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Failure to Detect *Helicobacter pylori* DNA in Semen Samples of Patients Referred to the Avicenna Infertility Clinic - Tehran, Iran

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Background: *Helicobacter pylori* (*H. pylori*) is a gram-negative, spiral-shaped, microaerophilic microorganism and a causative agent of many gastrointestinal tract diseases, as well as several extragastric infections. Several studies have suggested the possibility of sexual transmission of these bacteria.

Objectives: The aim of the current study Was to determine the possibility of detecting *H. pylori* DNA in semen samples from infertile men, compared with healthy controls.

Patients and Methods: One hundred infertile male patients and 100 age and gender-matched healthy controls have been enrolled in the study. Semen samples collected from each participant, undergone DNA extraction and polymerase chain reaction (PCR) assay to detect the *H. pylori*. The ß-actin PCR was performed to verify the accuracy of DNA extraction.

Results: Each sample was positive in the ß-actin PCR assay. None of the samples, from both patients and controls, showed positive PCR results. Consequently, statistical analysis was impossible to perform.

Conclusions: We could not confirm the presence of *H. pylori* DNA in semen samples, but this does not exclude the possibility of male urethral colonization by this organism. Further studies with similar results are necessary to certify this hypothesis.

Keywords: Helicobacter pylori; Polymerase Chain Reaction; Infertility; Semen

1. Background

Helicobacter pylori (*H. pylori*) is a gram-negative, spiral-shaped, microaerophilic bacterium which can cause gastritis, peptic ulcer and gastric cancer (1), as well as a number of extragastric infections, including vascular, respiratory, liver, skin, and kidney diseases, worldwide (2-4). Based on available literature, a *H. pylori* infection model, related to prostate and bladder diseases, has been hypothesized (5). In addition, an association between infection *with H. pylori* and poor sperm quality in infertile men, compared to uninfected individuals has been reported (6).

Several studies have reported that sexual behavior may be important in the transmission of *H. pylori* (7, 8). Different routes of transmission, including oral-oral, fecal-oral, fomite, iatrogenic, and vector borne have been proposed, but there is no definite report addressing the exact transmission pathway (9).

Other studies have detected *H. pylori* or it's DNA in oral cavities (10, 11). Since *H. pylori* can survive on the squamous epithelium of the mouth, inhabitation of this organism in the squamous epithelium of the vagina is expected. Furthermore, the role of vaginal tract, as a potential reservoir for sexually transmission of *H. pylori*, has been explained (12).

In the case of urethritis, vaginal and anal intercourses, as well as oral sex, are the main sexual routes of *H. pylori* transmission among males (13). Furthermore, oral sex is one of the main routes to spread common oral flora responsible for pathologic processes (14). Since *H. pylori* has been frequently detected in saliva, subgingival biofilm and dental plaque, the oral environment may be the potential medium for the oral-genital transmission of this organism (15-17).

Several investigations showed that uropathogens, including *Escherichia coli* could be sexually transmitted between sex partners (18), which could work similarly for *H. pylori* as well. According to these findings, in the large number of males with urethritis and no other detected microorganism, *H. pylori* may be the causative agent (19). Would the probability of sexual transmission of this organism be considered, then attempts to detect it in the genital tract could be important. On the other hand, the bacterial DNA isolation from genital secretions can strengthen the possibility of its sexual transmission. Although few studies have assessed the presence of *H. pylori* in vaginal secretions (20, 21), to date, no investigation has been undertaken to determine the presence of *H. pylori* DNA in semen.

Implication for health policy/practice/research/medical education:
This article may help other researchers to perform similar studies to evaluate the possible or exact role of *Helicobacter pylori* in infertility or STDs.

2. Objectives

The present study has examined the possibility of *H. pylori* DNA detection in semen samples collected from infertile men compared with healthy matched controls.

