Upper gastrointestinal tract abnormalities in patients referred for gastroscopy and the prevalence of Helicobacter pylori infection among them: a hospital based study in Sri Lanka

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Abstract

Upper gastrointestinal endoscopy is a common procedure in routine clinical practice. This study examines the types of upper gastrointestinal abnormalities, their correlation with histological changes and the prevalence of *Helicobacter pylori* (*H pylori*) infection among a group 251 Sri Lankan patients referred for upper gastrointestinal endoscopy.

Nine gastric mucosal biopsies were obtained from each patient for three diagnostic tests i.e. histology, rapid urease test and culture and 5 ml of peripheral venous blood was obtained for the detection of anti-*H.pylori* IgA and IgG. Three case control studies were performed by comparing patients with peptic ulcer disease, gastro-oesophageal reflux disease and gastritis with age and sex matched, hospital based control groups to asses the risk and protective factors for each disease entity. The quality of life of patient group was compared with a control group using the validated Sinhala translation of WHO Quality of Life BREF (WHO QOL BREF) questionnaire.

Approximately 86% of patients had histological evidence of chronic gastritis. There was a poor correlation between endoscopic and histological gastritis with more than 60% of patients with histological gastritis failing detection endoscopically. The prevalence of *H.pylori* determined by histology was 49.4%. When compared with histology which was taken as the gold standard (either Hematoxyline & Eosin or modified Giemsa positive) for the detection of *H.pylori*, all other diagnostic tests had low sensitivity and specificity. In the three case control studies, the middle socioeconomic group had lesser tendency to develop upper gastrointestinal diseases. While smoking had no effect, alcohol consumption, frequent use of certain groups of drugs and bad food habits significantly increased the risk of upper gastrointestinal diseases. Patients with upper gastrointestinal symptoms showed significantly low scores in physical and psychological domains of WHO QOL BREF.

A majority of patients referred for upper gastrointestinal endoscopy had a clinically relevant abnormality. There was relatively a low prevalence of *H.pylori* infection. Histology using H & E or modified Giemsa stain appeared to be the most reliable technique to detect *H.pylori*. Bad food practices, commonly used drugs and alcohol consumption were significant risk factors for upper gastrointestinal diseases and the symptoms significantly affect the quality of

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Background

Upper gastrointestinal complaints are very common among the general population. Every day, a large number of patients seek medical advice and undergo investigations at government and private sector hospitals for upper gastrointestinal complaints. Upper gastrointestinal endoscopy units in almost every tertiary care hospital in Sri Lanka are overcrowded with patients.

The patients present with a wide spectrum of complaints ranging from regurgitation to haematamesis. Endoscopic findings are either confined to oesophagus, stomach and duodenum or a combination of abnormalities in two or all three sites to a varying degree.

Helicobacter pylori (H.pylori) is a bacterium that inhabits the gastric mucosa and areas of gastric metaplasia in the duodenum. This organism is mostly responsible for chronic gastritis and it is a key etiological factor in the pathogenesis of peptic ulcer disease. Two Australian pathologists, Robin Warren and Barry Marshall isolated the particular organism from gastric tissue for the first time in 1982 (1).

The infection is acquired during childhood, persisting as chronic gastritis if the organism is not eradicated. The exact mode of transmission of the *H.pylori* infection is unclear, but intraepithelial clustering suggests person-to-person spread, either oral-oral or faeco-oral. Due to the progression of gastritis over the years, gastric mucosa undergoes a sequence of changes that may lead to glandular atrophy, intestinal metaplasia, increased risk of gastric dysplasia and carcinoma. The organism is also responsible for gastric mucosa associated B cell lymphoma.

The prevalence of *H.pylori* is high in developing countries ranging from 80%-90% of the population (2-4). More affluent countries show lower prevalence figures around 25-50% (5-7). Reported prevalence data from Sri Lanka showed a wide variation (8-11).

The prevalence of *H.pylori infection was studied in 251 patients referred for upper gastrointestinal endoscopy at the Teaching Hospital, Karapitiya* from November 2005 to June 2007. *Age of the subjects ranged from 15-84 years and 134 of them were males.*

Patients whose upper gastrointestinal endoscopy is contraindicated due to bleeding disorders, decompensated cirrhosis and those who were on anticoagulant therapy were excluded from the study. In addition to that patients who have taken specific *H.pylori* eradication therapy during the previous 6 months and who have consumed antibiotics active against *H.pylori* during the 4 weeks preceding the endoscopy were also excluded.

During the procedure, nine gastric biopsy specimens (antrum-5, corpus-4) were obtained for investigations. The ethical approval for the study was obtained from the Ethics Review Committee, Faculty of Medicine, Galle.

The association between endoscopic and histological abnormalities

A complete upper gastrointestinal endoscopy was performed in each patient. Of the 251 patients, 175 (69.7%) had abnormal findings in the stomach. The most frequently detected endoscopic abnormality was gastritis. Of the patients who had gastritis, 49 (67.1%), 22 (30.1%) and 2 (2.7%) had pan, antral and corporal gastritis respectively. Ninety (35.9%) patients had abnormalities in the esophagus while only 13 (5.2%) patients had endoscopically detected abnormalities in the duodenum. In one previous Sri Lankan study, gastritis with or without duodenitis was the most frequent abnormality (one third of cases) detected among patients who had non-specific upper abdominal symptoms (12). Duodenal abnormalities were less frequent among the sample population studied. Similarly, in another Sri Lankan study, duodenitis and duodenal ulcer contributed to 8% and 5% cases of upper gastrointestinal bleeding respectively (13).

Five gastric biopsies were obtained for histology. Three were collected from the gastric antrum 2-3 cm away from the pylorus from the distal parts of greater and lesser curves. Two biopsies were obtained from the corpus in a similar manner. Formalin fixed biopsy specimens were processed and stained with both Haematoxylin & Eosin (H & E) and modified Giemsa stains. The histological findings of gastric mucosa were interpreted according to the updated Sydney system (14).

Histological abnormalities were detected in 222 (88.4%) patients. Altogether 217(86.5%) patients had chronic gastritis. Among them 182(83.9%), 29 (13.7%) and 6 (2.8%) had pan, antral and corporal gastritis respectively. Of the 217 patients with histological chronic gastritis, 71 (32.7%) were correctly detected endoscopically while 146 (67.3%) were not detected endoscopically (sensitivity = 0.33, specificity = 0.94) (kappa=0.096).Two patients having gastritis endoscopically had normal gastric mucosa histologically.

