA ($P > 0.05$, $t = 0.933353$) for neoplastic and adjacent non-neoplastic lesions. It is to be noted that only for UEA-1 lectin almost reverse type of graphic presentation for neoplastic and adjacent non-neoplastic lesions

were evident,which was statistically highly significant. No such distinct differentiations were evident for PNA or CEA.

The regional differentiation of staining for non-neoplastic mucosa for UEA-1 was very distinctly evident (Table 4). Out of 13 cases of proximal colon 11 cases were mildly and 2 cases were moderately positive. But in case of distal colon only 1 case was mildly positive ($P < 0.01$), the rest were all negetive. No such regionality of staining was observed for the other lectins or CEA.

$=7.40126$), the neoplastic sites did not show much difference in their degree of staining (Figure 5).

According to Hamada's criteria[23] for the localization of staining it was evident that for neoplastic lesions UEA-1 predominently stained the SWOP type (60.57%) and the stromal type (28.84%). On the contrary for adjacent non-neoplastic mucosa SWP type (57.15%) and the apical type (35.71%) predominated (Table 5). For PNA lectin the cytoplasmic types predominated for both the types of lesions. Diffuse

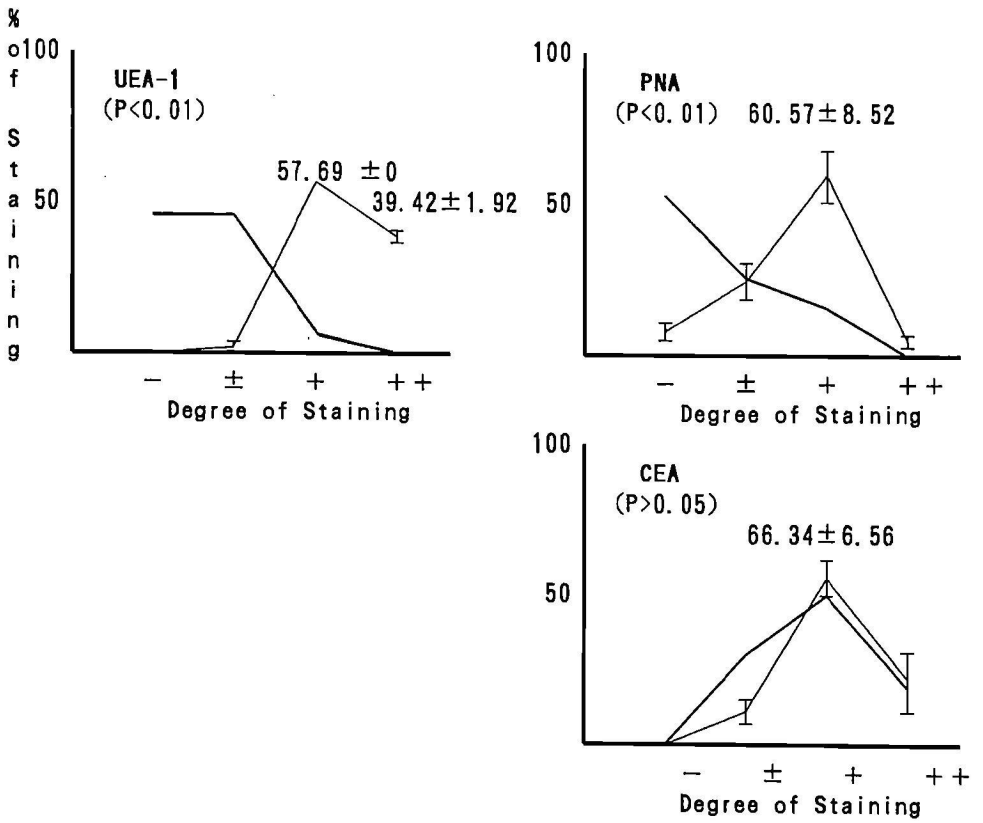

Figure 4. Comparative graphic representation of neoplastic tissue and adjacent non-neoplastic mucosa according to their degree of staining for UEA-1, PNA and CEA.(Thick line: non-neoplastic mucosa and thin line: neoplastic lesion). (Mean±SD).

