Research Article

The Relationship between *Helicobacter pylori* and Beta-2 Microglobulin in Humans

Abdullah Özgür Yeniova,¹ Metin Kucukazman,¹ Naim Ata,² Kursat Dal,² Ayşe Kefeli,¹ Sebahat Başyigit,¹ Bora Aktaş,¹ Kadir Okhan Akın,³ and Yaşar Nazlıgül¹

¹ Department of Gastroenterology, Kecioren Teaching and Research Hospital, Kecioren, 06380 Ankara, Turkey

² Department of Internal Medicine, Kecioren Teaching and Research Hospital, Kecioren, 06380 Ankara, Turkey

³ Department of Clinical Biochemistry, Kecioren Teaching and Research Hospital, Kecioren, 06380 Ankara, Turkey

Correspondence should be addressed to Abdullah Özgür Yeniova; zgryeniova@yahoo.com.tr

Received 10 March 2014; Revised 11 August 2014; Accepted 12 August 2014; Published 27 August 2014

Academic Editor: Jeffrey A. Frelinger

Copyright © 2014 Abdullah Özgür Yeniova et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

H. pylori is related to various gastrointestinal diseases. β_2 Microglobulin (β_2 M) is an intrinsic element of major histocompatibility complex (MHC I). Serum β_2 M level may increase in inflammatory states. The aim of current study is to evaluate the relationship between β_2 M and *H. pylori* bearing CagA strains. **Methods.** *H. pylori* status was determined by histopathology of samples taken from stomach. CagA status and β_2 M level were measured from blood samples of patients. Eradication therapy was administered to the patients with *H. pylori* infection. β_2 Microglobulin levels were measured before and after treatment. **Results.** 35 (29.2%) *H. pylori* (–) patients and 85 (70.8%) *H. pylori* (+) patients were included in the study. There were 52 (43.3%) patients with CagA negative and 33 (27.5%) patients with CagA positive *H. pylori* infection. The mean serum β_2 M level was 1.83 mg/L in *H. pylori* (–) group, 1.76 mg/L in *H. pylori* (+) CagA (–) group, and 1.93 mg/L in *H. pylori* and CagA (+) group ($P > 0.05$). Serum β_2 M levels (1.82 versus 1.64 mg/L $P < 0.05$) were decreased after eradication. **Conclusion.** *H. pylori* and CagA status did not affect β_2 M level. Relationship between low grade systematic inflammation and *H. pylori* should be investigated to find out new predictors for diseases associated with inflammation.

1. Introduction

Discovery of *Helicobacter pylori* (*H. pylori*) changed the management and treatment of many gastrointestinal diseases. Depending on the geographic location, *H. pylori* is an inhabitant of half of the world population's stomach. *H. pylori* plays a crucial role in the development of gastrointestinal diseases such as chronic gastritis, gastric ulcer and duodenal ulcer mucosa associated lymphoid tissue lymphoma (MAL-TOMA), and gastric adenocarcinoma [1, 2].

Despite its high prevalence, most of *H. pylori* infected subjects are asymptomatic. However some of them do not remain in gastritis stage and progress to gastric ulcer, duodenal ulcer, or gastric cancer [3]. Factors that contribute to disease progression have been recognised in the past decade. Cytotoxin-associated antigen (CagA) is one of the examples of *H. pylori* virulence factors that are

encoded by genes of *H. pylori* “pathogenicity island” [4]. CagA is associated with significant inflammation in gastric mucosa.

β_2 -microglobulin (β_2 M) is the light chain domain of major histocompatibility complex (MHC I) [5]. It plays a crucial role in antigen presentation by chaperoning the assembly of the complex [6, 7]. β_2 M is synthesized by all nucleated cells but is released from lymphocytes mainly so it can be a marker for inflammation [8]. β_2 M is bound noncovalently, so it can be detected as free monomer in serum, urine, and cerebrospinal fluid. After degradation of MHC I molecules, α heavy chain remains in the cell but β_2 M is released into circulation. Therefore, β_2 M serum level is associated with cell turnover and renal function. Impaired glomerular function and increased cell turnover raise β_2 M levels. Elevated serum β_2 M level is poor prognosis of multiple myeloma, lymphomas, and leukemias [9–11].

We hypothesized that CagA positive *H. pylori* infection can be linked with increased levels of β_2 M. Relationship between β_2 M and *H. pylori* has been evaluated in the past decades [12–14] but CagA positivity in *H. pylori* infection and its association with β_2 M have not been evaluated.