Analysis of our study showed that endoscopy had only 32.7% sensititivy in detecting histologically manifested gastritis. However it showed 94% specificity. Poor to moderate sensitivity of endoscopy in detecting gastritis was seen irrespective of its topographic distribution. This observation shows that endoscopic gastritis does not always coincide with histological gastritis. Various gastroscopic features may be interpreted as signs of gastritis, but the significance of such features in relation to histomorphology is less clear in many cases. The macroscopic features of gastritis recorded include erythema, inflammation and erosions of the gastric mucosa, reduced height and increased distance between rugae in the gastric corpus, and presence of visible vessels. Results showed that morphological features recorded were insensitive to detect gastritis in a majority of cases and if gastritis is suspected those patients should be subjected to biopsy and histological

Table 1 Sensitivity and specificity of different *H.pylori* diagnostic techniques when histology (H & E or modified Giemsa) was taken as the gold standard

Test	True Positives	True Negatives	False Positives	False Negatives	Sensitivity	Specificity
Serology (n=125)	3	63	2	57	5%	97%
Culture (n=62)	2	32	1	27	7%	97%
H&E†alone (n=251)	91	127	0	33	73%	100%
Giemsa alone (n=251)	101	127	0	23	81%	100%

† Hematoxylin and Eosin

Table 2 Sensitivity and specificity of *H.pylori* positivity in antrum and corpus when compared with the "gold standard" in which two staining methods are taken together

Test	True Positives	True Negatives	False Positives	False Negatives	Sensitivity	Specificity
H.pylori in antrum (H&E†alone)	88	127	0	36	71%	100%
H.pylori in corpus (H&E† alone)	35	127	0	89	28%	100%
H.pylori in antrum (Giemsa alone)	98	127	0	26	79%	100%
H.pylori in corpus (Giemsa alone)	91	127	0	33	73%	100%

Hematoxylin and Eosin

Table 3 The association between drug usage and upper gastrointestinal diseases among patients and controls

Peptic ulcer disease		Controls	Cases	P*	OR(95% CI)	P**
Antirheumatic drugs	never/infrequent use	110(98.2%)	32(76.2%)		1	
	used in the recent past	2 (1.8%)	10(23.8%)	<0.001	17.18 (3.58 -82.48)	<0.001
Analgesics	never/infrequent use	109(97.3%)	28(66.7%)		1	
	used in the recent past	3 (2.7%)	14(33.3%)	<0.001	18.17(4.88 - 67.62)	<0.001
Antibiotics	never/infrequent use	112 (100%)	34(81.0%)		1	
	used in the recent past	0	8 (19.0%)	<0.001	46.6(9.48-229.3)	<0.001
Gastro-oesophageal reflux						
Antirheumatic drugs	never/infrequent use	266(98.9%)	107(83.6%)		1	
	used in the recent past	3(1.1%)	21(16.4%)	<0.001	17.4(5.08 - 59.5)	<0.001
Analgesics	never/infrequent use	264(98.1%)	105(82.0%)		1	
	used in the recent past	5(1.9 %)	23 (18.0 %)	<0.001	11.6(4.28-31.2)	<0.001
Antibiotics	never/infrequent use	266(98.9%)	116(90.6%)		1	
	used in the recent past	3 (1.1%)	12 (9.4%)	<0.001	9.17 (2.54-33.12)	<0.001
Gastritis						
Antirheumatic drugs		282(98.6%)	140(82.4%)			
	used in the recent past	4 (1.4%)	30 (17.6%)	<0.001	15.1(5.2 - 43.6)	<0.001
Analgesics	never/infrequent use	280(97.9%)	131(77.1%)		1	
	used in the recent past	6 (2.1%)	39 (22.9%)	<0.001	13.8 (5.7 - 33.6)	<0.001
Antibiotics	never/infrequent use	283(99.0%)	145(85.3%)		1	
	used in the recent past	3 (1.0%)	25 (14.7%)	<0.001	16.21 (4.82 -54.50)	<0.001

P*= contrasts proportions in two groups and calculated using Chi-squared test.
P**= P for odds ratios.

Table 4 The association between social habits and upper gastrointestinal diseases among patients and controls

Peptic ulcer disease		Controls	Cases	P*	OR(95% CI)	P**
Smoking	never	82(73.2%)	30(73.2%)		1	
	ever	30(26.8%)	11(26.8%)	1	1.00(0.45 -2.24)	0.98
Alcohol consumption	never	84(75.0%)	25(61.0%)		1	
	ever	28(25.0%)	16(39.0%)	0.09	1.92(0.89 4.10)	0 .11
Gastro-oesophageal reflux						
Smoking	never	209 (77.7%)	92 (72.4%)		1	
	ever	60 (22.3 %)	35 (27.6 %)	0.26	1.33(0.82 - 2.15)	0.26
Alcohol consumption	never	203 (75.5%)	82 (64.6%)		1	
	ever	66 (24.5%)	45 (35.4%)	0.031	1.69 (1.07 - 2.6 7)	0.03
Gastritis						
Smoking		217(75.9%)	118(69.4%)		1	
	ever	69 (24.1%)	52 (30.6%)	0.081	1.39 (0.91 - 2.12)	0.15
Alcohol consumption	never	211(73.8%)	102(60.0%)		1	
	ever	75 (26.2%)	68 (40.0%)	0.002	1.88 (1.25 - 2.8 1)	0.002

P*= contrasts proportions in two groups and calculated using Chi-square test.
P**= P for odds ratios

examination even though endoscopic findings are negative.

Prevalence of H.pylori infection

The prevalence of H. pylori infection was determined by histology. A particular patient was identified as positive when either H & E or Giemsa stained histology demonstrated morphological evidence of H. pylori in at least one biopsy specimen.

Overall prevalence of H. pylori in the study sample was 49.4%. This contradicts the popular belief that H.pylori infection is very high among patients in developing countries. Fernando et al (15) have shown a similar prevalence of H.pylori (46%) among 100 consecutive Sri Lankan patients with upper gastrointestinal disease. Other studies from Sri Lanka have reported similar low prevalence figures of 30-40% (16-18). In contrast to above studies, one Sri Lankan study using PCR reported a higher prevalence of 75.4% of infection among 57 dyspeptic individuals (9). Histologically, 52.5% of patients with chronic gastritis and 41.4% patients with gastric ulcers had H.pylori. According to the distribution of gastritis, the prevalence of H. pylori in pangastritis and antral gastritis was 53.3% and 48.3%, respectively. The number of duodenal abnormalities was too small to determine the prevalence of infection in different duodenal diseases. Even though H.pylori is the key etiological factor in chronic gastritis, only 52.5% of our patients with chronic gastritis had *H.pylori*. These figures are relatively low when compared with other Asian countries (2-4). *H.pylori* infection is known to be associated with low socioeconomic status, overcrowding, unhygienic practices and poor sanitation. Sri Lanka has better health care indices compared to other South Asian countries and that may explain the low prevalence observed. Furthermore, some culinary and medicinal plants used in Sri Lankan cooking (e.g. tumeric, cumin, ginger etc) have shown bactericidal and anti-adhesive properties against *H.pylori* (19).

The patients with histological evidence of gastritis (52.5%) had a higher prevalence of *H.pylori* infection when compared to patients with other diseases (P=0.012). Furthermore, patients with histological evidence of pangastritis (53.3%) had higher prevalence of *H.pylori* when compared to patients with antral gastritis (48.3%), but the difference was not statistically significant (P=0.61).

Hence, it seems that *H.pylori* infection in Sri Lankan patients is associated with histologically manifested gastritis but not with its topographical distribution. Small sample size may have limited the significance of our analysis. The well known association between antral gastritis and *H.pylori* infection observed in other studies published in Asian and non-Asian countries was not apparent in this study (20, 21).

Sensitivity and specificity of diagnostic tests to detect H.pylori infection.

Several tests are available for the diagnosis of *H.pylori* infection. Depending on the need of endoscopy they are categorized into two groups; endoscopic dependent and endoscopic independent tests. Endoscopic dependent tests include histology, rapid urease test and culture. Endoscopic independent tests include urea breath test, serology and *H.pylori* stool antigen tests. Molecular diagnostic tests can either be dependent or independent of endoscopy depending on the type of material used for the diagnostic purposes.

Four diagnostic tests were used to detect the infection in this study i.e histology, rapid urease test, culture and serology.

