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Helicobacter pylori infection in children with sickle cell disease: IgG versus combined IgA and IgG serology.

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ABSTRACT

Serological tests are cheap and non-invasive, so they are used in the diagnosis of Helicobacter pylori infection and more preferred than other invasive methods. Although the diagnostic utility of IgG antibodies to H. pylori is well established, the usefulness of IgA tests is less well documented. Determine the prevalence of H. pylori infection and find the frequency of IgA-positive IgG-negative cases in children with SCD, thus assessing the clinical utility of IgA testing in those patients. Forty children with SCD having a mean age of 10.3years ±5.07 years were recruited from the pediatric hematology clinic of the new children's hospital Cairo University. They underwent clinical evaluation including duration of illness, recurrent abdominal pain (RAP) and peptic like features. They were also subjected to H. pylori serological tests using IgA and IgG antibodies. Thirty normal subjects matched for age and sex served as control group and for whom IgG and IgA serology was done. The prevalence of H. pylori in SCD children was 52.5% using IgG antibody test and 65% using combined serology(IgG + IgA), the same for controls, from 26.7% prevalence to 40%. A high frequency of IgA-positive IgG-negative cases was detected in both patients and controls (12.5% and 13.33% respectively).The age of the patients and their duration of illness were strongly associated to H. pylori seropositivity while RAP and peptic like features were not. Great care should be taken not to underestimate the prevalence of H. pylori infection in children with SCD from the results of IgG serology and it's mandatory to do IgA antibodies in any patient with suspected H. pylori infection and having negative IgG antibodies.

Keywords: Sickle cell disease- Helicobacter pylori- IgA- IgG

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INTRODUCTION

Helicobacter pylori (H. pylori) has been discovered by Warren and Marshall(1)two decades ago. Since then, it was proved that it plays a significant role in the development of chronic gastritis, peptic ulcer diseases, mucosa-associated lymphoid tissue lymphoma, and gastric cancer(2). Seroepidemiologic studies reported that infection with H. pylori is very common all over the world and most infections are acquired during childhood(3).

An association between gastritis or peptic ulcer disease and sickle cell disease (SCD) has been reported (4-6). SCD is a genetic disorder of hemoglobin formation that is inherited as autosomal recessive sickle cell gene(7).As result of anemia, chronic hypoxia, and infarct in SCD that may enhance the growth and survival of H pylori and the fact that H pylori infection has been claimed in many cases of gastritis and gastric ulcer pains(8, 9),the organism may play a role in recurrent abdominal pain that occurs in SCD patients. However, very few studies investigated this relationship (5, 6, 10), to give an adequate treatment of this morbid condition.

Serological tests are available, cheap, non-invasive and easy to perform; for which reasons they are used in the diagnosis of H. pylori infection and more preferred than other invasive methods(11, 12).The IgG antibody level to H. pylori is usually increased and may be a marker for H. pylori infection; also because of its high sensitivity and specificity it has been widely used in epidemiologic studies and many physicians didn't further diagnose their patients for H. pylori infection after an IgG negative serology(13, 14).

Although the diagnostic utility of IgG antibodies to H. pylori is well established, the usefulness of IgA tests is less well documented. Authors reported some H. pylori infected individuals who were positive for IgA but negative for IgG antibodies(15), making the evaluation of IgA titers the only serological method of confirmation in diagnosis of H. pylori infection.

Therefore, the aim of the present study was to determine the prevalence of H pylori infection and to compare the commercially available ELISA for IgG versus the combined IgG and IgA for the diagnosis of H. pylori infection in children with SCD. We were also aiming to investigate the relationship between H. pylori and recurrent abdominal pain, age, duration of illness and number of transfusions among a sample of SCD children in Egypt.

PATIENTS AND METHODS

Setting:

This is a cross-section study which was carried out in the pediatric hematology clinic of the new children's hospital (Abulrish) Cairo University during the period of February and March 2015.Fortypatients with SCD in the pediatric age group from (1-18 years) with a mean age of 10.3years ±5.07yearswereenrolled.

