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International variation in *Helicobacter pylori* infection and rates of oesophageal cancer

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ARTICLE INFO

Article history:
Received 2 January 2008
Accepted 18 January 2008
Available online 14 February 2008

Keywords:
Helicobacter pylori
Oesophageal cancer

ABSTRACT

Aim: To examine the association between gastric atrophy, *Helicobacter pylori* and CagA status, and ratio of oesophageal squamous cell carcinoma to oesophageal adenocarcinoma (OSCC:OAC) amongst international heterogeneous populations.

Methods: Standardised protocols were used to collect and process questionnaire data and serum samples for PgA and PgC levels and *H. pylori* and CagA serology. The OSCC:OAC were used to construct appropriate models.

Results: There were significant correlations between the OSCC:OAC and both rates of low PgA:PgC ratios and serological markers of *H. pylori* infection, in males, but not females. A significant correlation between OSCC:OAC and overall CagA-seropositivity was seen in males, but not females, but this was not independently associated with increasing OSCC:OAC.

Conclusions: In males, populations with higher rates of gastric atrophy or *H. pylori* infection have a higher OSCC:OAC. CagA seropositivity rates seem to have no additional effect.

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0959-8049/$ - see front matter
doi:10.1016/j.ejca.2008.01.018

1. Introduction

Since the 1970s the incidence of gastro-oesophageal reflux has risen dramatically.[1] There has also been a rapid increase in the incidence of adenocarcinomas of the distal oesophagus. Oesophageal adenocarcinoma (OAC) now accounts for at least 50% of these cases in some populations.[2] It is well recognised that the incidence of OAC in males is higher than in females. There has also been a suggestion that the gender difference is increasing, with estimates of up to fivefold difference in age-standardised rates between males and females for OAC.[2,3] In contrast to the increasing OAC rates over the last 30 years, there has been a decrease in the incidence of gastric carcinoma[3] and peptic ulcer disease.[1] The latter two diseases are strongly associated with *Helicobacter pylori* infection[4]. In 1994, *H. pylori* was classified as a definite cause of human gastric cancer.[5] Although 30–50% of populations in developed countries are still infected with *H. pylori*, rates of infection have declined significantly.[6] This decrease in *H. pylori* infection may be contributing to the rising rates of OAC, although the exact mechanisms for this association are unclear.

The development of gastric atrophy and subsequent hypoacidity with *H. pylori* infection may protect against reflux oesophagitis.[7] Clinical studies indicate an inverse association between *H. pylori* infection and reflux oesophagitis,[7] Barrett's oesophagus[8,9] and oesophageal adenocarcinoma.[8,9] Approximately 50% of *H. pylori*-infected subjects have some degree of atrophic gastritis, with up to 15% progressing to develop advanced corpus gastritis.[7] There are two main types of pepsinogen – A (PgA – also known as PgI) and C (PgC – also known as PgII). Both are produced by the chief and mucous neck cells in the gastric fundus and corpus, but only PgC is produced by the pyloric glands in the antrum. PgA is, therefore, an indirect measure of the corpus mass. With the progression of gastritis, the ratio between PgA levels and PgC levels decreases markedly. A low PgA:PgC ratio is an established predictor of chronic atrophic gastritis.[10] Both low serum levels of PgA (less than 25 ng/ml) and low PgA:PgC ratio (less than 2.5) are associated with an increased risk of gastric cancer.[11]

The outcome of *H. pylori* infection is determined by host, bacterial factors and environmental factors such as diet. There is an increasing evidence that infection with strains of *H. pylori* that possess the *cag* pathogenicity island (PAI) are associated with increased risk of atrophic gastritis,[12] peptic ulcer disease[13] and gastric cancer.[14,15] In contrast, in some studies *cag* PAI-positive *H. pylori* infections have been negatively associated with OAC[16] and Barrett's oesophagus,[8,17] compared to *cag* PAI-negative *H. pylori* infections. However, other studies have found no evidence of a protective effect of *cag* PAI-positive *H. pylori* infections on OAC.[14,18]

The EUROGAST study was set-up to investigate the relationship between upper gastrointestinal cancer rates and biological markers of gastritis in randomly selected, international populations at varying risk of gastric cancer. The aims of the analysis reported in this paper were threefold: (1) to investigate the relationship between PgA:PgC ratios (as a surrogate marker for the presence or the absence of chronic atrophic gastritis) and ratio of oesophageal squamous cell carcinoma:oesophageal adenocarcinoma (OSCC:OAC); (2) to investigate the association between *H. pylori* infection rates and OSCC:OAC; and (3) to establish whether rates of CagA seropositivity altered any association observed between *H. pylori* infection rates and OSCC:OAC. The reason for using OSCC:OAC is explained in Section 2.