Histology was performed in all patients. Formalin fixed biopsy specimens were processed and stained with both H & E and modified Giemsa stains. The histological findings of gastric mucosa were interpreted according to the updated Sydney system (14).

Urease test was performed in 210 patients. For this purpose two biopsy specimens each from antrum and corpus were immersed in homemade urea solution (22). The solution contained a mixture of urea, sodium chloride, potassium dihydrogen phosphate and phenol red as the indicator. Urease produced by *H.pylori*, converted urea to ammonia giving a change in colour in the solution. A positive test was indicated by yellow to pink colour change observed within 6-12 hours after the immersion of the biopsy specimens.

Culture was performed in 62 patients. Two biopsy specimens each from antrum and corpus were collected into 0.9% sterile saline stored at 4^0C. Soon after the collection the specimens were inoculated into Columbia agar base (CM331) supplemented by laked horse blood (SR 48) and *H.pylori* selective supplement (SR 147). The culture plates were incubated for 3 days at 37^0C under microaerophilic conditions (5%O_2, 10% CO_2, 85% N_2) using special gas kits (CampyGen CN0025A). On the 4^{th} day, positive cultures were identified by colony morphology, Gram stain and biochemical testing (positive uraese, catalase and oxidase activity). Positive colonies appeared discrete, translucent and non coalescent and Gram stain revealed Gram negative curved bacilli. Subcultures were performed as required.

Out of the 251 patients, a randomly selected subgroup of 125 underwent Enzyme –Linked Immuno-Sorbent Assay (ELISA) for the detection of anti-H.pylori IgA and IgG antibodies in plasma. For this purpose, 5 ml of peripheral venous blood was collected to EDTA bottles. The blood samples were centrifuged, plasma was separated and anti H.pylori IgA and IgG were measured using ELISA test kits (Human Gesellschaft fur Biochemica und Diagnostica GmbH, Germany).

Histologically, the prevalence of *H.pylori* in this referred sample was 49.4%. Culture isolated *H.pylori* from three patients. Positive urease activity was demonstrated in 67 patients either or both in antrum and corpus. Four patients were positive for IgG only while one patient was positive for both IgG and IgA.

The sensitivity and specificity of each diagnostic test was calculated taking histology (either H & E or Giemsa positive) as the gold standard. According to the gold standard **the sensitivity and specificity of diagnostic methods are summarized in table 1.**

Histology was the most sensitive method for the detection of *H.pylori* among these patients. Giemsa staining showed a marginally better sensitivity when compared to H & E. Urease test showed a moderate sensitivity and specificity while culture and serology had lower sensitivity. As histology was taken as the reference, both staining methods showed 100% specificity. Serology and culture also showed a very high specificity while urease test had the lowest specificity.

There is no gold standard for the detection of *H.pylori* in clinical practice. We considered histology as the gold standard in our study. Modified Giemsa stain was used as a special stain to improve the sensitivity and specificity of diagnosing *H.pylori* as recommended by the updated Sydney system (14). As the chosen gold standard was based on these two techniques, both stains, as expected, showed 100% specificity. Our findings support the fact that modified Giemsa stain is reliable, less technically demanding and easily reproducible amongst many available staining techniques for *H.pylori* (23).

According to our data, histology from multiple biopsies representing different areas of the stomach increases the detection of *H pylori*. If endoscopist has a practical limitation in obtaining multiple biopsies, a single biopsy obtained from antrum and stained with modified Giemsa gives an acceptable sensitivity to detect the organism (Table 2).

The rapid urease test when compared to histology, showed relatively low specificity and sensitivity, 32% and 68% respectively. This is not in line with many other studies where the rapid urease test has reported high specificity and sensitivity (24,25).

Table 5 The association between food habits and upper gastrointestinal diseases among patients and controls

		Controls	Cases	P*	OR(95% CI)	P**
Peptic ulcer disease						
Consumption of tea	None/occasional	11 (9.8%)	13(31.0%)		1	
	⩾ 2 cups /day	101(90.2%)	29(69.0%)	0.002	0.24 (0.10 -0.59)	0.002
Consumption of acidic/spicy foods	infrequent	51(46.4%)	19(46.3%)		1	
	frequent	59 (53.6%)	22(53.7%)	1	1.00(0.49 - 2.05)	0.98
Missing/delaying meals	no	71(64.5%)	13(31.7%)		1	
	yes	39(35.5%)	28(68.3%)	<0.001	3.92(1.82 - 8.43)	<0.001
Frequent intake of fruits/vegetables	no	81(73.6%)	22(53.7%)		1	
	yes	29(26.4%)	19(46.3%)	0.019	2.41(1.14 -5.09)	0.03
Frequent intake of starchy foods	no	63(57.3%)	26(63.4%)		1	
	yes	47(42.7%)	15(36.6%)	0.495	0.77 (0.37 -1.62)	0.58
Satisfactory intake of dairy foods	no	92(82.9%)	33(80.5%)		1	
		19 (17.1%)	8 (19.5%)	0.732	1.17(0.47 -2.94)	0.81
Gastro-oesophageal reflux						
Consumption of tea	None/occasional	22 (8.2%)	33(25.%)		1	
	⩾ 2 cups /day	247(91.8%)	95(74.2%)	<0.001	0.25(0.14-0.46)	<0.001
Consumption of acidic/spicy foods	infrequent	123(46.1%)	44(34.6%)		1	
	frequent	144(53.9%)	83(65.4%)	0.038	1.61(1.04 -2.49)	0.038
Missing/delaying meals	no	164(61.4%)	32(25.2%)		1	
	yes	103(38.6%)	95(74.8%)	<0.001	4.72 (2.95 -7.57)	<0.001
Frequent intake of friuts/vegetables	no	184(68.9%)	76(59.8%)		1	
	yes	83(31.1%)	51(40.2%)	0.088	1.48(0.96-2.31)	0.089
Frequent intake of starchy foods	no	159(59.6%)	61(48.0%)		1	
	yes	108(40.4%)	66(52.0%)	0.039	1.59(1.04 - 2.44)	0.043
Satisfactory intake of dairy foods	no	212 (9.1%)	103(81.1%)		1	
		56 (20.9%)	24(18.9%)	0.69	0.88(0.52 -1.50)	0.68
Gastritis						
Consumption of tea	None/occasional	24 (8.4%)	43 (25.3%)		1	
	⩾ 2 cups /day	262(91.6%)	127(74.7%)	<0.001	0.27(0.15 - 0.47)	<0.001
Consumption of acidic/spicy foods	infrequent	128(45.1%)	52 (31.1%)		1	
	frequent	156(54.9%)	115(68.9%)	<0.002	1.82 (1.21 -2.71)	0.004
Missing/delaying meals	no	171(60.2%)	43 (25.7%)		1	
	yes	113(39.8%)	124(74.3%)	<0.001	4.36 (2.87 - 6.64)	<0.001
Frequent intake of friuts/vegetables	no	192(67.6%)	104(62.3%)		1	
	yes	92 (32.4%)	63 (37.7%)	0.15	1.26 (0.85 - 1.88)	0.25
Frequent intake of starchy foods	no	171(60.2%)	81 (48.5%)		1	
	yes	113(39.8%)	86 (51.5%)	0.01	1.61 (1.09 - 2.36)	0.016
Satisfactory intake of dairy foods	no	223(78.2%)	133(79.6%)		1	
	yes	62 (21.8%)	34 (20.4%)	0.41	0.91(0.57 - 1.47)	0.81

P*= contrasts proportions in two groups and calculated using Chi-square test.
P**= P for odds ratios

