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CANDIDA CARRIAGE IN THE ALIMENTARY TRACT OF LIVER TRANSPLANT CANDIDATES[1]

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Thirty randomly selected patients with advanced chronic liver disease, which had been evaluated for possible liver transplantation, were sampled endoscopically at 7 alimentary tract locations to assess the frequency and amount of *Candida* carriage. Eighty-one percent (127/156) of the samples obtained contained *Candida* and 53% (82/156) yielded high counts (>300 CFU/ml). The most predominant *Candida* species isolated at each site was *Candida albicans*, which accounted for 103 (64%) of the 160 fungal isolates. The other *Candida* species isolated included *C tropicalis* 30 (19%), *C krusei* 16 (10%), and *C glabrata* 11 (7%).

Although the number of sites at which yeast was present and the quantities of yeast at each site varied widely among the patients studied, 100% of the patients had *Candida* in at least one site of the gastrointestinal tract. Eighty-six percent (24/28) of the duodenal aspirates contained *Candida* and 50% (14/28) of the duodenal samples contained greater than 300 CFU/ml. A positive culture from the stomach was a reliable predictor of the presence of *Candida* in the duodenum ($P=0.0001$), but a positive culture at no other site readily predicted the presence of *Candida* at yet another site. Importantly, there was no correlation between the presence or absence of *Candida* in either oral or rectal swabs and colonization at other anatomic sites within the gastrointestinal tract.

These findings are important in liver transplantation, particularly in those cases in which the bowel has been opened to create a choledochojejunostomy anastomosis. The operative attempts to reduce gastrointestinal fungal carriage using oral antifungal agents may be justified before liver transplantation in an effort to lower the risk of posttransplantation fungal infections, particularly in those patients expected to have a Roux-en-Y choledochojejunostomy biliary reconstruction.

Systemic fungal infections occur frequently after antibiotic therapy and surgery as well as during immunosuppression,

neoplastic diseases, and long hospitalization (1–4). Patients undergoing liver transplantation have many of these risk factors, and frequently develop fungal infections. A previous study from the University of Pittsburgh demonstrated that the rate of invasive fungal infections in liver transplant patients was 16% and most of these infections were due to candidiasis (5, 6). It has been suggested that intestinal colonization with *Candida* may be the source of these invasive *Candida* infections (3, 7, 8). There are experimental models demonstrating that animals treated with antibiotics and then "challenged" with yeast develop not only a high gastrointestinal colonization rate, but also visceral dissemination (9). Also, visceral dissemination was more common in animals that were given antibiotics and immunosuppressive agents (10, 11).

Various studies have examined the question of *Candida* carriage in the digestive tract, but only 2 have actually sampled multiple portions of the alimentary tract of living subjects (12, 13). Most such studies have been limited to only 1 or 2 sites, usually the oropharynx and rectum (14–16). In the present study, *Candida* carriage at 7 different anatomic sites within the gastrointestinal tract was sampled to determine the rate and amount of *Candida* carriage in candidates for liver transplantation.

MATERIALS AND METHODS

Patient selection. Samples were obtained from 30 randomly selected patients, who had been evaluated for liver transplantation, at Presbyterian University Hospital between April and August 1988. None of the patients had been taking antifungal agents during or before (3 months) the study period.

Specimen collection. All subjects were prepared for an endoscopic examination of their upper and lower gastrointestinal tract by ingestion of 3–4 L of iso-osmotic solution (Golytely; Braintree Laboratories, Inc., Braintree, MA) the evening before the examination. As many as 7 different sites were sampled per individual patient. Oral and rectal swabs as well as aspirates obtained by endoscopy of the esophagus, stomach, duodenum, cecum, and sigmoid colon were collected.

The upper gastrointestinal tract samples were collected in "reverse" order to minimize the introduction of flora from proximal sites to more distal sites by the endoscope or by the irrigating saline used to collect samples. The endoscope (Olympus T2) was passed under direct vision into the duodenum and duodenal contents were aspirated. If no fluid was present in the duodenum, 60 ml of saline were introduced and aspirated 1 min later. The endoscope was then withdrawn into the stomach and gastric material was aspirated. Finally, the endoscope was withdrawn into the esophagus and an esophageal sample was collected after a wash of the area with 60 ml of saline.