The purpose of this study was to evaluate the relationship between β_2 M and CagA status of *H. pylori* infection. We also aimed to investigate the effect of eradication on β_2 M levels.

2. Material and Methods

The patients with dyspepsia who were admitted to the gastroenterology outpatient clinic between June 2012 and December 2012 were evaluated. Patients between 18 and 65 years of age who were referred to upper endoscopy from outpatient clinic were enrolled in the study. Patients who used antibiotics or nonsteroidal anti-inflammatory drugs for any reason in the past two weeks were excluded from the study. Local ethical committee approved the study protocol and informed consent was obtained from each enrolled patient.

Also all patients were evaluated for prior chronic diseases. Routine hemogram tests and biochemical parameters which include alanine amino transferase (ALT), aspartate amino transferase (AST), alkaline phosphatase (ALP), gamma glutamyl transpeptidase (GGT), total and direct bilirubin, urea, creatine, and serum electrolytes were performed for all participants. Patients with abnormal values and patients with coronary artery disease, liver failure, kidney failure, cerebrovascular disease, and malignant disease which may contribute to serum levels of β_2 M were also excluded from the study.

All patients who fulfilled the criteria underwent upper gastrointestinal tract endoscopy and patients who were taken biopsies from antrum and corpus were considered eligible for assessment. Other patients for whom biopsy material could not be obtained were also excluded from the study. We also excluded patients with gastric cancer and gastric and duodenal ulcer determined in endoscopy. Samples from antrum and corpus were transported in formalin contained solutions and were embedded in paraffin blocks. Four-micrometer-thick sections from each specimen were stained with hematoxylin-eosin for histopathologic evaluation and with Giemsa for *H. pylori* evaluation. Not only diagnosis of *H. pylori* infection but also assessment of intensity of *H. pylori*, inflammation, activation, atrophy, and intestinal metaplasia according to Sydney classification has been determined from the stained specimens. Pathologists who were blinded to patients reported the histopathological assessments as normal (0), mild (1), moderate (2), and severe (3) for each parameter of Sydney classification.

Fasting blood samples were obtained by the venipuncture of the large antecubital veins of the studied patients without stasis, after a 12-hour fast for measured β_2 M and *H. pylori* CagA status.

Comparison of serum levels of β_2 M before and after the eradication therapy was another aim of the current study. Participants who were diagnosed with *H. pylori* infection were administered *H. pylori* eradication therapy for 14 days.

Eradication regimen was a combination of esomeprazole, 20 mg b.i.d., colloidal bismuth subcitrate, 600 mg b.i.d., tetracycline, 500 mg q.i.d., and metronidazole, 500 mg t.i.d. Six weeks after the last day of therapy, C14 urea-breath test (Heliprobe, Kibion AB Uppsala, Sweden) was performed for the patients.

Blood samples of patients who were negative for urea-breath test were collected. The samples were then centrifuged immediately; the plasma was separated and stored at -80°C . In order to avoid variation, all samples were studied on the same day and using the same kit. Commercial kit (DIA.PRO Diagnostic Bioprobes Srl, Milano, Italy) was used for analysis of *H. pylori* CagA status according to the manufacturer's instructions. β_2 M analysis was performed by using commercial kit (DiaSorin, Saluggia, Italy) according to the manufacturer's instructions.

SPSS version 15.0 statistical software was used for statistical analyses. Descriptive results were reported as mean $\pm$ standard deviation and median with minimum and maximum values. Results were analysed for normal distribution by using Kolmogorov-Smirnov. Student's *t*-test, Mann Whitney U, and Kruskal Wallis test were used where appropriate. Wilcoxon test was used to determine the comparison between serum levels of β_2 M before and after the eradication therapy. Correlation between serum levels of β_2 M and parameters of Sydney classification was determined by Spearman's correlation test. *P* value <0.05 was accepted as statistically significant.

3. Results

A total of 120 patients were included in the study. There were 68 (56.7%) female and 52 (43.4%) male patients. *H. pylori* infection was positive in 85 (70.8%) and negative in 35 (29.2%) patients. There were 52 (43.3%) patients who were CagA negative and 33 (27.5%) patients who were CagA positive in *H. pylori* infection positive group.

There was no statistically significant difference between *H. pylori* positive and negative (36.7 versus 37.2 resp.; $P > 0.05$) or CagA positive and negative ($P > 0.05$) subjects with regards to age and gender.