Table 6 Comparison of the quality of life of patients with upper gastrointestinal symptoms and the control group on the dimensions of the WHOQOL-BREF

Domain			Mean	SD	95% Confidence Interval for Mean		F	P-value
					Lower	Upper		
Physical	Patient	(n=126)	54.4	17.7	51.3	57.5	45.0	0.000
	Control	(n=200)	67.1	16.0	64.9	69.4		
Psychological	Patient	(n=123)	62.1	17.6	58.9	65.2	15.7	0.000
	Control	(n=179)	69.4	14.6	67.3	71.6		
Social relationship	Patient	(n=121)	60.6	21.7	56.7	64.5	1.2	0.283
	Control	(n=169)	63.3	21.0	60.1	66.5		
Environment	Patient	(n=124)	63.0	15.0	60.3	65.6	1.5	0.221
	Control	(n=176)	65.0	13.0	63.0	66.9		

Table 7 Effect of the *H.pylori* density on the quality of life among the symptomatic patients

Domain			Mean	SD	95% Confidence Interval for Mean		F	P-value
					Lower	Upper		
Physical	None	n=68	56.6	17.2	52.5	60.8	1.8	0.166
	Mild	n=24	53.0	18.1	45.4	60.7		
	Moderate	n=25	48.9	18.3	41.3	56.5		
Psychological	None	n=67	65.1	16.5	61.1	69.1	3.2	0.044
	Mild	n=23	59.4	19.3	51.1	67.8		
	Moderate	n=24	55.2	17.3	47.9	62.5		
Social relationship	None	n=65	68.0	18.7	63.4	72.6	10.1	0.000
	Mild	n=23	55.8	21.8	46.4	65.2		
	Moderate	n=25	48.7	19.0	40.8	56.5		
Environment	None	n=67	66.2	14.5	62.6	69.7	5.2	0.007
	Mild	n=23	60.1	17.2	52.7	67.5		
	Moderate	n=25	55.7	11.5	50.9	60.4		

Since the urease solution was sterile and sterility was maintained during the biopsy procedure, contamination is unlikely for false positive urease test. Studies have shown that hypochlorhydric patients could harbor many urease-positive bacteria in gastric mucosa other than *H. pylori* (26). The strong urease activity they possess could be the reason for false positive results.

Even though many studies have shown the sensitivity of urease test to be around 80-90%, the sensitivity observed is lower. The sensitivity can vary with the site chosen for biopsy due to patchy distribution of the infection. In one study the gastric angle site was positive in 100%, while the prepyloric and corpus sites were positive in 87% and 84.4%, respectively (27). Hence a false negative test could occur due to sampling error. Satarasinghe, *et al* (16) have postulated that Sri Lankan *H.pylori* strains are different from strains found elsewhere that they have shorter survival and they produce inconspicuous rapid urease results. Infection with *H. pylori* induces several antibodies; anti *H.pylori* IgG, IgA and less frequently IgM. Anti *H.pylori* IgM can be detected shortly after infection is acquired and IgG and IgA antibodies indicate a chronic infection. In most studies the prevalence of infection has been determined by IgG-type antibodies. Detection of serum IgA or IgM is known to have poor discriminatory value when compared to serum IgG.

Some investigators have reported a subset of patients who are positive for IgA but negative for IgG antibodies for *H.pylori* (28).

In serology, the two cases with false positive results observed may have had a previous infection with *H.pylori* which resulted in persistently elevated antibody levels. Due to the patchy distribution of *H.pylori* a sampling error could also have been the cause for the false positive results. A similar picture was observed in elderly people where the progression of atrophic gastritis has spontaneously eliminated the organism while a detectable level of antibody was observed in serum (29).The false negative results could be due to many reasons. It was reported that certain test kits were not successful to detect the infection in certain populations (30). In this study plasma instead of serum was used. Assurance was given by the manufacturer that both plasma and serum would give the same result when the test kit was used (31).

Culture of bacterium which is urease, oxidase and catalase positive from gastric biopsy specimens is a definitive proof of *H pylori* infection. However, the ability to isolate the organism from infected subjects varies widely among laboratories. That makes culture the most technically demanding *H pylori* diagnostic test. Biopsy specimens must be rapidly transferred to the laboratory in chilled transport medium. Upon the receipt, the sample is ground or minced to produce a homogenate which is inoculated on to freshly prepared media. When these fastidious requirements are met, culture yields positive results.

Even after fulfilling all these requirements, the sensitivity of culture varies among laboratories. Moayyedi, and Dixon (32) have performed *H.pylori* culture with the sensitivity and specificity of 90.5% and 99.2% respectively. In one study that compared eight different methods for detection of *H.pylori*, culture revealed 55.9% sensitivity with 100 % specificity (33).

Administration of drugs such as antibiotics, omeprazole or Bismuth– containing drugs, three months prior to the culture are likely to provide negative results (34, 35). Recent users of proton pump inhibitors were not excluded in our study and this may have contributed to negative cultures. Furthermore, commonly used non-steroidal anti-inflammatory drugs (NSAIDs) can also inhibit the growth of *H.pylori* in vitro (36).

Cost of *H.pylori* diagnostic tests

Of the four tests performed, histology was the most expensive test. It costs around 4 - 5 US Dollars (USD) per test to purchase consumables. Approximately 2 hours was required to complete the test for one patient. The estimated cost for man power for one test was 2 USD and therefore the total cost of the test was between 67USD. Many western countries, where the prevalence of infection is low, do not use histology in their routine clinical practice. However, in Sri Lanka endoscopy and histology are performed free of charge in most of the government owned tertiary care hospitals around the country. Hence, the cost factor has not limited the diagnostic utility of this investigation.

Serology was the second most expensive test. On average the consumable cost for one serology test was 3.8 USD. Out of the total time required to perform the test, approximately one hour was utilized for the direct involvement of man power. The estimated cost for man power per one serology test was approximately 1 USD and the total cost was 4.8 USD.

Culture was third in place when the total cost was compared. But it was more time consuming than serology. It required around 2.9 USD for consumables to perform the culture for one patient. On average, it required about 1.5 hours to perform the culture. The approximate cost for man power to perform one culture test was 1.6 USD. The total cost was 4.5 USD.

Urease test was the cheapest of all tests. Since the home-made urease solution was used, it required less than 0.2 USD to prepare 100 ml of the solution and the time spent was approximately one hour. One milliliter of this solution was required per test, therefore the approximate cost of man power to perform one urease test was 1 USD. Hence the total cost for urease test was 1.2 USD.

There were certain limitations in direct and indirect cost calculations. For histology, when calculating the reagent volumes required for staining techniques, total calculated volume was more than the true volume that was required. The reason is that once a staining procedure was performed, reagent tanks were refilled without waiting for the tanks to be emptied. So the estimate tends to be more than the actual value. Furthermore, in all four diagnostic tests there were varying time periods with minimal personnel involvement e.g. tissue processing time in histology, incubation periods in serology and culture. This was not taken into consideration when calculating indirect costs. So the estimated time was less than the actual number of hours taken to perform the test.

The risk factors of peptic ulcer disease, gastro-oesophageal reflux disease and gastritis - three case control studies

Upper gastrointestinal diseases are known to be associated with many risk factors. At the same time there are many protective factors as well. However, Sri Lankan studies are scarce on this aspect. There are

various beliefs and practices in the society based on personal experiences of individuals but no documented data are available. The impact of several possible risk and protective factors in three upper gastrointestinal diseases; peptic ulcer disease, gastro-esophageal reflux and gastritis was studied by comparing the patient group with a control group.

