Journal of AIDS and HIV Research Vol. 3(4), pp. 85-87, April 2011
Available online http://www.academicjournals.org/jahr
ISSN 2141-2359 ©2011 Academic Journals

Short Communication

Screening for the presence of *Helicobacter pylori* in stool of HIV- positive patients

Hossein Samadi Kafil[1]*, Fatemeh Farahi Jahromi[1], Bahareh Hajikhani[2], Shahin Najar Pirayeh[3] and Mohammad Aghazadeh[4]

[1]Student Research Center, Faculty of Medicine, Tarbiat Modares University, Tehran, Iran.
[2]Avicenna Research institute, Shaheed Beheshti University of Medical sciences, Tehran, Iran.
[3]Department of Medical Bacteriology, School of Medicine, Tarbiat Modares University, Tehran, Iran.
[4]Infectious Disease and Tropical Medicine Research Center, Tabriz University of Medical Sciences, Tabriz, Iran.

Accepted 24 February, 2011

Helicobacter Pylori **is a bacillus that naturally colonizes humans, living in gastric mucus. Association of** ***H. pylori*** **colonization of stomach with chronic gastritis, peptic ulcer and gastric malignancies has been well documented. In this study, we aimed to find presence of** ***H. pylori*** **in stool of HIV infected patients by PCR. 43 patients who had confirmed HIV-infection were subjected. Specific primers for** ***hpaA*** **(flagellar sheath adhesin) and** ***ureB*** **(urea amidohydrolase), of** ***H. pylori*** **were designed and, presence of the genome of** ***H. pylori*** **investigated by PCR method. 35 of the patients (81.39%) had CD4+ count below 200 and** ***H. pylori*** **was found in 30 patients (69.76%). Results show from screening by** ***H. pylor*****i in stool of HIV-infected patients that prevalence of this bacterium in these patients is high. This prevalence is similar to prevalence of** ***H. pylori*** **in HIV-non infected population.**

Key words: *Helicobacter pylori*, HIV, flagellar sheath adhesin (*hpaA)*, urea amidohydrolase *(ureB)*, gastric.

INTRODUCTION

Helicobacter pylori (*H. pylori*) is a gram negative, spiral, flagellate bacillus that naturally colonizes humans, living in their gastric mucus. There is 20% prevalence of infection with *H. pylori* among adolescents in United States in comparison to infection rates exceeding 90% in the developing countries (Frenck et al., 2003). Association of *H. pylori* colonization of stomach with chronic gastritis, peptic ulcer and gastric malignancies has been well documented (Everhart, 2000). However, despite the high rate of infection, only a small fraction of infected subjects go beyond development of gastritis and develops peptic ulcer or gastric malignancies. In Iran, 80% of population is infected with *H. pylori* (Massarrat et al., 1995). In contrast to immunocompetent subjects in HIV- infected patients, *H. pylori* related gastritis has been noted to occur less frequently (that is, in 5 to 59% of cases) in adult patients with the acquired immunodeficiency syndrome (AIDS) (Francis et al., 1990; Marano et al., 1993; Edwards et al., 1991; Battan et al., 1990). Previous studies attribute responsibility to microbial treatment and an impaired gastric acid secretion which are highly frequent in AIDS patients and could inhibit *H. pylori* colonization (Lake-Bakaar et al., 1988; Welage et al., 1995). The aim of present study was to screen for the presence of *H. pylori* in stool of HIV infected patients by PCR to find rate of co-infection of HIV and *H. pylori*.

*Corresponding author. E-mail: amirh_samadi@yahoo.com. Tel: +989127184735. Fax: +982182884555.

MATERIALS AND METHODS

Participants in this study comprised 43 patients who had confirmed HIV-infection and hospitalized or regularly visited hospital for their infection follow up. All samples were collected from sexual infection control center, Imam Khomeini Hospital, Tehran, Iran. Because of restrictions for access data of patients according to private system of Hospital, we had problem to complete all demographic information. Samples were collected by collecting stools of patients in sterile containers and formalin. *H.pylori* 26695 was used as positive control.

