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## Duodenal Brush Border Enzymes in Helicobacter Pylori Infection

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**Design, setting, participants & measurements:** One hundred and nine patients, age range 20-84 years, mean age 56 years were included in the study. They presented to the endoscopy suite of UCHG with upper gastrointestinal symptoms. The duodenal bulb was entirely normal and with no evidence of inflammation on endoscopic examination. Biopsies from the antrum were processed for histology and bacteriological culture. Two biopsies from the duodenal bulb were taken from each patient and were sealed in Para-film and stored at -20C0 until assayed for brush border enzymes.

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# Duodenal Brush Border Enzymes in Helicobacter Pylori Infection

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**Results:** Biopsies from duodenal bulb were assayed for the brush border enzymes, alkaline phosphatase (AP), and disaccharides {lactase (lac) and sucrase (Suc)} using the modified method of Dahlqvist and Kelly, micro-plate method as performed by Nugent. They were divided into 2 subgroups, consisting of 60 patients (HP-positive) who had evidence of H. pylori infection on histology and/or on culture and 49 patients (HP-negative) without evidence of H. pylori infection. All patients had normal looking duodenal mucosa on endoscopic examination. The difference in the results of AP activity between the H. pylori positive (mean  $\pm$  SD 8.26  $\pm$  4.8) and H. pylori negative groups (mean  $\pm$  SD 9.1  $\pm$  7.7) was highly statistically lower in the former group ( $p < 0.0001$ ). The lactase enzyme activity in patients with H. pylori positive (mean  $\pm$  SD 9.4  $\pm$  8.3) and H. pylori negative (mean  $\pm$  SD 8.6  $\pm$  7.6) was significant between the groups ( $p = 0.036$ ).

There was no statistically significant difference in the Sucrase activity between H. pylori positive and negative groups (mean  $\pm$  SD 30.3  $\pm$  22.3 and 28.95  $\pm$  22.1), ( $p = 0.138$ ).

**Conclusion:** AP and lactase enzymes are significantly lower in patients with H. pylori infection and normal duodenal mucosa on endoscopic examination denoting a probable cytopathic effect of the bacterial on the brush membrane enzymes.

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## I. INTRODUCTION

Helicobacter pylori are an accepted cause of chronic active gastritis and commonly associated with both gastric and duodenal ulcer. Moderate to severe gastritis increases the relative risk of developing peptic ulceration and eradication of the bacteria reduces duodenal ulcer recurrence (1-4). Hypergastrinemia and increase parietal cell mass or response to stimulation would result in an increase acid load to the duodenum. The resultant gastric metaplasia would be a target of H. pylori infection and the development of duodenitis and duodenal ulcer. The effect of H pylori on the duodenal brush border membrane enzymes have not been studied extensively in patients with normal looking duodenal mucosa. However, brush border enzymes, lactase, sucrase, maltase, leucine amino-peptidase and gamma glutamyl transpeptidase have been studied in the past in various disorders (5-7). This is a prospective longitudinal study of 109 patients presented to University College Hospital Galway, Ireland with upper gastrointestinal symptoms for endoscopy. Duodenal brush border enzymes are measured between the H. pylori positive and negatives to evaluate if there is difference between the 2 groups.

## II. SUBJECTS AND METHODS

One hundred and nine patients, age range 20-84 years, mean age 56 years were included in the study. They presented to the endoscopy suit of UCHG with upper gastrointestinal symptoms. The duodenal bulb was entirely normal and with no evidence of inflammation on endoscopic examination. Biopsies from the antrum were processed for histology and bacteriological culture. Two biopsies from the duodenal bulb were taken from each patient and were sealed in parafilm and stored at -20C<sup>ο</sup> until assayed for brush border enzymes.

### a) Estimation of duodenal enzymes

The micro-titer plate method for estimation of protein, alkaline phosphatase, and disaccharides (lactase and sucrase) in biopsy materials of human small intestine; a modified method of Dahlqvist et al(8) and Kelly et al(9) as used by D.W. Nugent et al(10), Department of Biochemistry, University College Galway (UCG) was adopted for the assay. The biopsy samples from the duodenal bulb of 109 patients with normal looking duodenal mucosa on endoscopic examination

and with no history of celiac disease or gastroenteropathies known to affect the duodenal mucosal brush border membrane enzymes. The biopsies were weighted and homogenized in 0.3 ml of distilled water using a Vertis homogenizer (The Vertis Company, Gardiner, New York 12525).

#### b) Quality controls

Aliquots of pooled biopsy homogenates containing high, medium, and low levels of the enzymes were stored frozen at  $-20\text{C}^{\circ}$ . An aliquot of each was thawed and included in each batch of samples for analysis.

#### c) Plate reader

Absorbencies were measured with a Dynatech MR 5000 automatic micro-plate reader (UK) fitted with an appropriate filter. A 410 nm filter was used for alkaline phosphatase assays, 450 nm filter for the disaccharides and 570 nm filter for the protein assays. The results were calculated as described by Nugent et al (10).

#### d) Protein assays

Homogenate protein estimates were performed using the micro-titer plate method of Nugent et al (10). The reagents necessary for the micro-titer plate method for protein assays are:

- BCA protein assay reagent (code Nr. 23225) pierce contains: Reagent (A) sodium carbonate, sodium bicarbonate, and BCA detection reagent and sodium tartarate in 0.1 M NaOH.
- Reagent (B) is 4%  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  (10x1 ml ampoules, 2 mg/ml albumin standard).

The methods for protein, alkaline phosphatase, and disaccharides (sucrase and lactase) assays as done by Nugent et al (10) were adapted for calculation of the enzymes.