There was no significant difference in mean serum β_2 M levels (1.82 mg/L versus 1.83 mg/L resp.; $P > 0.05$) between patients with and without *H. pylori* infection. There was also no significant difference in mean serum β_2 M levels between *H. pylori* negative patients group, *H. pylori* positive-CagA negative group, and *H. pylori* positive-CagA positive group (1.83 mg/L versus 1.76 mg/L versus 1.93 mg/L, $P > 0.05$ resp.) (Figure 1).

Sydney classification parameters were evaluated in biopsy specimens which were conducted from antrum and corpus. The highest value of these parameters was used in correlation analysis of *H. pylori* intensity, acute and chronic inflammation, atrophy, intestinal metaplasia, and serum β_2 M level. There was no significant correlation between Sydney classification parameters and serum β_2 M levels ($P > 0.05$) (Table 1).

Eighty-five patients who were *H. pylori* positive were administered *H. pylori* eradication therapy. Ten patients were

FIGURE 1: β_2 M level according to *H. pylori* and CagA status.

TABLE 1: Correlation between β_2 M level and parameters of Sydney classification.

Parameters	<i>r</i>	<i>P</i>
HP	-0.039	0.671
Neutrophil	-0.036	0.695
Mononuclear cell	-0.009	0.922
Atrophy	0.003	0.973
Intestinal metaplasia	0.132	0.150

lost to followup for biopsy. Four patients had to give up the therapy due to nonspecific adverse effect of therapy. A total of 17 patients were lost to followup for urea-breath test control. Urea-breath test could be performed to 54 patients. Urea-breath test was positive in 4 and negative in 50 patients. Intent to treat ratio was 50/71 (70%) while 50/54 (92.5%) per protocol. Blood samples were obtained from 50 patients for β_2 M level after eradication therapy. β_2 M levels (1.82 versus 1.64 mg/L, resp.; $P < 0.05$) were significantly decreased after eradication therapy.

4. Discussion

The current study showed no association with *H. pylori* infection and serum β_2 M level. Although *H. pylori* infection causes inflammation of gastric mucosa, it has no effect on serum β_2 M levels. *H. pylori* infection with CagA strains did not have any effect on β_2 M level. Although patients with CagA positive *H. pylori* infection had the highest serum β_2 M levels, it was not statistically significant. Severity of gastric inflammation did not correlate with serum β_2 M; however,

serum β_2 M levels were significantly decreased after *H. pylori* eradication.

H. pylori infection leads to accumulation of neutrophils and lymphocytes in the gastric mucosa. This accumulation progresses into chronic inflammation because *H. pylori* cannot be eradicated from mucosa by immune system. This local inflammation may trigger a systematic response in the host. *H. pylori* is suggested to be related with low grade inflammation [15]. This low grade inflammation regarding *H. pylori* infection is associated with diabetes mellitus and atherosclerosis [16, 17]. CagA is one of the most virulent factors of *H. pylori*. CagA positive *H. pylori* causes more serious gastric mucosa damage. *H. pylori* infection with CagA strains was associated with coronary artery diseases and cerebrovascular disease related with low grade inflammation more than CagA negative *H. pylori* infection [18, 19].

As serum β_2 M level reflects the activated lymphocytes, it is also considered as a marker of inflammation. There is growing evidence regarding the relationship between β_2 M and diseases associated with systemic inflammation. One study determined that elevated β_2 M levels reflect the severity of Crohn disease [20]. β_2 M levels were found to be elevated and correlated with disease severity in peripheral artery disease [21]. It is considered that hypoxia shed β_2 M from cell membranes. Also β_2 M level predicts cardiovascular mortality and all-cause mortality in general population, in population with renal impairment, and in elderly population [22–24]. Considering the association of β_2 M and inflammatory states, we hypothesized that β_2 M may increase in *H. pylori* infection, especially with CagA positive strains.

Previous studies have reported the same results on the relationship of serum β_2 M level and *H. pylori* infection. Our study had reiterated these previous results. First study in the literature was from pre-*H. pylori* era. In this study, serum β_2 M levels were measured in patients with malign and benign diseases of stomach. Elevated levels were determined in both groups of diseases but patients with gastric adenocarcinomas and gastric ulcer had elevated serum β_2 M levels more frequent than patients with gastritis and duodenal ulcer. But the difference was not statistically significant and *H. pylori* was not discovered yet when this study was published [12].