Inclusion criteria:

1- Patients with SCD who come to the clinic for routine follow up
2- Patients who are in a stable state and not requiring hospital admission

Exclusion criteria:

Patients with SCD who had received proton pump inhibitors, H2-receptor antagonists, amoxicillin, metronidazole, or clarithromycin within 1month prior to the study.

Ethical clearance was obtained from the hospital and National Research Centre (NRC) ethics committee. The ethics committee approved this work. All procedures were carried out in accordance with the Declaration of Helsinki 1975, revised 1983.

Subjects:

All patients were subjected to a thorough history and physical examination with a special emphasis on:

1- Positive consanguinity
2- Duration of illness
3- Number of blood transfusions
4- History of recurrent abdominal pain (RAP): RAP is characterized by three or more episodes of abdominal pain that occur over at least three months and are severe enough to require medical attention and interfere with normal activities, such as school attendance and performance, social activities, and participation in sports(16).
5- Features of peptic-like or dysmotility-like dyspepsia such as nausea/vomiting, pain relieved by food or antacid, night pain, abdominal bloating or distension, or hematemesis(16).
6- Patient's genotype taken from the patients' files.

Serology Test for Helicobacter pylori

Approximately 2 mL of venous blood was collected from each patient. The sample was centrifuged for 2 minutes to isolate serum, which was frozen until assayed. Using a test kit Immuno spec KIT Cat No E30-145, quantitative determination of immunoglobulin G to H. pyloriin sera was done by enzyme-linked immunosorbent assay (ELISA). The cut-off point is 10IU/ml. above 10 IU/ml the case is considered positive for H. pylori infection and below 10IU/ml it is negative. Assessment of Helicobacter pylori IgA was performed using CALBIOTECH ELISA KIT Cat No HP014A. The cut-off point is 0.9IU/ml above which the case is positive for H. pylori infection and below this value it is negative.

The subjects were matched for age and sex with 30 controls of the genotype AA recruited from the general outpatient clinic and who come for treatment of URTI, conjunctivitis, otitis media and UTI and for whom IgG and IgA serology was done.

Statistical analysis

The data were collected and studied using SPSS 20 statistical program. The mean, standard deviation (SD), minimum, maximum and range were calculated for all quantitative variables. The quantitative data were examined by Kolmogrov Smirnov test for normality.

Serological comparison between cases and controls was done using one way ANOVA test. Qui square (X^2) test was used to study the association between the test variables and the H. pylori IgG and combined IgG & IgA. Correlation between correlation between the test variables and H. pylori IgG &IgA antibodies was done using Pearson's correlation test.

Level of significance was considered at P-value < 0.05 in the all used tests.

RESULTS

The present study was carried out on 40 children with SCD recruited from the pediatric hematology clinic of the new children's hospital Cairo University, for whom serological diagnosis of helicobacter pylori infection was done using IgG and IgA antibodies.

Demographic and serological data of SCD children as well as serology of controls are described in (table 1), in which the mean age of patients was 10.23±5.03years, duration of illness 8.09± 4.54 years, hemoglobin (Hb) level 9.01±1.51mg/dl and reticulocytic (Retics) index 6.14±4.64.

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Table 1: Demographic data of the studied group

		N	Minimum	Maximum	Mean± SD
Age (years)		40	1.00	18.00	10.23±5.03
Duration of illness (years)		40	0.50	17.00	8.09±4.54
Hb (mg/dl)		40	5.40	12.00	9.01±1.51
Retics index		37	0.20	20.70	6.14±4.64
SCD H Pylori IgG (IU/mL)	Negative	19	3.70	9.5	7.01±1.78
	Positive	21	11.30	81.50	28.71±19.07
Control H Pylori IgG (IU/mL)	Negative	22	3.10	9.6	6.29±3.50
	Positive	8	13.20	23.00	16.60±3.51
SCD H Pylori IgA (IU/mL)	Negative	18	.30	.80	0.54±0.51
	Positive	22	.90	2.00	1.17±0.31
Control H Pylori IgA (IU/mL)	Negative	20	.20	.80	0.45±0.19
	Positive	10	.90	1.90	1.18±0.32

Serology of SCD patients revealed a mean IgG titer of 28.71±19.09 U/L in positive cases, while IgG titer of positive controls was 16.6±3.51IU/mL. As regards IgA titer the mean was 1.17±0.31IU/mL in positive cases and 1.18±0.32IU/mL in positive controls.SS genotype was present in 28(70%) of patients, SB in 11(27.5%) and SC genotype was present in only one patient (2.5%).

