

Effect of Helicobacter pylori and smoking on gastric prostaglandins

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Antral mucosal biopsies were taken at endoscopy in 64 successive patients with upper abdominal symptoms of whom 16 were smokers. Helicobacter were detected histologically in 30 and were strongly associated with the presence of gastritis ($p < 0.0001$). H.pylori were found in 6 out of 7 patients with duodenal ulcer, 6 out of 10 with gastric ulcer and 10 out of 24 which were endoscopically normal. There was no correlation between H.pylori and smoking. Prostaglandin (PG) concentrations were measured by radioimmunoassay in antral biopsies frozen in liquid nitrogen within seconds of collection. The concentration of PGE2 ranged from 34pg/mg protein to 1660 pg/mg and of 6-keto F1a from 30 pg/mg protein to 656 pg/mg. There was no correlation between PG concentration and histological inflammation. The concentration of PGE2 was significantly reduced in specimens from patients with H.pylori and that of PGE 6-keto F1a in those from smokers $p < 0.05$. H. pylori and smoking may contribute to the development of peptic ulcer by reducing the cytoprotective effects of gastric PGs. [Turk J Med Res 1993; 11(1): 15-20]

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As knowledge of the factors involved in the pathogenesis of chronic gastritis and peptic ulceration grows, it becomes apparent that the process is extremely complex. The role of gastric acid is now well understood, and its effects are commonly moderated by the use of H2 receptor antagonist drugs. More recently attention has been directed to cytoprotective mechanisms which maintain the integrity of the gastro-duodenal mucosa. These include mucus and bicarbonate production and changes in mucosal blood supply and cellular turnover (1). All these factors are believed to be influenced by the local effects of PGs which possess both cytoprotective and acid inhibitory properties (2). Prostaglandins have been shown to be effective in the prevention of gastric mucosal injury caused by alcohol and other irritants (3) and are effective in the treatment of both gastric and duodenal ulcers at antisecretory doses (4).

Non-steroidal anti-inflammatory drugs (NSAIDs) which inhibit prostaglandin production are well known to increase the frequency of peptic ulceration (3).

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Other factors may also influence the pathogenesis of peptic ulceration and gastritis. These include biliary reflux (5), smoking and possibly the presence in the stomach of the organism Helicobacter pylori (6,7). Biliary reflux is believed to impair cytoprotective mechanisms by dissolving the mucus bicarbonate barrier and thus permitting acid to penetrate the mucosa more easily. The reasons for the effects of smoking and H. pylori are less clearly understood, although it is noteworthy that infection by the latter is strongly associated with neutrophil infiltration of surface and foveolar epithelium. In this study we have attempted to investigate the mechanism of both smoking and C. pylori on gastric cytoprotection by direct measurement of PG concentrations in human gastric mucosa.

PATIENTS AND METHODS

Sixty four successive patients, 34 men aged 23 to 78 (mean 53) and 30 women aged 21 to 83 years (mean 51), undergoing elective endoscopy for the investigation of abdominal symptoms were included in the study. Written consent was given by all and smoking and drinking habits, drug history and relevant symptoms, including epigastric pain, abdominal distention, belching, nausea and vomiting, were recorded. Patients requiring emergency endoscopy and those on corticosteroids were excluded.

teroids, NSAIDs or antibiotics were excluded. Patients were asked to refrain from smoking on the day of endoscopy. At endoscopy, the duodenum and stomach were visualised and the macroscopic appearances, including the presence or absence of erythema or gastric or duodenal ulcer recorded. Four pieces of tissue each 3 mm in diameter were taken from the antrum for analysis.

Light Microscopy

After the specimens were obtained, 2 pieces were fixed in 10% buffered formalin for light microscopy. The tissue was routinely processed, sections were cut at 6 μ m and stained with haematoxylin eosin (HE). Histological examination was performed initially without knowledge of the clinical and laboratory data.

The diagnosis of gastritis was based on the amount of inflammatory infiltrate in the lamina propria according to the standard criteria (8) (Figure 1). The presence or absence of neutrophil polymorphs within the epithelium or superficial lamina propria was recorded. The presence or absence of atrophy and intestinal metaplasia were also noted.