2. Materials and methods

2.1. Study population

The methodology of the EUROGAST study has been described in detail previously.[19] In brief, 17 study centres from 13 different countries were chosen to reflect the global range of gastric and oesophageal cancer incidence and mortality rates. In each centre, approximately 200 subjects were randomly selected from the general population using population-based registers, general practitioners' lists (United Kingdom), driver's licence rosters (United States), or health screening programmes (Greece) and invited by letter and/or telephone to take part in the study. Every centre in the study aimed to recruit 50 men and 50 women in each of two age groups, 25–34 years and 55–64 years. Non-respondents were followed up with letters, telephone calls and home visits. Individuals who refused to participate or were unable to be contacted were replaced by additional subjects of the same sex and in the same age group. Participants provided a blood sample and answered, by personal interview, a short standardised questionnaire concerning socio-demographic details. Blood samples were collected, processed, transported and stored at −80 °C according to a common protocol.

2.2. Oesophageal cancer data

Data from high quality cancer registries were available in the publication 'Cancer Incidence in Five Continents' (CI5VIII).[20] In centres with low reported numbers of oesophageal cancer, and high levels of 'unknown' histology a disproportionate effect occurs on the age-standardised rate (ASR) of individual types of cancer, making direct comparisons of rates statistically unsound. Therefore, although ASR of both OAC and oesophageal squamous cell carcinoma (OSCC) of the oesophagus were calculated for each EUROGAST study centre for a period from 1993 to 1997 for use in sensitivity analyses, the ratio of squamous carcinomas to adenocarcinomas of the oesophagus (OSCC:OAC) was calculated for each study centre so as to allow direct comparison. CI5VIII registries did not always exactly match the EUROGAST study centres. Therefore, where an exact match could not be made, a surrogate CI5VIII registry was selected as a source of oesophageal cancer incidence data. These collected data were from either geographical areas in which the relevant EUROGAST study centre was located, or from a geographically adjacent area.

2.3. Serology

Where enough total serum was available, samples from patients were analysed for *H. pylori* immunoglobulin G (IgG) and CagA IgG by Western blot analysis. This assay was validated using sera from subjects with histologically proven *H. pylori* associated chronic gastritis and subjects with

histologically normal gastric mucosa. PgA and PgC levels were measured using a radioimmunoassay. A PgA:PgC ratio of <2.5 was considered indicative of the presence of chronic atrophic gastritis.[10]

2.4. Data analysis

For each study centre, overall population seroprevalence of *H. pylori* and CagA IgG (independent of *H. pylori* status) was calculated by averaging the seroprevalence in the 2 age groups. In general, the incidence of *H. pylori* infection increases as a population gets older, therefore this procedure adjusted for any effect which may be due to different median ages that may exist between study centres, i.e it is an age-standardisation process. As gastric atrophy increases with age similar standardisation was undertaken when calculating 'overall' frequency of low PgA:PgC ratio for each study centre. As the OSCC:OAC is not normally distributed, it was log transformed prior to analysis. Models were created with the natural log of the sex-specific OSCC:OAC as the dependent variable and with frequency of low PgA:PgC ratio (<2.5), the seroprevalence of anti-*H. pylori* IgG and overall rates of CagA seropositivity as the independent variables.

Models were also constructed with the ASR of both OSCC and OAC as the independent variables with the above dependent variables to clarify whether any significant correlations seen with OSCC:OAC in the above models were due to changes in OAC rates only, OSCC rates only or both.

A number of socio-demographic variables were calculated including the following: highest level of education received (higher, secondary or primary education); smoking status (never, ex-smoker, current smoker); alcohol consumption (any versus none in the week before sample collection); and body mass index (weight[kg]/height[m]^2); all were classified into three groups: low, medium and high, separately for men and women within each study centre. Statistical Package for Social Sciences Software (SPSS 10.1 for Windows) was used for data analysis.

