Candida Albicans Colonization in Duodenal Ulcer Disease

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Özet: *DUODENUM ÜLSER HASTALIĞINDA CANDİDA ALBİCANS KOLONİZASYONU*

Bu çalışmanın amacı 20 sağlıklı kontrol ve 24 duodenum ülser hastasında mantar mevcudiyeti açısından bir fark olup olmadığını göstermektir. Mide suyunda imprint tekniği ile kontrollerde %65, hasta grupta %54.1 mantar bulunurken Candida albicans aynı örneklerde hasta grupta (%62.5) kontrol gruba (%25) göre anlamlı (p<0.05) olarak yüksek üretildi. Candida albicans, ülserin kenarından alınan doku örneğinde (%45), kontrollerin bulbusundan (%15) alınan örneklere göre daha yüksek izole edilmiştir. Histolojik olarak mantar sadece epitelin yüzeyinde görülmüştür. Duodenum ülser hastalarında Candida'nın en virülan türü bulunurken sağlıklı kontrol grubunda bulunmaması bunların ülser hastalığı patogenezinde H. ploriye benzer bir rolleri olabileceğinin işaretidir.

Anahtar kelimeler: Candidiazis, duodenum, peptik ülser

Summary: *The purpose of this study was to show whether there exists a difference in 20 healthy control cases and 24 duodenal ulcer patients with respect to the presence of fungi. Using the imprint technique, fungi were observed at a rate of 65% (controls) and 54.1% (patients) in the gastric juice while Candida albicans grew in the same specimens significantly (p<0.05) higher in the patients (62.5%) when compared with the controls (25%). The isolation rate of Candida albicans in the specimens taken from the edge of ulcer (45.8%) was higher than in the tissues obtained from the bulbus (15%) in controls. Histologically, fungi were seen only on the surface of the epithelium. The finding of the potentially most virulent species of Candida predominantly in the duodenal ulcer patients but not in the healthy controls indicates that they may have some kind of role in the pathogenesis of ulcer disease similar to H. pylori.*

Key words: Candidiasis, duodenum, peptic ulcer.

Duodenal ulcer (DU) results from an imbalance between defensive and aggressive factors in the mucosa and hyperacidity is generally acknowledged as being the central factor in the pathogenesis of this complex disease. The pathophysiologic abnormalities related to hyperacidity in DU are; increased parietal cell mass (1), increased basal secretory drive (2), enhanced postprandial secretory drive (3), and rapid gastric emptying (4), but considerable overlap exists with normal subjects. Some patients who have high acid output don't develop duodenal ulcers, whereas ulcers may occur in persons with normal acid output indicating that although gastric acidity in necessary, it is not sufficient for the development of ulceration.

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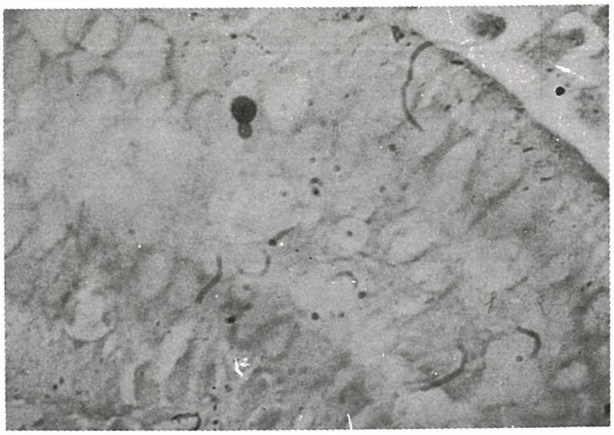
Several mechanisms are involved in the maintenance of mucosal integrity in an acid-peptic environment, including mucosal barrier function (5), cell renewal, mucus and mucosal bicarbonate secretion and mucosal blood flow. One of the most known important external etiologic agent which causes a derangement in some of these defensive factors is H. pylori (6). In duodenal ulcer patients cytotoxigenic strains of this spiral organism can colonize gastric metaplasia islands in the duodenum and this may lead to active duodenitis and ultimately to ulceration (7). Elimination of H. pylori alters the natural history of DU significantly in many patients but not in all of them (8). It is known that there is a relation between H. pylori recolonization and ulcer reappearance in some patients but another alternative in other patients can be the colonization of the mucosa with a different microorganism.