Samples were collected from the lower gastrointestinal tract using a fiberoptic colonoscope (Olympus). The colonic samples were col-

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398

lected, in order, from the sigmoid colon, ascending colon, and cecum. Again, if no spontaneous aspirate was obtainable, saline (60 ml) was introduced to the site in order to "bathe" the area for 1 min, and was then aspirated from the colon. Finally, oral and rectal swabs were collected after endoscopy. The swabs were premoistened with 0.1 ml of 0.9% saline before their use.

Quantitative methods. Initially, all fluid specimens, whether collected in situ or by saline lavage, were serially diluted 10- and 100-fold in saline. One tenth of 1 ml of each dilution as well as the undiluted specimens was inoculated onto Mycophil agar (BBL, Cockeysville, MD) containing 80 ug/ml gentamicin and 60 ug/ml meziocillin. In a preliminary experiment, this combination of antibiotics was shown to satisfactorily suppress overgrowth of contaminating enteric organisms.

The oral and rectal swabs were placed into containers of 0.9 ml of saline and were cultured similarly after preparing 10- and 100-fold dilutions. All primary samples and dilutions were plated using a spread plate technique and the plates were incubated at 30°C. The number of colonies on the plates was counted, and the concentration of yeast per milliliter (CFU/ml) in the sample was calculated. Plates with more than 300 colonies were counted at 24 hr using a stereomicroscope for accuracy and plates with less than 300 colonies were counted after 48 hr. An arbitrary limit of ≥ 300 CFU/ml was considered as an elevated yeast concentration, and lower concentrations were scored as "positive," as described by others (12). The specific identification of suspected *Candida* isolates was accomplished using cornmeal agar as described by Lodder (17).

C albicans and *C stellatoidea* were not distinguished in this study because of the known difficulty in separating these two "species" (18). Confirmation of *C albicans* isolates was accomplished by the "germ tube" method described by Berardinelli and Opheim (19). This method uses a medium consisting of 3 parts rabbit coagulase plasma and 2 parts trypticase-soy broth (BBL). Authentic strains of *C albicans* and *C tropicalis* were used as positive and negative controls. In addition, the identification of each *Candida* species was confirmed by the API 20C system (Analytab, Inc., Plainview, NY).

Storage. The effect of storage on yeast growth was evaluated. Aliquots were prepared of each sample from 4 different patients. These aliquots were either refrigerated at 4°C, or frozen at -10°C, for 1 and 7 days. The number of colonies that grew from these samples was counted at 24 and 48 hr and compared with counts obtained from the fresh samples.

Statistical methods. Statistical evaluation was accomplished using the general linear models procedure of SAS (SAS Institute Inc., Cary, NC) for regression and correlation analysis of all the data obtained from the 30 patients. Pearson correlation coefficients were calculated using Epistat (T.L. Gustafson, 1986) for analysis of data from the 12 patients with complete samples. These data were evaluated quantitatively with the proviso that values < 300 CFU/ml were treated as zero. A P-value of ≤ 0.05 was considered statistically significant (20).

