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Seroprevalence of Antibodies to *Helicobacter Pylori* Six Virulent Antigens in Asymptomatic Adult Beta Thalassemic Patients

Abstract

Introduction: The idea that Helicobacter pylori (H. pylori) might confer benefit to humans by impairing iron absorption has engendered considerable controversy among investigators. The aim of the present study was to compare the seroprevalence of anti H. pylori in asymptomatic beta thalassemia major (ß TM) patients and normal controls.

Materials and methods: 40 proven adults ß TM cases (group 1) and 40 age and sex-matched healthy controls (group 2) were the subject of the study. The mean age of adults' ß TM was 21.5±4.97 years. Twenty two (55%) were males and 18 (45%) were females. Serum samples from all patients and controls were examined for specific IgG antibodies to the following H. pylori six virulent antigens: cagA (cytotoxin-associated gene A), vacA (vacuolating cytotoxin A), UreA (urease A), p30 (OMP), p25 (OMP) and p19 (OMP) using H. pylori LINE Immunoblot Assay. Serum samples from all patients were examined also for HCV-RNA viral load by Real Time PCR.

Results: There was a statistically significantly higher seroprevalence of H. pylori among adult ß TM patients than among the controls [14/40 (35%) vs. 9/40 (22.5%)] respectively (X2=82.538, p=0.000). Antibodies against cagA and vacA were absent in the healthy seropositive controls. There was no statistically significant difference between H. pylori IgG-positive (14 cases) and negative (22 cases) ß TM as regards age (t=0.633, p=0.432), sex (x2=0.175, p=0.676), hemoglobin value (t=0.332, p=0.568), serum ferritin level (t=0.394, p=0.394) and presence of HCV. Antibodies to the six virulent antigens of H. pylori were higher in splenectomized than non splenectomized patients. Antibodies to cagA and vacA were significantly higher in splenectomized than non splenectomized patients (p=0.015 and 0.044 for cagA and vacA respectively).

Conclusion: There was higher H. pylori seroprevalence in asymptomatic β-TM cases than among controls, being more frequently in splenectomized than non splenectomized patients. No difference in serum ferritin level between H. pylori seropositive and seronegative cases.

Keywords: *H. pylori*; Beta thalassemia; cagA; vacA; HCV- RNA

Research Article

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Introduction

Thalassemias are the most common single gene disorder in the world. They are found at high frequencies in developing regions as well as in large multiethnic western cities due to expanding immigrant population [1]. ß thalassemias (ß TM) are an inherited group of conditions that result from an inadequate beta-globin synthesis of the hemoglobin molecule. There are approximately 240 million people worldwide who are heterozygous for β-thalassemia and approximately 200,000 affected homozygotes are born annually [2].

Patients usually present to medical attention in the first year of life with varying degrees of hemolytic anemia and expansion of the bone marrow. They require regular blood transfusions and iron-chelation therapy for survival [3]. Anemia and excess body iron can result in endocrine disorders (e.g., diabetes mellitus, hypogonadism, hypoparathyroidism, hypopituitarism), growth retardation, liver and cardiac failure and splenomegaly. The latter can worsen anemia and occasionally causes thrombocytopenia and neutropenia, thereby increasing the risk of infections and hemostatic disorders [4,5].

Infection-related mortality used to be the second leading cause of death after cardiac complication [6]. It has gradually become the leading cause of death in thalassemia in the modern era in western countries due, in part, to a significant reduction in the number of deaths from iron induced cardiac disease [7].

The increased phagocytic activity resulting from clearance of defective erythrocytes may reduce the capacity of the phagocytic system to defend against microorganisms and consequently overwhelms pattern recognition receptors, including Toll-like receptors. Severe anemia, itself, has also been observed as a risk factor for bacterial infections in thalassemia [8-10]. Other causes such as transfusion related immunomodulation, iron overload, some therapeutic interventions such as iron chelation therapy, central venous catheters and stem cell transplantation may contribute to infectious complications with resultant morbidity and mortality. The risk of severe bacterial infections in thalassemic

patients appears to be high, particularly after splenectomy [7].

Helicobacter pylori (*H. pylori*) are a gram-negative, microaerophilic, spiral, bacterial organism that infects around half of the world's population. The incidence rises steadily with age. In the UK, approximately 50% of the population (over 50 years of age) is infected with *H. pylori*. In the developing world, this figure may be as high as 90%. The vast majority of the infected population however remains asymptomatic [11].