Figüre 1: Fungi were seen as oval, budding yeast like cells located only the surface of epithelial cells. Mc Manus PAS, counter stained with light green.

Fungal infection in routine autopsy series is seen in 1%-4% of the cases (9) and is a relatively common finding in debilitated and immunosuppressed patients. On the other hand it was reported that by histologic examination, Candida infection was found to be positive in 33% of benign gastric ulcers in gastrectomized patients without known predisposing factors (10). Similarly, Candida species are often isolated from the peritoneal fluid cultures of patients with perforated peptic ulcers (11). For this reason we tried to investigate the role of fungal colonization in a small group of duodenal ulcer patients.

METHODS

A totol of 44 consecutive cases undergoing upper gastrointestinal endoscopy (Olympus, EVIS 200) during 1992 was included in the study. The control group (group 1) consisted of 20 healthy volunteers, 12 male and 8 female, with a mean age of 31. The patient group (group 2) consisted of 24 cases, 12 male and 12 female, with the diagnosis of DU and a mean age of 42. Both groups were analyzed and compared with each other for the presence of fungi in specimens taken from throat secretions, gastric juice, gastric corpus and bulbus (in duodenal ulcer patients from the periphery of the ulcer) mucosa. Imprints (stained by gram's solution) and cultures for Candida were made for all secretions while tissue specimens were examined for the presence of Candida by imprint, culture and histopathological techniques.

During endoscopy gastric juice specimens were first aspirated into a sterile syringe through a sterile ERCP cannula. Biopsy forceps which were sterilized by sporicidine solutions were used to take two biopsies from both the corpus of the stomach and the bulbus in all cases for culture, smear and histopathological examinations. The secretions taken from the throat and stomach were incubated on Sabouraud's dextrose agar (Difco) for 10 days at 37°C. Tissue specimens taken from the gastric corpus and bulbus were first divided in a sterile petri dish into two pieces and after mincing, one piece was incubated at 37°C in brain-heart infusion broth (Difco) with 1% glucose. Those found to be positive for any growth were subcultured on to the Sabouraud's dextrose agar. The other piece was used directly to make a smear where preparations stained with Giemsa and gram's solutions were examined for the presence of fungi. Growth of opaque, mounded, cream colored colonies with a distinctive yeast like odor in 2-4 days on Sabouraud's dextrose agar were considered positive for the presence of fungi and confirmed by the demonstration of yeast-like cells by staining the specimens prepared from typical colonies with gram's solution. Those which can grow on a medium with a pH of 1.55 (12), forming a germ tube with human serum and producing a chlamydospore on corn meal agar were considered to be as Candida albicans. API 20 C Aux (bio-Merieux, France) identification system was performed for the classification of Candida species which do not fulfill one of the criteria stated above.

The pH of gastric juice abtained from every case was measured by a glass electrode. All the subjects denied having taken drugs which can suppress gastric acid secretion (H_2R blockers, omeprazole or antacids), or steroids and antibiotics known to favor Candida overgrowth. Moreover, disease such as diabetes, neoplasia and hematologic disorders which are often found associated with gut mycosis were also excluded in our patients.

The endoscopic biopsy specimens were fixed in 10% formalin, dehydrated through using graded alcohol and then were embedded in paraffin. Four sections with 5 microns in thickness were

Table I: Results of fungi recovered from various parts of the body by different methods.

	THROAT		GASTRIC JUICE		CORPUS MUCOSA		BULBUS MUCOSA	
GROUP 1(n= 20)	n	%	n	%	n	%	n	%
Imprint	6	(30)	13	(65.0)	3	(15)	2	(10)
Mc Manus PAS stain	-	(-)	-	(-)	7	(35)	5	(25)
Fungi Culture	6	(30)	13	(65.0)	7	(35)	5	(25)
Candida albicans	4	(20)	5	(25.0)	5	(25)	3	(15)
Other Candida species	2	(10)	8	(40.0)	2	(10)	2	(10)
Gastric juice pH= 2.8								
GROUP 2(n=24)								
Imprint	7	(29)	13	(54.1)	-	(-)	9	(37.5)*
Mc Manus PAS stain	-	(-)	-	(-)	-	(-)	4	(16.6)
Fungi Culture	7	(29)	17	(70.8)	-	(-)	12	(50.0)*
Candida albicans	7	(29)	15	(62.5)*	-	(-)	11	(45.8)*
Other Candida species	0	(0)	2	(8.3)*	-	(-)	1	(4.2)
Gastric juice pH= 2.6								