Site wise comparison of staining between UEA-1 and CEA revealed that the main difference in degree of staining lays in case of the adjacent non-neoplastic mucosal sites ($P < 0.01$, t

cytoplasmic type of stainings were observed for Con-A lectin. As for WGA and CEA no specific type of staining pattern were observed, but WGA showed excessive background staining.

Table 4. Regional differention of UEA-1 lectin for non-neoplastic mucosa.

	− Negetive	± Mild	+ Moderate	++ Extensive
Proximal Colon (n=13)	0	11 (84.61)	2 (15.38)	0
Distal Colon (n=13)	12 (92.30)	1 (7.69)	0	0

Percentages are given in parenthesis.

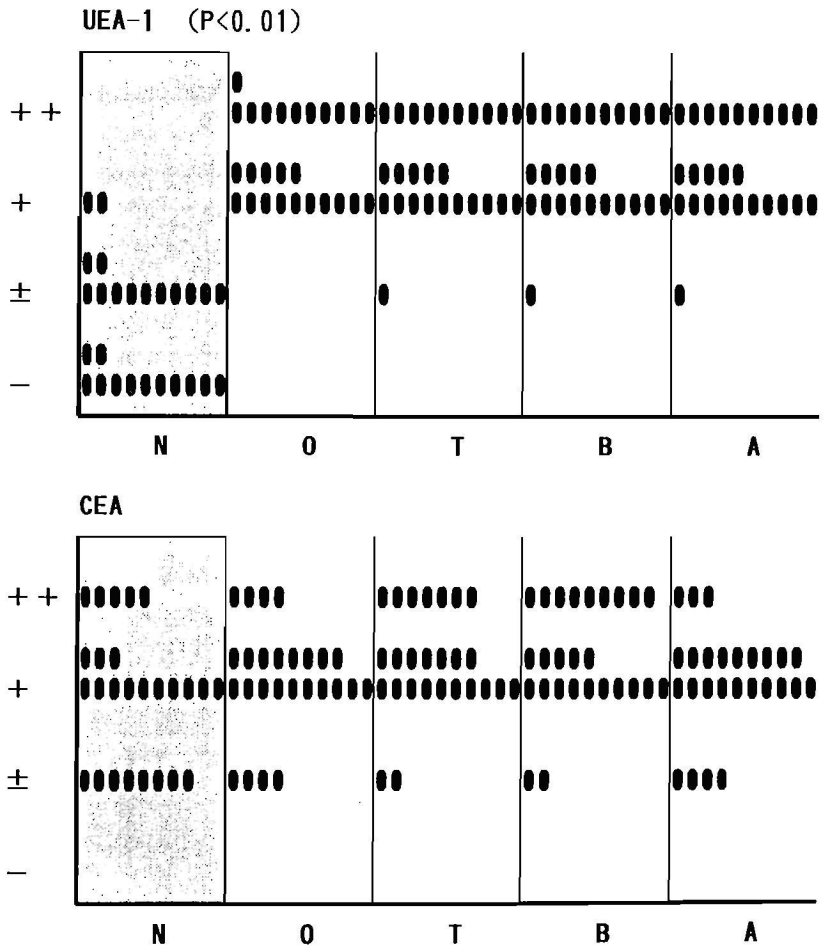

Figure 5. Comparison of UEA-1 and CEA staining according to site of lesions.

Except for UEA-1 no particular stining pattern was evident for the other lectins or CEA but it was quite distinctly observed that for all the lectins and CEA the SWOP and the stromal type predominated for the neoplastic lesions.

Table 5. Data of non-neoplastic mucosa and neoplastic lesion for the localization of staining.

