STUDY OF ENZYME ACTIVITIES IN THE DESCENDING PART OF THE DUODENUM IN PATIENTS OF DUODENAL ULCER

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ABSTRACT

The aim of this study was to measure the levels of lactase, sucrase, maltase, leucine amino peptidase and gamma-glutamyl transpeptidase in duodenum of Indian patients with duodenal ulcer. The effect of duodenum inflammation on these brush border enzymes has also been analysed in this study. Levels of lactase, sucrase, maltase, leucine amino peptidase and gamma-glutamyl transpeptidase were assessed in duodenal biopsies of 20 patients of duodenal ulcer and 20 non-ulcer dyspepsia. The duodenal biopsy specimens were also examined histopathologically for presence or absence of inflammation. There was no significant difference ($p>0.05$) in the activity of above mentioned enzyme levels in both the groups. Only levels of gamma-glutamyl transpeptidase were significantly decreased in patients of duodenal ulcer with duodenal inflammation. This study shows that only the levels of gamma-glutamyl transpeptidase were significantly decreased in patients of duodenal ulcer with inflammation but no change in duodenal enzymes due to duodenal ulcer as compared to non-ulcer dyspepsia.

KEYWORDS

Inflammation, duodenal ulcer, leucine aminopeptidase

INTRODUCTION

Hypochondriac syndrome has been reported in patients with duodenal ulcer (1). Incidence and risk factors of rebleeding from gastric and duodenal ulcers have also been reported (2). Prevalence and a markedly higher association of *H.pylori* infection with perforated duodenal ulcer have been documented (3, 4) in the literature. Digestive and transport functions of the small intestine in patients with complicated duodenal ulcer following organ-sparing operations have been shown (5). Proteolytic activity of duodenal mucosa in elderly and senile patients in the presence of duodenal hemorrhage from ulcer has also been studied (6). The brush border enzymes, lactase, sucrase, maltase, leucine amino peptidase (LAP) and gamma glutamyl transpeptidase (GGT) have been studied in the past in various disorders (7, 8). Recently, the focus has been on the role of these enzymes in the mucosal defense aspect in the etiology

of peptic ulcer disease (9). Vetvik *et al.* (10) demonstrated significantly decreased levels of brush border enzymes (except sucrase) in patients of duodenal ulcer (DU) and low values of LAP in patients of duodenal ulcer showing duodenal inflammation on histopathology. This suggested that perhaps enzyme activity may have a role in the mucosal defense and a change in enzyme activity might have a role in the pathogenesis of duodenal ulcer (11, 12).

However, there is paucity of such studies from our country. Therefore, this study was undertaken to assess the enzyme levels in patients of duodenal ulcer and non-ulcer dyspepsia in Indian population. To assess the effect of duodenal inflammation on levels of these enzymes in Indian population was also planned.

MATERIALS AND METHODS

Following two groups of patients were enrolled in this study.

(1) Study Group

This group comprised of 20 consecutive patients of mean age 40.89 years, range 17-66 years, with endoscopically proven duodenal ulcer (DU). Thirteen were the males while 7 females.

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(2) Control Group

This group also comprised of 20 consecutive patients of mean age 38.73 years, range 18-64 years, presenting with dyspeptic symptoms but having normal esophageal, gastric and duodenal mucosa on endoscopy. In this group 14 were males and 6 were females.

Exclusion Criteria

Patients on any form of anti ulcer therapy or antibiotics over previous 2 weeks were not included in this study.

After taking consent, endoscopy was done in all subjects using a forward viewing flexible endoscope. At endoscopy, a detailed examination of esophagus, stomach and duodenum was made. Two pairs of biopsy specimens were taken from the descending part of the duodenum (D2). One pair of biopsy specimens was kept in normal saline and stored at -20°C until the enzymes estimation. Estimation of all the enzymes and histopathological assessment were done within 2 weeks of obtaining samples by endoscopy.