*: Mean value significantly different from the control (group 1) ($p< 0.05$). GROUP 1: Healthy control group. GROUP 2: Duodenal ulcer patients.

prepared and two of them were stained with hematoxylin and eosin. The remaining two slides were stained with Mc Manus PAS stain which were also counter stained with light green in order to demonstrate the fungi (13). Tissue specimens taken from the stomach were classified as normal mucosa, chronic superficial gastritis (CSG), chronic atrophic gastritis (CAG) and if the latter two were in the active phase this was also noted separately (14). Duodenal mucosa was classified as normal mucosa or chronic duodenitis (CD) (15). If the specimens had a granulation tissue consistent with peptic ulcer, this was also noted separately. The presence of Helicobacter pylori (Hp) was also searched for in the tissue specimens and confirmed by the urease test.

Statistical analysis: To compare two independent proportions z-Approximation or Chisquare was used.

RESULTS

Imprints and cultures of the specimens taken from throat secretions revealed Candida in 6 (30%) cases in group 1 and in 7(29 %) cases in group 2, the difference being insignificant ($p>0.05$), Table I.

Fungi were found to be positive in the examination of the imprints which were made from gastric juice specimens in 13(65%) cases in group 1 and in 13(54%) cases in group 2, while the cultures of the same specimens recovered Candida in 13 cases (65%) and in 17(71%) cases respectively. Again the difference was found to be insignificant ($p>0.05$) when the patient and the control groups were compared with each other. On the other hand Candida albicans grew significantly ($p<0.01$) higher in the patient group (62.5%) than in the controls (25%). The other Candida species which grew in group 1 were C. tropicalis (4 cases), C. glabrata (2 cases), C. guilliermondii (1 case) and C. famata (1 case) while in group 2 only 1 case of C. tropicalis and C.crusei was recovered. The mean value of gastric juice pH was 2.8 in the control group and 2.6 in the patient group ($p> 0.05$).

The imprints of biopsy specimens taken from the normal appearing bulbus in group 1 revealed fungi in only 2(10%) cases while in group 2 fungi were detected in 9 (37.5%) patients ($p<0.05$). Candida grew in cultures of the tissue specimens taken from the bulbus of 5 (25%) cases in group 1 and of 12(50%) cases in group 2, ($p<0.05$) Similarly Candida albicans grew in only 3(15%) cases in group 1 and in 11(45.8%) cases in group 2, ($p<0.05$). The other Candida species which grew were C. guilliermondii (1 case) and C. famata (1 case) in group 1 and C. tropicalis (1 case) in group 2.

Table II: The histopathological results of group 1 (healthy controls) and group 2 (duodenal ulcer) patients and their relation to H. pylori positivity and Candida growth rates.

GROUP 1(n= 20)	STOMACH			DUODENUM		
	CSG(%)	CAG(%)	NGM(%)	CD(%)	DU(%)	NDM(%)
PATHOLOGY	10 (50)	3 (15)	7 (35)	11 (55)	0 (0)	9 (45)
Hp+	9 (45)	3 (15)	5 (25)	0 (0)	0 (0)	0 (0)
Candida+	2 (10)	3 (15)	2 (10)	2 (10)	0 (0)	3 (15)
GROUP 2(n= 24)						
PATHOLOGY	-	-	-	18 (75)	6 (25.0)	-
Hp+	-	-	-	6 (25)	3 (12.5)	-
Candida+	-	-	-	2 (8.5)	2 (8.5)	-

Hp+: H. pylori positivity diagnosed by the examination of biopsy specimens and urease test.
Candida+: Oval, budding yeast like cells located on the surface of epithelial cells demonstrated by Mc Manus PAS which was counter stained with light green
CSG: Chronic superficial gastritis, CAG: Chronic active gastritis, NGM: Normal gastric mucosa NDM: Normal duodenal mucosa.