2.5. Statistics

For each model created, linear regression was applied and Pearson's correlation coefficient was calculated. Confounding socio-demographic variables were tested by means of entering all of the relevant variables (in both a stepwise forward and backward manner) into a multivariate linear regression of each of the models. Sensitivity analyses were conducted to examine the effects of excluding centres with high numbers of known histology and/or low absolute numbers of reported cases of oesophageal cancer. All p values quoted are two-tailed. A p value of less than or equal to 0.05 was considered statistically significant.

3. Results

3.1. Oesophageal cancer incidence

Of the 17 centres originally investigated, three centres had no independently verified regional data on oesophageal cancer. Thus, 14 EUROGAST centres, with the relevant CI5VIII registry were used to calculate appropriate data. These are shown in Table 1.

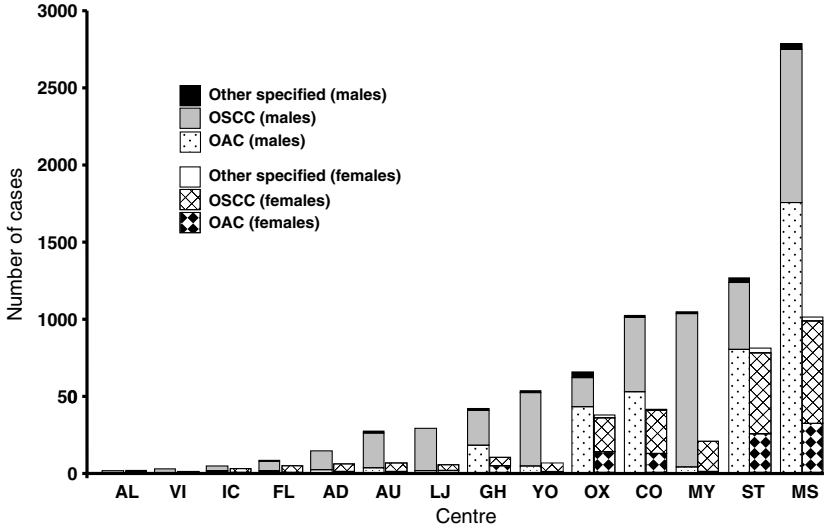
Within these 14 centres (Fig. 1), overall crude cancer incidence rates varied from 0.62/100,000 to 25/100,000 in males, and from 0.35/100,000 to 9.0/100,000 in females. The percentage of adenocarcinomas (as a proportion of histologically confirmed oesophageal cancers) varied from 3% to 66% in males and from 2% to 40% in females (Fig. 1).

3.1.1. H. pylori seropositivity

Both *H. pylori* and CagA serology results were available for 2212 subjects (1116 in the 25–34 age group and 1096 in the 55–64 age group) from the 14 centres studied. Of these, 1360 (61.5%) subjects tested were *H. pylori* +ve, of which 880 (64.7%; 448 male, 432 female) were also CagA +ve. There were a small number of subjects (n = 40; 1.8%; 18 male, 22 female) who were *H. pylori* –ve/CagA +ve. All of these subjects had a

Table 1 – CI5VIII centre used to calculate oesophageal cancer incidence data for each EUROGAST study centre included in analysis

Study centre	CI5VIII centre	Years of registry	Males			Females		
			OSCC:OAC	ASR OAC	ASR OSCC	OSCC:OAC	ASR OAC	ASR OSCC
Adamowka	Warsaw City	1993–1997	4.5	0.45	2.2	6.7	0.07	0.57
Algiers	Algiers	1993–1997	1.0	0.26	0.23	2.3	0.08	0.23
Augsburg	Saarland	1993–1997	5.7	0.94	5.3	8.7	0.07	0.98
Copenhagen	Denmark	1993–1997	0.90	2.5	2.5	2.3	0.37	1.1
Florence	Florence	1993–1997	3.6	0.29	1.3	13	0.04	0.50
Gaia	Vila Nova de Gaia	1993–1997	31	0.14	4.1	8.0	0.07	0.70
Ghent	Flanders (excl. Limburg)	1997–1998	1.2	2.2	2.9	1.4	0.33	0.54
Ljubljana	Slovenia	1993–1997	13	0.34	4.4	3.6	0.10	0.37
Minneapolis-St Paul	SEER	1993–1997	0.92	2.4	2.3	2.8	0.31	0.92
Miyagi	Miyagi Prefecture	1993–1997	25	0.44	11	39	0.04	1.7
Oxford	Oxford region	1993–1997	0.42	4.6	1.9	1.7	0.89	1.6
S. Region	Iceland	1993–1997	2.0	1.5	3.2	11	0.11	1.7
Stoke	West Midlands	1993–1997	0.54	3.8	2.0	2.1	0.74	1.8
Yokote	Yamagata Prefecture	1993–1997	10	0.78	7.8	8.7	0.05	0.62



