



C-Reactive Protein and Immunoglobulin Isotypes Concentrations in Helicobacter Pylori Sero-Positive Children Attending a Tertiary Health Institution in Uyo, Nigeria

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Abstract

Background: C-reactive protein (CRP) is an inflammatory acute phase protein known to support active immunological responses during infection or tissue injury.

Aim: This study aimed to determine the concentrations of CRP, IgM and IgG immunoglobulin isotypes in children with Helicobacter pylori infection with a view to assessing the extent of infection and host immune and inflammatory responses.

Method: seventy-four (74) *H. pylori* sero-positive children were assigned into 3 groups based on their predominant immunoglobulin isotypes, namely Group 1: IgM+ve, Group 2: IgM+ve/IgG+ve and Group 3: IgG+ve. The concentrations of CRP using immunoturbidimetric method, age and body temperature of the children were determined. History of abdominal symptoms were also taken.

Results: The results showed that the concentrations of C-reactive protein and IgM isotype of immunoglobulin were significantly higher in groups 1 and 2 compared to group 3 ($p < 0.000$) and correlated positively with each other ($r = 0.807$, $p = 0.000$) while IgG isotype was higher at the later phase and showed no correlation with C reactive protein. Abdominal symptoms were higher in (Group 3) the later phase of infection when the concentrations of CRP, IgM isotype and to some extent IgG isotype were declining.

Conclusion: Increased concentrations of CRP and immunoglobulin isotypes are not consistent with active bacterial invasion since abdominal symptoms are predominant at the late phase of *H. pylori* infection. Therefore, measurement of CRP along with immunoglobulin isotypes cannot actually be helpful in determining the extent of *H. pylori* invasion. However, the level of host immunological and inflammatory responses can be estimated.

Keywords

C-reactive protein; Immunoglobulin isotypes; Helicobacter Pylori; Inflammation; Immune Response

Introduction

Helicobacter pylorus is a human pathogen which infects nearly half of the world's population and frequently associated with chronic inflammation of the gastric mucosa [1]. Common consequences are peptic ulceration and gastric cancer in susceptible individuals [2]. The IgG seroprevalence of *H. Pylori* in Nigerian children had been reported to be 30.9% [3] Etukudoh). Infection with *H.pylori* is commonly acquired during childhood and if not treated, the host can carry the bacteria for life, mounting innate and adaptive immune responses which often do not clear the pathogen [4]. The ability of *H. pylori* to evade the host defense mechanisms of both innate and adaptive responses is the hallmark of its infection [5]. Studies have shown that specific T-helper (Th) cell subsets and their signature cytokines contribute to the control of infection and sustain the chronic inflammatory state (2) which precipitates increased C-reactive protein concentration.

Manifestation of symptoms in *H.Pylori* infections depends on the stage of infection and the effectiveness of host innate and adaptive immune responses. Synthesis of immunoglobulin against infectious agents is a major component of the adaptive response and the predominance of an immunoglobulin isotype has been known to indicate the type and stage of the host immune activities. For instance, the predominance of IgM with insignificant concentration of IgG suggests initial immune response to pathogen whereas the predominance of IgG with insignificant IgM suggests a late phase response. Between the two phases is the intermediate phase which is characterized by the presence of both IgM and IgG. C-reactive protein is an inflammatory acute phase protein synthesized by the liver and secreted into circulation as an innate complement to the immunological responses. This protein is higher in concentration during active inflammatory activities. Elevated concentration of C-reactive protein in serum has been shown to be a risk factor for many metabolic, inflammatory, cardio-vascular and some communicable and non-communicable diseases [6-9]. Measurements of CRP, though highly non-specific, are frequently used for evaluation of injury to body tissues or for the detection of inflammatory events. Patients with systemic inflammatory disease, severe infections, arthritis and liver diseases have been shown to have elevated serum CRP [10,11]. The assay methods for CRP are simple, less expensive and could represent a more convenient and cheaper method of assessing the progress or stage of immune and inflammatory responses of hosts during *H.pylori* infection. This study therefore aimed at determining the relationship between CRP and the different immunoglobulin isotypes during *H.pylori* infections with a view to evaluating its possible complementary role in the assessment of disease progression.

Materials and Methods

A total of 230 children of age 6 months-15 years attending the pediatric clinic of UUTH were recruited for studies after receiving informed consent from the parents or guardians. Blood samples were obtained from the children by venipuncture. Serum samples were separated after about two hours of clotting and retraction using bench top centrifuge at 3000 g for 5 minutes. Sera were stored frozen at -20°C till sufficient sample size was collected. The samples were assayed for IgM and IgG seropositivity using Vie Torch *H. pylori* IgM

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and IgG test kits respectively, the procedures were as given in the manufacturer’s manual.

