

CORRELATION OF SERUM ANTI-*HELICOBACTER PYLORI* IMMUNOGLOBULIN A (IGA) WITH HISTOLOGICAL PARAMETERS OF CHRONIC GASTRITIS IN IBADAN, NIGERIA

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ABSTRACT

Background: The seroprevalence of anti-*H. pylori* IgA antibodies has been reported to vary among populations and in relation to strains of *Helicobacter pylori* bacterium. However, there has been conflicting reports on the association between IgA serological status and the histological variables of chronic gastritis. This study was therefore conducted to clarify this relationship.

Method: Using an ELISA based commercial kit, anti-*H. pylori* IgA antibody tests were performed on 65 dyspeptic patients and 65 age- and sex-matched controls. The gastric biopsies of these patients were also examined histologically for the degrees of inflammation, activity, intestinal metaplasia and atrophy. The CagA status of the patients had been determined previously.

Results: There was an anti-*H. pylori* IgA antibody prevalence of 67.7% in dyspeptics and 56.9% in non-dyspeptic individuals. No correlations were observed between serum *H. pylori* IgA antibody and the graded parameters of chronic gastritis in dyspeptic patients, although twice more patients with mild gastric inflammation were found among IgA positive than among IgA negative patients. However, a statistically significant relationship was established between serum IgA positivity and the CagA status of the patients ($p = 0.028$).

Conclusion: The seroprevalence of anti-*H. pylori* IgA antibody is high in our environment. Serum IgA status may be associated with milder degrees of gastritis in our patients but a larger cohort of patients is needed to confirm this. There seems to be a good agreement between serum IgA and CagA statuses among dyspeptic patients.

INTRODUCTION

It is now established that *Helicobacter pylori* (*H. pylori*), which is believed to be the commonest bacterial infection of man, is the major causative agent of chronic gastritis.¹ This chronic inflammation of gastric mucosa which is histologically characterized by mucosal infiltration by plasma cells has been associated with detectable levels of specific anti-*H. pylori* antibodies of the IgA and IgG classes.² *Helicobacter pylori* infection is usually lifelong, especially in untreated individuals, and eventually results in atrophic changes, gastric ulcers and cancers.³ Specific antibodies of the IgA class are usually detected in about two-thirds of patients with raised IgG levels, and in a further 2-7% of IgG-negative patients.⁴

The IgA sero-prevalence of *Helicobacter pylori* is highly variable from population to population. From available literature, it appears the anti-*H. pylori* antibody sero-

prevalence is higher in developing countries than in developed countries.⁵ This is because *H. pylori* infection is associated with low socioeconomic status and low standards of hygiene that more often characterize the developing than the developed countries.⁶

Soluble cellular antigens such as urease and heat shock protein,⁷⁻¹⁰ a vacuolating cytotoxin,^{11,12} and, more recently, a 128-kDa protein (CagA) associated with cytotoxin production¹³ have been suggested as possible inducers of an inflammatory reaction in the gastric mucosa^{14,15} and could explain how bacteria living in the mucus layer can produce histological lesions in the full thickness of the mucosa.³ In addition, it has been suggested that intensity and specificity of the mucosal immune response may correlate with the level of tissue inflammation.¹⁶

It has been established that subjects seropositive for CagA protein more often have IgA antibody than CagA negative subjects.¹⁷⁻²¹ Even though *H. pylori* stimulate both local and systemic antibody responses, the role of IgA antibody with respect to bacterial colonization and gastric inflammation is still controversial.

Following detailed search of the English literature, it seems that this is the first study in Nigeria and possibly in sub-Saharan Africa to have evaluated the association between anti-*H. pylori* IgA and histological parameters of chronic gastritis as most sero-prevalence studies on *H. pylori* in Nigeria focused on IgG levels.²²⁻²⁶ We therefore investigated the anti-*H. pylori* IgA serology in chronic gastritis by determining the sero-prevalence of *H. pylori* IgA antibody in dyspeptics and in the general population and the relationship between anti-*H. pylori* IgA antibody positivity and the histological variables in chronic gastritis. We also evaluated if the presence of serum *H. pylori* IgA antibody is associated more often with serum cag-A positive subjects than with cag-A negative ones.

MATERIALS AND METHODS

This was a prospective study of 64 consecutive adult patients with dyspeptic symptoms who underwent endoscopy at the Gastrointestinal and Liver Unit of the University College Hospital, Ibadan, Nigeria.