RESULTS

Thirty randomly selected patients being evaluated for a liver transplantation were sampled, and 156 specimens from different sites were obtained. These sites were the mouth (16), esophagus (28), stomach (29), duodenum (28), cecum (19), sigmoid (20), and rectum (16). Of the 156 specimens obtained, 81% (127/156) yielded positive cultures, and 53% (82/156) yielded high counts (greater than 300 CFU/ml). A total of 160 *Candida* isolates were cultured, as few patients had more than 1 *Candida* isolate per site sampled. The quantitative results for all 30 patients studied are shown in Figure 1: (a) 75% (12/16) of the oral specimens were positive for *Candida*, and in 38% (6/16) elevated concentrations were detected; (b) 75% (21/28) of the esophageal specimens were positive, and 54% (15/28) contained high counts; (c) 93%
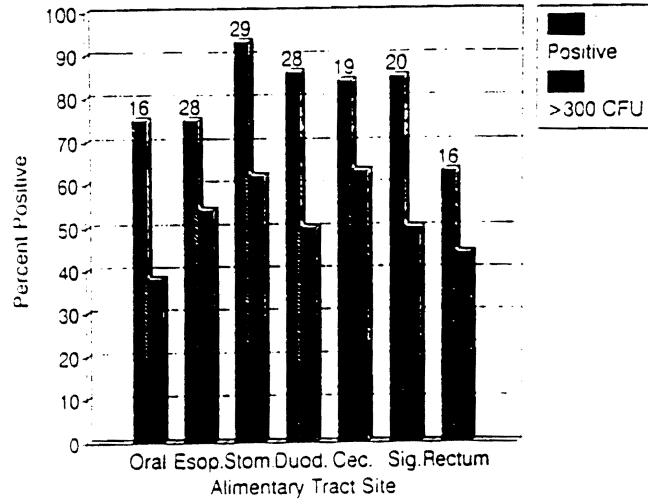

FIGURE 1. Recovery of yeast from various alimentary tract sites in 30 liver transplant candidates. Only 12 subjects were sampled at all 7 sites.

(27/29) of the gastric specimens were positive, and 62% (18/29) had high counts; (d) 86% (24/28) of duodenal aspirates were positive, and in 50% (14/28) there were high counts; (e) 84% (16/19) of cecal specimens were positive, and in 63% (12/19) there were high counts; (f) 85% (17/20) of sigmoid colon specimens were positive, and 50% (10/20) had high counts; (g) 63% (10/16) of the rectal specimens were positive, and 44% (7/16) had high counts.

Twelve of the 30 patients were sampled at all 7 anatomic sites. This group displayed the same frequency of fungal carriage as did the rest of the sample population. The fungal carriage among the 12 patients was variable, and did not show common qualitative or quantitative patterns (Fig. 2). Of the 12 patients, only 2 had elevated concentrations (> 300 CFU/ml) at all 7 sites. Three patients were found to have some growth from the mouth and rectal samples and had at least 1 other site that was positive for yeast. Three patients were found to have no growth of yeast at either the mouth or the rectal swab sample; however, each one of them had high
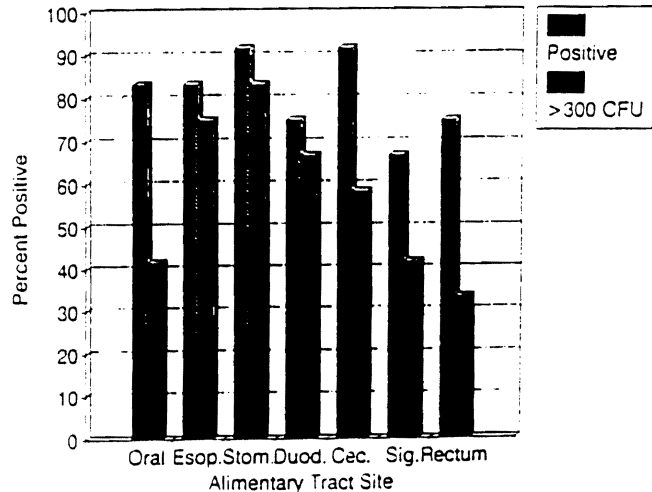

FIGURE 2. Recovery of yeast from the alimentary tract in 12 liver transplant candidates who were sampled at all 7 sites.

colony counts in the duodenal sample. The colonization profile of one of these patients (patient 17) is shown in Figure 3.

The duodenum was the major site of interest, due to its proximity to the surgical site during liver transplantation. Therefore, it was treated as a dependent variable during the statistical analysis. A correlation between gastric carriage and duodenal carriage of yeast was found ($P=0.0001$, $r^2=0.48$). There were no correlations of yeast carriage between any other anatomical sites involved in this study.