Two studies have reported that serum β_2 M levels have no relationship with *H. pylori* infection. Also correlation between serum β_2 M levels and *H. pylori* severity and intensity according to Sydney classification cannot be found. Akay et al. compared serum β_2 M levels of 30 patients with *H. pylori* and 22 patients without *H. pylori* infection. They failed to find a statistically significant difference in the mean of serum β_2 M levels between two groups. *H. pylori* intensity had increasing effect on serum β_2 M levels but it was not statistically significant [13].

Dincer et al. have conducted a study that aimed to find a correlation between serum β_2 M levels and parameters of Sydney classification for three regions of stomach (incisura angularis, antrum, and corpus). They were not able to find a correlation, but although it was not significant, biopsies from corpus showed that moderate and severe inflammation caused elevated serum β_2 M levels compared to mild inflammation [14].

While the previous reports did not determine difference in the mean of serum β_2 M levels, tissue concentrations of β_2 M did differ. In the same study, Conz et al. showed that 19 of 30 patients had subendothelial β_2 M but none of the control group had subendothelial β_2 M. Also one study conducted on uremic dialysis patients showed accumulation of β_2 M in gastric mucosa with *H. pylori* infection. Patients without *H. pylori* infection did not show this finding [25].

Our study had some limitations. Although the present study had the highest number of patients among the previous studies, the patient number was low. Tissue concentrations of β_2 M were not measured. These limitations may be a reason for the nonsignificant results.

5. Conclusion

To the best of our knowledge, this is the first report that investigated the association between β_2 M, CagA, and eradication therapy. Although there is no association, this possible relationship should be investigated by further studies with more patients. It is important to find reliable biomarkers for systemic inflammation and possible associations with chronic infections like *H. pylori* because diseases associated with systemic inflammations' severity and extension can be predicted with these biomarkers.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