CLOs were recognised H&E sections as haematoxyphilic curved structures, usually lying close to the surface or foveolar epithelium. In order to aid histological identification of the organisms additional sections were stained using Steiner's silver technique (Figure 1).

All results are based on the histological findings.

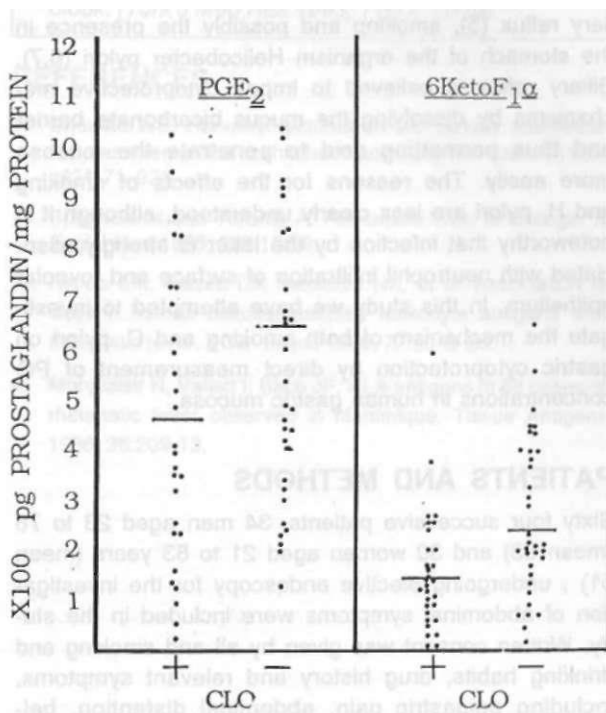


Figure 1. Prostaglandin concentration in antral biopsies from patients with and without *H. pylori*.

Prostaglandin Analysis

Biopsies obtained from the antrum were frozen in liquid nitrogen (N₂) within 20-30 seconds. The specimens were stored at -70°C until all the specimens to complete the study had been collected. They were then all extracted and analysed in one batch. No demonstrable change in the prostaglandins E₂ and 6 keto F₁α have been found when specimens were stored for at least 3 months at -70°C.

Extraction: All manipulations were carried out at 0-4°C. 2 biopsy specimens from each subject were homogenized by hand in a small glass homogenizer containing 1 ml 0.05 M Tris buffer pH 7.2. 2 ml of ice cold acetone was added. The precipitated proteins were centrifuged down and analysed for protein content. The supernatant was extracted with twice the volume of Petroleum Ether (40-60°C). The Petroleum Ether layer was discarded. The aqueous layer was brought to pH 3.0 with dilute formic acid and extracted with twice the volume of ethyl acetate. The ethyl acetate layer was separated off and the aqueous layer further extracted with twice the volume of ethyl acetate.

The ethyl acetate layers were combined and taken to dryness under N₂. Samples were suspended in radioimmunoassay buffer (0.1 M phosphate buffer containing sodium chloride 0.9%, sodium azide 0.1%) and submitted to assay using the following: anti sera E₂ with a specificity of 100% and cross reactivity with E₁ of 4%; anti serum 6 keto F₁α with a specificity of 100% and cross reactivity of 3% with E₂ (Biosys S.A. France). Each sample was analysed in duplicate and the results expressed as pg of PG per mg protein.

RESULTS

Sixty-four successive patients were studied. *H. pylori* were detected by light microscopy in 30 patients (47%). In the antral biopsies the organisms were seen on the surface epithelium and within gastric foveolae. A striking feature in the antral biopsies was the absence of the organisms in areas with intestinal metaplasia, even there was colonisation of the adjacent surface epithelium.

A strong association was found between the presence of *H. pylori* in antral biopsies and gastritis (Table 1). The organisms were found in only 3 of 35 histologically normal antral biopsies (9%), and histological gastritis was found in only 2 of 34 *H. pylori* negative biopsies (6%). The association between histological gastritis and the presence of *H. pylori* was highly significant ($p < 0.0001$, Fisher's exact test), but there was no relation between the presence of polymorphonuclear leucocyte (PMLs) infiltration in the mucosa and PG levels.