A hospital based control group (n=350) was selected from attendees to the out patients department of the same hospital seeking treatment for brief illnesses. Those who sought medical advice, those who have taken medication for, and those who have experienced any upper gastrointestinal symptom during the previous year were excluded from the study. Two controls were selected for one case approximately after stratifying for age (within ten years) and sex.

Patients from both test group and control group were interviewed and basic information such as age, sex, occupation, level of education were recorded with presenting complaint, past medical history, drug history, social habits, food habits, sanitary practices etc. At the end of the interview, a brief clinical examination was performed. The height and weight were measured and blood pressure and pulse rate were recorded while resting.

Risk and protective factors for peptic ulcer disease

The impact of risk factors on the development of peptic ulcer disease has been shown to vary among different populations. Factors such as *H. pylori*, smoking, alcohol use, and NSAIDs use are well documented risk factors for peptic ulcer disease (37, 38).

Cases for this part of the study were selected from the study sample. There were 56 patients with peptic ulcer disease (male/female: 31/25) and they were compared with 112 controls (male/female: 62/50).

Cases and controls had significant differences with regards to socio economic status, type of drinking water and physical activity. Smoking and alcohol consumption were not different between the two groups. When compared with the higher socio economic group, people in the middle group had lesser tendency to develop peptic ulcers. People who did not drink boiled cooled water regularly had lesser chance of having ulcers when compared with people who regularly drank boiled cooled water. Similarly less physical activity was seen as a risk factor for ulcer disease. Anti-rheumatic and analgesic use increased the risk of peptic ulcer disease. Apart from frequent fruit/vegetable consumption and missing/delaying meals which increased the risk of peptic ulcer disease, other food habits examined in this study showed no association.

Studies have shown that aspirin has a significant impact on both duodenal ulcer and gastric ulcer (38). Even though smoking has not being a significant risk factor among the group of patients used in this study, it is a well established risk factor for peptic ulcer disease among both men and women (39, 40). In this analysis, those who belonged to the middle social class had a less chance of having a peptic ulcer disease. One reason for this could be due to the less stress they face by being in the middle social class. Räihä *et al*(40) suggested that stress is a significant predictor of peptic ulcer disease. They reported an association between the self – reported stress involved with daily activities and peptic ulcer disease. So the less mental stress generated by being in the middle social class may have been a protective factor.

Adaptation of healthy practices had no significant protective effect against peptic ulcer disease among our patients. In this study, healthy practices such as personal hygiene, proper disposal of faeces and hygienic garbage disposal were considered. Deficiency of most of these practices has an effect on transmission of *H.pylori* infection. There are many studies that highlighted the importance of sanitary practices on transmission of *H.pylori* infection (41, 42).

Gastro-oesophageal reflux disease

Gastro-oesophageal reflux disease was diagnosed according to the criteria described by Devault and Castell (43).There were 142 patients with gastro-oesophageal reflux (Male/Female: 75/67) and they were compared with 269 controls (Male/female: 147/122).

When compared with the higher socioeconomic class, subjects in the middle class had a lesser risk in developing the reflux disease. Compared with regular consumers of boiled cooled water, people who never consumed boiled cooled water had a lesser risk of getting the disease. While smoking had no effect, alcohol consumption increased the risk of reflux. Consumption of tea more than twice a day reduced the risk of reflux while intake of anti-rheumatics, analgesics or antibiotics and missing/delaying meals predisposed the reflux disease. While frequent consumption of fruit/vegetables had no effect, frequent intake of spicy or starchy food increased the risk of the disease.

Obesity has long been considered to cause gastro-oesophageal reflux. The mechanism by which obesity causes reflux is not clear, although there is some limited data suggesting that hiatus hernia may be the causal link between obesity and reflux (44). However in this study there is no significant difference between the mean body weight of the cases and controls.

Consistent with the studies reported, alcohol consumption has been a significant risk factor in our group of patients (45).

Among the bad food practices, missing or delaying meals frequently was a significant risk factor identified in this study. Many studies have shown an association between food habits and reflux disease. In some studies, the prevalence of reflux disease was significantly lower in subjects taking fruit and vegetables frequently (45, 46). But this association was not seen among our subjects.

Sedentary life style was identified as a significant risk factor in this study. Compared to very active category, subjects in less active groups had more chance of developing reflux disease. Studies carried out elsewhere support this protective role of physical activity on reflux disease (44, 45). Some suggest that physical activity at work appears to be a risk factor for frequent reflux symptoms, whereas recreational physical activity appears to be beneficial against the development of reflux disease (47).

Being in the middle social class was a protective factor for gastro-oesophageal reflux disease. Less stress associated with middle class life style would be the plausible explanation. In previous studies, psychological, physical and social stresses were known to increase reflux symptoms (46, 48).

Gastritis

Apart from the etiological agent *H. pylori,* other risk factors of gastritis are not well known. Factors that promote non *H.pylori* gastritis except alcohol have not been studied extensively. There are different beliefs among people that some life style practices induce gastritis.

There were 217 patients with histologically confirmed gastritis (Male/Female: 117/100).They were compared with 286 controls (Male/Female: 160/126).

In our sample of patients, being in the middle social class, frequent consumption of tea and healthy practices during day to day activities were significant protective factors against the development of gastritis. Bad food practices such as frequent consumption of acidic/spicy food, frequent missing or delaying meals and frequent consumption of starchy foods were seen as significant risk factors for the development of gastritis. Sedentary life style and consumption of alcohol too were significant risk factors for gastritis. Drinking of ordinary water instead of boiled cooled water had a significant protective effect against the development of gastritis. Use of drugs in the recent past such as anti-rheumatic drugs, analgesics and antibiotics appeared to be significant risk factors for the development of gastritis. Alcohol induced gastritis is a well known phenomenon in etiology of gastritisas shown in many studies (49, 50). This study also showed that alcohol is a significant risk factor.

There are some studies that favour the increased association between acidic and spicy foods with gastritis. Atisook *et al*(51) showed that dietary habits of Thai had a greater influence on gastritis. Intake of hot and spicy food and capsicum was related to gastritis in the Thai population. They also suggested that seafood and fruit may be protective against mucosal injury induced by hot and spicy food.

The use of drugs such as NSAIDs and analgesics has been shown to increase the risk of gastritis. Atisook *et al.* (51) has identified NSAIDs as another risk factor inducing gastritis among Thais. Their study suggested that the nationwide incidence of pangastritis correlated well with common use of aspirin and non prescribed medications.

There are no studies to support that physical activity has a direct relationship with gastritis. Yet there are evidence to suggest that excessive physical exertion result in upper gastrointestinal disturbances that has been observed in long distance runners (52). Since recreational runners were not affected by such disturbances, regular physical activity may not generate gastrointestinal disturbances (53). The psychological stress associated with sedentary life style may have contributory effects on gastritis. In the group of gastritis patients used for the study, those who belonged to the middle social class produced less stress and therefore more protective against gastritis.

The association of drug usage, social habits and food habits with the three upper gastrointestinal diseases is summarized in tables 3, 4 and 5 respectively.

