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 (β TM) patients and normal controls.

Materials and methods: 40 proven adults β 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’ β 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 β 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) β 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

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]. β-thalassemia (β 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 dearance 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
Seroprevalence of Antibodies to Helicobacter Pylori Six Virulent Antigens in Asymptomatic Adult Beta Thalassemic Patients

H. 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 cholecytisitis, increasing the risk or exacerbating heart diseases and in impairing iron absorption, it is important to study it in β TM patients.

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

The study was conducted on 40 adults beta thalassemia major (β 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’ β 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 β 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 Biosysstem, 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 Immuno blot, 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 gene A), vacA (vaculating 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.

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 β TM patients than among the controls (14/40 (35%) vs. 9/40 (22.5%)) respectively (X²=82.538, p=0.000). However, there was no statistically significant difference between H. pylori positive cases and controls as regards age (t=0.057, p=0.813) and sex (x²=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 no statistically significant difference between total H. pylori IgG-positive (14 cases) and negative (22 cases) β TM cases as regards age (t= 0.633, p=0.432), sex (x²=0.175, p=0.676), hemoglobin value (t=0.332, p=0.568). However, there was no statistically significant difference between H. pylori positive cases and controls as regards age (t=0.057, p=0.813) and sex (x²=0.006, p=0.94).

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 cases. Antibodies to cagA, vacA and p25 were significantly higher in splenectomized than non splenectomized patients (x²=5.934*, p=0.025 for cagA, x²=4.038*, p=0.044 for vacA and x²=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.](image)

![Figure 2: Comparison of antibodies to six virulent antigens of *H. pylori* in seropositive β TM patients by splenectomy.](image)

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%       |

Citation: Hamed NA, Ghallab O, Morsi MG, Hassona E, Elshohy I (2015) Seroprevalence of Antibodies to *Helicobacter Pylori* Six Virulent Antigens in Asymptomatic Adult Beta Thalassemic Patients. J Microbiol Exp 2(6): 00071. DOI: 10.15406/jmen.2015.02.00071
Seroprevalence of Antibodies to Helicobacter Pylori Six Virulent Antigens in Asymptomatic Adult Beta Thalassemic Patients

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 No. | HCV Positive "n=27" | HCV Negative "n=13" | \( \chi^2 \) | p |
|-----------------------------------------------|----------|---------------------|---------------------|---------|---|
| CagA                                          | 14       | 10                  | 7.14                | 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 persons [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) β-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. Giacci 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.

Citation: Hamed NA, Ghallab O, Morsi MG, Hassona E, Elkholy I (2015) Seroprevalence of Antibodies to Helicobacter Pylori Six Virulent Antigens in Asymptomatic Adult Beta Thalassemic Patients. J Microbiol Exp 2(6): 00071. DOI: 10.15406/jmen.2015.02.00071
References