#### e) Calculations

The mean sample of absorbance (sample absorbance - blank) is compared to the standard curve.

Enzyme Unit/ml sample =  $\mu\text{moles p-nitrophenol/ml}$

Enzyme Units/gram protein = Enzyme unit/ml sample  $\div$  protein concentration of sample (mg/ml).

#### f) Disaccharides assay calculations

The unit of lactase and sucrase activity is defined as the amount of the enzyme that will liberate one  $\mu\text{mole}$  of glucose from the  $\mu\text{mole}$  of substrate per minute under the assay conditions specified.

Lactase and sucrase activities are expressed as units/g homogenate protein.

The mean sample absorbance (sample absorbance - sample blank) is compared to the standard curve.

Enzyme units/ml sample =  $\mu\text{mole glucose/ml} \div 60$

Enzyme units/g protein = Enzyme units/ml sample  $\div$  protein concentration of sample (mg/ml).

#### g) Statistical methods

The results of brush border enzyme analysis were analyzed using the Mann-Whitney U test. We used the SYSTAT computer package (SYSTAT 1990, inc). The difference in the alkaline phosphatase, sucrase and lactase between H. pylori positive and negative was estimated. A p value of 0.05 was taken as significant.

### III. RESULTS

Biopsies from duodenal bulb of 109 patients were assayed for the brush border enzymes using the modified method of Dahlqvist et al (8) and Kelly et al (9), micro-plate method as performed by Nugent et al (10). They were divided into 2 subgroups, consisting of 60 patients (HP-positive) who had evidence of H. pylori infection on histology and/or on culture and 49 patients (HP-negative) without evidence of H. pylori infection. All patients had normal looking duodenal mucosa on endoscopic examination. The difference in the results of alkaline phosphatase activity between the H. pylori positive (mean  $\pm$  SD  $8.26 \pm 4.8$ ) and H. pylori negative groups (mean  $\pm$  SD  $9.1 \pm 7.7$ ) was highly statistically lower in the former group ( $p < 0.0001$ ).

The lactase enzyme activity in patients with H. pylori positive (mean  $\pm$  SD  $9.4 \pm 8.3$ ) and H. pylori negative (mean  $\pm$  SD  $8.6 \pm 7.6$ ) was significant between the groups ( $p = 0.036$ ).

There was no statistically significant difference in the sucrase activity between H. pylori positive and negative groups (mean  $\pm$  SD  $30.3 \pm 22.3$  and  $28.95 \pm 22.1$ ), ( $p = 0.138$ ).

### IV. DISCUSSION

H. pylori is capable of inflicting a cytopathic effects on the gastro-duodenal mucosa either through the release of substances secreted by the bacteria like phospholipase A and its cytotoxic metabolites, lysolecithin, which was found to be high in patients with H. pylori infection (11) and/or through the stimulation of the body immune response, both cellular and humoral components.

In this study the effect of antral H. pylori colonization on duodenal brush border enzymes has been investigated. The level of alkaline phosphatase and lactase were significantly lower in patients with H. pylori infection. There was no significant difference in the level of sucrase enzyme between the 2 groups. There are scarce reports of similar studies on endoscopically normal duodenal mucosa for comparison. The first data on the duodenal mucosal enzyme activities in patients with duodenal ulcer was reported by Vetvik et al (12). These investigators have found that most membrane enzymes activities were decreased in the duodenum of DU patients. Their findings are not unexpected due to the cytopathic effects on the cell membrane of the lysosomal enzymes and other inflammatory mediators

from the local inflammatory infiltrate. This process may disrupt the attachment of the membrane brush border enzymes. Our results were the first to demonstrate decreased membrane enzyme activities in patients with *H. pylori* infection and with endoscopically normal mucosa.

The results of alkaline phosphatase and lactase in this study suggest that the organism *H. pylori* may have cytopathic effect on the brush border cells of the duodenal mucosa through the release of substances like phospholipase A2 and its toxic metabolite, lysolecithin. These substances are reported by Langston et al (11) to be higher in patients with *H. pylori* infection. Recent quantitative histological analysis of duodenal biopsies of dyspeptic patients with no endoscopic duodenitis has shown increased polymorph and mononuclear cell infiltrate in duodenal bulb mucosa relative to controls (13, 14) suggesting subclinical inflammation which is insufficient to produce a lesion recognizable endoscopically (13). Toxic substances may be carried from the antrum to exert their effect on the duodenal mucosa even before the macroscopic appearance of duodenal inflammation. The nature of these substances needs to be further elucidated and characterized. The disruption of the cyto-skeletal membranes of the duodenal cells results in loss of membrane-bound duodenal enzymes. Consequently, this may have other deleterious effect on the integrity of the duodenal cells either directly or indirectly through the chemical substances like bile acids and the alkaline nature of the environment as a result of elevated pH. The inflammatory process which develops through the activation of the local and systemic components of the immune system of the infected individual will further contribute to the duodenal mucosal damage. The inflammatory process is probably important for *H. pylori* colonization of the duodenal mucosa. Healing of these inflammatory areas result in the development of gastric metaplasia in the duodenum. The gastric metaplasia may then be colonized by *H. pylori*. The limitations of this study are that it was carried out on a small sample size and in one center which may affect its significance. Repeating the study on a large sample size would add weight to the validity of the results.

## V. CONCLUSION

Alkaline phosphatase and lactase enzymes are significantly lower in patients with *H. pylori* infection and normal duodenal mucosa on endoscopic examination denoting a probable cytopathic effect of the bacterial on the brush membrane enzymes.

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