Measurement of quality of life in patients with upper gastrointestinal symptoms; a comparative study

Upper gastrointestinal symptoms are very common in clinical practice. However the effects of these long term symptoms on the physical, psychological, social and environmental well being are not well known. There are sufficient studies to indicate that upper gastrointestinal symptoms are associated with poor QOL in affected individuals. However head to head comparisons of these studies are not possible as they have different entry criteria and have used different scales. In this study, the validated Sinhala translation of World Health Organization Quality of Life BREF (WHO QOL BREF) questionnaire was used on 126 patients of the same patient group and 200 controls to study the impact of these symptoms (54). As it contains only 26 questions representing the four domains, this scale is convenient to be used in busy endoscopy setups. Further, it takes less time to fill the information and the patient acceptance was also good.

Both groups completed the questionnaire that asses QOL in four domains; physical, psychological, environmental and social relationship.

The overall QOL for patient group was 59.9 whereas the overall QOL for control group was 66.6 (p <0.001). Results showed that in all four domains, patients scored less than controls indicating that patients had poor QOL when compared to controls. Differences in physical (p <0.001) and psychological (p <0.001) domains were wider and were statistically significant. Although the differences in social relationship and environment domains showed similar trends, they were not statistically significant (Table 6). Most of the available scales were confined to measure the quality of life in specific disease categories such as thegastro-oesophageal reflux disease, but not in a broader spectrum of people with common symptoms. Madisch *et al* (55) described the impact of heartburn on patients' Health-Related Quality of Life (HRQOL) using the validated Quality of Life in Reflux and Dyspepsia questionnaire (QOLRAD). The questionnaire had domains for daily functioning, impaired vitality, emotional distress and sleep disturbance. They found that overall HRQOL was impaired across all domains and stated that there was consistent evidence to believe that heartburn substantially impairs all aspects of health-related quality of life. Kinoshita *et al* (56) also reported that as in Western countries, QOL of the Japanese patients with gastro-oesophageal reflux disease was significantly decreased in comparison to those of healthy individuals.

The effect of density of *H.pylori* colonization on quality of life.

The association between *H.pylori* density and quality of life of the patients within the group was also studied. The density of *H.pylori* colonization within the patient group was graded as none, mild and moderate.

There was no significant difference in the mean scores of physical (P=0.31) domain. However there was a significant difference in the mean scores of social (P<0.001), environmental (P=0.01) and psychological (P=0.044) domains depending on the degree of *H.pylori* colonization (Table7). This might be due to the known association of*H.pylori* infection with poor socioeconomic status and unhealthy environment (57, 58). People who belonged to less privileged groups probably had higher tendency to carry *H.pylori* and therefore when their QOL was analyzed the particular association of poor socioeconomic status and unhealthy environment with the disease might have been revealed.

The significant effect of *H.pylori* density on the psychological domain could be related to the stress. We can hypothesize that when the *H.pylori* density is more, the severity of dyspeptic symptoms would be more resulting in psychological stress.

Moayyedi *et al* (59) found that *H. pylori* is significantly associated with dyspepsia and suggested that the organism may be responsible for 5% of upper gastrointestinal symptoms in the community. Animal studies have demonstrated that *H. pylori* infection influences the development of gastric mucosal injury in the early phase of stress exposure (60). Therefore stress and *H.pylori* seem to exert their effects in a vicious cycle. Yet a controversial theory put forward by Stone et al (61) indicates that *H. pylori* infection does not play an important role in overall symptoms of dyspepsia in a community.

Conclusions and recommendations

This study reveals that more than 80 % of the patients referred for upper gastrointestinal endoscopy had an abnormality. Although national guidelines for endoscopy were not developed, our findings show that current referral system is appropriate and yields more positive than negative results. This reflects that clinicians use the endoscopy service judiciously to avoid unnecessary referrals. Furthermore, endoscopy provided information which could influence the short term and long term management of many patients. Therefore it is a very beneficial investigation for our clinical settings.

Among all the abnormalities detected, gastric abnormalities were commoner. Most common abnormalities found were gastritis and gastric ulcers. The third most common abnormality was gastro-oesophageal reflux disease. Duodenal abnormalities were rare among our patients.

In the sample population, 86% of patients had histological evidence of gastritis but endoscopy missed the diagnosis in about 60% of the patients with histological gastritis. Hence endoscopically normal mucosa does not exclude gastritis. If gastritis is suspected on clinical grounds or is considered in the differential diagnosis, a biopsy should be obtained for histological examination even if gastric mucosa appears to be normal on endoscopy.

The overall prevalence of *H.pylori* infection among the patients was 49.4%. This is very much less than expected. Most of the developing countries in Asia especially our neighbouring countries such as India, Pakistan and Bangladesh record high prevalence rates. Even more affluent countries in the region such as China and Japan also have reported relatively high prevalence rates. Our prevalence figures are closer to those of Western countries.

The prevalence of infection among different disease categories was found to be low in the sample studied. Reports from other countries, both developed and less developed, indicate that the infection is highly prevalent among the patients with gastritis and gastric ulcers. In the sample population, the prevalence of infection was around 50% for both disease categories. The low prevalence of infection, question the role of *H.pylori* as an etiological agent in upper gastrointestinal diseases in our country. Factors that prevent *H.pylori* colonization of the gastric mucosa should be explored further. If food or hygiene related factors are found to be responsible for the low prevalence of infection among Sri Lankans, they can be used to control the infection in other populations.

Generally the disease is known to be related to low socio-economic status and poor sanitation. Improvement of socio-economic status and sanitary practices in the recent past may have limited the transmission of infection. Most Sri Lankans have easy access to healthy drinking water. In all three case control studies, people who drank water without boiling had a low prevalence of major three upper gastrointestinal diseases. Whether unboiled water has a factor which prevent *H.pylori* colonization and whether it gets destroyed during the process of boling should be considered.

There may be certain genetic factors that prevent the infection. Since the majority of the study sample constituted of Sinhalese, a genetic factor protecting them from infection is a possibility. However there is no such evidence to prove this theory. Certain culinary and medicinal herbs used in Sri Lanka might play a role against *H.pylori*. As demonstrated by studies their bactericidal and anti- adhesive properties against *H.pylori* could be protecting Sri Lankan people from colonization by this bacterium. We recommend further studies in this area.

Since *H.pylori* is associatedwith a little more than 50% of people with gastritis and gastric ulcers, blind anti *H.pylori* therapy in these cases is not justifiable. Therapy should be given only to those with demonstrable *H.pylori* preferably in the histology specimen stained by the modified Gimsa staining. Histology appears to be the most reliable way of detecting the organism in our settings. Endoscopist should make an attempt to obtain multiple biopsies representing different areas of the stomach to increase the detection of *H.pylori* and to get a better idea about the musocal changes. If endoscopist has a practical limitation in obtaining multiple biopsies, single biopsy obtained from antrum and stained with modified Gimasa can be recommended. The appropriateness of the urease test which is the second most common method of detecting *H.pylori* in our hospitals needs to be reconsidered. Although histology takes more time and relatively expensive, it appears to be the best method for the detection of the organism. The yieldof the histology can be augmented by having the services of a histopathologist who has a special interest in this area due to the difficulty in recognizing the slender, thin organism unless special attention is given to locate them.

