

● **HELICOBACTER PYLORI AND HYPOCHLORHYDRIA IN ASYMPTOMATIC BRAZILIAN MEN.** L.L. Braga, B.J. Marshall, H. Moreno, D. Menzes, R.W. McCallum, R. L. Guerrant, University of Virginia, Charlottesville, VA 22908 and Universidade Federal do Ceara, Fortaleza, Brazil.

In the state of Ceara, Brazil, gastric carcinoma is the most common malignancy of men. Asymptomatic *H.pylori* (HP) infection is also common in Brazil and is a suspected risk factor for gastric carcinoma. The AIM of this study was to determine the prevalence of *H.pylori* in healthy Brazilian men and to test for a correlation between acid secretion and presence of the organism. **METHOD.** Healthy men were recruited on the beach at Fortaleza, a city of 2,000,000 people in northeastern Brazil. Subjects gave informed consent and were paid \$18.00 U.S. After an overnight fast, subjects completed a questionnaire detailing the presence or absence of dyspeptic symptoms. A nasogastric tube was then passed and position in the stomach was confirmed with auscultation and aspiration. After 4 15-minute baseline collections, they were given 0.04 mg/kg of histamine and three more collections were made. All subjects then underwent endoscopy at which biopsies were taken from the antrum and body mucosa for histology from the antral mucosa for a CLOtest rapid urease test. **RESULTS:** The mean age of the subjects was 33 years (range 19-67). Only one subject was negative for *H.pylori*. This 31-year-old male was the only subject who did not accept payment for the experiment. His endoscopy and biopsies were normal with a negative CLOtest. BAO was .79 and PAO was 7.9 meq/hour. In the other 19 subjects histology revealed *H.pylori* with associated active gastritis (sensitivity 100%). In 18 of these the CLOtest was positive (sensitivity 95%, specificity 100%). Of the 19 HP+ subjects, 17 had a normal endoscopy, 1 had antral erosions and 1 had corpus erosions. PAO in these two subjects was 9.4 and 8.5 meq/hour, respectively. Among the 17 HP+ subjects with normal endoscopy, 4 were achlorhydric with BAO and PAO < 2 meq/hr. In 5 others PAO was less than 5 meq/hr. **CONCLUSION:** Among poor Brazilian men, the prevalence of *H.pylori* approaches 100%. Asymptomatic Brazilians with *H.pylori* are commonly hypochlorhydric and 20% have histamine fast achlorhydria. Achlorhydria and gastritis may be risk factors for gastric carcinoma.

● **QUANTITATIVE IMAGE CYTOMETRY TO EVALUATE THE ADENOMA-CARCINOMA SEQUENCE IN THE HUMAN COLON.** RS Bresalier, KL Chew, SB Ho, JD Hom, BH Mayall, MH Sleisenger & YS Kim. GI Res Lab, VA Med Ctr & Depts Med & Lab Med, Univ of Calif., SF, CA.

The adenoma-carcinoma sequence assumes that colonic neoplasms result from sequential mucosal changes which are associated with morphologic abnormalities culminating in invasive carcinoma. Quantitative image cytometry provides an objective & reproducible means of cytomorphometric analysis which may be used to assess tumor grade & study tumor progression. We have employed image cytometry to evaluate the evolution of changes in nuclear size, shape & chromatin distribution in 15 normal and 52 early neoplastic colonic lesions including tubular adenomas <1cm, adenomas >2cm, adenomas with CIS, and Dukes B well-differentiated carcinomas. 4 μ tissue sections were stained with azure A Feulgen, & 12 features in 100 epithelial nuclei examined in each section using the TAS-Plus image cytometer. Regression analysis demonstrated a highly significant progression in measurements of nuclear size, shape and DNA content from benign to malignant lesions.

	Nl	Adenomas		Dukes B		Regression	
		<1cm	>2cm	CIS	WD CA	r	p
Area (μ ²)	33.2	33.9	43.6*	62.4**	68.7**	.80	<.001
Perimeter (μ)	23.1	25.4	28.9**	33.4**	33.1**	.81	<.001
Max diameter (μ)	8.4	10.2**	11.4**	12.9**	11.7**	.73	<.001
Min diameter (μ)	5.6	4.8	5.4	6.9**	8.1**	.80	<.001
DNA (units)	1.7	2.4*	2.7**	4.4**	4.7**	.76	<.001

*p<.02, **p<.005 vs. nl (pairwise comparisons). Patterns of chromatin clumping, condensation & heterogeneity also correlated with progression at the <.001 level. A stepwise discriminant analysis using these parameters ("jackknife classification") correctly classified 87% of lesions including 93% of normals, 97% of carcinomas, 100% of benign adenomas (<1cm>2cm), and 100% of small adenomas. 4/5 cases of focal CIS in adenomas were classified as malignant (1 was classed as "large adenoma"). Hyperplastic polyps (n=5) were all classed as benign (3-"hyperplasia, 1-"nl", 1-"small adenoma"). **Conclusion:** Image cytometry provides an objective quantitative confirmation of the evolution of nuclear changes in the progression from normal mucosa to invasive cancer. This method may be useful in clinically differentiating benign and early neoplastic lesions.