			Cytoplasmic		
		APICAL	SWP	SWOP	STROMAL
UEA-1	Normal (n=14)	5 (35.71)	8 (57.15)	1 (7.14)	0
	Carcinoma (n=104)	0	11 (10.57)	63 (60.58)	30 (28.85)
PNA	Normal (n=12)	0	11 (91.67)	1 (8.33)	0
	Carcinoma (n=95)	16 (16.84)	56 (58.95)	18 (18.95)	5 (5.26)
WGA	Normal (n=22)	0	6 (27.27)	15 (68.18)	1 (4.55)
	Carcinoma (n=104)	0	0	10 (9.62)	94 (90.38)
CONA	Normal (n=26)	0	4 (15.38)	12 (46.16)	10 (38.46)
	Carcinoma (n=104)	0	2 (1.93)	32 (30.77)	70 (67.30)
CEA	Normal (n=26)	4 (15.38)	8 (30.76)	14 (53.84)	0
	Carcinoma (n=104)	7 (6.73)	17 (16.34)	59 (56.73)	21 (20.19)

Percentages are given in parenthesis.

Discussion

We have performed a comparative study both quantitatively and qualitatively for neoplastic and their adjacemt non-neoplastic tissues between four different types of lectins and CEA to evaluate the specificity and efficacy of lectin histochemical staining on human colorectal tissue.Various reports are available regarding on human colorectal tissue. Various reports are available regarding certain lectins and its role in human colorectal malignant transformation [2,3,6,7,11-16,24-30] but no definite comparative syudies are done comparing the lectin histochemical staining with that of standard colorectal tumor marker.

It is evident that lectins are glycoproteins in nature found both in plants and animals that bind to specific carbohydrate moieties [31]. Therefore, we selected four different types of lectins from four different groups of carbohydrate specificities [17]. There has been various types of opinion regarding the non-neoplastic mucosa adjacent to the tumor. Filipe in 1969 for the first time named this adjacent mucosa as the transitional mucosa [32]. It has been reported that the histologically normal mucosa adjacent to carcinoma of the large intestine is often histochemically abnormal [33-35]. This transitional mucosa is charecterized by an increase of silomucins, usually accompanied by a decrease or absence of sulphomucins of the large intestine [27,36]. The extent of this abnormal transitional mucosa

varies from 3.4 to 19.5 cm from the tumor edge[37]. Some authors described this transitional mucosa morphologically and histochemically abnormal and suggestive of primary or an early feature of malignant transformation or a nonspecific cellular response to an unknown stimuli[35,36,38,39]. But others concluded from their studies that the features of transitional mucosa as a secondary effect of the tumor itself can not be excluded[11,23,27,40,41]. Confirmatory evidence is, however lacking and the significance of these proclamations still remains speculative. From our study we found that UEA-1 lectin to be extremely non-reactive to this adjacent non-neoplastic mucosa, which was suggestive of that this adjacent non-neoplastic mucosa not to be a site of primary or an early feature of malignant transformation. When a malignant transformation occurs in a cell, it starts to proliferate and increase in size, during this procedure biochemical changes also occurs within these malignant cells. The cells adjacent to this neoplastic tissue may be influenced biochmically by the process of diffusion or by any other unknown process. Therefore we are of this opinion that the histochemically abnormal mucosa adjacent to the neoplastic tissue is not a feature of early malignant transformation. The UEA-1 lectin staining was supportive to this hypothesis and observed to be a very localized indicator of neoplastic and non-neoplastic tissue.

The regional differentiation of UEA-1 lectin for normal proximal and distal colonic mucosa has been reported[4,14,30]. Terminal positions on the oligosaccharide side chains are generally occupied by silic acid or fucose. These two sugars compete with each other for occupation of the terminal position. As UEA-1 is specific to a terminal fucosyl residue, the lack of UEA-1 binding with distal colonic mucosa may be related to the abscence or low fucose content in the distal colonic region[13]. The results of our study confirms these reports and there by further reconfirms that the adjacent non-neoplastic mucosa does not posses any potential malignant charecteristics.