H. pylori was present in 6 out of 7 biopsies from patients with duodenal ulcer (86%) and 6 out of 10 of those with gastric ulcer (60%), 8 out of 14 cases of

Table 1. Relation of histologic findings with H.pylori

Histology	H.Pylori Positive (n=30)				H.Pylori Negative (n=34)			
	Present	%	Absent	%	Present	%	Absent	%
Chronic inflammation	27	90	3	7*	2	6	32	94*
Neutrophil polymorphs	20	67	10	33	0	0	34	100*
Atrophy	12	40	18	60	2	6	32	94*
Intestinal metaplasia		23	23	77	2	6	32	94*

* p<0.0001 Fisher's Exact Test

Table 2. Relation of endoscopic appearance with H.pylori

Endoscopic Appearance	H.Pylori			
	Positive (n=30)		Negative (n=34)	
		%		%
Normal	10	42	14	58
Gastritis	10	44	13	56
Gastric ulcer	6	60	4	40
Duodenitis	8	57	6	43
Duodenal ulcer	6	86	1	14

duodenitis (57%) and 10 of 23 with gastritis (44%). 10 of 24 endoscopically normal (42%) were also H.pylori positive (Table 2).

The presence of H. pylori was also evaluated with regard to symptoms (Table 3) but no significant association was discovered. Nor was there any relationship between H. pylori and smoking and drinking habits (Table 4).

Sixty two of the biopsies obtained for PG analysis were processed for PGE2 and 6-keto F1. (the biopsies of one patient were lost, and one patient was excluded from PG analysis because he was taking Imipramine and thyroxine). The concentration of PG found in the tissues was widely variable. The concentration of PGE2 ranged from 34 pg/mg protein to 1660 pg/mg (Figures 1,2). The concentration of both PGE2 and 6-keto F1a in the mucosa was reduced in the presence of H. pylori (Figure 1). However, using the analysis of variance test, only in the case of 6-keto F1, did this difference reach statistical significance (p<0.05).

Smoking habits affected mucosal PG concentration. Prostaglandin levels were reduced in smokers, including those with and without detectable H. pylori, as compared to non smokers (Figure 2) but which in the case of PGE2 reached statistical significance (p<0.05).

DISCUSSION

Smoking is well known both to increase the frequency of gastritis and peptic ulceration and to impair the effectiveness of drugs which promote ulcer healing (8), but the mechanisms involved are poorly understood. Helicobacter pylori was first seen in the human stomach in 1906 (9) but not cultured by Marshall until

1982 (10). There is now a considerable body of evidence that H. pylori is associated with acute gastric inflammation primarily affecting the antrum and pyloric region of the stomach. There is a statistical relationship between such gastritis and H. pylori infection, a correlation between the number of organisms and the leucocytes infiltrating the mucosa, improvement in gastritis after therapy effective against H. pylori and a single report of a previously healthy subject ingesting a culture of H. pylori and developing acute gastritis (7,11-14).

Many authors now accept that H. pylori has a role in the pathogenesis of acute gastritis and our results strongly support this contention, H. pylori being found in only 9% of histologically normal biopsies and histological gastritis being found in only 6% of H. pylori negative biopsies.

The role of H. pylori in the pathogenesis of peptic ulcer is less well established. The frequency with which the organism has been found in patients with peptic ulcer has varied from as low as 46% (14) to 100% (15). However, drugs known to inhibit H. pylori accelerate ulcer healing (16-18).

The mechanism by which gastric mucosal infection could cause peptic ulcer disease is still uncertain, it is not yet clear whether the organism is a commensal, an opportunist or a primary pathogen (7). It has been suggested that H. pylori by producing antral gastritis might affect gastric motility (13), but this is as yet unproven.

There is no ideal way of measuring PG content in human tissues. The trauma of taking a biopsy and homogenising the tissue will itself activate the cyclooxygenase pathway leading to the spontaneous generation of PGs. Attempts to get round this problem by measuring PG synthesis by cell cultures or tissue homogenates are often inaccurate, if degradation is not simultaneously measured and although measurement of both has been attempted (19) the disadvantage of all in vitro determinations remains that the concentrations of precursors and cofactors may be very different to those obtaining in vivo.