Enzyme assays

Protein was taken as the reference determinant for tissue enzyme activities, which was determined by the method of Lowry *et al.* (13). Lactase, sucrase and maltase were determined by measuring the D-glucose liberated from the respective sugars using glucose-oxidase-peroxidase system as described by Dahlqvist (14). Leucine aminopeptidase (LAP) was measured by the method of Goldberg and Ruttenberg (15). Brief outline of this method is that 0.5 ml buffered substrate was taken in different tubes. To each tube 0.1ml of the enzyme was added, mixed well and incubated in a water bath for 30 minutes at 37°C. 0.5ml of 2N HCl was then added to stop enzyme reaction. To each tube 1.0ml of 0.1% NaNO₂ was added, after waiting for exactly 3 min at room temperature, 1.0 ml of 0.5% ammonium sulfamate was added. After 3 minutes, 2 ml of coloring reagent was added. Tubes were allowed to stand for 45 minutes and O.D. was read at 580nm. Blank and standards (β -naphthylamine, 1-6 g) were also run simultaneously. Gamma glutamyl transpeptidase (GGT) was determined by the method described by Naftalin *et al.* (16). Brief outline of this method is that 0.5 ml buffered substrate was taken in different tubes. To each tube 0.1 ml of the enzyme was added, mixed well and incubated in a water bath for 20 min at 37°C. 2 ml of glacial acetic acid was then added to stop enzyme reaction. To each tube, 1.0 ml of 0.1% NaNO₂ was added, after waiting for exactly 3 min at room temperature, 1.0 ml of 1% ammonium sulfamate was added. After 3 min, 1 ml of coloring reagent was added. Tubes were allowed to stand for 45 min and O.D. was read at 540 nm. Blank and

standards (p-nitroaniline 0.02-0.06 moles) were also run simultaneously.

Histopathological examination

Biopsy specimens were examined for histopathology to observe the change in villous architecture, surface and crypt epithelium. Duodenitis was defined according to the criteria reported earlier (17). The presence of infiltration by inflammatory cells in the lamina propria was taken as a most important criterion for defining duodenitis (10).

Statistical analysis : One-way analysis of variance was applied to localize differences between groups.

RESULTS

Histological examination was as given below in two groups

Study Group (Duodenal ulcer patients)

Histology was done only in 16 patients (11 males, 5 females with a mean age of 39.5 years, range 18 to 65 years) out of 20 patients in this group. Ten out of these 16 patients had inflammation while 6 had no-inflammation. Two specimens out of remaining four were lost during processing and two were inadequate for opinion.

Control Group (Non-ulcer dyspepsia)

In this group, histology was done in 16 patients (12 males and 4 females) with a mean age of 40.5 years (range 19 to 64 years). Eight out of these 16 had inflammation while 8 had no-inflammation. One specimen out of remaining 4 was lost during processing while two were inadequate for opinion in

Table 1. Enzyme activities in human duodenal mucosa (D2) in study group (DU) and non-ulcer dyspepsia. Values are given as Mean $\pm$ SE

Enzymes	Study Group (DU) (n=20)	Non-ulcer dyspepsia (NUD) (n=20)
Lactase U/g Protein	7.67 $\pm$ 1.31	10.75 $\pm$ 1.39
Sucrase U/g Protein	40.38 $\pm$ 10.58	41.61 $\pm$ 4.63
Maltase U/g Protein	75.09 $\pm$ 11.06	85.38 $\pm$ 10.47
LAP g/mg Protein	123.77 $\pm$ 15.09	118.50 $\pm$ 13.40
GGT g/mg Protein	82.71 $\pm$ 11.27	89.39 $\pm$ 6.83

this group.