* NB Ghent (GH) cases accumulated only over 2 years (1997-98).

Fig. 1 – Cumulative crude oesophageal cancer incidence rates by subtype and gender within the 14 centres included in the final analysis. Centre codes: AD, Adamowka; AL, Algiers; AU, Augsburg; CO, Copenhagen; FL, Florence; GH, Ghent; IC, Iceland; LJ, Ljubljana; MS, Minneapolis-St Paul; MY, Miyagi; OX, Oxford; ST, Stoke; VI, Vila Nova de Gaia; YO, Yokote.

serum PgA:PgC ratio of less than 2.5 indicating gastric atrophy. Twenty-eight of these 40 subjects were in the younger age group. Data on *H. pylori* and CagA seropositivity rates in all EUROGAST study centres have already been published.[21]

3.2. *Association with oesophageal cancer histological ratio*

3.2.1. *Serum pepsinogens*

For males, but not females, there was a statistically significant inverse association between population prevalence of a low PgA:PgC ratio (i.e. less than 2.5) and OSCC:OAC ($p < 0.002$ for males; $p = 0.09$ for females; Fig. 2A and B).

3.2.2. *H. pylori* and CagA seropositivity

Again for males, but not females, there was a significant correlation between the OSCC:OAC and the population prevalence of *H. pylori* +ve subjects ($p = 0.01$ males; $p = 0.14$ females; Fig. 3A and B). A significant correlation between prevalence of CagA +ve subjects (regardless of *H. pylori* status) and OSCC:OAC was also observed in males ($p = 0.02$), but no statistically significant association was seen in females ($p = 0.65$) (data not shown).

This association seen between prevalence of CagA +ve subjects and OSCC:OAC in male subjects could reflect the high proportion of those subjects who were *H. pylori* +ve also being CagA +ve. Therefore, the proportion of *H. pylori* +ve subjects (i.e. *H. pylori* +ve/CagA +ve plus *H. pylori* +ve/CagA –ve) within a given population that were also CagA +ve (i.e. *H. pylori* +ve/CagA +ve) was also used as an independent variable for the constructed model. No correlation was observed in males with this model ($p = 0.87$, Fig. 3C), implying that the presence of positive CagA serology was not independently

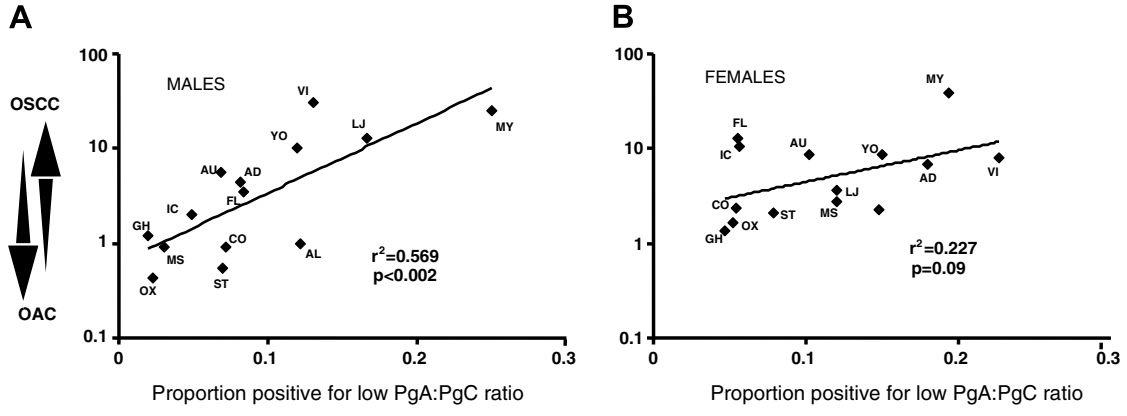


Fig. 2 – Models (unadjusted) showing natural log of sex-specific OSCC:OAC ratios as the dependent variable and proportion of population positive for low (<2.5) PgA:PgC ratio as the independent variable. Pearson's correlation coefficient was calculated for each model.

