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Correlation of Cag-A serological status with histological parameters of chronic gastritis among dyspeptic patients in south western Nigeria

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Abstract

Background: The aim of this study was to determine the sero-prevalence of Cag-A strains of *Helicobacter pylori* in both dyspeptic and non-dyspeptic individuals and also correlate the serological status of Cag-A strain of *H. pylori* with the various graded histological variables of chronic gastritis in the dyspeptic patients.

Methods: Using helicobacter p120 Cag-A enzyme linked immunosorbent assay, Cag-A serology test was carried out on 65 dyspeptic patients and 65 age and sex matched non-dyspeptic controls. The gastric biopsies of the patients were also histologically examined to ascertain the presence, nature and degree of the following histological variables of gastritis: colonisation by *H. pylori*; inflammation, intestinal metaplasia and mucosal atrophy. The CagA serological status was then correlated with the graded variables.

Results: A prevalence of 46.2% and 58.8% seropositivity for Cag-A strain of *H. pylori* was found among dyspeptic patients and control individuals respectively. Cag-A seropositive patients accounted for nine(81.8%) of the 11 cases with moderate to severe activity and 75% of both cases with mucosal atrophy and cases with intestinal metaplasia.

Conclusion: Infection with Cag-A positive *Helicobacter pylori* was equally prevalent among both dyspeptic patients and control subjects studied. CagA seropositivity, however, appeared to be associated with higher inflammatory activity in the mucosa of patients with chronic gastritis and may be associated with intestinal metaplasia and mucosal atrophy in *H. pylori*-induced chronic gastritis.

Keywords: *CagA status H. pylori chronic gastritis Nigeria*

Abstrait

Contexte: Le but de cette étude était de déterminer la séroprévalence des souches du Cag-A s de l'Helicobacter pylori tant chez les individus dyspeptiques que chez des non-dyspeptiques et aussi en corrélation avec le statut sérologique de Cag-A, une souche de H. pylori avec diverses variables histologiques graduées de la gastrite chronique chez les patients dyspeptiques.

Méthodes: L'utilisation d'Helicobacter p120 Cag-A, un dosage immunoenzymatique, le Cag-A, un test de sérologie sur 65 patients dyspeptiques âgés de 65 ans et sur les contrôles de sexe non-dyspeptiques correspondant a été fait. Les biopsies gastriques des patients ont également été histologiquement examinés pour déterminer la présence, la nature et le degré de suivantes variables histologiques de gastrite: la colonisation par H. pylori, l'inflammation, la métaplasie intestinale et l'atrophie de la muqueuse. Le statut sérologique du Cag-A a ensuite été corrélé avec les variables gradués.

Résultats: Une prévalence de 46,2% et 58,8% pour la séropositivité Cag-A, une souche de H. pylori a été retrouvée chez les patients dyspeptiques et contrôle les individus respectivement. CAG-A Les patients séropositifs du CAG-A représentaient neuf (81,8%) des 11 cas avec une activité modérée aux activités dure et 75% des cas avec à la fois de cas d' atrophie de la muqueuse et des cas de métaplasie intestinale.

Conclusion: L'infection par le Cag-A Helicobacter pylori positif était également répandue à la fois chez les patients dyspeptiques et les sujets cotrolés étudiés. CagA séropositivité, cependant, semblait être associée à l'activité inflammatoire élevée dans la muqueuse des patients souffrant de gastrite chronique et peut être associé à une métaplasie intestinale et à l'atrophie de la muqueuse de H. pylori-inclus à la gastrite chronique.

Introduction

Helicobacter pylori is a gram negative micro-aerophilic spiral bacillus that colonizes between 20% - 80% of the world's population and causes chronic gastritis, peptic ulceration, gastric adenocarcinoma, and gastric lymphoma [1]. It was therefore classified in October 1994 by the World Health Organisation as a category I carcinogen [2].

Its prevalence among middle-aged adults is over 80% in many developing countries, as compared with 20 to 50% in industrialized countries [2,3]. In Nigeria, the prevalence rate of infection with *H. pylori* has been reported to be between 60.5% and 94.5% depending on the region and on the method of evaluation used [4-8]. While the prevalence of the infection has dropped signiûcantly in many parts of

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North America, Western Europe and Asia (especially Korea), no such decline has been noted in the developing world [9]. The overall prevalence of infection is strongly correlated with socioeconomic conditions [10].