Enzyme activities in duodenal mucosa

A comparison of the enzymes activities in study group (duodenal ulcer n=20) and control group (non-ulcer dyspepsia n=20) showed no significant difference ($p>0.05$) in the levels of lactase, sucrase, maltase, LAP & GGT enzymes in patients with duodenal ulcer as compared to controls, non-ulcer dyspepsia (Table 1). When levels of all enzymes in inflammation and no-inflammation conditions were compared in study group (duodenal ulcer), only the levels of GGT were significantly decreased, $p<0.05$ (Table 2) in inflammation condition. There was no significant change in lactase, sucrase, maltase and LAP enzyme activities between patients with inflammation as compared to no-inflammation in study group. In contrast, there was no significant change in enzyme activities of any of the enzymes between inflammation and no- inflammation condition in control group (Table 2).

DISCUSSION

In our study, we have not been able to demonstrate any significant difference in the enzyme levels in patients of duodenal ulcer when compared to patients with non-ulcer dyspepsia. This is unlike the study of Vetvik *et al.* (10) who demonstrated a significant decrease in the levels of all brush border enzymes (except sucrase) in patients of duodenal ulcer. In our study, we took endoscopically normal patients who presented with dyspeptic symptoms, as controls. It would have been ideal to take healthy persons as controls but it was ethically non justifiable. This may be the reason that there was no difference in enzyme

activity in both the groups in our study.

The level of GGT was significantly decreased in patients with duodenal ulcer who had duodenal inflammation on histopathological examination in our study. The reason of this may be that GGT is present superficially in brush border membrane and due to inflammation and duodenal ulcer (double insult) in study group it could have decreased but not in non-ulcer dyspepsia in this study. GGT is present in the brush border membrane and is an important enzyme of the glutamyl cycle for amino acid uptake (18). Studies in animal models have shown decreased levels of dipeptidases with starvation (19-21) but GGT was found to be increased in patients of malnutrition (22). It was suspected that the increase in GGT in patients of malnutrition could be an adaptive phenomenon to augment the absorptive capacity of intestinal cells. In the presence of duodenal inflammation (in the present study) when membrane integrity is destroyed due to presence of an ulcer, the double insult (duodenal inflammation and ulceration) could possibly result in decrease levels of this brush border enzyme. Unlike in our study, Vetvik *et al.* (10) did not demonstrate a significant difference in GGT activity. They noted a significantly low value of LAP in duodenal ulcer patients with inflammation, which was not observed in our study. The reason of this may be that the inflammation in their case may be more as compared to inflammation in our study.

Our study has shown change only in GGT enzyme levels of duodenal mucosa in patients of duodenal ulcer with inflammation but no change in disaccharidases, LAP and GGT levels due to duodenal ulcer in Indian patients.

Table 2. Enzyme activities in duodenal mucosa (D2) of patients with & without duodenal inflammation in study group (DU) and patients with non-ulcer dyspepsia. Values are given as Mean $\pm$ SE

Enzymes (n=10)	Study group (n=16)		NUD group (n=16)	
	Inflamm. (n=10)	No Inflamm. (n=6)	Inflamm. (n=8)	No Inflamm. (n=8)
Lactase (U/g protein)	7.85 $\pm$ 1.78	8.37 $\pm$ 2.67	13.80 $\pm$ 2.68	9.80 $\pm$ 1.46
Sucrase (U/g protein)	35.64 $\pm$ 6.00	31.45 $\pm$ 10.00	47.15 $\pm$ 8.53	43.63 $\pm$ 6.27
Maltase (U/g protein)	72.32 $\pm$ 7.19	68.56 $\pm$ 16.77	94.98 $\pm$ 21.74	63.54 $\pm$ 12.37
LAP (g/mg protein)	122.01 $\pm$ 13.93	118.14 $\pm$ 23.17	120.78 $\pm$ 19.34	104.22 $\pm$ 20.52
GGT (g/mg protein)	62.07 $\pm$ 5.11*	103.70 $\pm$ 20.63	83.22 $\pm$ 12.44	98.07 $\pm$ 9.84

*p < 0.05

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