The test kits were ELISA based assay methods for quantifying the immunoglobulin isotypes. Samples with IgM and IgG concentrations above 0.60 IU and 20 IU were respectively considered as sero-positive for *H.pylori* IgM and IgG. Patients were also examined for history of any abdominal symptoms namely: frequent vomiting, abdominal pain, heart burns, evening colic and diarrhea. Abdominal symptoms were noted depending on how many of the above symptoms were observed in a patient. Seventy-four (74) of the recruited children who were seropositive for either IgM or IgG or both, and were divided accordingly into the three groups based on the predominant immunoglobulin isotype seropositivity as follows:

Group 1 was 16 IgM sero-positive patients, with insignificant IgG; Group 2 was 28 patients with both IgM and IgG seropositivity; and Group 3 were 30 IgG seropositive patients with insignificant IgM.

C-reactive protein concentrations were assayed using the CRP quantitative latex immunoturbidimetric method. The reagent was manufacturer by Giesse Diagnostic Sac, Roma-Italy. The test is based on reaction between antigen (CRP) and latex particles coated with anti-CRP antibody. The protocol was as described by the manufacturer’s instruction.

Statistical analysis

Data were analysed using SPSS software version 20.0. Independent t-test or analysis of variance post-hoc were used to compare means between the groups. Regression analysis and Pearson-bivariate correlation analysis were performed to established relationships among parameters and characteristics of the patients. P-values ≤ 0.050 were considered significant.

Results

The concentrations of serum C-reactive protein and anti-*H.pylori* IgM and IgG isotypes of seropositive children seen in UUTH pediatric clinic were measured and the results were presented in Table 1. Of the 74 *H.pylori* seropositive children in the study, 30 were IgG+ve; 28 were IgG+ve/IgM+ve while 16 were IgM+ve. About 11 (14.86%) patients were without abdominal symptoms while 63 (85.14%) presented with at least one of the 5 symptoms namely; evening colic, heart burns, frequent vomiting, abdominal pain and diarrhea.

Percentage abdominal symptom score was significantly higher in group 3 (16.9%) compared to those of group 1 (3.9%) and group 2 (4.7%).

Abdominal pain and frequent vomiting were the predominant symptoms which were present respectively in 45.10% and 41.17%

of seropositive children across the groups and were also significantly higher in group 3.

The mean age of the children, in month, was highest in group 3, followed by group 2 and least in group 1. The difference in age across the groups was significant (F=6.04, P=0.004). Also, there were corresponding increase in weight across the groups in the same order (F=4.11, P=0.020). Body temperature of the patients were not significantly different within and across the groups (F=1.48, P=0.234).

The result also showed that the concentration of anti *H.pylori* IgM in group 3 was significantly lower than those of groups 1 and 2 (P=0.000). The IgM concentrations in groups 1 and 2 were not significantly different. On the other hand, patients in group 1 had significantly lower concentration of anti-*H.pylori* IgG compared to group 2 and 3 (P=0.000), the highest concentration of IgG (63.14 ± 23.06) was observed in group 2. The IgG concentration (49.38 ± 20.59) for group 3 was lower than those groups 2. The pattern of C-reactive protein levels was similar to that of anti *H.pylori* IgM, with significantly lower concentration in group 3 compared to groups 1 and 2. C-reactive protein levels were observed to decrease with increasing age of children similar to that of IgM. Correlation analysis between CRP and anti *H.pylori* IgM was strongly positive and significant (r=0.807, p=0.000), however, anti *H.pylori* IgG and CRP showed a negative correlation which was not significant (r=-0.105, p=0.367). The concentrations of CRP in serum correlated negatively with ages of sero-positive patients (r=-0.209, p=0.019) and abdominal symptoms (r=-0.114, p=0.328). There was a significant positive correlation between serum CRP concentration and body temperature of patients (r=0.287, P=0.038) while IgM and IgG showed no relationship with temperature. Linear model regression analysis indicated that positive CRP did not significantly contribute to abdominal symptoms, but negative IgM contributed significantly (22.68%, CI=95%, P=0.048) to abdominal symptoms. CRP correlated negatively with all the individual abdominal symptoms except frequent vomiting which was observed to increase by 7.24% following a unit increase in CRP (CI=95%, P=0.297).

Discussion

H.pylori is known to cause continuous gastric inflammation in almost all infected persons [12]. The inflammation was reported to be chronic, initially consisting of neutrophil recruitment, followed by T and B lymphocytes, plasma cells and macrophages, along with epithelial cell damage [13]. *H. pylori* showed strict trophism for gastric mucosa or sites in which there is gastric metastasis. The infection was predominant in the lumen of the stomach and attached to the host cells. On attachment to cells, *H. Pylori* injects into the epithelial cells some bacterial components. A bacterial DNA segment called the Cag

Table 1: Patient’s characteristics and concentrations of serum immunoglobulin isotypes and C-reactive protein in *H. pylori* seropositive children.