Stools were suspended in 400 ml of TE buffer (10 mM Tris-HCL, 1 mM EDTA (Ethylenediaminetetraacetic acid), pH 8.0, filtrated and subjected for DNA extraction. Suspension was placed at 80°C for

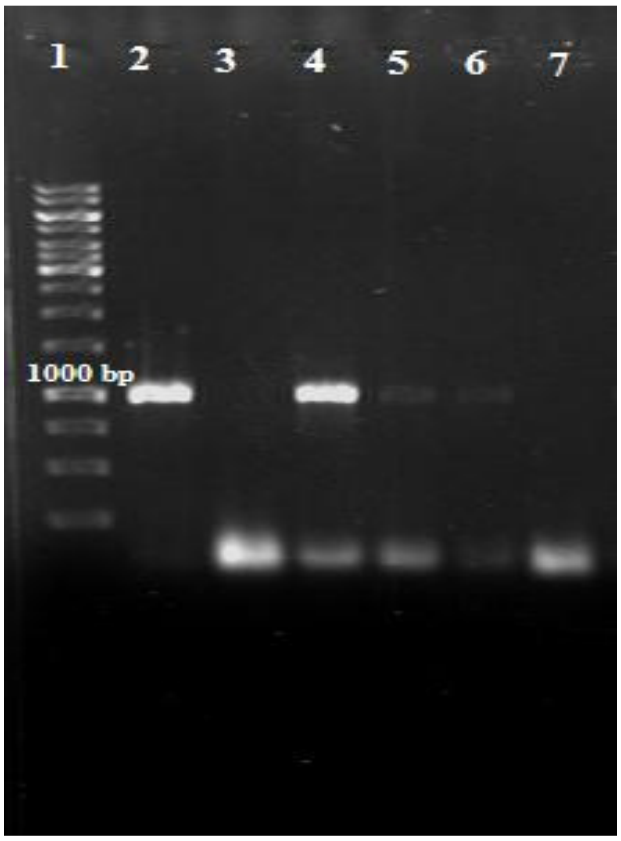

Figure 1. Confirming presence of *H.pylori* genome in stool of HIV-infected patients by screening *ureB*; 1: ladder; 2: positive control (*Helicobacter pylori* 26695); 3: negative control; 4.5.6: positive; 7: negative.

20 min to kill the bacteria; DNA was extracted by lysozyme, SDS (Sodium dodecyl sulfate), proteinase K and CTAB (Cetyl trimethylammonium bromide). Extracted DNA after sedimentation with isopropanol and washing with 70% ethanol, was resolved in 100 µl TE buffer (Asgharzadeh et al., 2008, 2011).

PCR (Polymerase chain reaction) was performed for two conserve gene of *H. pylori* containing *hpaA* (Flagellar sheath adhesin) and *ureB* (urea amidohydrolase). Primers which were used for finding genome of *H. pylori* were *hpaA* (Forward: 5'-ATAAAGCTTTCGGTGGTGGAACGATG-3; Reverse: 5'-TATCTCGAGTTGTCGGTTTCTTTTGC-3') and *ureB* (Forward: 5'-AACCCATATGTCTGCAATCAATCATGC-3'; Reverse: 5'-TTGGAGCTCGCTCACTTTATTGGCTG-3'). Primers were designed for this study by the information available in pubmed (http://www.ncbi.nlm.nih.gov/pubmed/, *hpaA* GeneID: 898975, *ureB* GeneID: 899104) and using Genrunner software (version 3.5). PCR was performed in 50 µl volumes that contained 20 to 200 ng DNA, 0.5 µM of specific primers, in the presence of 1.5 mM MgCl2 (for both primers), 200 µM of each dNTP (Cinnagen, Iran) and 2U DNA polymerase (Cinnagen, Iran). DNA was amplified by allele specific PCR. An initial denaturation of 7 min at 94°C was followed by 35 cycles of denaturation at 94°C for 30s, annealing at 55°C for 30s (for both genes) and extension at 72°C for 1 min, followed by a final extension at 72°C for 10 min. Positive control consisted genome of *H. pylori* 26695 and negative control consisted of the PCR components on reaction mixtures lacking *H. pylori* DNA. PCR products were analyzed in agarose gels (1.5%) and after staining with 0.5 µg ml-1 ethidium bromide, they were visualized under UV light.

The amplicons were 850 bp and 1017 bp long for the *hpaA* and *ureB* respectively. Data were compared by chi-square test (or Fishers exact test). *P values* below 0.05 were considered significant.

RESULTS

The 43 patients (38 males and 5 females; age range 20 to 52) had confirmed HIV-infection and any gastric ulcer background. The peripheral CD4+ lymphocyte count of patients were documented from their file; 35 of the patients (81.39%) had CD4+ count below 200. Most of the patients had been given antibiotic therapy (75%). There was no significant relationship between CD4+ count and presence of *H. pylori* (*P value* > 0.05). By screening presence of *hpaA* and *ureB* in stool of HIV-infected patients, *H. pylori* was found in 30 patients (69.76%). Results were confirmed by presence of both *hpaA* and *ureB* (Figure 1).