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. Base line bio data were obtained. 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 two endoscopic biopsies were fixed in 10% formaldehyde and transferred to the histopathology laboratory of the hospital for processing. Four micron

thick paraffin sections were stained with routine Haematoxylin and Eosin for the diagnosis of chronic gastritis. Sections were examined microscopically for the histological changes of gastritis and two of the histological variables (degree of chronic inflammation and activity) were graded based on the revised Sydney System,²⁷ while the mucosal atrophy and intestinal metaplasia were graded as either present or absent.

Five millilitres of venous blood was collected from all the recruited patients and 64 randomly selected age- and sex-matched controls for IgA serology testing. Serological analysis was performed in the serological unit of the Department of Virology of the institution. The presence/ absence of serum anti-*H. pylori* IgA antibodies to *H. pylori* immunodominant antigens was determined by Enzyme Linked Immune-Sorbent Assay (ELISA) [(Dia.Pro Diagnostic Bioprobes srl Milano Italy)] and the results were recorded as either positive or negative. The CagA status of the same set of patients and controls had been determined previously with a similar commercial ELISA kit.

Data were analysed using Statistical Package for Social Sciences, version 16.0 (SPSS Inc. Chicago Illinois). Results were presented as means \pm 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 .

The study was conducted in compliance with the guidelines of the Helsinki declaration on biomedical research in human subjects. Informed consent was obtained and the confidentiality of the patients' identity and personal health information was maintained.

RESULTS

The ages of the patients ranged from 20 years to 78 years, with an average of 47.7 ± 16.7 years. There were 31 males and 33 females giving a ratio of 1:1.06. Forty-four (44) patients were sero-positive for the IgA antibody to *Helicobacter pylori* while 20 were sero-negative, giving a 67.7% sero-prevalence level for anti-

| Degree of inflammation | Mild inflammation | Moderate inflammation | Severe inflammation | Total |
|------------------------|-------------------|-----------------------|---------------------|-------|
| <i>H. pylori</i> IgA | | | | |
| Negative | 9 | 7 | 4 | 20 |
| Positive | 23 | 16 | 5 | 44 |
| Total | 32 | 23 | 9 | 64 |

P=0.64

Table 1: Serum IgA and degree of inflammation

| Neutrophilic Infiltrate | Nil | Mild | Moderate | Severe | Total |
|-------------------------------|-----|------|----------|--------|-------|
| <i>H. pylori</i> IgA negative | 13 | 4 | 1 | 2 | 20 |
| Positive | 27 | 10 | 6 | 1 | 44 |
| Total | 40 | 14 | 7 | 3 | 64 |

$P=0.43$

Table 2: Serum IgA and Activity

H. pylori IgA in chronic gastritis patients. However, among the control group 37 cohorts (56.9%) were seropositive while 27 (41.5%) were sero-negative individuals. This difference in proportion was however not statistically significant ($P = 0.68$)

The anti *H. pylori* IgA seropositivity/ seronegativity ratio was greater among patients having low grade (mild) chronic gastritis (2.6:1) when compared with patients having higher grades (moderate and severe)

| Atrophy | Absent | Present | Total |
|-------------------------------|--------|---------|-------|
| <i>H. pylori</i> IgA negative | 19 | 1 | 20 |
| Positive | 42 | 2 | 44 |
| Total | 61 | 3 | 64 |

$P=0.94$

Table 3: Serum IgA and Mucosal atrophy

of chronic gastritis (1.9:1). Amongst both categories of patients having low grade (mild) chronic gastritis and those having higher grades (moderate and severe) chronic gastritis, a greater percentage elicited anti-*H. pylori* IgA immunological reaction (71.9% & 65.6% respectively). The ratio of subjects with lower grade (mild) inflammation compared to higher grade inflammation (i.e. moderate and severe inflammation) was slightly higher in IgA positive than IgA negative patients (1.1: 0.82). However, these differences in ratios and proportions did not show statistical significance. ($P = 0.59$)

Overall, serum IgA antibody reaction was more frequent (61.4%) among patients without detectable activity in relation to chronic inflammation in gastric mucosa compared to sero-prevalence (38.6%) among those having varying grades of activity. This difference in proportion was however not statistically significant ($P = 0.43$). Amongst both categories of patients having low grade (mild) activity and those having higher grades

(moderate and severe activity, a greater percentage elicited anti-*H. pylori* IgA immunological reaction (71.4% & 70.0% respectively). However, this difference in proportion was also not statistically significant ($P = 0.94$)

Only four patients were found to have intestinal metaplasia, three of whom were IgA positive. This histological parameter was also not significantly associated with IgA serological status in our study. Similarly, mucosal atrophy was not significantly associated with serum IgA status as only three patients were found to have mucosal atrophy on histology. Two of them were also IgA positive.