Table 1 lists the percentile distribution of the various *Candida* species in each site of sampling. *C albicans* was the predominant species isolated at all sites (103/160, 64%). The other isolated species were *C tropicalis* (30/160, 19%), *C glabrata* (11/160, 7%), and *C krusei* (16/160, 10%). While the first 3 species were isolated from all 7 sites, *C krusei* was isolated from esophagus, stomach, and duodenum only. In 12 of 30 patients, and in 25 of 156 specimens (25/156, 16%), more than 1 *Candida* species was isolated. Seven specimens obtained from 3 patients grew 3 different species each (*C albicans, C tropicalis,* and *C krusei*).

Nineteen of these 30 patients subsequently underwent a liver transplantation. Of these 19 patients, 9 (47%) had positive cultures of different body fluids for *Candida* during the first 3 months after liver transplantation, and 6 (6/9, 67%) still carried the same *Candida* species that was isolated originally from their gastrointestinal tract before transplantation. The time between preoperative and postoperative samples from these 7 patients varied from 25 to 39 days.

Storage of specimens was studied on a limited number of early specimens. Specimens stored at 4°C were found to be stable for up to 24 hr. A minimal reduction in counts was noted in the specimens refrigerated for 1 week. Freezing produced a marked reduction in colony count, and the greatest reduction occurred after 7 days of freezing. Therefore, all specimens were cultured immediately after collection, and, if stored, they were refrigerated for 1 night only.

Mycobacterium chelonei was detected in 8 samples from 5 patients. These samples included 2 esophageal, 1 duodenal, 1 cecal, 3 sigmoid, and 1 rectal. These patients did not differ from the others in terms of severity of liver disease, survival, or frequency and type of *Candida* isolated.

TABLE 1. Frequency of recovery of various *Candida* species from gastrointestinal tract specimens in liver transplant candidates

Site (n)[a]	No. of specimens positive for (%)			
	C albicans	*C tropicalis*	*C krusei*	*C glabrata*
Mouth (13)	10 (77)	2 (15)	0	1 (8)
Esophagus (31)	18 (58)	6 (19)	5 (16)	2 (6.5)
Stomach (36)	21 (58)	7 (19)	6 (17)	2 (60)
Duodenum (33)	18 (55)	8 (24)	5 (15)	2 (6)
Cecum (17)	13 (76)	3 (18)	0	1 (6)
Sigmoid (19)	15 (79)	2 (10.5)	0	2 (10.5)
Rectum (11)	8 (73)	2 (18)	0	1 (9)
Total (160)	103 (63)	30 (19)	16 (10)	11 (7)

[a] Number of isolates at each site.

DISCUSSION

Candida carriage within the alimentary tract has been investigated by several different authors. Cohen et al. (12) studied normal volunteers using oral swabs and samples of jejunal and ileal contents as well as feces. An increase in both the rate of recovery and concentration of organisms from the mouth to the rectum was suggested. Stone et al. (13) measured *Candida* concentration in patients with penetrating abdominal wounds and quantified the fungi found in the mouth, rectum, and ostomy sites. He found high rates of carriage in the mouth (53%), stomach (69%), duodenum (72%), jejunum (21%), ileum (47%), colon (65%), and rectum (31%). Although the number of cultures obtained in their study was reported the number of patients studied at each alimentary tract site was not, making it difficult to compare their results with the present report.