Parameters	Groups 1 (N=16) IgM+ve	Group 2 (N=28) IgM+ve/IgG+ve	Group 3 (N=30) IgG+ve	F-value	P. Value
1gG(Iu/ml)	6.70 ± 2.80 ^a	63.14 ± 23.06 ^b	49.57 ± 20.59 ^b	49.38	0.000
1gM(IU/ml)	1.27 ± 0.35 ^a	1.23 ± 0.51 ^a	0.28 ± 0.17 ^b	62.47	0.000
CRP (mg/l)	28.27 ± 9.79 ^a	30.67 ± 14.42 ^a	6.55 ± 3.79 ^b	46.81	0.000
Age (month)	28.67 ± 18.47 ^a	55.71 ± 27.01 ^b	73.47 ± 29.48 ^c	6.04	0.029
Weight (kg)	12.36 ± 4.35 ^a	17.64 ± 7.0.13 ^b	20.16 ± 8.22 ^b	4.11	0.040
Temp °C	37.98 ± 1.94	38.24 ± 1.30	37.55 ± 1.06	1.48	0.234
Abdominal Sympt.	3.9 ± 1.86 ^a	4.7 ± 2.01 ^a	16.9 ± 4.53 ^b	5.68	0.037

Note: Data are presented as Mean ± SD; different superscripts indicate significant difference at p<0.050.

pathogenic island encodes proteins that provide a type IV secretion apparatus (cag) which allows bacterial macromolecules to translocate into the host epithelial [14].

H.pylori infection induces vigorous systemic and mucosal humoral response. During infection with *H.pylori* the number of immunoglobulin producing cells increases as a result of enhanced production of cytokines [14,15]. IgG and IgM were also detected along with activated complement [16].

In this study, significantly higher concentrations of anti *H.pylori* IgM were observed in younger children (group 1) compared to the older ones (groups 2 and 3). As the infected children increased in ages, the concentration of IgM decreased while that of IgG increases to a peak and began to decline with increasing age. These findings are consistent with normal immunological response where IgM isotype is the predominant immunoglobulin against an antigen at primary exposure. In this case primary exposure is encountered at younger ages. Similarly, serum CRP levels were higher in younger children and followed similar pattern as the IgM. Normally CRP concentration is elevated during acute phase inflammatory response against injurious agents which normally are triggered by increased production of IL-1, IL-6 and TNF- α by immune and inflammatory cells.

We noted a significant positive correlation between CRP and anti *H.pylori* IgM but no relationship with IgG. It is traditional to expect higher frequency of symptoms during active inflammatory responses to infections. Paradoxically, in this study patients with increased concentration of anti *H.pylori* IgM and CRP, which would indicate active immune and inflammatory activities, were not showing corresponding increase in abdominal symptoms. Anti *H.pylori* IgG levels were also not significantly correlated with abdominal symptoms in this study.

Although CRP levels strongly correlated positively with body temperature of patients, its relationship with abdominal symptoms was strongly negative. These findings suggest that abdominal symptoms are not necessarily associated with the initial active immune and inflammatory response. The positive correlation of CRP and body temperature of children may reflect the effect of interleukins, interferon and TNF- α on the temperature regulatory centres of the body, which also enhances the synthesis and release of CRP by the hepatocytes. The higher percentage of abdominal symptoms seen in group 3 with insignificant levels of CRP and IgM suggest that the initial immunological and inflammatory host response to *H.pylori* infection was not capable of eradicating the infection and hence the symptoms predominate at the time the host defense activities were down regulated, i.e. when the initial of IgM, IgG and CRP levels were declining. Velin and Michette [14] had reported that the IgG, IgM and activated complements produced during *H.pylori* infection does not lead to eradication of the infections but may contribute to tissue damage and gastric parietal cells atrophy.

Normally, immature T-helper (Th0) cells can differentiate into two functional subtypes namely Th1 cells, secreting IL-2 and interferon- γ and Th2 cells, secreting IL4, IL-5 and IL-10. Th2 cells stimulate is cells in response to extracellular pathogens while the Th1 cells are mostly induced in response to intracellular pathogens. *H.pylori* infection is non-invasive (extracellular) and so would be expected to induce a strong humoral Th2 phenotype response which is capable of eradicating the bacteria. Paradoxically, *H.pylori*-specific gastric mucosal T cells generally presented a Th1 phenotype [17,18]. The *H.pylori* induced switch in T cell phenotype is a probable

mechanism by which the bacteria evade the initial host immune and inflammatory responses and persist for decades in our stomach [19,20]. The predominance of symptoms in older children (group 3) when the activities of immune and inflammatory responses against *H.pylori* were declining, as evident by reduction in immunoglobulin isotypes and CRP concentrations, is consistent with earlier findings reported by Lundgren et al. [21] that *H.pylori* infection generated regulatory T cells (Treg) that prevent infection-induced immunopathology but may also increase, the load of infection and prolong pathogen persistence by suppressing protective immune responses. Therefore, the declining levels of CRP and immunoglobulin isotypes in *H.pylori* infected patients in this study does not represent declining bacterial activities but may be evidence of increasing suppressor T cell activities.

Conclusion

The study showed that the concentrations of C-reactive protein and IgM isotype of immunoglobulin were higher at the early stage of *H.pylori* infection and correlated positively with each other while IgG isotype was higher at the later phase and showed no correlation with C reactive protein. Abdominal symptoms were higher in the later phase of infection when the concentrations of CRP, IgM isotype and to some extent IgG isotype were declining. Therefore, measurement of CRP along with immunoglobulin isotypes cannot actually be helpful in determining the extent of *H.pylori* invasion. However, the level of host immunological and inflammatory responses can be estimated.

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