DISCUSSION

The AIDS caused by the HIV virus has already claimed nearly 21 million lives and more than 33.2 million people are currently living with this infection worldwide (UNAIDS, 2008). The prevalence of *H. pylori* in patients with HIV is a controversial subject and has been reported to be remarkably lower than that found in non-HIV infected individuals (Chiu et al., 2004; Blondon et al., 1998; Nielsen et al., 1995; Fabris et al., 1997). In this study, we aimed to screen presence of *H. pylori* in HIV infected patients who hospitalized or visited our hospital. For finding *H. pylori* we used amplification of two conserve gene of *H. pylori* to finding this bacterium in stool of HIV-infected patients.

Results for these lower rates remain unclear (Shelton et al., 1998; Benz et al., 1993). In some studies, difference in ratio of CD4\CD8 in gastric mucosa with and without *H. pylori* infected HIV-patients is considered a responsible agent. According to the hypothesis, CD4 lymphocytes, which are depleted in AIDS patients, might be associated with a different presentation of *H. pylori* infection (Yamaoka et al., 2002; Scarpellini et al., 2001; Bamford et al., 1998). Another hypothesis is that the frequent use of antibiotics by HIV-infected patients could lead to *H. pylori* eradication from gastric mucosa and explains the lower prevalence described in this population (Isomoto et al., 1999; Edwards et al., 1991).

In some new articles, screening of the presence of *H. pylori* by culture, found that *H. pylori* grew from 48.9% of individuals (Urdez et al., 2004). In another study, *H. pylori* was also found in 41.1 to 51% patients (Olmos et al., 2004; Fabris et al., 1997), including higher rate of *H. pylori* infection among patients with HIV\AIDS who had

advanced immunosuppression. Prevalence of *H. pylori* in our studied region according to previous studies was 80% (Massarrat et al., 1995). In our study we used PCR method for screening presence of *H. pylori* in stool of HIV-infected patients especially in immunosuppressed patients and in 69.76% of the patients, we found *H. pylori*. This result was close to prevalence of *H. pylori* in our population (Massarrat et al., 1995). We tried to use two conserve genes of *H. pylori* to remove possible false positives and all results confirmed by presence of both genes (Figure 1). This finding can be interpreted that *H. pylori* is present in gastric of HIV-infected patients but cannot reveal its clinical symptoms and cannot develop disease. This result is in agreement with results of studies of Olmos et al. (2004), however, they found that HIV-infected patients with *H. pylori* have a higher mean CD4 count than HIV-infected patients without *H. pylori*, but, in our study we didn't find any difference in CD4 count between these two groups. Probably, frequent use of antibiotic in HIV-infected patients has a role in eradicating or controlling *H. pylori* infection but cannot completely eradicate *H. pylori* from gastric of these patients.

In conclusion, our results showed, by screening *H. pylori* in stool of HIV-infected patients that, prevalence of this bacterium in these patients is high. In fact, this prevalence is similar to prevalence of *H. pylori* in population, but why this bacterium cannot show gastric symptoms in these patients is not clear and more studies are needed to clarify it.

ACKNOWLEDGEMENTS

This study was supported by Student Research Center, Faculty of Medicine, Tarbiat Modares University with grant number 5234297. We thank staff of HIV and sex related infections controlling center of Imam Khomeini University Hospital for their cooperation in this study. We also thank Dr. Hossein Navidinia and Dr. Mohammad Momenian for their helpful comments.

REFERENCES

Asgharzadeh M, Kafil HS, Roudsary AA, Hanifi GR (2011). Tuberculosis transmission in Northwest of Iran: Using MIRU-VNTR, ETR-VNTR and IS6110-RFLP methods. Infect. Genet. Evol., 11(1): 124-131.

Asgharzadeh M, Kafil HS, Khakpour M (2008). Comparison of mycobacterial interspersed repetitive unit-variable number tandem repeat and IS6110–RFLP methods in identifying epidemiological links in patients with tuberculosis in Northwest of Iran. Ann. Microbiol., 58: 333-339.

Bamford KB, Fan X, Crowe SE, Leary JF, Gourley WK, Luthra GK, Brooks EG, Graham DY, Reyes VE, Ernst PB (1998). Lymphocytes in the human gastric mucosa during Helicobacter pylori have a T helper cell 1 phenotype. Gastroenterology, 114(3): 482-492.

Battan R, Raviglione MC, Palagiano A, Boyle JF, Sabatini MT, Sayad K, Ottaviano LJ (1990). *Helicobacter pylori* infection in patients with acquired immunedeficiency syndrome. Am. J. Gastroenterol., 85(12): 1576-1579.

Benz J, Hasbach H, Brenden M, Eidt S, Fätkenheuer G, Schrappe M, Geisel J, Goossens H, Mauff G (1993). Humoral and cellular immunity in HIV positive and HIVnegative *Helicobacter pylori* infected patients. Zentralbl. Bakteriol., 280(1-2): 186-196.