References

- [1] J. Parsonnet, G. D. Friedman, D. P. Vandersteen et al., "Helicobacter pylori infection and the risk of gastric carcinoma," *The New England Journal of Medicine*, vol. 325, no. 16, pp. 1127–1131, 1991.
- [2] A. C. Wotherspoon, C. Ortiz-Hidalgo, M. R. Falzon, and P. G. Isaacson, "Helicobacter pylori-associated gastritis and primary B-cell gastric lymphoma," *The Lancet*, vol. 338, pp. 1175–1176, 1991.
- [3] A. Nomura, G. N. Stemmermann, P.-H. Chyou, G. I. Perez-Perez, and M. J. Blaser, "Helicobacter pylori infection and the risk for duodenal and gastric ulceration," *Annals of Internal Medicine*, vol. 120, no. 12, pp. 977–981, 1994.
- [4] A. Covacci, S. Censini, M. Bugnoli et al., "Molecular characterization of the 128-kDa immunodominant antigen of Helicobacter pylori associated with cytotoxicity and duodenal ulcer," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 90, no. 12, pp. 5791–5795, 1993.
- [5] J. W. Yewdell, E. Reits, and J. Neefjes, "Making sense of mass destruction: quantitating MHC class I antigen presentation," *Nature Reviews Immunology*, vol. 3, no. 12, pp. 952–961, 2003.
- [6] K. M. Paulsson, P. Wang, P. O. Anderson, S. Chen, R. F. Pettersson, and S. Li, "Distinct differences in association of MHC class I with endoplasmic reticulum proteins in wild-type, and β_2 -microglobulin- and TAP-deficient cell lines," *International Immunology*, vol. 13, no. 8, pp. 1063–1073, 2001.
- [7] M. Zijlstra, M. Bix, N. E. Simister, J. M. Loring, D. H. Raulet, and R. Jaenisch, " β_2 -microglobulin deficient mice lack CD4⁺8⁺ cytolytic T cells," *Nature*, vol. 344, no. 6268, pp. 742–746, 1990.
- [8] M. Bethea and D. T. Forman, " β_2 -Microglobulin: Its significance and clinical usefulness," *Annals of Clinical and Laboratory Science*, vol. 20, no. 3, pp. 163–168, 1990.
- [9] R. Bataille, B. G. M. Durie, and J. Grenier, "Serum beta2 microglobulin and survival duration in multiple myeloma: a simple reliable marker for staging," *British Journal of Haematology*, vol. 55, no. 3, pp. 439–447, 1983.
- [10] E. H. Cooper and T. Plesner, "Beta-2-microglobulin review: its relevance in clinical oncology," *Medical and Pediatric Oncology*, vol. 8, no. 4, pp. 323–334, 1980.
- [11] S. Molica, D. Levato, N. Cascavilla, L. Levato, and P. Musto, "Clinico-prognostic implications of simultaneous increased serum levels of soluble CD23 and β_2 -microglobulin in B-cell chronic lymphocytic leukemia," *European Journal of Haematology*, vol. 62, no. 2, pp. 117–122, 1999.
- [12] S. A. Rashid, E. H. Cooper, A. T. R. Axon, and G. Eaves, "Serum β_2 -microglobulin in malignant and benign diseases of the stomach and pancreas," *Biomedicine Express*, vol. 33, no. 4, pp. 112–116, 1980.
- [13] H. Akay, A. Akay, and S. Köklü, "Serum and tissue beta-2 microglobulin levels in patients with Helicobacter pylori infection," *Digestive Diseases and Sciences*, vol. 53, no. 2, pp. 358–362, 2008.
- [14] N. Dincer, F. Topal, K. Karaman et al., "Relationship of the intensity of helicobacter pylori and severity of inflammation with beta-2 microglobulin levels in serum according to the updated sydney system," *Southern Medical Journal*, vol. 103, no. 11, pp. 1092–1096, 2010.
- [15] L. Jackson, J. Britton, S. A. Lewis et al., "A population-based epidemiologic study of helicobacter pylori infection and its association with systemic inflammation," *Helicobacter*, vol. 14, no. 5, pp. 108–113, 2009.
- [16] D. Rajasekhar, G. Subramanyam, S. A. Latheef, V. Vanajakshamma, A. Srilatha, and A. Chaudhury, "Infectious aetiology in acute coronary syndromes," *Indian Journal of Medical Microbiology*, vol. 20, pp. 83–87, 2002.
- [17] R. Pellicano, F. Franceschi, G. Saracco, S. Fagoonee, D. Roccarina, and A. Gasbarrini, "Helicobacters and extragastric diseases," *Helicobacter*, vol. 14, no. 1, pp. 58–68, 2009.
- [18] S. Zhang, Y. Guo, Y. Ma, and Y. Teng, "Cytotoxin-associated gene-A-seropositive virulent strains of Helicobacter pylori and atherosclerotic diseases: a systematic review," *Chinese Medical Journal*, vol. 121, no. 10, pp. 946–951, 2008.
- [19] S. Zhang, Y. Guo, Y. Ma, and Y. Teng, "Relationship between the cytotoxin-associated gene-A status of H pylori strains and cerebral infarction in European Caucasians and Chinese Han: a meta-analysis," *World Journal of Gastroenterology*, vol. 14, no. 8, pp. 1286–1292, 2008.
- [20] M. Zissis, A. Afroudakis, G. Galanopoulos et al., "B2 microglobulin: is it a reliable marker of activity in inflammatory bowel disease?" *The American Journal of Gastroenterology*, vol. 96, no. 7, pp. 2177–2183, 2001.
- [21] A. M. Wilson, E. Kimura, R. K. Harada et al., " β_2 -Microglobulin as a biomarker in peripheral arterial disease: proteomic profiling and clinical studies," *Circulation*, vol. 116, no. 12, pp. 1396–1403, 2007.

- [22] C.-L. Cheung, K. S. L. Lam, and B. M. Y. Cheung, "Serum β -2 microglobulin concentration predicts cardiovascular and all-cause mortality," *International Journal of Cardiology*, vol. 168, pp. 4811–4813, 2013.
- [23] S. Liabeuf, A. Lenglet, L. Desjardins et al., "Plasma beta-2 microglobulin is associated with cardiovascular disease in uremic patients," *Kidney International*, vol. 82, no. 12, pp. 1297–1303, 2012.
- [24] S. Shinkai, P. H. M. Chaves, Y. Fujiwara et al., " β 2-microglobulin for risk stratification of total mortality in the elderly population: comparison with cystatin C and C-reactive protein," *Archives of Internal Medicine*, vol. 168, no. 2, pp. 200–206, 2008.
- [25] P. A. Conz, S. Dante, D. Bernardini, V. Bertoncello, G. La Greca, and P. A. Bevilacqua, "Beta-2-microglobulin and *Helicobacter pylori* infection in uraemic dialysed patients," *Journal of Gastroenterology and Hepatology*, vol. 7, no. 2, pp. 191–193, 1992.