1. Weatherall DJ (2010) The inherited diseases of hemoglobin are an emerging global health burden. Blood 115(22): 4331-4336.
2. Cao A, Saba L, Galanello R, Rossetti M (2007) Molecular diagnosis and carrier screening for β-thalassemia. JAMA 297(15): 1273-1277.
3. Weatherall DJ, Clegg JB (2001) The thalassemia syndromes. (4th edn). Blackwell Science, Oxford, Malden, MA, UK.
4. Muncie H, Campbell JS (2009) Alpha and β thalassemia. Am Fam Physician 80(4): 339-344.
5. Borgna-Pignatti C, Cappellini MD, De Stefano P, Del Vecchio GC, Forni GL, et al. (2005) Survival and complications in thalassemia. Ann N Y Acad Sci 1054: 40-47.
6. Vento S, Caine L, Cesario F (2006) Infections and thalassemia. Lancet Infect Dis 6(4): 226-253.
7. Cappellini MD, Cohen A, Porter J, Taher A, Viprakasit V (2014) Guidelines for the management of transfusion dependent thalassemia. (3rd edn). Thalassaemia international federation, Cyprus.
8. Wiener E, Wanachiwawin W, Chinprasertsuk S, Sripanyaaphinyo U, Mawas F, et al. (1996) Increased serum levels of macrophage colony stimulating factor (M-CSF) in alpha- and beta thalassemia syndromes: correlation with anemia and monocyte activation. Eur J Haematol 57(5): 364-369.
9. Ozinsky A, Underhill DM, Festenot JD, Hajjar AM, Smith KD, et al. (2000) The repertoire for pattern recognition of pathogens by the innate immune system is defined by cooperation between toll-like receptors. Proc Natl Acad Sci USA 97(25): 13766-13771.
10. Realdi G, Dore MP, Fastame L (1999) Extradigestive manifestations of Helicobacter pylori infection. World J Gastroenterol 20(6): 1379-1401.
11. Helicobacter pylori impairs iron absorption in infected individuals. Dig Liver Dis 36(7): 455-600.
12. Ciacci C, Sabbatini F, Cavaliaro R, Castiglione F, Di Belia S, et al. (2004) Helicobacter pylori infection and extragastric disorders in children: A critical update. World J Gastroenterol 10(6): 1379-1401.
13. Realki G, Dore MP, Fastame L (1999) Extradigestive manifestations of Helicobacter pylori infection: fact and fiction. Dig Dis Sci 44(2): 229-236.
14. Kato N, Yokosuka O, Omato M, Hosoda K, Ohto M (1990) Detection of Hepatitis C virus ribonucleic acid in the serum by amplification with polymerase chain reaction. J Clin Invest 86(5): 1764-1747.
15. Tajana FK, Miroslava K, Vladimir P, Slavko G, Vesna GC (2009) The World Medical Association. Declaration of Helsinki 50(2): 124-132.
16. Christoforidis A, Vlachaki E, Perifanis V, Frida-Michaelidou I, Tsatra I (2008) Can Helicobacter pylori infection be proven beneficial to patients with beta-thalassaemia major? Med Hypotheses 71(4): 616-617.
17. Rajendra J, Devanarayana NM, de Silva HJ (2009) Helicobacter pylori infection in children. Saudi J Gastroenterol 15(2): 86-94.
18. Jaber SM (2006) Helicobacter pylori seropositivity in children with chronic disease in Jeddah, Saudi Arabia. Saudi J Gastroenterol 12(1): 21-26.
19. Reiby GKY, Nesrin MH, Badr AMM (2015) Prevalence of Helicobacter pylori infection among β-thalassemia major children with recurrent abdominal pain at Suez Canal University Hospital. Egyptian Journal of Haematology 40: 74-79.
20. Graham DY, Malaty HM, Evans DG, Evans DJ, Klein PD, et al. (1991) Epidemiology of Helicobacter pylori infection in an asymptomatic population in the United States. Effect of age, race and socioeconomic status. Gastroenterology 100(6): 1495-1501.
21. Chiesa C, Pacifico L, Anania C, Poggioigile E, Chiarelli F, et al. (2010) Helicobacter pylori therapy in children: overview and challenges. Int J Immunopharmacol Pharmacol 23(2): 405-416.
22. Baclì YI, Aral YZ, Covut IE, Polat Y, Turk M, Acinìs N (2011) The frequency of Helicobacter pylori infection in beta thalassemia major patients with recurrent abdominal pain. Pak J Med Sci 2011 27: 316-319.
23. Granström M, Tindberg Y, Bellenow M (1997) Seroepidemiology of Helicobacter pylori infection in a cohort of children monitored from 6 months to 11 years of age. J Clin Microbiol 35(2): 466-470.
24. Karimi M, Hadi Imanieh M, Ghiam AF, Hashemi Z (2005) Investigation of Helicobacter pylori infection in beta-thalassaemia major patients with recurrent abdominal pain. Eur J Gastroenterol Hepatol 17(12): 1363-1367.
25. Sheng C-F, Liu B-Y, Zhang H-M, Zheng X (2015) Overwhelming post-splenectomy infection. Genetics and Molecular Research 14(1): 2702-2706.
26. Singer DB (1973) Postsplenectomy sepsis. Perspect Pediatr Pathol 1: 285-311.
27. Borgna-Pignatti C, Rugolotto S, De Stefano P, Zhao H, Cappellini MD, et al. (2004) Survival and complications in patients with thalassemia major treated with transfusion and deferoxamine. Haematologica 89(10): 1187-1193.
28. Gacci C, Sabatini F, Cavaliaro R, Castiglione F, Di Belia S, et al. (2004) Helicobacter pylori impairs iron absorption in infected individuals. Dig Liver Dis 36(7): 455-600.
29. Berg G, Bode G, Blettner M, Boeing H, Brenner H (2001) Helicobacter pylori infection and serum ferritin: A population-based study among 1806 adults in Germany. Am J Gastroenterol 96(4): 1014-1018.
30. Muhisen K, Barak M, Shinaidei L, Nir A, Bassal R, et al. (2009) Helicobacter pylori infection is associated with low serum ferritin levels in Israeli Arab children: a seroepidemiologic study. J Pediatr Gastroenterol Nutr 49(2): 262-264.
31. Cardenas VM, Prieto-Jimenez CA, Mulca YD, Rivera JD, Dominguez DC, et al. (2011) Helicobacter pylori eradication and change in markers of iron stores among noniron-deficient children in El Paso, Texas: an etiologic intervention study. J Pediatr Gastroenterol Nutr 52(3): 326-332.
32. Beutler E (2007) Heparin mimetics from microorganisms? A possible explanation for the effect of Helicobacter pylori on iron homeostasis. Blood Cells Mol Dis 38(1): 54-55.