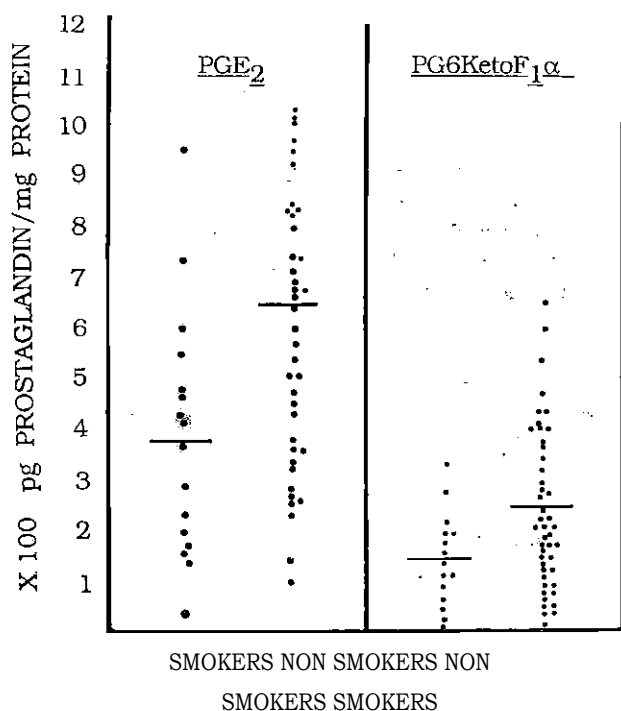
Of the techniques available we believe that the direct measurement is likely to be most representative. Hawkey and Rampling (20) have argued that direct tissue measurements of PGs are unreliable in that the

Table 3. Relation of symptoms to presence of *H. pylori*

Symptoms	H. Pylori Positive (n-30)				H. Pylori Negative (n-34)			
	Present	%	Absent	%	Present	%	Absent	%
Pain	19	63	11	37	21	62	13	38
Belching	17	57	13	43	24	71	10	29
Distention	14	47	16	53	21	62	13	38
Vomiting	4	13	26	87	6	18	28	82

Table 4. Relation of social habits to presence of *H. pylori*

Habit	H. pylori Positive		H. pylori Negative	
	Present	Absent	Present	Absent
Smoking	12	18	8	26
Drinking	15	15	19	15

**Figure 2.** Prostaglandin concentrations in antral biopsies from smokers and non-smokers.

GPs measured are not those present in the resting tissue, but those generated in the biopsy and homogenisation process. However, if PG concentrations are to be measured as an index of the capacity of a tissue to react to a noxious stimulus, then the production of less PG during homogenisation is likely to reflect a similar reduction in the potential response to other stimuli. Furthermore, the precursors and cofactors available are those present in the tissue itself and not from an extraneous source. The concentration of PG in tissue homogenised in a standard fashion is the single

determination most likely to reflect all these various factors and we have attempted to overcome the difficulties involved by standardising the site of the gastric biopsies, by immediately fixing the tissues in liquid nitrogen, by measuring the most stable of the relevant metabolites and by assaying all the biopsies together under identical conditions. The reliability of the technique is supported by the profound falls in gastric PGs we have demonstrated using this technique in subjects taking NSAIDs (21).

Despite these precautions, there was still a variation in the PG concentrations in these gastric biopsies which may well have represented the effects of differing rates of spontaneous PG generation. Nevertheless, it was possible to demonstrate a significant fall in PGE₂ concentration in smokers, and in 6-keto F₁ levels in patients with *H. pylori*.

Several studies have attempted to evaluate PG metabolism in patients with peptic ulcer, with conflicting results. One study of gastric ulcer reveals high mucosal prostaglandin levels (22) whereas others have reported low levels (23,24). Similar discrepancies have been apparent in patients with duodenal ulcer whether PGs have been measured directly in the mucosa (22,23,25,26), synthesis by cell culture (27) or mucosal homogenates (28). Crampton et al (19) measured both synthesis and degradation rates of PGs in vitro and found reduction in PGE₂ synthesis in patients with gastric ulcer, but not those with duodenal ulcer. In none of these studies was any cognisance taken of the presence of *H. pylori* or smoking habits, but it seems clear that PG deficiency alone is not the major factor leading to the development of peptic ulcer, and indeed one study (29) has suggested that PG production is only relevant when related to gastric acid secretion.