It is further suggested of that neoplastic glycoprotein with alpha-l-fucosyl residue is produced or the terminal carbohydrate structure of glycoprotein present in the non-neoplastic mucosa is altered to bind freely with UEA-1 after the neoplastic transformation occurs[3]. As for the neoplastic tissues our result reflected with that of various other authors[5,26]. Various authors is of the same opinion that CEA is a normal glycoprotein constituent of the epithelial cells of the normal human colorectal mucosa[24-26,42-44], and the difference between the neoplastic tissues and the non-neoplastic mucosa is actually only quantitative, not qualitative[23,45,46]. Some are of the opinion that the demonstration of CEA is a reliable indicator of malignant change in colonic mucosa[47]. Our findings were direct cotradictory to theirs. In our study UEA-1 lectin was competible to CEA for neoplastic tissues but for adjacent non-neoplastic mucosa CEA showed very nonspecific results and proved to be a poor indicator of non-neoplastic tissues. By paired two tailed t-test UEA-1 showed highly significant results for adjacent non-neoplastic mucosa to that of CEA.

As for the localization of staining UEA-1 exhibited quite significant staining pattern. On the other hand CEA did not show any definite type of staining pattern for neoplastic and non-neoplastic tissue. The prominent cytoplasmic staining of the lectins observed in this study may explain the presence of glycoproteins with different sugar residues in the Golgi apparatus. It is at this site that a variety of sugars are added on to the oloigosaccharide side chain of the protein core[48,49]. Ultrastuctural visualization of peanut lectin (PNA) conjugate in the golgi cisterne has been demonstrated[28]. The enhanced and diffuse cytoplasmic staining of neoplastic tissue by most of the lectins might be attribut-

able to the disorganized and dispersed nature of the organelles responsible for biosynthesis of glycoproteins within the cytoplasm[12]. The internal GlcNAc residues are typical components of hyaluronic acid, which explains for the intense binding of WEA lectin to the connective tissue stroma[16]. Mannose is present in cell membranes and is especially abundant in serum glycoproteins, probably explains the extensive cytoplasmic staining pattern of Con-A lectin[4].

It is therefore appears that the glycoprotein modifications of all lectins associated with neoplastic transformations are not always a specific indicator of the potential state of the colorectal mucosa. But from our study it was evident that UEA-1 lectin was much more localized, specific and reliable for the malignant transformation of the colonic tissue in comparison to the carcinoembryonic antigen, CEA. Finally the potential usefulness of UEA-1 lectin expression and its clinical representation as a predictive indicator of biological behavior of adenocarcinoma of the large bowel needs further study.

References

1) Klein PJ, Osmers R, Vierbuchen M, Ortmann M, Kania J and Uhlenbruck G : The importance of lectin binding sites and carcinoembryonic antigen with regard to normal, hyperplastic, adenomatous and carcinomatous colonic mucosa. Rec Res Cancer Res 79 : 1-9, 1981.

2) Cooper HS : Peanut lectin binding sites in large bowel carcinoma. Lab Invest 47 : 383-390, 1982.

3) Yonezawa S, Nakamura T, Tanaka S and Sato E : Glycoconjugate with Ulex europaeus agglutinin-1 binding sites in normal mucosa, adenoma and carcinoma of tha human large bowel. J Natl Cancer Inst 69 : 777-781, 1982.

4) Boland CR, Kim YS : Lectin markers of differentiation and malignancy. Prog Res Ther 29 : 253-266, 1984.

5) Nakamura T, Yonezawa S, Tanaka S and Sato e : Lectin histochemistry in colorectal adenomas and early carconomas. Pathol and Clin Med 3 : 317-322, 1985.

6) Rhodes JM, Black RR, Savage A : Glycoprotein abnormalities in colonic carcinomata and hyperplastic polyps shown by lectin peroxidase histochemistry. J Clin Pathol 39 : 1331-1334, 1986.

7) Yuan M, Itzkowitz SH, Bolond CR, Kim YD, Tomita JT, Palekar A, Bennington JL, Trump BF and Kum YS : Comparison of T-antigen expression in normal, premalignant and malignant human colonic tissue using lectin and antibody immunohistochemistry. Cancer Res 46 : 4841-4847, 1986.