The percentages of seropositive and seronegative H pylori IgG, IgA and combined, of both groups (cases and controls) are illustrated in (table 2 and figure1) where 21(52.5%) of cases with SCD were positive for H pylori IgG alone, while26 (65%) of SCD patients were positive for both IgG and IgA antibodies indicating that 5(12.5%) of cases had negative IgG but positive IgA serology. Concerning the control group 8 (26.7%) children were positive for H pylori IgG alone, while 12 (40%) of them were positive for both IgG and IgA antibodies indicating that 4(13.33%) of controls were IgG negative but IgA positive. From a total number of 70 children (patients + controls) we have 9 (12.85%) cases with IgA-positive and IgG-negative results.

Table 2: Serological comparison between cases and controls

Category		H pylori serology		Total	P value
		Negative	Positive		
IgG	SCD	19 (47.5%)	21 (52.5%)	40	0.03*
	Control	22 (73.3%)	8 (26.7%)	30	
IgA	SCD	18 (45%)	22(55%)	40	0.03*
	Control	21 (70%)	9 (30%)	30	
Combined IgG & IgA	SCD	14 (35%)	26 (65%)	40	0.03*
	Control	18 (60%)	12 (40%)	30	

* Significant

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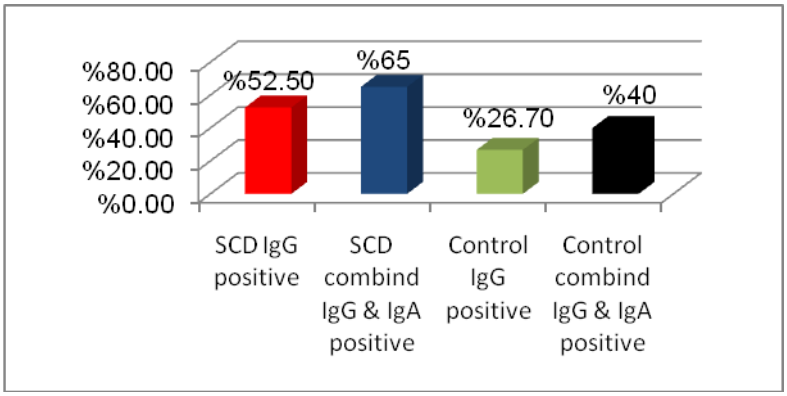

Figure 1: H. Pylori prevalence in SCD and control

In (Table 2, Figure 1) we also recorded a statistically significant difference between cases and controls where the percentage of seropositive cases was significantly higher than controls (P<0.05) regarding IgG, IgA and combined serology.

Table 3: association between the test variables and the H pylori IgG and combined IgG & IgA