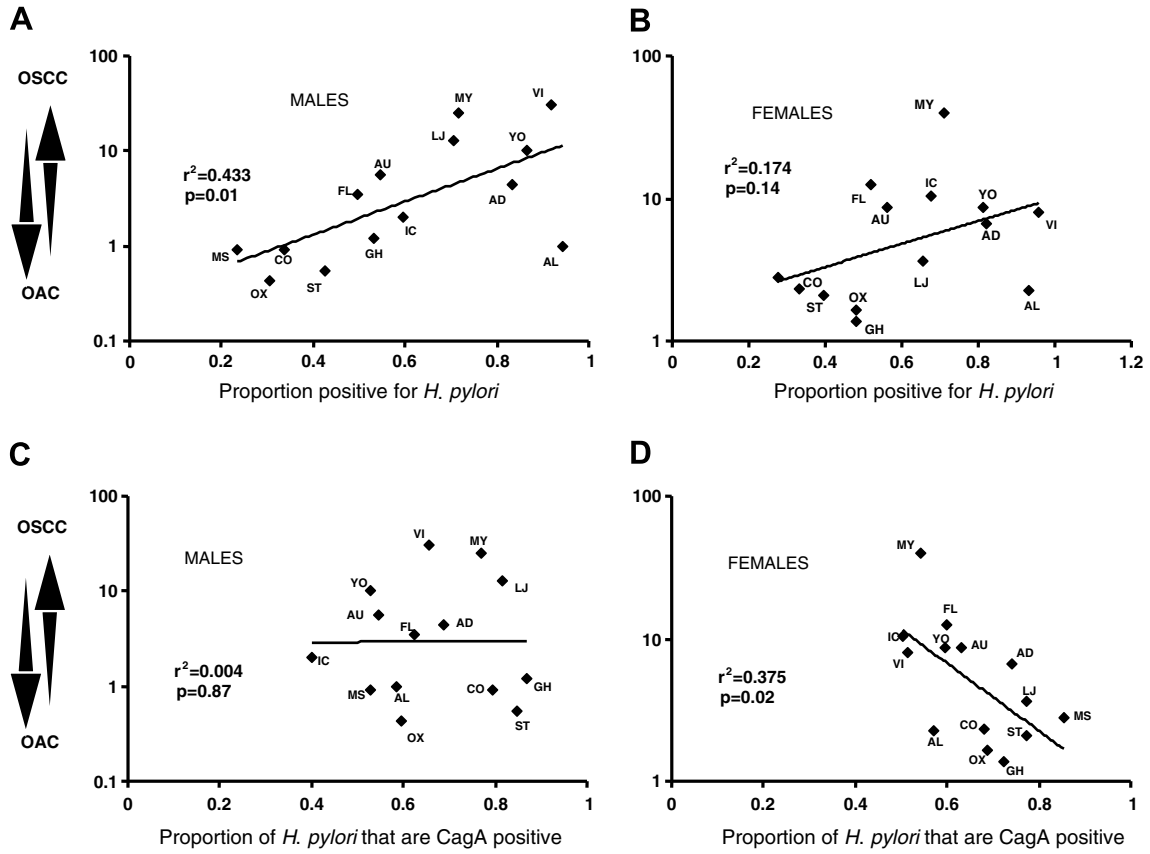


Fig. 3 – Models (unadjusted) showing natural log of sex-specific OSCC:OAC ratios as the dependent variable and either (A, B) proportion of population with positive *H. pylori* serology as the independent variable or (C, D) proportion of those with positive *H. pylori* serology who also have positive CagA serology as the independent variable. Pearson's correlation coefficient was calculated for each model.

associated with OSCC:OAC, but merely a reflection of the population prevalence of *H. pylori* seropositivity. In females, there was a strong inverse correlation between OSCC:OAC and *H. pylori* +ve/CagA +ve serology ($p = 0.02$, Fig. 3D).