The present study examined yeast carriage in patients who were being evaluated for liver transplantation. Although this group of patients was randomly selected for the study, they were representative of the liver candidate population at our institution. A comparison of their severity of liver disease expressed by their UNOS status to a group of 50 consecutive liver transplant patients, performed in 1988, showed that the 2 patient groups were equivalent in term of their UNOS distribution. Liver transplant patients are known frequently to develop candidiasis after transplantation. Many investigators believe that the source of invasive *Candida* infections is the patient's own colonizing yeast. Increased colonization was found to be associated with long hospital stay, prolonged courses of antibiotics, and the use of antacids (21–25). In the present study, 100% of patients were shown to carry *Candida* when multiple sites in the gastrointestinal tract were sampled. These observations confirm that patients with multiple risk factors for fungal colonization and infection, including multiple prior hospital admissions, treatment with antimicrobial agents, and the use of antacids, have widespread *Candida* carriage in their alimentary tract. No common pattern of yeast carriage in these patients was found. A potential confounding variable in this study was the fact that a laxative (Golytely) was used in the preparation of each subject for the endoscopic studies. Since most of the gastrointestinal contents were evacuated as a result of this preparation, reduced numbers of organisms would be expected. The data therefore, represent minimal estimates of the frequency and intensity of yeast colonization from sites, which without such preparation may have been more heavily populated before their preparation for endoscopy. Sites with lower levels o
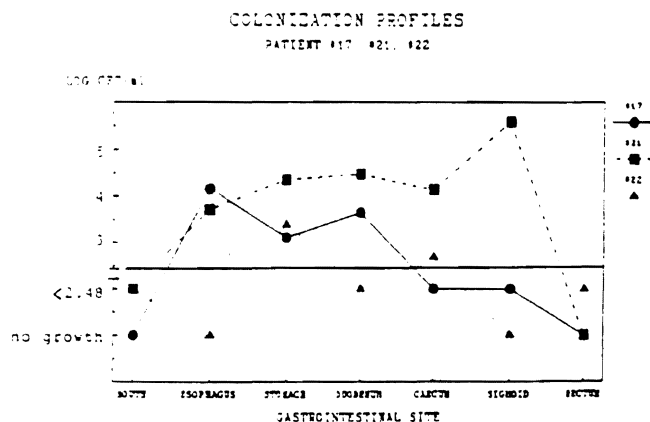

COLONIZATION PROFILES
PATIENT #17 #21 #22

FIGURE 3. Quantitative patterns of gastrointestinal carriage of yeast in 3 liver transplant candidates. Although yeast was not recovered from oral and rectal swabs of patient 17, this subject was colonized in other gastrointestinal sites.

colonization may have become artificially negative as a result of the cleansing procedure used.

Twelve patients who were sampled at all 7 sites demonstrated great variations in their carriage pattern. This demonstrates that information on the mycoflora of a particular alimentary tract site of interest is required if meaningful data are to be obtained. The present results indicate that a significant correlation between yeast counts in the stomach and those in the duodenum exists ($P = 0.0001$); however, only 48% of the variation in duodenal yeast counts could be attributed to gastric colonization ($r^2 = 0.483438$) (20).

Despite efforts to reduce site to site contamination, it is entirely possible that the results obtained merely indicate carryover of organisms from the stomach to the duodenum or from the duodenum to the stomach during the sampling procedure. We know of no way to avoid this problem. Clinically, oral and rectal swabs appear to be inadequate for assessing colonization at sites other than the mouth and rectum. A recent case report supporting this statement describes a patient who was found to have negative fungal stool cultures, but in whom *C albicans* was isolated from endoscopic samples obtained from duodenal and jejunal plaques (26).

Six of 9 subjects in the present study who went on to receive a liver transplant were independently found to harbor *Candida*, up to 89 days after their original investigation. One patient had in his sputum a different *Candida* species as compared with the *Candida* originally isolated from the alimentary tract.

These results suggest that the high point prevalence of *Candida* carriage found in this study may not be a transient event, but rather is likely to be a stable feature of this group of patients with advanced liver disease. In a previous study of *Candida* colonization among liver transplant candidates who subsequently underwent liver transplantation, a high frequency of *Candida* colonization was observed in surveillance cultures collected every 2 weeks for a period of up to 158 days. Sixty-two percent (32/52) of the patients studied showed persistent *Candida* colonization of the mouth and/or rectum as evidenced by repeated positive cultures and *C albicans* was the most frequently isolated species (91%) (S. Kusne, personal observation, 1988). Taken together, these data indicate that it can be assumed that this unique patient population is highly colonized by *Candida*. Also, the finding among the *Candida* species in this study that *C krusei* and *C glabrata* were isolated in 10% and 7%, respectively, is important because of the propensity of these *Candida* species to be resistant to azole antifungals such as fluconazole. These observations could form the basis of future studies to assess the effect of oral antifungal agents for prophylaxis in this population. Reduction of *Candida* colonization of the gastrointestinal tract before transplantation may be important in cases in which the bowel is entered for creation of biliary anastomosis.