H. pylori have been associated with the development of extragastric disorders including iron deficiency anemia, chronic idiopathic thrombocytopenic purpura, growth retardation and diabetes mellitus [12]. The postulated role of *H. pylori* in the pathogenesis of extragastric disorders is based on the fact that persistent infection induces a chronic inflammatory and immune response that is able to induce lesions both locally and remote to the primary site of infection [13].

Because of the association of *H. pylori* with extragastric disorders such as cholecystitis, increasing the risk or exacerbating heart diseases and in impairing iron absorption, it is important to study it in ß TM patients.

The aim of the present study was to compare the seroprevalence of *H. pylori* in asymptomatic ß TM patients and normal controls to improve their quality of life.

The study was conducted on 40 adults beta thalassemia major (ß TM) cases (group 1) and 40 age and sex-matched healthy controls (group 2) without any manifestations of recurrent abdominal pain. Patients were recruited from the Hematology outpatient clinic at Alexandria Main University Hospital during the period of March 2014 to July 2015 among those who came for routine follow-up. The mean age of adults' ß TM patients was 21.5±4.97 years. Twenty two (55%) were males and 18 (45%) were females.

A detailed medical history and physical examination were carried out to all patients with special emphasis on age, sex, duration of blood transfusion, duration of iron chelating agent, age at splenectomy, complications of iron overload and all information about abdominal pain if present. All patients had proven ß TM on the basis of complete blood count, hemoglobin electrophoresis from the first years of life and were on regular blood transfusion and iron chelating therapy. Evaluation of serum ferritin level was carried out in all cases as a part of their routine evaluation. Hemoglobin level was evaluated in all controls.

Serum samples from all patients were examined for HCV-RNA viral load by Real Time PCR (AB: Applied Biosystem, USA) [14]. Serum samples from all patients and controls were examined for *H. pylori* antibodies to six virulent *H. pylori* antigens *by* LINE Immuno Assay (*Helicobacter pylori* LINE IgG / IgA Line Immunoblot, Virotech genzyme, USA). It was a quantitative detection of *H. pylori* specific IgG respectively IgA antibodies in human serum to the following six virulent antigens: cagA (cytotoxin-associated geneA), vacA (vacuolating cytotoxin A), UreA (urease A), p30 (OMP), p25 (OMP) and p19 (OMP), according to the manufacturer instructions [15]

Interpretation criteria of Helicobacter pylori LINE IgG / IgA Line Immunoblot assay:

- No band or only one band of p30, p19: Negative
- Only one band of vacA, ureA, p25: Borderline
- CagA or arise from ≥2 bands of vacA, p30, ureA, p25, p19: Positive (Type I: virulent, Type II: non virulent; cagA, vacA negative)

All cases were informed of the study objectives and gave their informed consent prior to their inclusion in the study. The research protocol was approved by the local ethics and research committees of the participating hospital.

Statistical analysis

Statistical package for the social science (SPSS, version 21.0; SPSS Inc., Chicago, Illinois, USA) was used in the evaluation of the data. Quantitative data were expressed as mean±SD while qualitative data were expressed as values and percent. Independent "t" test was used to evaluate the difference between two mean" values. "chi- square analysis" was used for qualitative data. $p<0.05$ was accepted as significant.

Results

Table 1 shows the clinical and laboratory data of the studied thalassemic patients (group 1) and controls (group 2). Both groups are of matched age and sex. Figure 1 shows seroprevalence of *H. pylori* antibodies among thalassemic cases and controls. Fourteen thalassemic patients (35%) were positive for *H. pylori*, 4 (10%) were borderline and 22 (55%) were negative. Eight patients were males and 6 were females. Nine (22.5%) of the controls were seropositive for *H. pylori*. Five of controls were males and 4 were females. There was a statistically significantly higher seroprevalence of *H. pylori* among adult ß TM patients than among the controls (14/40 (35%) vs. 9/40 (22.5%)) respectively (X^2=82.538, p=0.000). However, there was non statistically significant difference between *H. pylori* positive cases and controls as regards age (t=0.057, p= 0.813) and sex (x^2=0.006, p=0.94).

Table 2 shows the percent of *H. pylori* specific IgG antibodies to the six virulent *H. pylori* antigens in thalassemic patients. Antibodies against cagA and vacA are absent in the healthy seropositive controls. There was non statistically significant difference between total *H. pylori* IgG-positive (14 cases) and negative (22 cases) ß TM cases as regards age (t= 0.633, p=0.432), sex (x^2=0.175, p=0.676), hemoglobin value (t=0.332, p=0.568) and serum ferritin level (t=0.394, p=0.394).