All the strains of *H. pylori* cause gastric inflammation, however, only 15% of infections result in peptic ulceration and only 0.5%–2% in gastric adenocarcinoma [9]. Who develops disease depends upon three main factors namely: the virulence of the infecting *H. pylori* strain; the type and extent of the host immune response to infection, and modulating cofactors such as smoking and diet [9]. Of the virulence factors studied the most important is the approximately 140 kDa Cytotoxin associated antigen A (Cag-A) protein, which is encoded by an gene that forms part of the cag Pathogenocity Island (*cag-PAI*) a region in the *H. pylori* genome that encodes other virulence factors like cag E, F, G, H, L, M, and N. This gene is present in approximately 50 to 70% of *H. pylori* strains [11]. The prevalence rate of CagA-positive strains among chronic gastritis patients is also highly variable from one geographic region to another. The reported figures vary from about 30-40% in Amsterdam, Mexico and Malaysia to between 59-97% in several other regions [12-16]. In Nigeria, data on the prevalence of CagA-positive *H. pylori* strain is sparse with three studies reporting prevalence rates of 68-90% among dyspeptic patients [17-19].

An increasing number of studies have shown a close association between CagA antibodies and the development of duodenal ulcer and gastric cancer [20-22]. *H. pylori* strains with *cag*-PAI tend to cause a more intense inflammatory response of the gastric mucosa, leading to increased tissue damage and higher risk of developing atrophic gastritis, precancerous lesions, and gastric cancer [23,24]. The CagA protein is the main molecule injected into epithelial cells by a type IV secretion system; it is a highly immunogenic protein which is responsible for many of the cytotoxic and proinflammatory activities. CagA is regarded as a marker for the *cag*-PAI [25].

Infection with CagA positive strains of *H. pylori* have been shown to be positively correlated with clinical outcome among Caucasians [26] but the association in Asians and Africans have not been consistent [27,28]. Given the high prevalence of *H. pylori*-associated chronic gastritis in Nigeria [29,30], the reported high prevalence of CagA strains in Nigerian patients with gastro-duodenal diseases [17-19], the reported association of the CagA strains with severe forms of gastritis and adverse clinical outcomes [20,23,24,31], we investigated the prevalence of CagA-positive *H. pylori* strains. We also studied the association between CagA seropositivity and the histological forms of chronic gastritis in Nigerian patients in view of the dearth of this knowledge. This is the first study in Nigeria to our knowledge to have studied the association of this virulence factor with histological parameters.

Materials and methods

This is a prospective serological study involving 65 consecutive adult patients with dyspeptic symptoms who underwent upper gastrointestinal endoscopy at the Gastrointestinal and Liver Unit of the Department of Medicine, University College Hospital, Ibadan, Nigeria and the Serology unit of the Department of Virology of the institution.

The patients who were previously treated for *H. pylori* infection or who had received antibiotics, proton pump inhibitors or bismuth compounds in the preceding 4 weeks were excluded. Oesophago-gastro-duodenoscopy (OGD) was performed on all the participants using Olympus (GFIXQ20) or Pentax (FG29W) forward-viewing Oesophago-gastro-duodenoscope. A minimum of two gastric antral mucosal biopsies were taken from each patient for histology.

The endoscopic biopsies were fixed in 10% formaldehyde and transferred to the histopathology laboratory for processing. Four micron thick paraffin sections were stained with routine Haematoxylin and Eosin for diagnosis of chronic gastritis. Slides were examined microscopically for the histological changes of gastritis and two of the histological variables (degree of chronic inflammation and activity) were graded according to the semi-quantitative ordinal scale of the updated Sydney classification [32] while the mucosal atrophy and intestinal metaplasia were graded as either present or absent.

The presence/absence of serum anti-CagA immunoglobulin G (IgG) to Helicobacter Hp-120 protein (120kD CagA protein) by enzyme linked immunosorbent assay (ELISA) and the results were recorded as either positive or negative. Anti-CagA serology test was also performed on blood samples obtained from 65 randomly selected age- and sex-matched healthy controls.

The data was analyzed using Statistical Package for Social Sciences, version 16.0 (SPSS Inc. Chicago Illinois). The results were presented as means ± standard deviation for quantitative variables and number (percentages) for qualitative variables. Categorical variables were compared with Pearson's Chi-square. Significant P-value was taken as <0.05.

Ethical approval was obtained from the University of Ibadan/University College Hospital instritional review committee.

Results

The 65 patients recruited in this study were between the ages of 20 and 78 years with an average age of 47.7 ± 16.7 yrs. There were 32 males and 33 females giving a ratio of about 1:1(Table 1)

Table 1: Patients' gender and CagA sero-status

Sex	Male	Female	Total
CagA test^{-ve}	17	18	35
CagA test^{+ve}	15	15	30
Total	32	33	65

A total of thirty patients were positive for the anti-CagA while thirty-five were negative, giving a 46.2% and 53.8% prevalence of anti-Cag seropositivity and seronegativity in chronic gastritis patients respectively. However, among the control group, thirty-eight cohorts (58.5%) were seropositive as against twenty-seven (41.5%) seronegative individuals.

