Immunochromatographic detection of Helicobacter pylori antibodies in paediatric patients in Asaba, Nigeria.

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Abstract

Background: Helicobacter pylori has been implicated as one of the aetiological agents of gastrointestinal infections in children in developing countries. Paediatric patients attending Federal Medical Centre (FMÇ) and General Hospital (GH) Asaba, Nigeria, were serologically assessed for the infection.Methodology: 100 blood samples were aseptically collected by venepuncture from subjects and controls, and screened for Helicobacter pylori antibodies, using an immunochromatographic assay method.Results: An overall high prevalence rate of 55% was recorded. At the FMC, 8(20%) and 2(20%) prevalence was recorded for subjects and controls respectively, while at the GH, 14(35%) and 4(40%) were seropositive for the subjects and controls respectively.Conclusion: A causal relationship between gastrointestinal complaints and Helicobacter infection as well as asymptomatic carriage of infection amongst children in the study area has been serologically demonstrated. There is urgent need for routine screening of paediatric patients presenting with abdominal pains.

INTRODUCTION

The taxonomy of the genus Campylobacter went through extensive revision in the late 1980s. The organism, Helicobacter pylori is a Gram negative curved bacillus that colonizes gastric mucosa in humans. It was first isolated from a gastric mucosa biopsy specimen in 1983 [1]. It plays an important role in the development of peptic ulcer disease [2,3]. About half of the adults may be infected with this pathogen in developed countries while this figure could be as high as 90% in developing countries [4].

Predisposing factors such as overcrowding of people, poor sanitation and living conditions can be found in most developing countries. [4] have reported that close person to person contact in childhood is an important determinant of seroprevalence of H. pylori in adulthood, suggesting that the infection is transmitted directly from one person to another and may be commonly acquired in early life. The helicobacters are one of the major bacterial causes of enteritis worldwide and their prevalence makes them important from both clinical and economic perspectives. Accurate identification of these organisms is therefore a desirable objective, to provide important clinical and epidemiological information in a developing country such as Nigeria.

Several diagnostic methods such as the pulsed-field-gel-electrophoresis [5], PCR [6], protein profiling [7] etc, have been used. These methods, no doubt, require special technologies, high cost and trained manpower factors that put them out of the ready reach of routine laboratory use in most third world countries. While the combination of culture and histological examination of gastric biopsy specimens has been considered the “gold standard” for the diagnosis of Helicobacter pylori, serological diagnosis has also been proven to be more sensitive [8,9]. All patients colonized with H. pylori elicit a local antibody response against antigens covering the surface and flagella of the organism. In the majority of the cases this antibody response is detectable in the serum. These circulating antibodies can be detected by a variety of techniques.

Currently, the majority of routine serodiagnostic tests for H. pylori measure lgG antibodies. Locally, the antibodies are mostly of the lgA class, but the circulating antibodies are primarily of the lgG class, usually the lgGI, lgG2) and lgG4 subclasses. [10]. A specific lgG response at least as great as that found in adults can also be detected in infected children [11].

Here we report the prevalence of Helicobacter pylori antibodies among children within Asaba, a busy metropolitan city in Delta region of Nigeria. The objective of study was also to assess the evidence for a cause – and –

effect relationship between H. pylori infection and gastrointestinal complaints among children visiting two major hospitals in Asaba.

SUBJECTS AND METHODS

The subjects comprised of 100 children (aged 1 year – 16 years), who visited both the Federal Medical Centre (FMC) and the General Hospital (GH), Asaba. The children were first registered at the out-patients department by their mothers before being referred to the paediatrician for treatment. 80 of the children were clinically suspected of having gastrointestinal complaints while the remaining 20 children were apparently healthy, but needed medical check-up.

SAMPLE COLLECTION

The consent of the hospitals's medical directors and the childrens,s parents were obtained, before obtaining blood from the patients. About 5ml of blood was aseptically collected from each child by venepuncture and put into EDTA sample container; plasma samples were extracted, properly labeled and stored in a -200 C freezer at the end of each day.

TEST PRINCIPLE

World-wide Diagnostics Helicobacter pylori test reagents were used. The H.pylori test is a lateral flow immunochromatographic screening test. Helicobacter pylori antigens are precoated onto membrane as a capture reagent on the test band region. During the assay, the specimen (Plasma) was allowed to react with H. pylori antigens gold conjugate. The mixture then moves laterally in the membrane chromatographically to the test region with immobilized antigens of H.pylori. If H.pylori antibodies are present in the specimen, two colored bands are formed on the test region. One colored band in the test region indicates a negative result.