However, we found a significant relationship between seropositivity for anti-*H. pylori* Cag-A and anti-*H. pylori* IgA sero-status. Of the 29 cag-A seropositive patients,

| Intestinal metaplasia | Absent | Present | Total |
|-------------------------------|--------|---------|-------|
| <i>H. pylori</i> IgA Negative | 19 | 1 | 20 |
| Positive | 41 | 3 | 44 |
| Total | 60 | 4 | 64 |

$P=0$

Table 4: Serum IgA and Intestinal Metaplasia

25 were found to be also IgA antibody positive, with a p value of 0.028.

DISCUSSION

A major characteristic feature of *H. pylori* associated chronic gastritis is the presence of mononuclear cells, particularly plasma cells, infiltrating the lamina propria alongside increased epithelial expression of secretory component.^{28,29} The resultant local and systemic humoral responses to *H. pylori* infection may be correlated, since eradication of *H. pylori* leads to

concomitant reductions in tissue inflammation³⁰ and decreases the levels of serum antibodies.³¹

The high overall anti-*H. pylori* IgA prevalence of 56.9% and the 67.7% among our healthy controls and dyspeptic patients respectively, are similar to the values found in other studies for developing countries, and contrasts with the lower values obtained for developed countries.^{6-10, 32-34} Difference in socioeconomic levels and standards of hygiene and therefore the differences in the prevalence of *H. pylori* infections have been advanced as reason for the variation in IgA seroprevalence in populations. Nigeria shares with other developing countries many demographic features important to the persistent infection with *H. pylori*, e.g., low socioeconomic conditions and low standards of hygiene, hence the high prevalence values found in this study.

It is also possible that the variable antigenicity of the different strains of *H. pylori* in the different studies could contribute to the variation in seroprevalence in some of the populations cited. For example, Mattson *et al*⁵ found that infection with *Helicobacter pylori* gives rise to specific B-cell responses against a number of putative virulence factors of *H. pylori*, e.g., urease, flagellin, and different bacterial surface antigens, locally in the gastric mucosa. In that study, most of the infected subjects had IgA antibody secreting cells (ASCs) reacting with *H. pylori* membrane proteins, flagellin, and urease, while none of the non-infected subjects had any detectable *H. pylori*-reactive ASCs. Furthermore, half of the infected subjects also had ASCs reacting with a *Helicobacter*-specific 26-kDa protein, while only a few of them had ASCs reacting with neutrophil-activating protein, the neuraminylactose-binding haemagglutinin HpaA, or lipopolysaccharides purified from different *H. pylori* strains.

If the systemic response to *H. pylori* is same as this pattern of local gastric mucosal response, it is possible that anti-*H. pylori* IgA studies using one or two of this variety of antigens will give different prevalence rates and severity of histological parameters even in the same cohort.

Akhiani *et al*,³⁶ using laboratory mice demonstrated that IgA deficient mice exhibited significantly less colonization of the gastric mucosa and more severe gastric inflammation than wild type mice. They demonstrated that IgA antibodies counteracted gastric inflammation.

Generally, secretory IgA antibody has been viewed as an immune barrier which inhibits the entrance of external foreign antigenic molecules. However, recent

studies^{37, 38} have suggested two additional functions: to neutralise intracellular microbial pathogens directly within epithelial cells; and to bind antigens in the mucosa and excrete the immune complexes through the adjacent epithelium into the lumen to rid the body of locally produced immune complexes.

This finding has also found support in human subjects as demonstrated by Perez-Perez *et al*⁹, who found that IgA responses to *H. pylori* whole cell sonicate and to *vac* antigens were inversely related to chronic inflammatory responses in American patients. Similarly, Futagami *et al*¹⁰ demonstrated that strong serum IgA responses were associated with type I gastritis (superficial, mild) while weak signals were associated with more severe gastritis (type II), in Japanese patients. However, Kreuning *et al*¹¹ who investigated the relationship between IgG and IgA titres against the *H. pylori* in serum and the severity of gastritis in asymptomatic Dutch subjects found a direct correlation between the IgA *H. pylori* antibody absorbance index and the severity of gastritis in the antrum and corpus of the stomach.