Category		IgG serology			Combined IgG & IgA serology			Total
		Negative	Positive	P value	Negative	Positive	P value	
Age group	< 5	6 (15%)	2 (5%)	0.02*	4 (10.0%)	4 (10.0%)	0.1	8 (20.0%)
	5-11	9 (22.5%)	6 (15.0%)		7 (17.5%)	8 (20.0%)		15 (37.5%)
	> 11	4 (10.0%)	13 (32.5%)		3 (7.5%)	14 (35.0%)		17(42.5%)
Sex	Male	12 (30.0%)	11 (27.5%)	0.4	10 (25%)	13 (32.5%)	0.1	23 (57.5%)
	Female	7 (17.5%)	10 (25%)		4 (10.0%)	13 (32.5%)		17 (42.5%)
Consanguinity	Negative	9 (22.5%)	6 (15%)	0.2	6 (15%)	9 (22.5%)	0.7	15 (37.5%)
	Positive	10 (25.0%)	15 (37.5%)		8 (20.0%)	17 (42.5%)		25 (62.5%)
Life time transfusion	No	1 (2.5%)	2 (5.0%)	0.2	1 (2.5%)	2 (5.0%)	0.8	3 (7.5%)
	< 10	8 (20.0%)	5 (12.5%)		5 (12.5%)	8 (20.0%)		13 (32.5%)
	10-30	7 (17.5%)	5 (12.5%)		5 (12.5%)	7 (17.5%)		12 (30.0%)
	> 30	3 (7.5%)	9 (22.5%)		3 (7.5%)	9 (22.5%)		12 (30.0%)
History of recurrent abdominal pain	Yes	5 (12.5%)	3 (7.5%)	0.3	4 (10.5%)	4 (10.5%)	0.3	8 (20.0%)
	No	14 (35%)	18 (45%)		10 (25.5%)	21 (52.5%)		32 (80.0%)
Peptic-like features	Yes	3 (7.5%)	2 (5.0%)	0.5	2 (5%)	3 (7.5%)	0.8	5 (12.5%)
	No	16 (40.0%)	19 (47.5%)		12 (32.5%)	23 (57.5%)		35 (87.5%)
Genotype	S/Beta	6 (15.0%)	5 (12.5%)	0.4	5 (12.5%)	6 (15%)	0.5	11 (27.5%)
	SS	12 (30.0%)	16 (40.0%)		9 (22.5%)	19 (47.5%)		28 (70%)
	SC	1 (2.5%)	0 (0.0%)		0 (0%)	1 (2.5%)		1 (2.5%)

* Significant

The association between different clinical variables and H. pylori IgG and combined IgG and IgA antibodies is displayed in (Table 3) in which we found that the older age group (>11years) is strongly related to H. pylori IgG seropositivity (P=0.02). On the other hand sex difference was not associated with IgG or combined IgG & IgA serology. There was also no association between recurrent abdominal pains and peptic-like features in children with SCD and seropositive IgG or combined IgG +IgA antibodies. Concerning life-time transfusion and genotype, despite a non-significance we recorded that the SS genotype (70%) in SCD patients was associated with a higher IgG and combined IgG + IgA seropositivity. The highest number of blood transfusion (>30 times) was associated with the highest IgG and combined seropositivity. It is noticed also in all clinical variables that H. pylori IgG alone dropped some infected cases which were picked up by the combined IgG and IgA antibodies.

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Table 4: Interrelationship between IgG, IgA and combined serology

Category		IgG Serology			Combined IgG & IgA serology			Total
		Negative	Positive	P value	Negative	Positive	P value	
IgA serology	Negative	14 (35.0%)	4 (10.0%)	0.001*	14 (35.0%)	4 (10.0%)	0.000*	18 (45%)
	Positive	5 (12.5%)	17 (42.5%)		0 (0.0%)	22 (55.0%)		22 (55.0%)
IgG serology	Negative	———————			14 (35.0%)	5 (12.5%)	0.000*	19 (47.5%
	Positive				0 (0.0%)	21 (52.5%)		21 (52.5%)
Combined IgG & IgA serology	Negative	14 (35.0%)	0 (0.0%)	0.000*	----------			14 (35.0%)
	Positive	5 (12.5%)	21 (52.5%)					26 (65.0%)

*Significant

An interrelationship between H. pylori IgG, IgA and combined IgG + IgA in patients with SCD is exhibited in (table 4) in which the results of IgG and IgA serology were similar in 31 cases where 14 patients were negative IgG and negative IgA and 17 cases had positive IgG and IgA serology. However, in 5 cases we found that they were IgG negative but IgA positive and this association was highly statistically significant. As regards the relation between the IgG and combined serology, we recorded 14 patients with negative IgG and negative combined serology, and 21 patients with positive IgG and positive combined serology, while in 5 cases the IgG was negative but the IgA antibodies were positive, giving a total number of 26 patients with positive combined serology (IgG + IgA) and this relation had also a high statistical significance. So when using the IgG only we were able to discover 80.76% of H. pylori infected cases with SCD($\frac{\text{IgG}}{\text{Combined IgG\& IgA}}$ x 100) and 19.23% of H. pylori infected patients will not be diagnosed.

