Helicobacter pylori acquisition rates and the associated risk factors amongst newlywed couples; a prospective cohort study in Tehran, Iran

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Abstract

Background The rates and routes of *Helicobacter pylori* transmission, in a high prevalent country like Iran, with gastric cancer as the leading cause of male cancer mortality is of essence. Here, we have studied the *H. pylori*-associated risk factors and the likelihood of interspousal transmission.

Methods In a cohort of 686 young prewed couples, questionnaires were self-administered and serum samples were collected, for assessment of risk factors and sero-status of *H. pylori*, at baseline and follow-up. Of the 475 *H. pylori* single- or double- seronegative couples, 201 returned for follow-up. The average follow-up duration was 2.2 (SD 0.6) years, with a total of 560.1 person-years. Logistic regression and Cox regression models were used to estimate the odds ratios (ORs) and hazard ratios (HRs).

Results The risk of infection was higher in men than women (OR:1.3, 95%CI:1.0-1.8) and among metropolitan than rural residents (OR=1.4, 95%CI:1.1-1.9). The risk of infection was significantly higher among those with three siblings (OR=1.6, 95%CI:1.1-2.2), and four or more siblings (OR=1.4, 95%CI:1.0-1.9), in reference to those with one or no siblings. *H. pylori* acquisition occurred in 10.9% (27/247) of the *H. pylori* seronegative participants. The risk of acquisition was significantly higher in older aged (HR=1.2, 95%CI: 1.1-1.3) and higher educated (HR=0.2, 95%CI:0.1-0.9) participants, than younger and illiterate subjects, respectively. Our analysis did not find any evidence for interspousal transmission (HR=1.0, 95%CI: 0.4-2.2).

Conclusion Although we detected *H. pylori* acquisition in the young adult Iranian population, our findings did not support interspousal transmission, as a mode of adult *H. pylori* aquisition.

Keywords: *H. pylori*, acquisition, transmission, risk factors, spouses, couples, serology

Introduction

Helicobacter pylori (*H. pylori*) is classified as a group 1 carcinogen by the International Agency for Research on Cancer (IARC) [1]**.** It is one of the most common bacterial pathogens in humans [2], which upon colonization of the gastric epithelium, causes a variety of gastrointestinal diseases, including gastritis, duodenal, gastric ulcer and gastric cancer [3]. The prevalence of *H. pylori* is much higher in the developing countries, as compared to the developed countries [4], where it is less prevalent among the younger generations [5]. Studies on *H. pylori* seroprevalence revealed that up to 86% of the people living in South Asia [6], nearly 70% of Africans [7], and 22%–43% of the populations in Northern Europe are estimated to be infected with *H. pylori* [7], while the global average of *H. pylori* prevalence is estimated to be 58% [8]. Gastric cancer is the most common cause of cancer mortality among Iranian males, with *H. pylori* infection as the prominent risk factor [9]. Among all potential risk factors for gastric cancer, control of *H. pylori* infection seems to be the most promising measure for prevention [10].

Although *H. pylori* is the most widespread infection worldwide, the reservoir of the bacteria and how it is spread, has not yet been fully elucidated. Both fecal-oral and oral-oral spread, as well as waterborne transmission, have been suggested as the main routes [11], which may vary according to lifestyle habits [12]. Person-to-person transmission can occur in horizontal or vertical fashions [12]. The horizontal mode is when contamination is transmitted via external sources outside of the family, whereas the vertical mode refers to familial transmission between parents, children, and spouses [12]. Most studies show that transmission of *H. pylori* infection occurs vertically during childhood [13-15], and mother-to-child, as well as inter-siblings transmission, are the most established modes [16, 17]. However, there is some evidence that acquisition and even clearance of infection may also occur vertically or horizontally during adulthood [18, 19], which depends on the circumstances of the community [12].

An obvious source of familial transmission amongst adults could be an infected spouse [20-26]. A number of studies have investigated bacterial transmission amongst couples [20-34]. Most of these studies originate from developed countries, with a low prevalence rate of *H. pylori* infection [5, 35]. Cross-sectional studies comparing the prevalence of *H. pylori* infection, in index subjects and spouses can suggest, but not prove, spouse-to-spouse transmission. Longitudinal studies, following the acquisition of infection provide reliable evidence for or against the partner's role in the spread of *H. pylori* infection.

In Iran, young couples typically remain engaged for approximately 6 to 12 months prior to the wedding ceremony and cohabitance. Here, we have carried out a cohort study to evaluate the prevalence of *H. pylori* seroprevalence among prewed couples, in order to evaluate adult *H. pylori* acquisition and assess the incidence rate of spouse-to-spouse transmission amongst discordant *H. pylori* positive/negative and concordant double-negative couples. We also estimated the role of other variables in the transmission process, including demographic factors and socio economic status (SES).