The degree of inflammation were graded into mild, moderate and marked according to the revised Sydney System [32]. Thirty-two patients (49.2%) had mild inflammation in their gastric biopsies, twenty-four (36.9%) had moderate chronic gastritis while nine (13.8%) had severe gastritis (Table 2).

Table 2: CagA Sero-status and degree of inflammation

Degree of inflammation	Mild	Moderate	Severe	Total
CagA test^{-ve}	17	12	6	35
CagA test $^{+ve}$	15	12	3	30
Total	32	24	9	65

The ratio of subjects with more severe inflammation (i.e. Moderate and severe inflammation) as compared to mild inflammation was about equal in both CagA- positive and -negative groups (1/1.1). This variable did not show statistical significance with CagA serological status. ($X^2 = 0.75$; p value = 0.69).

Table 3: CagA Sero-status and activity

Neutrophilic infiltrate	Nil	mild	Moderate	Severe	Total
CagA test^{-ve}	28	5	1	1	35
CagA test^{+ve}	12	9	7	2	30
Total	40	14	8	3	65

The activity was graded into nil, mild, moderate and severe. Overall, the gastric biopsies of CagA positive patients had relatively higher grades of activity than those from seronegative patients (81.8% and 18.2% respectively of cases with moderate to severe grades of activity) and these differences were statistically significant ($X^2 = 12.06$; $p < 0.01$) (Tables 3).

Table 4: CagA sero-status and intestinal metaplasia

Intestinal metaplasia	Absent	Present	Total
CagA test^{-ve}	34	1	35
CagA test^{+ve}	27	3	30
Total	61	4	65

Intestinal metaplasia and mucosal atrophy were graded as either present or absence. The reason being that there were very few of the gastric biopsies (7 in total) showing these changes. From our findings, more CagA-positive patients had intestinal metaplasia and mucosal atrophy in their gastric biopsies than CagA negative patients (intestinal metaplasia: 3(75%) versus 1(25%); mucosal atrophy 3(75%) versus 1(25%)), although the difference was not statistically significant($X^2 = 1.43$; p value = 0.23) (Tables 4 and 5).

Table 5: CagA sero-status and atrophy

Atrophy	absent	Present	Total
CagA test^{-ve}	34	1	35
CagA test^{+ve}	28	3	30
Total	62	4	65

Discussion

Gastric cancer is a multi-staged process evolving over a period of time. It is initiated following a persistent *H. pylori* infection, with an initial chronic active inflammation, then evolving through stages of atrophic gastritis, intestinal metaplasia, dysplasia and then gastric cancer, with the progression of the disease taking decades. The major role played by *H. pylori* infection at the initial steps of disease, can result in tissue damage, leading to alterations in cell cycle and damage to DNA [33,34]. This risk in the course of chronic aggression to the gastric epithelia has been found to be facilitated by presence of *H. pylori* virulence factors in conjunction with other cofactors including host cytokine gene polymorphism [35,36].

An association of *H. pylori* virulence factors with an increasing risk for duodenal ulcer and gastric cancer has been observed, particularly with *H. pylori* strains possessing the CagA pathogenicity island *cag*-PAI [37]. Most studies show increased odds ratios in the 2–3 range, but some studies show increased

risk of cancer with CagA strains *H. pylori* infections with odds ratios as high as 28.4 (95% CI: 3.7–217.1) [38]. CagA strains have also been associated with increased inûammation [39,40], cell proliferation, and metaplasia [41] of the gastric mucosa. It is currently known that CagA can activate a number of signal transduction pathways that resemble signalling by growth factor receptors, and, simultaneously, CagA is involved in binding and perturbing the function of the epithelial junctions, resulting in aberrations in tight junction function, cell polarity, and cellular differentiation [42].