Comparison between *H. pylori* positive cases as regards presence of HCV and splenectomy was shown in Figure 2 and Table 3 respectively. Higher number of antibodies to all six virulent antigens of H pylori was present in splenectomized versus non splenectomized patients. Antibodies to cagA, vacA and p25 were significantly higher in splenectomized than non splenectomized patients (x^2=5.934*, p=0.015 for cagA, x^2=4.038*, p=0.044 for vacA and x^2=5*, p=0.025 for p25).

There was a statistically significantly higher seroprevalence of *H. pylori* among adult β-TM patients than among the adult controls (14/40 (35%) vs. 9/40 (22.5%)) respectively. Christoforidis et al also compared anti-*H. pylori* IgG among 40 asymptomatic β-TM patients with a mean age of 27.2±9.7 years and 30 age and sex-matched controls in Greece. No significant difference was

observed in his study between the prevalence of *H. pylori* IgG in β-thalassemia patients (15%) compared with controls (20%) [16].

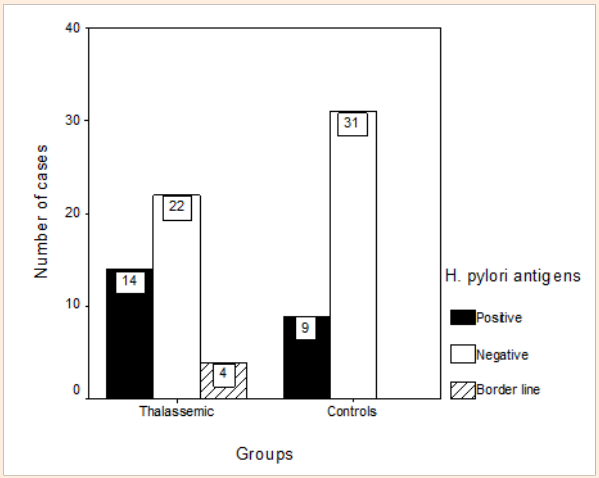

Figure 1: Seroprevalence of H. pylori antibodies among thalassemic cases and controls.

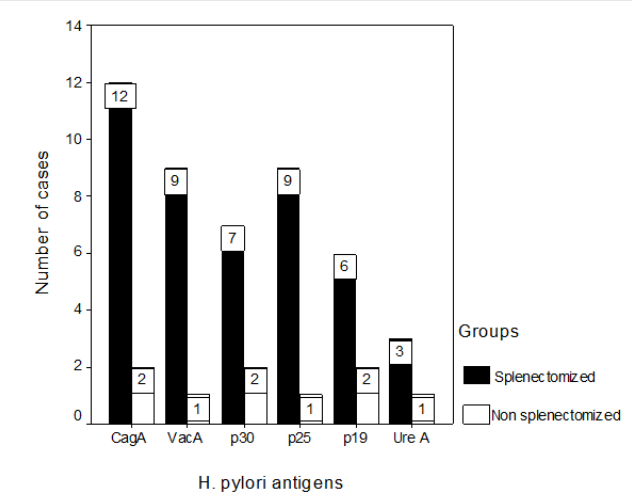

Figure 2: Comparison of antibodies to six virulent antigens of H. pylori in seropositive β TM patients by splenectomy.

Host iron is known to be essential for pathogen growth [17]. However, there have been inconsistent reports on the roles of iron in the acquisition of *H. pylori* infection [17-19]. Perhaps, the iron overload in thalassemia patients might have contributed to the high prevalence of *H. pylori* infection observed in our study.

Table 1: Clinical and laboratory data of thalassemic patients and controls.
Values are mean ± SD; *Significantly different as compared with controls; p is significant if ≤0.05.

Parameter	Cases (n=40)	Controls (n=40)	Test of significance	p value
Age (yrs)	21.5±4.97	21.425±3.87	t=0.308	0.58
Sex				
Male	22 (55%)	20 (50%)	x^2=0.201	0.654
female	18 (45%)	20 (50%)		
No. of splenectomized patients	24			
Presence of HCV	27			
Complications of iron overload	12			
Hb (gm/dl)	6.73±0.85	13.49±1.08	t=6.966*	0.01
Serum ferritin (mg/dl)	4379.25±2213.27	66.95±18.49	t=55.577*	0

Table 2: *Helicobacter pylori* specific IgG antibodies to the six virulent *H. pylori* antigens in thalassemic patients.

H. pylori antigens	***H. pylori*** positive cases (no.14)	Percentage %
Cag A(+ve)	14	100.00%
Vac A(+ve)	9	64.30%
p30 (+ve)	10	71.14%
p25 (+ve)	10	71.14%
p19(+ve)	8	57.14%
UreA (+ve)	6	42.90%

Table 3: Comparison of antibodies to six virulent antigens of H pylori in seropositive thalassemic patients by HCV.