Many risk factors for gastritis other than *H.pylori* were identified in this study. There are commonly used drug groups such as analgesics, anti-rheumatics and antibiotics that were identified as significant risk factors for gastritis in the sample population. Many of these drugs can be obtained in Sri Lanka without a doctor's prescription. Therefore inappropriate use of these drugs is very common among Sri Lankan people. Alcohol was identified as a significant risk factor for gastritis. Many bad food habits practiced by Sri Lankans also act as significant risk factors. Skipping and delaying meals, frequent use of spicy and acidic foods, and consumption of starchy foods contributed significantly. This study shows that there are many factors that can be used to prevent people from geting gastritis other than eradicating *H.pylori*. Discouraging the indiscriminate use of drugs, modification of lifestyle practices and proper eating habits are important components in the management of gastritis. Skipping and delaying meals are common practices among school children and working class. Reflux symptoms are common among these groups and they should be advised to take meals at regular intervals.

According to the study presented here, upper gastrointestinal symptoms cause significant morbidty. The symptoms have a significant impact on the physical and psychological aspects of quality of life. This aspect of life is often forgotten when dealing with these patients. They should be treated to relieve their symptoms promptly and should be followed up to make sure that symptoms are kept under control. This would minimize their symptoms and improve the quality of life. Further these patients should be motivated during consultations to improve their morale.

The findings of this study would be helpful in planning the management of individual patient, improving the yield of endoscopic examination and advising the community on measures to be taken to prevent or minimize upper gastrointestinal diseases. The information could be useful to clinicians and health policy makers. This information can be passed on to the teachers and parents who can educate and reinforce proper eating habits among children. Furthermore this data can be used in planning future studies in this area.

Future studies

In Sri Lankan setup the most widely used investigation to diagnose *H.pylroi* infection is histology using routine H & E staining. The test is invasive, requires endoscopy and therefore an additional burden to the hospital setup where there is usually long queues at endoscopy units. Furthermore, histology is time consuming and costly even though it is being done at almost all tertiary care units free of charge. Introduction of a non invasive test is beneficial to diagnose patients without endoscopy. Using such a test asymptomatic people can be screened, the response to treatment can be monitored and subsequently patient compliance will be better. Value of serology in this context is poor since it has yielded low sensitivity and specificity values. *H.pylori* stool antigen test is such a non invasive test that is widely used in other countries where prevalence of infection is low. The validity of this test is currently being studied.

We also intend to study the prevalence of *H.pylori* infection in different geographic locations i.e Eastern Province of Sri Lanka and among a different ethnic group; Muslims. In multi-ethnic countries *H.pylori* has been studied extensively. There has been a variation in prevalence figures, strains responsible, virulence markers, antimicrobial susceptibility etc. Therefore, it will be an important aspect of*H.pylori* infection to be studied in Sri Lanka.

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References

1. Marshall BJ, Warren JR. Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. *Lancet* 1984;1(8390):1311-5.
2. Prasad S, Mathan M, Chandy G, et al. Prevalence of Helicobacter pylori in southern Indian controls and patients with gastroduodenal disease. *Journal of Gastroenterology and Hepatology* 1994;9:501-506.
3. Kazi JI, Jafarey NA, Alam SM, et al. Association of Helicobacter pylori with acid peptic disease in Karachi. *Journal of the Pakistan Medical Association* 1990;40: 240-241.
4. Sarker SA, Nahar S, Rahman M, et al. High prevalence of cagA and vacA seropositivity in asymptomatic Bangladeshi children with Helicobacter pyloriinfection. *Acta Paediatrica* 2004 ;93(11):1432-6.
5. Moayyedi P, Feltbower R, Brown J, et al. Effect of population screening and treatment for Helicobacter pylori on dyspepsia and quality of life in the community: a randomised controlled trial. Leeds HELP Study Group. *Lancet* 2000; 355(9216):1665-9.
6. Straten E, Jimenez S. The prevalence of Helicobacter pylori antibody in different age groups in Central Texas. *Clinical Laboratory Science* 2012;25(2):102-6.
7. Oleastro M, Pelerito A, Nogueira P, et al. Prevalence and incidence of Helicobacter pylori Infection in a healthy pediatric population in the Lisbon area. *Helicobacter* 2011;16(5):363-72.
8. Fernando D. Helicobacter pylori and peptic ulcer disease in Sri Lanka. *Ceylon Medical Journal* 1992; 37: 15-17.
9. Fernando N, Holton J, Vaira D, et al. Prevalence of Helicobacter pylori in Sri Lanka as Determined by PCR. *Journal of Clinical Microbiology* 2002;40: 2675-2676.
10. Hewavisenthi SJDS, Suranimala HDC, Alwis WHD. Helicobacter pylori associated gastric pathology. The College of Pathologists Sri Lanka Annual Academic Sessions 2002; 9.
11. Waidyarathne EI, Mudduwa LKB, Lekamwasam JDVC, et al. Helicobacter pylori detection techniques:comparison of sensitivity, specificity and cost. *Galle Medical Journal* 2012; 17(2):1-7.
12. Lekamwasam JDVC, Waidyarathne E. An audit of patients referred for gastroscopy. Annual Academic Sessions of Galle Medical Association 1999;1:19.
13. Lekamwasam JDVC, Priyanthi DN, Anandagoda M. Gastroscopic abnormalities found in patients with upper GIT bleeding. Annual Academic Sessions of Galle Medical Association 2002; 2: 6.
14. Price AB. The sydney system: histological division. *Journal of Gastroenterology and Hepatology*1991;6:209-222.
15. Fernando D. Helicobacter pylori and peptic ulcer disease in Sri Lanka. *Ceylon Medical Journal* 1992;37:15-17.
16. Satarasinghe RL, Jayamaha DH, Vidyathilake HMS, et al. Do our Helicobacter pylori differ from those found in the west, in their ability to produce urease enzyme? - A preliminary observation. *Journal of the Ceylon College of Physicians* 2000;33:121-3.
17. Ambalavanar DC, Pradeepkumar G. CLO test positivity in patients with acid peptic disorders in Jaffna. *Ceylon Medical Journal* 2001;46:158.
18. Satarasinghe RL, Fernando HRR, Sivakumar T. Do our Helicobacter pylori have shorter survival in their natural history? An observation based on qualitative analysis of urease production and histology. *Galle Medical Journal* 2003;4:2.
19. O'Mahony R, Al-Khtheeri H, Weerasekera D, et al. Bactericidal and anti-adhesive properties of culinary and Medicinal plants against Helicobacter pylori .*World Journal of Gastroenterology* 2005;11:7499-7507.
20. Bayerdörffer E, Lehn N, Hatz R, et al. Difference in expression of Helicobacter pylori gastritis in antrum and body. *Gastroenterology* 1992;102:1575-1582.
21. Misra V, Misra SP, Dwivedi M, et al. A topographic study of Helicobacter pylori density, distribution and associated