Unlike Perez-Perez *et al*⁹ and Akhiani *et al*⁶, on the one hand, and Kreuning *et al*¹¹ on the other hand, who respectively found inverse and direct correlation between IgA seropositivity and degrees of mucosal inflammation, we did not find such relationship.

When we examined our result from the point of view of studies which suggest that serum IgA counteracted inflammation⁴², we found that the anti *H. pylori* IgA seropositivity/seronegativity ratio was greater among patients having low grade (mild) chronic gastritis (2.6:1) when compared with patients having higher grades (moderate and severe) of chronic gastritis (1.9:1). Furthermore, we observed that the ratio of subjects with lower grade (mild) inflammation compared to higher grade inflammation (i.e. moderate and severe inflammation) was slightly higher in IgA positive than IgA negative patients (1.1: 0.82). These differences in ratios and proportions were however not statistically significant. We are of the opinion that the use of a larger cohort might help us to conclude on the relationship between IgA serology and the severity of gastritis.

Our finding of no significant correlation between neutrophilic infiltrate in chronic gastritis and the presence of serum anti-*H. pylori* IgA antibody is in agreement with the works of Kreuning *et al*⁹ and Perez-Perez *et al*⁹ who also found no statistically significant relationship between the two parameters. Perhaps, this is the true picture. Otherwise, we may need a larger cohort to prove the actual relationship

between the parameters. It is noteworthy that the above studies cited used small numbers of cohorts (48 and 82 respectively), similar to our number of cohorts of 64.

Furthermore, no relationship was established between serum anti-*H. pylori* IgA antibody status and gastric mucosal atrophy and intestinal metaplasia. This contrasts with the findings of Yamamoto *et al*³ and Li *et al*⁴ who investigated the relationship between severity of *H. pylori* gastritis and serum anti-*H. pylori* antibodies in Japanese patients, and found a correlation between these histological variables and IgA status.

Yamamoto *et al*³ found a significant association between serum IgA titres and the development of atrophic gastritis. Li *et al*⁴, on the other hand found that in the absence of histological diagnosis of *H. pylori* in the gastric mucosa in chronic gastritis anti-*H. pylori* serum IgA is significantly higher in patients with severe intestinal metaplasia than in those with mild intestinal metaplasia. However, in the presence of *H. pylori*-like organisms in the gastric mucosa no such relationship was demonstrated. As is the case of the other histological parameters, our use of a small cohort might explain our inability to establish a relationship between the variables in question.

However, a statistically significant relationship was established between *cagA* status and serum IgA status. This is in concordance with the findings of Rautelin *et al*⁷ who found a direct relationship between IgA and *cag A* statuses. With this finding of significant *cag-A* status relationship with IgA serology, one would expect serum IgA positive status to be associated with more intense mononuclear and neutrophilic infiltrates, higher number and degrees of mucosal atrophy and intestinal metaplasia.¹⁸⁻²¹ But this is not the case in this and other studies.^{36,39,40} The relationship between IgA status and *cag-A* may, therefore, not be a simple one. It is possible that other host and bacterial genetic factors come into play to determine the severity and course of chronic gastritis. For example, Akhiani *et al*⁶ established that interleukin-10 (IL-10) polymorphism may influence the course of the disease in IgA positive and IgA- negative mice. Also, in the absence of IL-12 (IL-12 polymorphism), the normal Th1 response to *H. pylori* infection is known to be replaced by Th2 host immune activity. Th1 response is associated with strong immune activity and mucosal damage while Th2 response appears to be protective against mucosal damage.⁴⁵⁻⁴⁷ In addition, host genetic polymorphisms in the IL-1 β gene and the IL-1 receptor-antagonist gene (IL-1RN) have also been found to lead to increased gastric mucosal levels of IL-1 β in individuals infected with *H. pylori*^{48,49} and increased levels of

inflammation.⁴⁸⁻⁵² The interplay between these host genetic factors and the inflammatory mediators in the determination of host susceptibility to adverse outcomes of *Helicobacter pylori* infection in our population shall form the basis of our future research thrust using a larger cohort.

CONCLUSION

We conclude that the seroprevalence of anti-*H. pylori* IgA antibody is high in our environment *pylori* and IgA sero-status does not discriminate between symptomatic and asymptomatic individuals. Serum IgA status may be associated with milder degrees of gastritis in our patients but a larger cohorts is needed to prove this. The serum *H. pylori* IgA activity in our dyspeptic patients is associated with the *cag-A* status of the *H. pylori* strain, however, the relationship between *cag-A* and IgA statuses might be a complex one.

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