TEST PROCEDURE

The test device was carefully removed from the sterile aluminium pouch bag. A drop (50ml) of patients' plasma was added into the sample well until it was completely absorbed; this was followed with a drop of chasing buffer solution. The preparation was then observed for 10-15 mins. A negative result was indicated when only one pink band showed up, thus indicating the absence of H.pylori antibodies. In a positive test, showing the presence of H.pylori antibodies in patients specimens, two pink bands were observed. A repeat test was performed whenever there was absence of colored bands.

RESULTS

A total of 100 children were screened for H.pylori antibodies. Table 1 shows the result of 50 patients screened at the FMC. Of the 40 subjects screened 8 (20%) were seropositive while 32 (80%) were seronegative. Of the 10 control subjects, 2 (20%) tested positive for H.pylori antibodies, while 8 (80%) were seronegative (Table 1).

The results of the 50 subjects screened at the GH are shown in Table 2. Out of the 40 subjects screened 14 (35%) tested positive for the antibodies while 26 (65%) were seronegative. 4 (40%) of the 10 control subjects were seropositive while 6 (60%) of them were seronegative for H.pylori antibodies.

Figure 1

Table 1 Results of subjects screened for antibodies at the Federal Medical Centre, Asaba.

	Number of Subjects Tested	Number (%) of Seropositive Subjects	Number (%) of Seronegative Subjects
	40	8 (20%)	32 (80)
	10(control)	2 (20%)	8 ((80)
Total	50	10	40

Figure 2

Table 2 Results showing prevalence of antibodies in subjects screened at the General Hospital, Asaba.

	Number of Subjects Tested	Number (%) of Testing Positive for *H.pylori* antibodies	Number (%) of Resting negative for *H.pylori* antibodies
	40	14(35)	26 (65)
	10(control)	4 (40)	6 (60)
Total	50	18	32

DISCUSSION

In the present study, infection with Helicobacter pylori in children has been assessed serologically. The preference in using serodiagnostic methods for the rapid, non-invasive, sensitive and specific detection of Helicobacter pylori is no longer in doubt [12,13]. Also, it has been reported that measurement of Helicobacter pylori antibodies is move sensitive than the conventional gold standard combination of culture and histology[14].

Of the 40 children screened at the Federal Medical Centre (Table 1), 8 (20%) tested seropositive, while of those screened at the General Hospital, Asaba, 35% showed

seropositivity for Helicobacter pylori antibodies. Thus, a relationship between gastrointestinal complaints in children screened and Helicobacter pylori infection has been established in this study. Our results are consistent with a study [15] that reported an association between Helicobacter pylori infection with gastritis. Helicobacter pylori can elicit the production of lgG antibodies in man during infection by this organism. It has been suggested that infection could be acquired early in childhood [16,4].

The faecal-oral route of transmitting infection may play a leading role for children's infection since children's poor sanitary habits make them easily vulnerable. We observe that the control specimens collected from the Federal Medical Centre and General Hospital, Asaba, tested seropositive (20% and 40% respectively) to Helicobacter pylori antibodies. This outcome is suggestive of asymptomatic carriage of infection. This finding is corroborated by Drumm [17], who reported that most children without any clinical symptoms of gastroenteritis tested seropositive to Helicobacter pylori.

In this study an overall high seroprevalence rate of 55% children with gastrointestinal complaints has been recorded. High prevalence rates have also been recorded by other workers from other locations. In a survey of 268 subjects in Maiduguri, Nigeria, 85% of the people screened had lgG antibodies to Helicobacter pylori [15]. In Abakaliki another Nigerian city, Ugwuja and Ugwu [18] found that 69 (26.3%) of the 262 patients screened had lgG antibodies. Also, at Ile-Ife, histologic and cultural examination of 138 patients showed that Helicobacter pylori was positive in 107 (77.5%) [19].

Overcrowding and poor sanitary conditions in most of the cities in developing countries often pave way for gastrointestinal pathogens to affect people living in these regions. Although there is yet no epidemic of Helicobacter pylori infection recorded in Nigeria, our results and other findings from other parts of Nigeria tend to suggest the existence of some measure of herd immunity, contributing a balance in the host-parasite relationship. However, to ensure that this balance is not some day dislodged in favour of the parasite, routine screening, especially for the paediatric patients, is necessary. Primary health care paediatricians may consider the urgent need to screen for Helicobacter pylori infection in children presenting with abdominal pains.

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