The relatively low prevalence (46.2%) of CagA positive *H. pylori* infection in dyspeptic patients in this study in Southwestern Nigeria is comparable to the reports by Amjad *et al* [13] in Malaysia, Paniagua *et al* [14] in Mexico, and Kuipers *et al* [12] in Amsterdam who found 39% to 43% prevalence of CagA positive *H. pylori* strains in patients with gastro-duodenal diseases. However, it is lower than that reported by Rochas *et al* [17] and Smith *et al* [18,19] who found a 68% to >90% prevalence of CagA positive strains among the patients in different parts of Nigeria predominantly in Lagos. The difference may be due to the more sensitive polymerase chain reaction-based method employed by Smith *et al* [18,19], to determine the genotype of *H. pylori* strains in patients with gastro-duodenal diseases. Our study used an ELISA-based serology test to investigate the presence of antibodies to CagA antigen in dyspeptic patients. The difference from the Rochas study, which also utilised an ELISA-based serology method may reflect a true loco-regional difference in prevalence of CagA positive strain, between our region (Southwestern Nigeria) and the Middle Belt of Nigeria. Furthermore this geographical variation is evident from several other studies from other parts of the world where the recorded prevalence are between 59% and 97% [15,16]. This phenomenon has partly be explained by the occurrence of CagA diversity in the same population [13].

Our finding of a statistically significant higher activity in the gastric biopsies of CagA positive patients is congruent with the results obtained by Yamaoka *et al* [43] Bhat *et al* [44], Soltermann *et al* [45] and Peek *et al* [39]. As elucidated earlier, CagA protein is associated with the stimulation of IL-8 secretion by gastric epithelial cells. IL-8 is a potent inflammatory cytokine mediating neutrophil chemotaxis [12,25,27].

The gastric biopsies of CagA seropositive patients showed more intestinal metaplasia and mucosal atrophy than those from seronegative patients in concordance with the findings of Oksanen *et al* [27], Soltermann *et al* [45] and Kuipers *et al* [12], even though the difference between the two groups of cohorts in our study was not statistically significant. Our small sample size may explain this insignificant difference this being the limitation of this study.

Contrary to the findings in other studies by Yamaoka *et al* [43], Bhat *et al* [44] and Peek *et al* [39] in which *H. pylori* CagA positivity was significantly associated with enhanced mononuclear cell infiltration in cases of *H. pylori* associated chronic gastritis, the relative proportion of cases with more severe chronic inflammation was lesser in CagA positive- patients than in CagA negative gastritis in this present study, even though the difference was not significant. This is also at variance with the present knowledge of the pathogenesis of CagA-positive and -negative *H. pylori* infections. However, Soltermann *et al* [45] found no correlation between *H. pylori* virulence status and gastric mucosa chronic inflammation. We may also need a larger number of cohorts to ascertain the relationship between CagA and the degrees of chronic inflammation in chronic gastritis in our patient population.

Environmental factors such as a high salt diet can potentially affect the disease outcome in *H. pylori*-infected persons through multiple mechanisms, and variation among strains in response to this environmental stimulus may be a relevant determinant of disease risk [46]. In Colombia, the consumption of high levels of salt (as measured by high urinary sodium to creatinine ratios) is associated with increased risk for precancerous gastric lesions (chronic atrophic gastritis, intestinal metaplasia and dysplasia) compared to what is observed in persons who consume lower levels of salt [47]. In some populations with a high incidence of gastric cancer (including China and Japan), dietary salt intakes have been reported to reach 55 g per day [48,49].

Loh *et al* [46] using global proteomic approach, recently demonstrated that CagA expression is significantly up-regulated when *H. pylori* is cultured in medium containing elevated salt concentrations. The study reported that a correlation between the levels of salt-responsive CagA expression and the severity of gastric lesions was only observed among strains demonstrating relatively low basal levels of CagA and not in strains expressing high basal levels. Based on their findings, they suggested that, in patients infected with strains that express low basal CagA levels, a high salt diet can potentiate strain pathogenicity, whereas in contrast, a high salt diet may have relatively little effect on disease outcome in patients who are infected with strains that express

high basal levels of CagA. It can therefore be inferred that our finding of relatively lower prevalence of Cag A positive strains and low correlation with precancerous gastric lesions of *H. pylori* in Southwestern Nigeria, that whereas our average salt consumption is relatively high [50] our patients are probably infected predominantly with strains that express high basal levels of CagA.

In conclusion, our findings suggest that CagA sero-status is not discriminatory between symptomatic and asymptomatic individuals with *H.pylori* Infection. CagA serostatus only showed a significant relationship with the extent of activity in the mucosa and gastric pits in *H. pylori*-induced chronic gastritis among dyspeptic patients in Ibadan, Nigeria. CagA serostatus may also be associated with intestinal metaplasia and mucosal atrophy. However, we may need a larger number of cohorts to prove this. In this regard a large scale multicentre molecular study is being planned to accurately elucidate the differential role of bacterial strains in the pathogenesis of *H. pylori* associated gastro-duodenal diseases in Nigeria.

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