8) Cooper HS , Farano P, Rebecca AC : Peanut lectin binding sites in colons of patients with ulcerative colitis. Arch Pathol Lab Med 111 : 270-275, 1987.

9) Kotake K : Lectin histochemistry of 1,2 dimethylhydrazine induced rat colonic neoplasia and human colorectal neoplasia. J Jap Surg Soc 89 : 534-546, 1987 (Japanese).

10) Lee YS : Lectin reactivity in human large bowel. Pathology 19:397-401, 1987.

11) Campo E, Condom E, Palacin A, Quesada E and Cardesa A : Lectin binding patterns in normal and neoplastic colonic mucosa. A study of Dolichos biflorus agglutinin, peanut agglutinin and wheat germ agglutinin. Dis Col Rect 31 : 892-899, 1988.

12) Lee YS : Lectin expression in neoplastic and non-neoplastic lesions of the rectum. Pathology 20 : 157-165, 1988.

13) Caccamo D, Telenta M and Celener D : Concanavalin A binding sites in fetal, adult, transitional and malignant rectosigmoid mucosa. Human Pathol 20: 1186-1192, 1989.

14) Caldero J, Campo E, Acaso C, Ramos J,

Panades MJ and Rene JM : Regional distribution of glycoconjugates in normal, tran sitional and neoplastic human colonic mucosa. Virchows Arch (Pathol Anat) 415 : 347-356, 1989.

15) Shah M, Shrikhande SS and Swaroop VS : Lectin binding in colorectal mucosa. Ind J Gastroenterol 8 : 331-333, 1989.

16) Carcinoembryonic antigen : Its role as a marker in the management of cancer-National institutes of health consensus development conference statement. Cancer Res 41 : 2017-2018, 1981.

17) Damjanov I : Biology of disease. Lectin cytochemistry and histochemistry. Lab Invest 57 : 5-20, 1987.

18) Blenkinsopp WK, Stewart BS, Blesovsky L, Kearney G and Fielding LP : Histopathology reporting in large bowel cancer. J Clin Pathol 34 : 509-513, 1981.

19) Dukes CE and Bussey HJR : The spread of rectal cancer and its effect on prognosis. Br J Cancer 12 : 309-320, 1958.

20) Morson BC and Sobin LH : Histological typing of intestinal tumors. Wold Health Organization, Geneva (International histological classification of tumors no.15) 1976.

21) Hsu SM, Raine L and Fanger H : Use of avidin-biotin-peroxidase complex in immunoperoxidase techniques : A comparison between ABC and unlabled antibody (PAP) procedures. J Histochem Cytochem 29 : 577-580, 1981.

22) Kuhlman WD, Peschke P and Wysster K : Lectin peroxidase conjugates in histopathology of gatrointestinal mucosa. Virchows Arch (Pathol Anat) 398 : 298-328, 1983.

23) Hamada Y, Yamamura M, Hioki K, Yamamoto M, Nagura H and Watanabe K : Immunohisochemical study of carcinoembronic antigen in patients with colorectal cancer. Cancer 55 : 136-141, 1985.

24) Burtin P, Martin F, Sabine MC and von Kleist S : Immunological study of polyps of the colon. J Natl Cancer Inst 48 : 25-29, 1972.

25) Lo Gerfo P and Herter F : Demonstration of tumor associated antigen in normal colon and lung. J Surg Oncol 4 : 1-7, 1972.

26) Khoo SK, Warner NL, Lie JT and Mackay IR : Carcinoembryonic antigen activety of tissue extracts. A quantitative study of malignant and benign neoplasms, cirrhotic liver, normal adult and fetal organs. Int J Cancer 11 : 681-687, 1973.

27) Penelope AD and Filipe MI : An ultrastructural and histochemical study of the mucous membrane adjacent to and remote from carcinoma of the colon. Cancer 37 : 2388-2398, 1976.

28) Sato A and Spicer SS : Ultrastructural visualization of galactosyl residues in various alimentary epithelial cells with the peanut lectin horseradish peroxidase procedure. Histochemistry 73 : 607-624, 1982.