In group 1, while the endoscopic diagnosis was normal, the histopathological evaluation of the stomach revealed chronic superficial gastritis (CSG) (50%), chronic active gastritis (CAG) (15%) and normal gastric mucosa (NGM) (35%), Table II. In specimens taken from the gastric corpus the overall Hp positivity in group 1 was 85% (9 cases in association with CSG, 3 with CAG and 5 with NGM). We were not be able to demonstrate the presence of fungi in biopsy specimens stained with hematoxylin and eosin so supplemental specimens were stained with Mc Manus PAS and counter stained with light green. With this technique we observed fungi in 7(35%) (2 cases in association with CSG, 3 with CAG and 2 with NGM) control cases in the stomach.

In the control group the histopathological evaluation of the bulbus revealed chronic duodenitis (CD) in 11 (55%) cases and normal duodenal mucosa (NDM) in 9 (45%) cases and we were unabe to demonstrate any Hp positivity with either method. Fungi were observed in only 5 (25%) cases. In group 2, the histopathological examination of the specimens taken from the edge of the ulcer in bulbus disclosed either CD (75%) or peptic ulcer (25%). The Hp positivity rate was found to be 37.5% while fungi was demonstrated in 4 cases (16.6%), which wasn't significantly ($p > 0.05$) different than the control group. Fungi were seen as oval, measuring 2x5 μm, budding yeast like cells located on the surface of epithelial cells, without any tissue invasion or abscess formation (Fig. 1).

When the patient group was subdivided with respect to the presence of Candida albicans in the duodenum we couldn't find any difference in terms of age, sex, aggressive nature (those with a history of three or more recurrence of the typical ulcer pain in one year) and the size of the ulcer (Table III).

DISCUSSION

Candida albicans is a member of the normal flora of the mucous membranes in the gastrointestinal, respiratory, and female genital tracts. Although it has a low virulence, it may gain dominance and be associated with pathologic conditions in different parts of the body. Within the gastrointestinal tract esophagus is the most preferred site for Candida colonization while stomach is the second and there are very few data about the duodenal colonization. The accepted standard for the diagnosis of Candidiasis in the gastrointestinal tract was the finding of infiltration by yeasts and/or hyphae of tissues in histological sections of biopsies and in general fungi located on the surface of epithelium were ignored (16). Histologically proven Candida contaminated gastric ulcer frequency ranges from

Table III: Age, sex and the size of the ulcer in the patient group when subdivided with respect to the presence of Candida albicans in the duodenum.

	C. albicans (+)	C. albicans (-)
N	11	13
Age	43.8	40.7
Sex	5 male/6 female	7 male/6 female
Ulcer > 10mm	4	6
Ulcer < 10 mm	7	7
Aggressive (+)*	7	8
Aggressive (-)	4	5

*: Those patients with a histroy of three or more recurrence of the typical ulcer pain in one year.

9.1% (17), through 16% (16), to 33% (10). In 15 duodenal ulcer patients Candidiasis occured in only 3 (20%) (18). It was claimed that demonstration of Candida in smears and cultures is not a reliable evindence for Candidiasis, as this organism is a common commensal and its presence doesn't imply a pathogenic role (17). Visualization of the fungus by histology is a less precise and less sensitive diagnostic method than culture but if found positive it can be a good clue to demonstrate the relation between the fungi and the changes in the tissue. On the other hand culture allows definitive identification of the pathogen and can detect small number of organisms. Keeping in mind the advantages and disadvantages of both techniques we searched for the presence of Candida with both methods and compared the results with a healthy control group.