3. Patients and Methods

3.1. Patients and Sampling

One hundred male patients with infertility problems (failure to impregnate their wives after a period of one year of unprotected regular sexual intercourses) and abnormal semen analysis reports referred to Avicenna Infertility Clinic, Teheran, IR Iran, were enrolled in the study. Their mean age was 34.68 years (ranged from 22 to 59 years). One hundred age-matched healthy controls were entered in to this study in the control group. Semen samples were collected from each individual and sent to Avicenna Research Center, Teheran, IR Iran, under suitable conditions.

3.2. DNA Extraction and PCR

The DNA was extracted from semen samples by using Chelex-100 resin (Sigma-Aldrich, St. Louis, MO, The USA) (22). Briefly, 50 μL of semen were mixed with 2 μL proteinase K (20 mg/mL), 2 μL dithiothreitol (DTT) and 150 μL Chelex-100 5% (Sigma-Aldrich, St. Louis, MO, USA) in a microtube. Tubes containing the mixture were incubated at 37°C for 90 minutes and then centrifuged for 15 seconds at 15500 g. The tubes were placed in boiling water for 8 minutes. After further centrifugation for 3 minutes, the supernatants containing the extracted DNA were transferred to new tubes. All extracted DNA were stored at -20°C until used. To evaluate the accuracy of semen DNA extraction, we performed ß-actin polymerase chain reaction (PCR) assay to check for the presence of human DNA, which would indicate the accuracy of the extraction procedure.

The PCRs were carried out with the primers that had previously been designed to amplify the *hpaA* gene of *H. pylori*. The primers sequences were as follows:

hpa-forward 5'- ATAAAGCTTTCGGTGGTGGAACGATG-3'

hpa-reverse 5'- TATCTCGAGTTGTCGGTTTCTTTTGC-3'.

The PCR amplification was performed in a total volume of 25 μL consisting of 0.2 mM dNTP, 2.5 mM $MgCl_2$, 50 pmol of each primer, 2.5 μL of 10X PCR buffer, 3 μL extracted DNA and 1.5 U Taq polymerase.

The PCR protocol consisted of initial denaturation at 98°C for 60 seconds followed by 30 cycles of denaturation at 95°C for 30 seconds, annealing at 55°C for 30 seconds, polymerization at 72°C for 60 seconds and a final extension for 8 minutes after the last PCR cycle. Positive and negative control reactions were used. Resulting PCR products were loaded on 1% (w/v) agarose gel containing ethidium bromide, followed by the analysis of the bands visualized under UV light.

3.3. Data Analysis

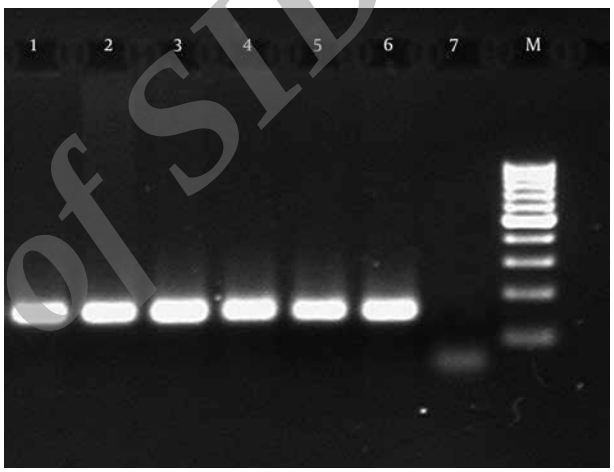
Data analysis was performed with the SPSS version 16 software (SPSS Inc., Chicago, IL, USA) and a $P < 0.05$ was considered as significant.

4. Results

In this study, semen samples collected from 100 infertile men and 100 controls were analyzed. The ß-actin PCR assay showed positive result (Figure 1), which confirmed that the DNA extraction procedure has been performed correctly. The PCR of positive controls also revealed a band of 850 bp, indicating the correct *hpaA* amplification (Figure 2).