gastritis. *Journal of Gastroenterology and Hepatology* 2000;15:737-743.
22. Fernando N, Weerasekera ID, Meedin F, et al. A pilot study of a low cost CLO test. Ceylon Medical Journal 2003;48:98-99.
23. Rotimi O, Cairns A, Gray S, et al. Histological identification of Helicobacter pylori: comparison of staining methods. *Journal of Clinical Pathology* 2000;53:756-9.
24. Haruma K, Okamoto S, Sumii K et al. Helicobacter pylori infection and gastroduodenal disease: a comparison of endoscopic findings, histology, and urease test data. *Hiroshima Journal of Medical Sciences* 1992;41:65-70.
25. Bermejo F, Boixeda D, Gisbert JP, et al. Rapid urease test utility for Helicobacter pylori infection diagnosis in gastric ulcer disease. *Hepatogastroenterology* 2002;49:572-5.
26. Brandi G, Biavati B, Calabrese C, et al. Urease-positive bacteria other than Helicobacter pylori in human gastric juice and mucosa. *American Journal of Gastroenterology.* 2006;101:1756-61.
27. Woo JS, el-Zimaity HM, Genta RM, et al. The best gastric site for obtaining a positive rapid urease test. *Helicobacter* 1996;1:256-9.
28. Kosunen, TU, Seppälä K, Sarna S, et al. Diagnostic value of decreasing IgG, IgA, and IgM antibody titres after eradication of Helicobacter pylori. *Lancet* 1992;11:893-895.
29. Fox JG, Correa P, Taylor NS, et al. Campylobacter pylori-associated gastritis and immune response in a population at increased risk of gastric carcinoma. *American Journal of Gastroenterology* 1989;84:775-81.
30. Miwa H, Kikuchi S, Ohtaka K, et al. Insufficient diagnostic accuracy of imported serological kits for Helicobacter pylori infection in Japanese population. Diagnostic. *Microbiology and Infectious Diseases* 2000;36:95-9.
31. Lee AS, Finkielman JD, Peikert T, et al. Agreement of anti-neutrophil cytoplasmic antibody measurements obtained from serum and plasma. *Clinical and Experimental Immunology* 2006;146:15-20.
32. Moayyedi P, Dixon MF. Any role left for invasive tests? Histology in clinical practice. *Gut* 1998;43(Suppl. 1):S51-5.
33. Kullavanijaya P, Thong-Ngam D, Hanvivatvong O, et al. Analysis of eight different methods for the detection of Helicobacter pylori infection in patients with dyspepsia. *Journal of Gastroenterology and Hepatology* 2004;19:1392-6.
34. Mirshahi F, Fowler G, Patel A, et al. Omeprazole may exert both a bacteriostatic and a bacteriocidal effect on the growth of Helicobacter pylori (NCTC 11637) in vitro by inhibiting bacterial urease activity. *Journal of Clinical Pathology* 1998;51:220-4.
35. Kung NN, Sung JJ, Yuen NW, et al. One week ranitidine bismuth sulfate versus colloidal bismuth subcitrate-based anti-Helicobacter triple therapy a prospective randomaized controlled trial. *American Journal of Gastroenterology* 1999;94:721-724.
36. Shirin H, Moss SF, Kancherla S, et al. Non-steroidal anti-inflammatory drugs have bacteriostatic and bactericidal activity against Helicobacter pylori. *Journal of Gastroenterologyand Hepatology* 2006;21:1388-93.
37. Salih BA, Abasiyanik MF, Bayyurt N, et al. Helicobacter pylori infection and other risk factors associated with peptic ulcers in Turkish patients: a retrospective study. *World Journal of Gastroenterology* 2007;13:3245-3248.
38. Bayyurt N, Abasiyanik MF, Sander E, et al. Canonical correlation analysis of factors involved in the occurrence of peptic ulcers. Digestive Diseases and Sciences 2006;52:140-146.
39. Schöön IM, Mellström D, OdØn A, et al. Peptic ulcer disease in older age groups in Gothenburg in 1985: the association with smoking. *Age and Ageing* 1991;20:371-376.
40. Räihä I, Kemppainen H, Kaprio J, et al. Lifestyle, stress, and genes in peptic ulcer disease: a nationwide twin cohort study. *Archives of Internal Medicine* 1998;158:698-704.
41. Chen SY, Liu TS, Fan XM, et al. Epidemiological study of Helicobacter pylori infection and its risk factors in Shanghai. *Chinese Journal of Internal Medicine* 2005;85:802-806.
42. Ahmed KS, Khan AA, Ahmed I, et al. Prevalence study to elucidate the transmission pathways of Helicobacter pylori at oral and gastroduodenal sites of a South Indian population. *Singapore Medical Journal* 2006;47:291-296.
43. DeVault KR, Castell DO. Updated guidelines for the diagnosis and treatment of gastroesophageal reflux disease. The Practice Parameters Committee of the American College of Gastroenterology. *American Journal of Gastroenterology* 1999;94:434-442.
44. Nilsson M, Lagergren J. The relation between body mass and gastro-oesophageal reflux. Best Practice and Research. *Clinical Gastroenterology* 2004;18:1117-1123.
45. Nocon M, Labenz J, Willich SN. Lifestyle factors and symptoms of gastro-oesophageal reflux—a population-based study. Alimentary Pharmacology and Therapeutics 2006;23:169-174.
46. Saberi-Firoozi M, Khademolhosseini F, Yousefi M, et al. Risk factors of gastroesophageal reflux disease in Shiraz, southern Iran. *World Journal of Gastroenterology* 2007;13:5486-5491.
47. Zheng Z, Nordenstedt H, Pedersen NL, et al. Lifestyle factors and risk for symptomatic gastroesophageal reflux in monozygotic twins. *Gastroenterology* 2007;132:87-95.
48. Chen HX, Xiong LS, Xu AG. The risk factors and impact of gastroesophageal reflux disease on quality of life in general population. *Chinese Journal of Internal Medicine* 2006;45:202-205.
49. Lieber CS. Gastric Ethanol Metabolism and Gastritis: Interactions with Other Drugs, Helicobacter pylori, and Antibiotic Therapy (1957-1997)—a review. *Alcoholism, Clinical and Experimental Research* 1997;21:1360-1366.
50. Franke A, Teyssen S, Singer MV. Alcohol-related diseases of the esophagus and stomach. *Digestive Diseases* 2005;23:204-213.
51. Atisook K, Kachinthorn U, Luengrojanakul P, et al. Histology of Gastritis and Helicobacter pylori Infection in Thailand: a Nationwide Study of 3776 Cases. *Helicobacter* 2003;8:132-141.
52. Riddoch C, Trinick T. Gastrointestinal disturbances in marathon runners. *British Journal of Sports Medicine* 1988;22:71-74.
53. Simons SM, Kennedy RG. Gastrointestinal problems in runners. *Current Sports Medicine Reports* 2004;3:112-116.
54. Somasiri KG, Gunawardena S, et al. Quality of life of patients with hypertension attending medical clinics for treatment. Annual Academic sessions of Galle Medical Association 2003.
55. Madisch A, Kulich KR, Malfertheiner P, et al. Impact of reflux disease on general and disease-related quality of life - evidence from a recent comparative methodological study in Germany. *Zeitschrift für Gastroenterologie* 2003;41:1137-1143.
56. Kinoshita Y, Miki M, Tanimura T. QOL of Japanese patients with GERD. *Japanese Journal of Clinical Medicine* 2007;65:1539-1544.
57. Nurgalieva ZZ, Malaty HM, Graham DY, et al. Helicobacter pylori infection in kazakhstan; effect of water source and household hygeine. *American Journal of Tropical Medicine*

58. Mitipat N, Siripermpool P, Jadwattanakul T, et al. The prevalence of Helicobacter pylori infection in patients with gastrointestinal symptoms in Chon Buri, Thailand. Southeast *Asian Journal of Tropical Medicine and Public Health* 2005;36:341-346.
59. Moayyedi P, Forman D, Braunholtz D, et al. The proportion of upper gastrointestinal symptoms in the community associated with Helicobacter pylori, lifestyle factors, and nonsteroidal anti-inflammatory drugs. Leeds HELP Study Group. *American Journal of Gastroenterology* 2000;95:1448-1455.
60. Yamamoto N, Sakagami T, Fukuda Y, et al. Influence of Helicobacter pylori infection on development of stress-induced gastric mucosal injury. *Journal of Gastroenterology* 2000;35:332-340.