Table 5: correlation between test variables and H pylori IgG &IgA antibodies

		H Pylori IgG	H Pylori IgA
Age (years)	Pearson Correlation	.389*	.526**
	Sig. (2-tailed)	.013	.000
Duration of illness (years)	Pearson Correlation	.360*	.550**
	Sig. (2-tailed)	.022	.000
Hb	Pearson Correlation	-.017-	-.118-
	Sig. (2-tailed)	.918	.467
Retics index	Pearson Correlation	-.059-	.135
	Sig. (2-tailed)	.729	.426
H Pylori IgG	Pearson Correlation	1	.355*
	Sig. (2-tailed)		.024
H Pylori IgA	Pearson Correlation	.355*	1
	Sig. (2-tailed)	.024	

* Significant

Correlation between the different clinical and laboratory variables and H. pylori IgG and IgA antibodies is presented in table 5 which shows a strong positive correlation between the age of the patients as well as duration of illness and the IgG and IgA antibodies (P<0.05) in IgG and (P<0.001) in IgA antibodies. Also a positive correlation is present between IgG and IgA antibodies (P=0.024)

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DISCUSSION

Epidemiology of H pylori infection has not been widely studied in adults and children with SCD; and its prevalence is still unknown, therefore there is limited data with which we can compare our results. The prevalence of H. pylori in our series was 65% with combined serology, which was convenient to a 67.8% prevalence of H pylori infection that has been reported in children with SCD(6). We reported also a significantly higher prevalence of H. pylori infection in SCD children than controls 65% versus 40% respectively (P<0.05). Similarly, Jaber (2006)(6, 17) studied chronic hemolytic anemia patients in Jeddah city Saudi Arabia and showed an increase in prevalence of H. pylori infection versus controls (31.7% versus 23.6%, p<0.01) .However, contrary to our results, some authors(6)found non-significant difference in H. pylori infection between SCD and non SCD children; but unlike our cases 71.9% of their children with IgG positive serology had an unsafe water supply. It's worthy to mention that the different prevalence between investigators (65%and 67.8% on one side and 31.7% on the other side) proves that the prevalence of H. pylori in SCD patients is not settled yet.

In the present work we recorded a rapid increase in the prevalence of H. pylori infection with age (P=0.02) and a strong positive correlation between the age of SCD patients and pylori IgG and IgA antibodies (P<0.05 and <0.001respectively). This was like other studies that showed an increase in H. pylori prevalence with age (18-20). Our results also concurs authors who reported significant association between prevalence of H. pylori in studied chronic hemolytic anemic children and increased age (6, 17).

A statistically significant correlation was found between duration of illness and H. pylori IgG and IgA antibodies (P<0.05 and <0.001 respectively). We, as well, recorded that the highest number of lifetime transfusion (>30) was associated with the highest frequency (22.5%) of IgG and combined (seropositivity. These results agree those of Jaber (2006)(17) who reported significant associations between H. pylori seroprevalence and duration of illness and number of blood transfusions in patients with chronic hemolytic anemia and concluded a strong relation with the severity of chronic disease.

Our children belonged to three forms of sickle hemoglobinopathy, SS (70%), S/beta (27.5%) and SC (2.5%). The term "sickle cell disease" includes the four most common forms of sickle hemoglobinopathy: sickle cell anemia (SCA; Hb SS), sickle-C disease (Hb SC), sickle-, B+ thalassemia (Hb SB+) and sickle B0 thalassemia (Hb SB0)(21). The SS(70%) genotype in our patients was accompanied by the highest H. pylori IgG and combined seropositivity (40% and 47.5% respectively), which was nearly in keeping with authors(6) who studied H. pylori infection in 118 children with SCD with 67.8% prevalence and found that 96.6% of them were of the SS genotype and only 3.4% were SC genotype; an issue that really needs more investigation.