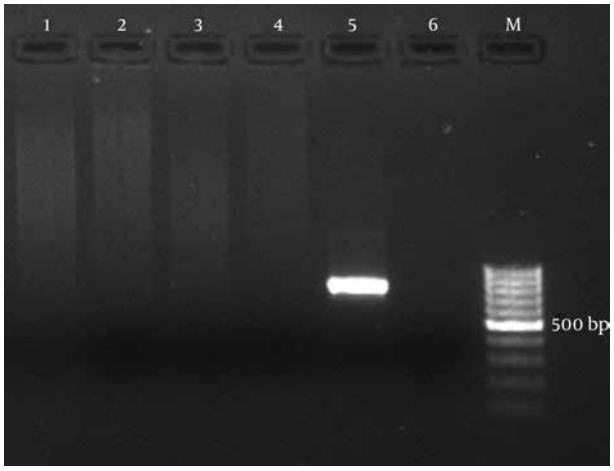
Nevertheless, the PCR assay of the *hpaA* gene did not show positive results in any of the evaluated samples from both patients and controls (Figure 2). Due to the absence of positive results, it was impossible to perform statistical analysis.

Figure 1. The ß-actin PCR Results



Lane 1-5, patients' samples; lane 6, positive control; lane 7, negative control; lane M, DNA marker (1 kb).

Figure 2. The *hpaA* PCR Results



Lane 1-4, patients' samples; lane 5, positive control; lane 6, negative control; lane M, DNA size marker (1 kb).

5. Discussion

Helicobacter pylori infection is very common and affects more than half of the world's population. For years, this organism has been known to cause only gastrointestinal pathology, but many studies have showed that *H. pylori* could be associated with several extra-gastric complications (5, 23, 24). The link between this organism and reproductive disorders has been also reported (24, 25).

It has been shown that infection with CagA-positive *H. pylori* strains in women is linked to an increased potential of early abortion and in men is related to poor sperm quality (6, 26, 27). Although several transmission pathways have been identified for this microorganism, the definite route of transmission is not completely understood. Recently, sexual transmission has also been proposed for this organism (19).

Since *H. pylori* was detected in saliva, dental plaque, vagina and rectum, it can be expected that these environments may act as a source of bacteria transmission. For example, it may colonize male urethra during sexual contacts. Some investigators have classified the sexual transmission routes of *H. pylori* as oral-oral, oral-anal, and oral-genital (12). Because *H. pylori* infection can trigger local and systemic inflammatory responses (28), the presence of the bacteria on the male urethra may cause chronic inflammation, which may lead to pathology.

Figura et al. (24) found a significantly increased prevalence of infection in infertile couples compared with the control group. Among the study groups, 50% of the sperm samples and all follicular fluid samples were positive for *H. pylori* antibodies (24). Most of the previous studies used serological methods to evaluate the association between *H. pylori* and different consequences. However, some studies have examined the presence of the organism outside the stomach by means of molecular or bacterial culture methods (16, 29, 30). Hence, in the current study, we used PCR assay to examine the presence of the organism in semen.

The PCR is a very sensitive and specific method, which can detect very small concentrations of organisms. By using specific primers, one can be sure that bacteria, if present, will be detected. Primers amplifying *H. pylori hpaA* gene were used in this study to detect *H. pylori* DNA in semen samples. The *hpaA* gene has been described to code a flagellar sheath protein. It has been found to be highly conserved among *H. pylori* isolates and, based on genomic studies, there are no significant sequence homologies between *hpaA* and the other known proteins (31, 32). Despite the use of sensitive and specific methods, we could not detect any *H. pylori* DNA in the collected samples. This may be due to several reasons, such as small sample size or very small bacterial load in selected samples, which could not be detected by PCR, as well as real absence of this bacterial colonization in male urethra.

In conclusion, we could not confirm the presence of *H. pylori* DNA in semen samples, but we cannot underestimate the possibility *H. pylori* colonization in male urethra, since this is the first study carried out on this issue, and there are no other similar reports to compare. More studies, with larger sample size and more sensitive detection techniques, such as real time PCR, should be performed to confirm whether *H. pylori* is indeed harbored in this environment.