An interesting and unexpected finding in this investigation was the isolation of *M chelonei* in some of the esophageal, duodenal, cecal, sigmoidal, and rectal samples from 5 patients. Although this bacterium has been reported to cause infections secondary to contaminated medical equipment (27, 3), we could not demonstrate contamination of our instruments, and we found no evidence of disease in any of these colonized patients. This finding has not been reported previously, but its significance is unclear to us.

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BILIARY ANASTOMOSIS AFTER LIVER TRANSPLANTATION DOES NOT BENEFIT FROM T TUBE SPLINTAGE

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T tubes are commonly used to splint biliary anastomoses after liver transplantation. Although several advantages are claimed for this approach, there is undoubtedly some iatrogenic morbidity associated with the use of T tubes in this situation. We have evaluated 120 consecutive biliary reconstructions after liver transplant, the majority of which were unsplinted end to end bile duct anastomoses. We have shown that biliary leakage and stricture rates are not significantly affected by T tubes. We have also shown that endoscopic retrograde cholangiopancreatography and percutaneous cholangiography are reliable posttransplant methods for cholangiography and stricture dilatation.

Routine T tube splintage of post-liver transplant biliary anastomoses is unjustified.

Liver transplantation is now an established means of treating many types of end-stage liver disease (1–3). Complications after reconstruction of the biliary tract make a significant contribution to postoperative morbidity and mortality in transplant patients (4–8). Most methods of biliary reconstruction, such as the gallbladder conduit, the choledochojejunostomy en Y, and duct to duct anastomoses have used T tube splintage (5, 7, 9). The claimed advantages of T tube splintage include the provision of radiological access for performing subsequent T tube cholangiography, control of bile leakage, and prevention of anastomotic strictures. Routine placement of T tubes may, however, be itself the cause of problems such as biliary tree sepsis, bile sludging or inspissation, and biliary leak. The preferred method of biliary reconstruction in our unit is now end to end, duct to duct anastomosis without T tube drainage (D-D).* In this article, we retrospectively review the results of 120 consecutive biliary anastomoses following liver transplantation and assess whether abandoning routine T tube splintage in direct duct to duct anastomosis has been detrimental to outcome.

PATIENTS AND METHODS

Between October 1988 and July 1992, 123 liver transplants were performed in 118 patients, at this unit. All transplants were performed by a single surgeon. As a crude measure of outcome, 73 (62%) patients are currently alive and 45 (38%) are dead. The 30-day mortality was 10% (12/118). Survival currently ranges from 1 day to 46 months, with a mean of just over 13 months (SD 11). Intraoperative death occurred in 3 cases, and in these patients biliary anastomosis was not performed. Thus there are 120 transplants with biliary reconstruction valid for assessment.

In cases in which the gallbladder conduit was used, anastomoses were fashioned between donor gallbladder and donor common hepatic duct, and between donor gallbladder fundus and recipient bile duct. Both anastomoses were splinted by a T tube. Where Roux-en-Y reconstruction was used, the donor bile duct was anastomosed end to side onto the Roux loop of jejunum without splintage. Direct duct to duct anastomosis was performed using interrupted 5/0 PDS sutures and, if a T tube was used, it was inserted into the recipient duct just above the duodenum distal to the line of anastomosis with one limb of the T tube crossing the anastomosis. All T tubes were routinely left in place for 3 months before removal. The donor gallbladder was excised immediately after all types of reconstruction except the gallbladder conduit.

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* Abbreviations: D-D, duct to duct anastomosis without T tube drainage; D-D+T, duct to duct anastomosis with T tube drainage; ERCP, endoscopic retrograde cholangiopancreatography.