The link between H pylori and recurrent abdominal pain in children is still controversial. While some studies reported an association between recurrent abdominal pain (RAP) and H. pylori (22-24); others found no association (25-28). The Canadian H. pylori study group and Sherman concluded that RAP is not an indication for testing for H. pylori (25, 26). We, like other authors reported no significant association between (RAPS) or peptic like features and H pylori infection in SCD children(6).

Our results, however, cannot exclude H. pylori as a cause of RAP in SCD patients. In a review of prevention and management of infection in sickle cell anemia, a high suspicion of H. pylori gastritis has been suggested when there is RAP(4). H pylori infection has also been implicated in a child(29), six children and eight adults (5) with SCD having RAP due to gastric or duodenal gastritis. This relationship requires larger and nation-wide multidimensional studies.

Biopsy-based methods such as histology and rapid urease test are the gold standard for H. pylori detection (30, 31), but both these methods require gastroscopy, and hence are expensive, time-consuming and not without complications. The importance of H. pylori makes it imperative to develop a safe, noninvasive and simple method of detection that should be easily available to all clinicians. As serological testing for only (IgG) antibodies has been used in studies which assessed H. pylori infection in patients with SCD(5, 6, 17, 29) ; while the clinical value of IgA antibodies remains controversial(32); so the main plan of our study was to determine the frequency of IgA-positive IgG-negative cases in children with SCD, thus assessing the clinical utility of IgA testing in those patients.

We reported 5 (12.5%) cases with SCD infected with H. pylori, had positive IgA but negative IgG antibodies, raising the prevalence from 52.5% to 65%; and in controls 4 (13.33%) raising prevalence from 26.7% to 40% in our study with a total of 9(12.85%) infected cases with +ve IgA and –ve IgG. Two studies have noted few patients with confirmed H. pylori infection and with only IgA antibodies (33, 34). Aromaa et al (1996) reported that IgA antibodies and low pepsinogen I levels increase the risk of gastric carcinoma(35). Moreover, IgA antibodies may appear earlier than IgG in patients who become reinfected (35). Some investigators have found that about 2% of cases exhibit an IgA response in the absence of IgG response (15, 33). However, Jaskowski et al(1997)(32)showed a higher frequency(7.2%) of IgA positive and IgG negative patients (38/824) cases with gastrointestinal disorders compatible with H. pylori infection and suggested that a large number of IgG negative patients have not been investigated. It is assumed that H. pylori are excluded by the clinician in the majority of these infected patients solely on the basis of a negative IgG antibody test.

Uritaet al (2004)(30)recorded 3% of his patients (3/101) were IgA positive and IgG negative and of eight non-infected patients in whom intestinal metaplasia was found by dye endoscopy four cases (50%) were IgA positive and IgG negative. Yamamoto et al (1995) have shown the IgA antibody to be 100% specific for H. pylori infection compared to histology(36).

Furthermore, Fallone et al(1995) proved that at one month post-eradication therapy IgA, but not IgG, detection may be a good method of assessing disappearance of H. pylori(37). They suggested more studies to determine whether serology alone is sufficient in certain clinical settings to determine subsequent clinical strategy.

In conclusion, the prevalence of H. pylori infection in our SCD children was 65% versus 40% in controls. A positive IgG and IgA antibodies was strongly related to the age of the patients and their duration of illness, but was not associated with RAP or peptic like features. In addition, a high frequency of IgA- positive IgG-negative results among H. pylori infected cases was detected in both patients and controls.

Hence, we recommend to extremely raise the awareness of all clinicians that great care should be taken not to underestimate the prevalence of H. pylori infection from the results of IgG serology and it's mandatory to do IgA antibodies not only in SCD but in other chronic diseases and in any individual with suspected H. pylori infection and having negative IgG antibodies. We as well agree to a limited extent, histology in SCD patients with gastrointestinal symptoms to assess the sensitivity and specificity of both serological tests (IgG and IgA) using histology as the gold standard. We also suggest longitudinal studies in H. pylori infected patients with SCD, to test the usefulness of IgA antibodies in establishing eradication as soon as one month after cessation of treatment. Finally, we need further and larger studies in Egypt to evaluate the higher frequency of IgA-positive and IgG-negative H. pylori infected patients relative to other investigators.

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