3.2.3. *Sensitivity analyses*

In males, sensitivity analyses did not alter the above findings. Replacement of OSCC:OAC with the ASR of OAC as the dependent variable did not alter the above findings in males, yet no correlation was seen in any model when ASR of OSCC was substituted as the dependent variable. For females, results were inconsistent, and depending on which sensitivity analysis was conducted there were variable levels of correlation or not. Although in the original models there was a strong inverse association between OSCC:OAC and *H. pylori* +ve/CagA +ve serology in females, this was less frequently significant than not during sensitivity analysis.

3.2.4. *Socio-demographic factors*

Construction of multivariate linear regression models using the mean population prevalence of the levels of the years of education, of smoking, of alcohol consumption and of body mass index (BMI) showed no significant effect on the original models constructed (data not shown).

4. Discussion

This study has demonstrated a statistically significant association in males between *H. pylori* infection rates and OSCC:OAC rates in 14 geographically diverse international populations. Whilst our data cannot wholly discriminate whether this is due to a negative correlation with OAC, a positive correlation with OSCC or both, it suggests that there is a significant inverse correlation between *H. pylori* infection rates and OAC. Our data also indicate that the development of gastric atrophy in males, as assessed using serum pepsinogen ratios and thus, by implication, a less acidic refluxate is inversely associated with OAC. The data imply that, in males, prevalence of CagA +ve subjects has no effect over and above the prevalence of *H. pylori* +ve subjects with regard to the rate of OAC, within any given population. The finding of the lack of association between CagA positivity and OAC is consistent with the recent publication showing lack of association between CagA positivity and acid secretion in *H. pylori* infection.[22] The female data are inconsistent and no meaningful conclusions can be drawn. Whilst this might reflect a genuine lack of association, it may simply be due to the smaller numbers of reported oesophageal cancers in women (Fig. 1).

Interpretation of geographical correlations, in studies such as this, cannot provide definitive evidence of cause and effect, because of the possibility of confounding factors affecting both the rates of *H. pylori* infection and OAC. Low socio-economic status is known to be strongly related to the risk of both *H. pylori* infection,[23] and oesophageal cancer.[24] Level of education, though not perfect, is often used as a surrogate measure of socio-economic status. In this study, the construction of multivariate linear regression models using socio-demographic data had no significant effect on the original models constructed. As serological markers of *H. pylori* infection are known to be associated with low socio-economic status,[23] it may be that serological evidence of *H. pylori* infection and socio-economic status are co-linear, and hence no additional effect of the level of education will be seen. This also raises the possibility that the effect noted on OSCC:OAC is due to low socio-economic status, but this does not explain the association with low PgA:PgC ratios. The lack of association with obesity rates as measured by BMI reflects that obesity is less important than factors such as *H. pylori* status, although the measurement of waist circumference (as a crude marker of adipose tissue distribution) may have been a better marker.[25]

Whilst it has been reported that there is no inverse association between *H. pylori* infection and risk of OAC[14]; the general consensus is that *H. pylori* is associated with a reduced risk of OAC[9,18] though the mechanisms for this association are controversial. One postulated mechanism is the development of atrophic corpus gastritis due to chronic *H. pylori* infection resulting in a less acidic refluxate. Whilst this theory seems to be supported by studies, which report a negative correlation between *H. pylori* infection and reflux oesophagitis,[26] and by the results of this study, other factors may be involved.[18] Other protective mechanisms which have been proposed include ammonia production by *H. pylori* (which will neutralise gastric acid) and *H. pylori*-induced elevation of gastrin mediating increases in lower oesophageal sphincter tone. In Ye et al.'s study of Swedish subjects[18], whilst *H. pylori* infection was statistically associated with a reduced risk of OAC, gastric atrophy (as indicated by a low level of pepsinogen A) was not-consistent with previous studies showing no decreased risk for OAC in patients with long-term achlorhydria secondary to pernicious anaemia.[27] Another possible explanation considers the distribution of *H. pylori* infection within the stomach and this is discussed below.