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Authors' Contribution

Bahareh Hajikhani developed the original idea and the protocol. Bahareh Hajikhani and Leili Chamani Tabriz abstracted and analyzed the data, wrote the manuscript, and performed the study supervision. Rezvan Bagheri, Nastaran Saeedi and Parisa Sadrpour collected the samples, developed the protocol and performed the laboratory tests.

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The authors had no financial interests related to the material in the manuscript.

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References

1. Hatakeyama M, Brzozowski T. Pathogenesis of Helicobacter pylori infection. *Helicobacter.* 2006;**11** Suppl 1:14–20.
2. Nagashima R, Maeda K, Yuda F, Kudo K, Saitoh M, Takahashi T. Helicobacter pylori antigen in the glomeruli of patients with membranous nephropathy. *Virchows Arch.* 1997;**431**(4):235–9.
3. Pellicano R, Mazzaferro V, Grigioni WF, Cutufia MA, Fagoonee S, Silengo L, et al. Helicobacter species sequences in liver samples from patients with and without hepatocellular carcinoma. *World J Gastroenterol.* 2004;**10**(4):598–601.
4. Tsang KW, Lam SK. Helicobacter pylori and extra-digestive diseases. *J Gastroenterol Hepatol.* 1999;**14**(9):844–50.
5. Al-Marhoon MS. Is there a role for Helicobacter pylori infection in urological diseases? *Urol J.* 2008;**5**(3):139–43.
6. Collodel G, Moretti E, Campagna MS, Capitani S, Lenzi C, Figura N. Infection by CagA-positive Helicobacter pylori strains may contribute to alter the sperm quality of men with fertility disorders and increase the systemic levels of TNF-alpha. *Dig Dis Sci.*;**55**(1):94–100.
7. Aceti A, Attanasio R, Pennica A, Taliani G, Sebastiani A, Rezza G, et al. Campylobacter pylori infection in homosexuals. *Lancet.* 1987;**2**(8551):154–5.
8. Perez-Perez GI, Witkin SS, Decker MD, Blaser MJ. Seroprevalence of Helicobacter pylori infection in couples. *J Clin Microbiol.* 1991;**29**(3):642–4.
9. Nabwera HM, Logan RP. Epidemiology of Helicobacter pylori: transmission, translocation and extragastric reservoirs. *J Physiol Pharmacol.* 1999;**50**(5):711–22.
10. Kignel S, de Almeida Pina F, Andre EA, Alves Mayer MP, Birman EG. Occurrence of Helicobacter pylori in dental plaque and saliva of dyspeptic patients. *Oral Dis.* 2005;**11**(1):17–21.
11. Souto R, Colombo AP. Detection of Helicobacter pylori by polymerase chain reaction in the subgingival biofilm and saliva of non-dyspeptic periodontal patients. *J Periodontol.* 2008;**79**(1):97–103.
12. Eslick GD. Helicobacter pylori infection transmitted sexually