Antibodies to six virulent antigens of *H. pylori*	Total	HCV				X^2	p
		Positive "n=27"		Negative "n=13"			
		No.	%	No.	%		
Cag A	14	10	71.4	4	28.6	0.152	0.697
VacA	9	6	66.7	3	33.3	0.004	0.952
p30	10	7	70	3	30	0.038	0.845
p25	10	7	70	3	30	0.038	0.845
p19	8	5	62.5	3	37.5	0.114	0.736
UreA	6	4	66.7	2	33.3	0.002	0.962

However, there was non statistically significant difference between anti *H. pylori* IgG positive cases and controls as regards age or sex. Epidemiological studies have demonstrated that the prevalence of *H. pylori* infection increases with advancing age and is higher in developing countries and among population with low socioeconomic background, probably due to conditions that favor the acquisition of infection, such as poor hygiene, crowded living conditions and absence or deficiency of sanitation [20]. It has been reported that in developing countries, *H. pylori* infection is usually acquired before 10 years of age and in the absence of antibiotic therapy, it generally persists for life [21].

Our result is in accordance with other studies showing that both seroprevalence and active infection increase with age in β-TM patients [22]. In literature, different results are indicated about gender. No relationship about gender was identified [16,23].

There was no significant difference between our *H. pylori* IgG-positive (14 cases) and negative (22 cases) β-TM as regards age, sex, hemoglobin value, serum ferritin level or presence of HCV infection. In our study, a significant relationship was found between seroprevalence and splenectomy only. Higher number of patients with antibodies to six virulent antigens of H pylori was present in splenectomized than non splenectomized patients. Antibodies to cagA and vacA were significantly higher in splenectomized than non splenectomized patients. In harmony with our result, Feiby GKY et al [19], Christoforidis et al [16] and Karimi et al [24] found a clear relationship between the prevalence of *H. pylori* infection and splenectomy. However, Balci et al. [22] did not find any relationship between *H. pylori* infection and splenectomy.

The spleen provides important host defense functions by removing circulating antigens and synthesizing opsonizing antibodies, tuftsin, immunoglobulins, principally immunoglobulin M, 7 complements, and endogenous cytotoxic factors [25].

In older studies, the risk of post splenectomy sepsis in β thalassemia is increased more than 30 folds than in non splenectomized person [26]. This makes us think that the immune response, which can weaken after splenectomy, may play a protecting role against *H. pylori* infection

Iron absorption increases several-fold in thalassemia patients who do not receive any transfusion. It has been estimated that iron absorption exceeds iron loss when expansion of red cell precursors in the bone marrow exceeds five times that of healthy individuals. In individuals who are poorly transfused, absorption rises to 3-5mg/day or more, representing an additional 1-2 g of iron loading per year [7]. However, even with the introduction of iron chelator desferrioxamine, cardiac failure due to iron overload still accounted for 67% of deaths in thalassemia major and many patients experienced endocrinopathies secondary to iron overload [27].

The idea that *H. pylori* might actually confer benefit to humans has engendered considerable controversy among investigators [16]. In our study, there was no statistically significant difference between *H. pylori* IgG-positive (14 cases) and negative (22 cases) ß TM as regards serum ferritin level. Studies regarding the involvement of *H. pylori* cagA strains in the alteration of the hosts' iron stores are controversial. Ciacci *et al* showed that impaired iron absorption in *H. pylori*-infected adult (>17 years) patients was not related to infection with cagA positive strains [28].

Data generated from a large adult German population-based study could not establish a risk excess for the reduction in the serum ferritin levels according to CagA seropositivity [29]. In contrast, a cross-sectional study found a higher prevalence of low serum ferritin (< 10ng/mL) among iron deficient subjects with cagA-positive strains than CagA-negative strains and *H. pylori*-negative subjects [30]. Of interest, a double blind randomized intervention trial on non-iron-deficient 3- to 10-year-old children in Texas, showed that eradication of *H. pylori* infection by cagA-negative strains was associated with a larger serum ferritin increase [31].

Beutler hypothesized that *H. pylori* subvert the human iron regulatory mechanism by producing hepcidin mimics in a manner that is useful to the micro-organism and deleterious to the host [32]. However, particular for patients with β-thalassemia this iron sequestration may be proven beneficial. This preliminary data need additional confirmation and further investigation [16].

Conclusion

There was a higher prevalence of *H. pylori* IgG in asymptomatic adult β -TM cases than among controls. *H. pylori* IgG was detected more frequently in splenectomized than non splenectomized patients. Other parameters like age, sex, Hb value, serum ferritin and HCV had no role.

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