The potential role of *cag* PAI status has also been considered. Incidence of oesophagitis, Barrett's oesophagus and OAC have all been shown to be decreased in those patients infected with *cagA* positive *H. pylori* versus those with both *cagA* negative infections and *H. pylori* negative controls.[8,16,17] Ye et al. suggested that CagA status was more important than *H. pylori* status per se.[18] The inverse association reported between CagA positive serology and OAC observed previously[8,16,17] was not confirmed in this study. These former studies involved patient populations from single centres. In contrast, the present international study included subjects from 14 globally diverse populations. In the studies of Vicari et al.[8] and Vaezi et al.,[17] patients with peptic ulcer disease were excluded. *H. pylori* strains which possess the *cag* PAI are known to be associated with duodenal ulceration[28]. Exclusion of such patients generates a potential inherent bias towards an inverse association between CagA status and gastro-oesophageal reflux related oesophageal disease. Indeed, recently published data based on the Swedish Inpatient Register confirms that patients with a history of duodenal ulceration have a significant 70% excess risk of OAC[29], indirectly supporting our hypothesis that hypoacidic reflux secondary to gastric atrophy is less likely to result in future development of OAC than acidic reflux. However, the same study did also show a strong excess risk of OSCC with previous gastric ulceration and it is highly likely that gastric distribution of *H. pylori* is a crucial determinant as to which (if any) type of oesophageal cancer a subject will develop. Whilst some inference of *H. pylori* distribution can be made from PgA:PgC levels, our study is not designed to look at this aspect.

In conclusion, this international population-based study demonstrates that in those populations with higher rates of *H. pylori* infection, the ratio of OSCC:OAC is higher in males. Prevalence, or otherwise, of CagA +ve serology within a population does not have any additional effect on this ratio. The pepsinogen data suggest that an increased incidence of gastric atrophy within a population may protect that population from developing OAC, although this may be an indirect marker of *H. pylori* distribution.

Conflict of interest statement

None declared.

Acknowledgements

Studies in the laboratory of JEC are supported by Yorkshire Cancer Research and the European Commission Sixth Framework Project INCA (LSHC-CT-2005-018704).

We thank Beena Neelam for expert technical assistance.

REFERENCES

1. El-Serag HB, Sonnenberg A. Opposing time trends of peptic ulcer and reflux disease. *Gut* 1998;**43**:327–33.
2. Powell J, McConkey CC, Gillison EW, et al. Continuing rising trend in oesophageal adenocarcinoma. *Int J Cancer* 2002;**102**:422–7.
3. Kocher HM, Linklater K, Patel S, et al. Epidemiological study of oesophageal and gastric cancer in south-east England. *Br J Surg* 2001;**88**(9):1249–57.
4. The Eurogast Study Group. An international association between *Helicobacter pylori* infection and gastric cancer. *Lancet* 1993;**341**:1359–62.
5. International Agency for Research on Cancer. Schistosomiasis, liver flukes and *Helicobacter pylori*. *IARC monographs on the evaluation of carcinogenic risks to humans*. Lyon: IARC; 1994.
6. Blaser MJ. Hypothesis: the changing relationships of *Helicobacter pylori* and humans: implications for health and disease. *J Infect Dis* 1999;**179**:1523–30.