The mean pH value of the gastric juice specimens in both groups were around 2.6 which served as a suitable milieu for the Candida to grow at frequency of 65% to 70.8%. This finding is against the speculation that cimetidine favors infection by Candida by causing a marked reduction in acid secretion, as we found a high incidence of fungal growth in a medium of low pH. A prospective study has failed to demonstrate that cimetidine therapy favored Candida colonization in the stomach (19) indicating that other factors, probably gastric motility or the normal immune response of the host are interacting in the colonization process. In gastric juice we demonstrated comparable rates of Candida positivity with using both the imprint (65%) and the culture (65%) methods within the control group but it was clear that the dominant species was not Candida albicans which can only be specified by culture (table 1). However, in gastric juice of DU patients the leading species was Candida albicans (62.5%), which has a potential of being more invasive by its ability to secrete different types of proteinases with respect to other Candida species (20). It was known that the pH optima for those proteinases range from 2.2 to 3.2, (within the limits of gastric juice pH) and they have a wide range of substrate specificities. In analogy with cytotoxigenic H. pylori strains, proteinases or other potentially toxic substances secreted from Candida albicans can alter the mucus barrier (which may increase H^+ back diffusion) or epithelial cell integrity, which is already disturbed by the peptic ulcer itself, leading to perpetuation of the ulcer disease in some patients. In accordance with this hypothesis it was reported that in duodenal ulcer patients treated with H_2R blockers, fungal colonization of gastric juice was associated with a delay in the rate of ulcer healing in 50% of cases (21). In the same study, prior to therapy, Candida was detected at a rate of 8% in the gastric juice of patients with DU. On the other hand the effect of long term maintenance therapy with H2R blockers on Candida growth (53.8%) in gastric juice was reported to be significantly high when compared with short-term treatment with H2R blockers on Candida growth (21.4%) in gastric juice. Drasar et al., detected Candida in gastric juice of 42 healthy subjects at a rate of 12% (22) which is far less than ours which can be explained by the difference in geographic location, sanitation or eating habits. Similarly in our country the H. pylori positivity rate in the gastric antrum of healthy young volunteers (medical students) is around 70% (23) (85% in our healthy controls) which is again very high when compared with the western studies giving a rate around 25% (24) for all adults, showing the importance of selecting different populations.

Within the control group the finding of different growth rates of Candida in gastric juice (65%) versus corpus mucosa (35%) may indicate that they represent different environmental conditions with respect to Candida growth. As we could not show any tissue invasion by Candida,

we assume that the positivity of Candida in the specimens taken from the corpus mucosa might represent Candida which is located in the mucin layer, on the surface of epithelial cells. In parallel with this assumption, we were able to see the fungi in the form of budding yeast like cells located only on the surface of epithelial cells. The finding of mainly yeast froms was related to the pH of the environment as we know that the fungi readily converts its cell shape to a hyphal form mostly above pH 6.5. It was interesting to find that mainly the Candida albicans species isolated in the gastric juice were also isolated in the corpus mucosa of same patients indicating that mucin layer can hamper the entrance of other Candida species onto the epithelial cells but not Candida albicans. In other words Candida albicans has a virulence factor (proteinases?) which may be responsible from this finding.

In the patient group, fungi (50%), mainly Candida albicans (45.8%) was isolated from the edge for ulcer located in bulbus. Only one case was associated with C. tropicalis, again demonstrating the importance of the presence of Candida albicans in the pathologic mucosa. This suggests that either the fungi which is a bystander in ulcerated tissue is Candida albicans or it is somewhat related to the development of duodenal ulcer. If we presume that Candida is an innocent bystander in an already pathologic tissue what is the reason of finding mainly Candida albicans but not the other species in DU patients? Or why Candida albicans is localized in the mucosa more easily than the other species in DU patients?

The concurrent finding of H. pylori and Candida in DU patients was not searched for extensively. In patients with DU disease Candidiasis was reported in 20% of cases without accompanying H. pylori and it was stated that the organisms prefer different environmental conditions for colonization and growth (18). In our patient group Candida albicans was recovered in 11 cases from the edge of the ulcer while in 5 of them H. pylori was simultaneously demonstrated indicating that they can grow together and perhaps act in collaboration in the pathogenesis of duodenal ulcer in some patients. Similarly in our control group H. pylori was demonstrated in 17 (85%) cases in the corpus mucosa, while Candida was observed simultaneously with the histopathologic method in 5, and with the culture method in 7 of them indicating again that they can colonize in the same environment.

In summary, we found a high but similar rates of fungi in gastric juice of healthy controls and duodenal ulcer patients using either the smear or the culture technique.

Mainly Candida albicans was isolated in gastric juice of the patients. Fungal growth rate in gastric mucosa was lower than in gastric juice but Candida albicans was isolated principally in those who had Candida albicans in the gastric juice. Similarly in the patient group Candida albicans grew from the ulcer edge significantly higher than in the patient group Candida albicans grew from the ulcer edge significantly higher than in the control group. All those findings are in favor of Candida albicans colonization in duodenal ulcer disease. Further studies are needed in order to find a relation between finding the fungi in the pathologic mucosa and the pathogenesis of duodenal ulcer as demonstration of only the fungi is not sufficient and some toxins or proteinases must be searched for. Similar to H. pylori, Candida albicans may alter the defensive factors in the gastrointestinal mucosa and be related to the perpetuation of duodenal ulcer disease in some patients.

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