Blondon H, Tsakiris L, Coste T (1996). Helicobacter pylori, gastric acid secretion, ulcer disease and HIV infection. Gastroenterol. Clin. Biol., 20(3):248-253.

Chiu HM, Wu MS, Hung CC, Shun CT, Lin JT (2004). Low prevalence of *Helicobacter pylori* but high prevalence of cytomegalovirus-associated peptic ulcer disease in AIDS patients: Comparative study of symptomatic subjects evaluated by endoscopy and CD4 counts. J. Gastroenterol. Hepatol., 19(4): 423-428.

Edwards PD, Carrick J, Turner J, Lee A, Mitchell H, Cooper DA (1991). *Helicobacter pylori*-associated gastritis is rare in AIDS: antibiotic effect or a consequence of immunodeficiency? Am. J. Gastroenterol., 86(12): 1761-1764.

Everhart JE (2000). Recent developments in the epidemiology of Helicobacter pylori. Gastroenterol. Clin. North. Am., 29(3): 559-578.

Fabris P, Bozzola L, Benedetti P, Scagnelli M, Nicolin R, Manfrin V, Scarparo C, De Lalla F (1997). H. pylori infection in HIV-positive patients. A serohistological study. Dig. Dis. Sci., 42(2): 289-292.

Francis ND, Logan RP, Walker MM, Polson RJ, Boylston AW, Pinching AJ, HarrisJR, Baron JH (1990). Campylobacter pylori in the upper gastrointestinal tract of patients with HIV-1 infection. J. Clin. Pathol., 43(1): 60-62.

Frenck RW, Clemens J (2003). Helicobacter in the developing world. Microbes Infect., 5(8):705-713.

Isomoto H, Mizuta Y, Fukushima K, Takeshima F, Miyazaki M, Murase K, Omagari K, Maeda T, Kamihira S, Tomonaga M, Kohno S (1999). Low prevalence of Helicobacter pylori in individuals with HTLV-I infection. Eur. J. Gastroenterol. Hepatol., 11(5): 497-502.

Lake-Bakaar G, Quadros E, Beidas S, Elsakr M, Tom W, Wilson DE, Dincsoy HP, Cohen P, Straus EW (1988). Gastric secretory failure in patients with the acquired immunodeficiency syndrome (AIDS). Ann. Intern. Med., 109(6): 502-504.

Marano BJ Jr, Smith F, Bonanno CA (1993). H*elicobacter pylori* prevalence in acquired immunodeficiency syndrome. Am. J. Gastroenterol., 88(5): 687-690.

Massarrat S, Saberi-Firoozi M, Soleimani A, Himmelmann GW, Hitzges M, Keshavarz H (1995). Peptic ulcer disease, irritable bowel syndrome and constipation in two populations in Iran. Eur. J. Gastroenterol. Hepatol., 7(5): 427-433.

Nielsen H, Andersen LP (1995). Serodiagnosis of *Helicobacter pylori* infection in patients with human immunodeficiency virus infection. APMIS, 103(10): 689-692.

Olmos M, Araya V, Pskorz E, Quesada EC, Concetti H, Perez H, Cahn P (2004). Coinfection: *Helicobacter pylori*/human immunodeficiency virus. Dig. Dis. Sci., 49(11-12): 1836-1839.

Scarpellini P, Carrera P, Cavallero A, Cernuschi M, Mezzi G, Testoni PA,Zingale A, Lazzarin A (2002). Direct detection of *Helicobacter pylori* mutations associated with macrolide resistance in gastric biopsy material taken from human immunodeficiency virus-infected subjects. J. Clin. Microbiol., 40(6): 2234-2237.

Shelton MJ, Adams JM, Hewitt RG, Morse GD (1998). Previous infection with *Helicobacter pylori* is the primary determinant of spontaneous gastric hypoacidityin human immunodeficiency virus-infected outpatients. Clin. Infect. Dis., 27(4): 739-745.

UNAIDS (2008). Report on the global AIDS epidemic (July 2008) http://data.unaids.org/pub/epislides/2007/2007epiupdate.en.pdf.

Urdez HE, Reglín NH, Escamilla AE, Manríquez MA (2004). Prevalence of *Helicobacter pylori* among Patients Infected with HIV. Rev. Med. IMSS, 42 (3): 211-210.

Welage LS, Carver PL, Revankar S, Pierson C, Kauffman CA (1995). Alterations in gastric acidity in patients infected with human immunodeficiency virus. Clin. Infect. Dis., 21(6):1431-1438.

Yamaoka Y, Kikuchi S, el-Zimaity HM, Gutierrez O, Osato MS, Graham DY (2002). Importance of *Helicobacter pylori* oipA in clinical presentation, gastricinflammation, and mucosal interleukin 8 production. Gastroenterology, 123(2): 414-424.