29) Bresalier RS, Boland CR and Kim YS : Regional differences in normal and cancer associated glycoconjugates of the human colon. JNCI 75 : 249-260, 1985.

30) Rhodes JM, Black RR and Savage A : Altered lectin binding by colonic epithelial glycoconjugates in ulcerative colitis and crohn's disease. Digest Dis Sci 33 : 1359-1363, 1988.

31) Goldstein IJ : What should be called a lectin. Nature 285 : 66, 1980.

32) Filipe MI : Value of histochemical reactions for mucosubstances in the diagnosis of certain pathological conditions of the colon and rectum. Gut 10 : 577-586, 1969.

33) Filipe MI : The mucous membrane of the normal human large intestine and the changes which occur in it immediately adjacent to proven carcinoma-A histochemical, autoradiographic and chemical study. Phd. Thesis, University of London, 1971.

34) Filipe MI and Cooke KB : Changes in composition of mucin in the mucosa adjacent to carcinoma of the colon as compared with the normal-A biochemical investigation. J Clin Pathol 27 : 315-318, 1974.

35) Filipe MI : Mucins in the human gastrointestinal epithelium : A review. Invest Cell Pathol 2 : 195-216, 1979.

36) Habib N, Salem R, luck RJ, Bolount MA, Rifaat MA and Wood CB : A histochemical method that predicts local recurrence after curative resection in carcinoma of the colon and rectum. Surg Gynecol and Obstet 159 : 436, 1984.

37) Wood CB, Dawson PM and Habib NA : The sialomucin content of colonic resection margins. Dis Col Rect 28 : 260-261, 1985.

38) Greaves P, Filipe MI and Branfoot AC : Transitional mucosa and survival in human colorectal cancer. Cancer 46 : 764-770, 1980.

39) Shamsuddin AKM, Weiss L, Phelps PC and Trump BF : Colon epithelium IV. Human colon carcinogenesis. Changes in human colon mucosa adjacent to and remote from carcinomas of the colon. J Natl Cancer Inst 66 : 413-419, 1981.

40) Isaäcson P and Attwood PRA : Failure to demonstrate specificity of the morphological and histochemical changes in mucosa adjacent to colonic carcinoma (transitional mucosa). J Clin Pathol 32 : 214-218, 1979.

41) Williams GT : Transitional mucosa of the large intestine. Histopathology 9 : 1237-1243, 1985.

42) Martin F anf Martin MS : Radioimmunoassay of carcinoembryonic antigen in extracts of human colon and stomach. Int J Cancer 9 : 641-647, 1972.

43) Rosai J, Tillak TK and Marchesi VT : Membrane antigens of human colonic carcinoma and nontumoral colonic mucosa; results obtained with a new isolation method. Int J Cancer 10 : 357-367, 1972.

44) Wagener C, Csaszar H, Totovic V and Breuer II : A highly sensitive method for the demonstration of carcinoembryonic antigen in normal and neoplastic colonic tissue. Histochemistry 58 : 1-11, 1978.

45) Huitric E, Laumonier R, Burtin P, Von Kleist S and Chavanel G : An optical and ultrastructural study of the localization of carinoembryonic antigen (CEA) in normal and cancerous human rectocolonic mucosa. Lab Invest 34 : 97-107, 1976.

46) Fritsche R and Mach JP : Isolation and characterization of carcinoembryonic antigen (CEA) extracted from normal human colon mucosa. Immunochemistry 14 : 119-127, 1977.

47) Isaacson P and Le Vann HP : The demonstration of carcinoembryonic antigen in colorectal carcinoma and colonic polyps using an immunoperoxidase technique. Caner 38 : 1348-1356, 1976.

48) Neutra M and Leblond CP : Synthesis of the carbohydrate of mucus in the golgi complex as shown by electron microscope, radioautography of goblet cells. From rats injected with glucose-H3. J cell Biol 30 : 119-136, 1966.

49) Phelps CF : Biosynthesis of mucus glycoprotein. Br Med Bull 34 : 43-48, 1978.