7. Manes G, Mosca S, Laccetti M, et al. *Helicobacter pylori* infection, pattern of gastritis and symptoms in erosive and nonerosive gastroesophageal reflux disease. *Scand J Gastroenterol* 1999;**34**:658–62.
8. Vicari JJ, Peek RM, Falk GW, et al. The seroprevalence of cagA-positive *Helicobacter pylori* strains in the spectrum of gastroesophageal reflux disease. *Gastroenterology* 1998;**115**:50–7.
9. Weston AP, Badr AS, Topalovski M, et al. Prospective evaluation of the prevalence of gastric *Helicobacter pylori* infection in pateints with GERD, Barrett's esophagus, Barrett's dysplasia, and Barrett's adenocarcinoma. *Am J Gastroenterol* 2000;**95**:387–94.
10. Broutet N, Plebani M, Sakarovitch C, et al. Pepsinogen A, Pepsinogen C, and gastrin as markers of atrophic chronic gastritis in European dyspeptics. *Br J Cancer* 2003;**88**: 1239–47.
11. Webb PM, Hengels KJ, Möller H, et al. The epidemiology of low serum pepsinogen A levels and an international association with gastric cancer rates. *Gastroenterology* 1994;**107**:1335–44.
12. Maaroos H-I, Vorobjova T, Sipponen P, et al. An 18-year follow-up study of chronic gastritis and *Helicobacter pylori*: association of CagA positivity with development of atrophy and activity of gastritis. *Scand J Gastroenterol* 1999;**34**:864–9.
13. Van Doorn L-J, Figueiredo C, Sanna R, et al. Clinical relevance of the cagA, vacA, and iceA status of *Helicobacter pylori*. *Gastroenterology* 1998;**115**:58–66.
14. Wu AH, Crabtree JE, Bernstein L, et al. Role of *Helicobacter pylori* CagA+ strains and risk of adenocarcinoma of the stomach and esophagus. *Int J Cancer* 2003;**103**:815–21.
15. Peek RM, Crabtree JE. Helicobacter infection and gastric neoplasia. *J Pathol* 2006;**208**:233–48.
16. Chow WH, Blaser MJ, Blot WJ, et al. An inverse relation between cagA+ve strains of *Helicobacter pylori* infection and risk of esophageal and gastric cardia adenocarcinoma. *Cancer Res* 1998;**58**:588–90.
17. Vaezi MF, Falk GW, Peek RM, et al. CagA-positive strains of *Helicobacter pylori* may protect against Barrett's esophagus. *Am J Gastroenterol* 2000;**95**:2206–11.
18. Ye W, Held M, Lagergren J, et al. *Helicobacter pylori* infection and gastric atrophy: risk of adenocarcinoma and squamous-cell carcinoma of the esophagus and adenocarcinoma of the gastric cardia. *J Natl Cancer Inst* 2004;**96**:388–96.
19. Forman D, Webb P, Parsonnet J. *H. pylori* and gastric cancer. *Lancet* 1994;**343**(8891):243–4.
20. Parkin DM, Whelan SL, Ferlay J, Teppo L, Thomas DB. Cancer incidence in five continents, vol. VIII. Lyon: IARC Scientific Publications; 2002.
21. Webb PM, Crabtree JE, Forman D. Gastric cancer, cytotoxin-associated gene A-positive *Helicobacter pylori*, and serum pepsinogens: an international study. The Eurogast Study Group. *Gastroenterology* 1999;**116**(2):269–76.
22. Derakhshan MH, El-Omar E, Oien K, et al. Gastric histology, serological markers and age as predictors of gastric acid secretion in patients infected with *Helicobacter pylori*. *J Clin Pathol* 2006;**59**(12):1293–9.
23. Rosenstock SJ, Andersen LP, Rosenstock CV, et al. Socioeconomic factors in *Helicobacter pylori* infection among Danish adults. *Am J Public Health* 1996;**86**(11):1539–44.
24. Bosetti C, Franceschi S, Negri E, et al. Changing socioeconomic correlates for cancers of the upper digestive tract. *Ann Oncol* 2001;**12**(3):327–30.
25. Corley DA, Kubo A, Levin TR, et al. Abdominal obesity and body mass index as risk factors for Barrett's esophagus. *Gastroenterology* 2007;**133**(1):34–41.
26. Raghunath A, Hungin AP, Wooff D, et al. Prevalence of *Helicobacter pylori* in patients with gastro-oesophageal reflux disease: systematic review. *BMJ* 2003;**326**:737.
27. Ye W, Nyrén O. Risk of cancers of the oesophagus and stomach by histology or subsite in patients hospitalised for pernicious anaemia. *Gut* 2003;**52**:938–41.
28. Chen XJ, Yan J, Shen YF. Dominant cagA/vacA genotypes and coinfection frequency of *H. pylori* in peptic ulcer or chronic gastritis patients in Zhejiang Province and correlations among different genotypes, coinfection and severity of the diseases. *Chin Med J (Engl)* 2005;**118**(6):460–7.
29. Bahmanyar S, Zendehdel K, Nyren O, et al. Risk of oesophageal cancer by histology among patients hospitalised for gastroduodenal ulcers. *Gut* 2007;**56**(4):464–8.