● **KI-RAS MUTATIONS IN PRIMARY AND METASTATIC NEOPLASIA.** GDBren, T Gomez, JRCoggins, DJScarpenter, DKerr, HTLynch, DAAhlquist, SMThClinic, Rochester, MN and Creighton Univ, Omaha

As a result of recent advances in molecular biology techniques, such as the polymerase chain reaction, several types of genetic events are now being detected in colorectal cancer. One of the more frequent events that has been shown to occur is the presence of mutations within the proto-oncogene *Ki-ras*. We determined the frequency of *ras* point mutations in sporadic colorectal cancer (composed of 8, 2, and 31 pts in Dukes A, B₁, B₂, C₁, C₂, and D) and a number of other subgroups including familial adenomatous polyposis (FAP, 12 pts), hereditary nonpolyposis colorectal cancer (HNPCC, 7 pts), and metastatic lesions in both colon (4) and liver (15). **METHODS:** DNA from normal colonic mucosa and from cryostat sectioned adenoma and carcinoma tissue were analyzed with the use of a PCR-based assay combined with allele-specific oligonucleotide hybridization to detect all mutations found in codons 12 and 13. **RESULTS:** No mutations were found in any of the 100 tissue examined. However, *ras* mutations were found in 100% of adenomas and carcinomas from these same pts.

Frequency of *ras* Point Mutations

	Adenomas	Carcinomas	Adenomas + Carcinomas
Sporadic (Dukes A-D)	44/125 (35)	8/7	
HNPCC	1/8 (12)	1/1	
FAP	2/6 (33)	1/1	
Metastatic Lesions	14/20 (70)		

Although Dukes stage D showed the highest frequency of mutations, there was no statistically significant difference between the various Dukes stages (p=0.08). Additionally, the proportion of mutations found in adenomas from primary carcinomas from HNPCC pts were lower than in the sporadic cases (data ns). However, the frequency of mutations found in metastatic tissue was higher than in primary cancers (p=0.003). The frequency of *ras* mutations is higher in cancer than in primary colorectal neoplasms and warrants further investigation.

● **ALTERATIONS IN MUCIN TYPE GLYCOPROTEINS IN HUMAN COLON CANCER METASTASES.** RS Bresalier, SB Ho & YS Kim. VA Med. Ctr., Dept. of Med., Univ. of Calif., SF, CA.

Alterations in mucin type glycoproteins on the cell surface & secreted glycoproteins associated with colorectal carcinogenesis, & methods in determining which cells are capable of metastasis. We have used avidin immunoperoxidase immunohistochemistry to study the expression of the mucin core carbohydrates (GalNAc6Ser/Thr, T (Galβ3GalNAc-Ser/Thr), 6GalNAc-Ser/Thr), & the oncodevelopmental carbohydrate sialylT₂ difucosyl LeX in 9 primary carcinomas & 18 metastases from colorectal cancer patients. In 10 primary cecal tumors & 18 associated metastases formed after growth of the human colon carcinoma LS174T & its derivatives in athymic nude mice (1987;39:625-30). Specific probes included the villoso (Tn) MAb AH9-16 (T), MAb TKH2 (sialylT₂ difucosyl LeX). 7/9 (78%) primary carcinomas expressed the T antigen, while only 4/22 (18%) metastases expressed this structure. This finding is similar to that reported by us using the T-specific lectin PNA. A group of 10 primary cecal tumors & 18 associated metastases were pretreated with neuraminidase & probed with both MAb AH9-16 & PNA. Desialylation increased expression in all cases, but this was most evident in the primary. Tn was expressed in 70% of carcinomas & increased in 80% of metastatic foci examined. SialylT₂ difucosyl LeX was increased in 45% of primary carcinomas & unchanged in 45% & decreased in 10%, while sialylT₂ difucosyl LeX was increased in 60%, unchanged in 30%, & decreased in 10% of metastases. 80% of primary tumors showed homogeneous expression of one or more antigens & 60% of metastases were heterogeneous. Homogeneous expression of T antigens was observed in cecal tumors formed after growth of LS174T in nude mice also demonstrated decreased expression of T/enhanced expression of sialylT₂ difucosyl LeX in primary & metastases. **Conclusion:** Primary & metastatic colorectal cancers differ in their expression of mucin-associated carbohydrate structures. This may be due to alterations in glycosylation which can enhance or mask epitope expression.