via oral-genital contact: a hypothetical model. *Sex Transm Infect.* 2000;**76**(6):489–92.
13. Burstein GR, Zenilman JM. Nongonococcal urethritis--a new paradigm. *Clin Infect Dis.* 1999;**28 Suppl 1**:S66–73.
14. Edwards S, Carne C. Oral sex and transmission of non-viral STIs. *Sex Transm Infect.* 1998;**74**(2):95–100.
15. Anand PS, Nandakumar K, Shenoy KT. Are dental plaque, poor oral hygiene, and periodontal disease associated with Helicobacter pylori infection? *J Periodontol.* 2006;**77**(4):692–8.
16. Eskandari A, Mahmoudpour A, Abolfazli N, Lafzi A. Detection of Helicobacter pylori using PCR in dental plaque of patients with and without gastritis. *Med Oral Patol Oral Cir Bucal.*;**15**(1):e28–31.
17. Gebara EC, Faria CM, Pannuti C, Chehter L, Mayer MP, Lima LA. Persistence of Helicobacter pylori in the oral cavity after systemic eradication therapy. *J Clin Periodontol.* 2006;**33**(5):329–33.
18. Foxman B, Zhang L, Tallman P, Andree BC, Geiger AM, Koopman JS, et al. Transmission of uropathogens between sex partners. *J Infect Dis.* 1997;**175**(4):989–92.
19. Eslick GD. Non-gonococcal urethritis, Helicobacter pylori infection and fellatio: a new menage a trois? *Microbiology.* 2004;**150**(Pt 3):520–2.
20. Martin-De-Argila C, Garcia Arata I, Boixeda D, Sancha M, Canton R, Baquero F. Failure to detect Helicobacter pylori in vaginal secretions. *Clin Microbiol Infect.* 1998;**4**(7):412–3.
21. Minakami H, Hayashi M, Sato I. Does Helicobacter pylori colonize the vagina of pregnant women? *J Infect.* 2000;**41**(1):112–3.
22. Chandler JE, Canal AM, Paul JB, Moser EB. Collection frequency affects percent Y-chromosome bearing sperm, sperm head area and quality of bovine ejaculates. *Theriogenology.* 2002;**57**(4):1327–46.
23. Afifi RA, Ali DK, Shaheen IA. A localized case-control study of extra-gastric manifestations of Helicobacter pylori infection in children. *Indian J Pediatr.*;**78**(4):418–22.
24. Figura N, Piomboni P, Ponzetto A, Gambera L, Lenzi C, Vaira D, et al. Helicobacter pylori infection and infertility. *Eur J Gastroenterol Hepatol.* 2002;**14**(6):663–9.
25. Kurotsuchi S, Ando H, Iwase A, Ishida Y, Hamajima N, Kikkawa F. The plausibility of Helicobacter pylori-related infertility in Japan. *Fertil Steril.* 2008;**90**(3):866–8.
26. Ambrosini G, Andrisani A, Fiore C, Faggian D, D'Antona D, Ragazzi E, et al. Anti-Helicobacter pylori antibodies in cervical mucus: a new cause of infertility. *Eur J Obstet Gynecol Reprod Biol.*;**155**(2):157–60.
27. Moretti E, Collodel G, Campagna MS, Franci MB, Iacoponi F, Mazzi L, et al. Influence of Helicobacter pylori Infection on Levels of Ghrelin and Obestatin in Human Semen. *J Androl.*
28. Crabtree JE. Role of cytokines in pathogenesis of Helicobacter pylori-induced mucosal damage. *Dig Dis Sci.* 1998;**43**(9 Suppl):46S–55S.
29. Iriz E, Cirak MY, Engin ED, Zor MH, Erer D, Ozdogan ME, et al. Detection of Helicobacter pylori DNA in aortic and left internal mammary artery biopsies. *Tex Heart Inst J.* 2008;**35**(2):130–5.
30. Yucebilgili K, Mehmetoglu T, Gucin Z, Salih BA. Helicobacter pylori DNA in gallbladder tissue of patients with cholelithiasis and cholecystitis. *J Infect Dev Ctries.* 2009;**3**(11):856–9.
31. Bolin I, Lonroth H, Svennerholm AM. Identification of Helicobacter pylori by immunological dot blot method based on reaction of a species-specific monoclonal antibody with a surface-exposed protein. *J Clin Microbiol.* 1995;**33**(2):381–4.
32. Jones AC, Logan RP, Foynes S, Cockayne A, Wren BW, Penn CW. A flagellar sheath protein of Helicobacter pylori is identical to HpaA, a putative N-acetylneuraminyllactose-binding hemagglutinin, but is not an adhesin for AGS cells. *J Bacteriol.* 1997;**179**(17):5643–7.