There was no relationship between smoking and *H. pylori* presence, or between smoking and histological gastritis (Table 4). Despite a wide variation in mucosal PG concentrations there was a significantly lower level of PGE₂ in smokers than non-smokers. Our finding of low PG concentrations measured directly in the gastric mucosa of smokers is in keeping with other reports. Reduced PG concentrations were found in the gastric aspirate of subjects who smoked (30). In active smokers 6 keto F₁α synthesis was reduced in vitro in antral and fundal mucosal biopsies (31). It is possible that smoking may adversely affect one or more of the

mucosal defence mechanisms. There is some evidence that smoking may alter prostanoid synthesis (32), and decrease pancreatic and gastric ulcer. Smoking has also been shown to alter PG metabolism in other organ systems, for example, aspirin prevented the smoking induced platelet aggregate formation in non-smokers, and cigarette smoke reduced prostacyclin production by rat aorta invitro (33). It is therefore reasonable to suggest that smoking may impair prostaglandin production by the human gastric mucosa.

As our findings on the effects of smoking on gastric PGs are consistent with other studies it seems possible that the lower concentrations of PGs found in patients with *H. pylori* may also be a genuine phenomenon. It is perhaps surprising that PG concentrations should be lower in histologically inflamed tissue but no correlation existed between PML infiltration in the mucosa and PG levels. The mechanisms by which *H. pylori* might inhibit the cyclooxygenase system are unknown, and it is equally possible that the finding is a consequence of enhanced PG degradation.

These findings suggest that smoking or colonisation of *H. pylori* are not the direct cause of gastritis and peptic ulceration but that they may possibly represent permissive factors, reducing the mucosal concentration of PGs in such a way as to render the mucosa more liable to damage from stomach acid.

Acid secretion in duodenal ulcer patients overlaps considerably with that of normals but when acid secretion and duodenal prostaglandin production were both compared together separation of patients from normals was more complete (29). It seems likely that inflammation and ulcers in the stomach and duodenum may be the consequence of an imbalance between aggressive factors (e.g. acid, pepsin and bile) and the mucosal defences. The association of smoking and *H. pylori* with ulcers is not absolute as aggressive factors may overcome normal defences (e.g. in Zollinger Ellison Syndrome). Conversely, mucosal defensive impairment by smoking or *H. pylori* may allow ulceration to occur even when gastric acid secretion is normal or only slightly increased.

Helicobacter pylori ve sigaranın gastrik prostaglandinler üzerine etkisi

Endoskop! laboratuvarında endoskopiye alınan 64 hastadan 4'er adet antral biyopsi alınıp 2'si histoloji laboratuvarına (gastritis ve H.pylori muayenesi için) gönderildi. Diğer iki parça hemen sıvı hidrojenle -70°C'da dondurulup PG analizi için laboratuvara gönderildi.

Işık mikroskopunda incelenen 64 biyopsinin 30'unda (%47) H.pyloridis tespit edildi. Bakteri kolonileri genellikle epitel yüzeyinde ve mukus altında toplanmış olup, intestinal metaplazi bölgelerinde bakteri bulunmaması dikkati çekti.

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*H.pyloridis mevcudiyeti ile histolojik gastritis (lenfosit infiltrasyonu) arasında çok kuvvetli ilişki bulundu ($p<0.0001$, Fisher kesin ki kare testi). Aynı zamanda 7 duodenal ülserli hastanın 6'sında (%86) ve 10 mide ülserli hastanın 6'sında (%60) *H.pyloridis* pozitif bulundu.*

H.pyloridis pozitif hastaların antral biyopsilerinde PGEz ve 6-keto F^a (PGIz türevi) bakteri negatif hastalara göre genelde düşük bulundu İse de ancak 6-keto F^a düşüklüğü istatistiki olarak anlamlı seviyeye ulaştı ($p<0.05$). Sigara içenlerde de her iki PG düşük bulunmuş olmasına rağmen ancak PGEz istatistiki olarak anlamlı idi ($p<0.05$).

Sonuç olarak sigara içme ve C.pyloridis mevcudiyetinin gastrik PG sentezinde yetersizliğe veya parçalanmada hızlanmaya sebep olarak gastritis ve ülser oluşumunda önemli rol oynadığı, diğer ifade ile "Cytoprotection" ı bozarak gastritis ve ülserin ortaya çıkmasına katkıda bulunduğu kanaatine varılmıştır.

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