Materials and methods

Study population

We recruited a cohort of prewed (to-be-married) couples (686 couples, 1372 individuals) attending Farmanfarmayan Health Center (45 couples) or the Genetics Counselling Clinic at Imam Khomeini Hospital (641 couples), Tehran, Iran. After a pilot study in 2008, we recruited the cohort members between 18 April 2009 and 28 July 2011. The inclusion criteria included: a) current residence of Tehran, for follow-up feasibility, and b) no history of *H. pylori* eradication treatment within the last six months, before recruitment.

Sociodemographic variables

Upon recruitment, every participant signed a written informed consent and completed a self-administered questionnaire, containing questions about demographic information including age, gender, longest place of residence, childhood socioeconomic status (i.e., parents' education, occupation, and assets), number of siblings, level of education, type of employment, prior history of *H. pylori* eradication treatment. The level of education of the participants' parents was categorized based on having a high school diplomas, as: 1) neither parents, 2) one parent, or 3) both parents. The parents' occupation was categorized as follows: both parents unemployed (class I); both manual workers or one manual worker, the other unemployed (class II); both self-employed, or one self-employed, the other of class I or II (class III); both office employees, or one office employee, the other of classes I-III (class IV); and both managers, or one manager, the other of classes I-IV (class V). We also grouped the parents' assets based on house and car ownership as 1) low (no house or car), 2) medium (house or car), and 3) high (both house and car).

H. pylori serostatus

Blood samples were collected from each consenting participant. Sera were isolated and stored at –20ºC until further analysis. Serum IgG antibodies against *H. pylori* were quantitated, using CapitaTM *H. pylori* IgG ELISA kit (Trinity Biotech, USA). Sera were identified as positive, negative, or borderline.

H. pylori serostatus was evaluated at two time points: 1) at recruitment (baseline) and 2) upon follow-up. The study design is depicted in Figure 1.

Follow-up

Our study population constituted individuals residing in the Tehran metropolitan city. The recruited couples (n = 686) were grouped into four categories, according to their *H. pylori* serostatus at baseline, as: 1) both negative (-/-) (n = 139), 2) discordant results (+/- or -/+) (n = 336), or 3) both positive (+/+) (n = 211). The first 3 groups who were at risk for *H. pylori* acquisition, were followed (Figure 1)

Out of the 1372 participants, 758 (55.2%) individuals were baseline-positive for *H. pylori* infection. As mentioned above, 422 individuals (211 couples) were not followed due to *H. pylori* seropositivity of both partners. Of the remaining 475 couples, 274 (548 individuals) were lost to follow-up. The loss to follow-up was mainly due to the lack of motivation and time for participation. This left us with 201 couples (402 individuals), who were followed for an average period of 2.2 (± SD = 0.6) years, equivalent to a total of 560.1 person-years. The latest follow-up date 17 Feb 2013.

Statistical analyses

We applied unconditional logistic regression models to estimate odds ratios (OR) and 95% confidence intervals (CI) to measure the association between *H. pylori* sero-status and various risk factors. The stepwise backward elimination approach was used to fit the model and to select variables that were associated with *H. pylori* serostatus in the final model. Compared to the full model, the nested model using the likelihood ratio test was used, and the variables were dropped if they did not significantly reduce the fit. We included age as a continuous variable in the model and calculated the *p* values for trend. The Cox regression model estimated the hazard ratio (HR) and corresponding 95% CI of *H. pylori* serostatus for different risk factors, including the *H. pylori* serostatus of their spouses at baseline, subject's education, and the socioeconomic status of the parents. Since *H. pylori* infection occurs mainly during childhood, we included parent's socioeconomic status (SES) instead of the subject's SES in the multivariable model, when we analyzed risk factors of *H. pylori* infection at baseline. However, both the SES of both the parents as well as the subjects were used to study risk factors of *H. pylori* infection during the cohort follow-up. Stata, version 16 (Stata Corp, College Station, Texas 77845 USA, licensed to Tampere University) was used for statistical analyses.

Sensitvity anlysis

The sensitivity and specificity rates of the CapitaTM *H. pylori* IgG ELISA kit, reported by the manufacturing company, is 100% and 75%, respectively. Therefore some detected results during the

follow-up could be false-positives and lead to bias. Therefore, we have performed a sensitivity analysis and studied the results taking into account the sensitivity and specificity of the kit.

Search and Data Sources

Keywords were identified based on *H. pylori* transmission, and searches were made using MeSH to find synonymous keywords. The literature search has been carried out via online electronic databases, including PubMed, Web of Science, and Scopus. EndNote X9 was used as the reference management software.

Results

The average age of the participants at baseline was 23.8 (SD ± 4.3) years. *H. pylori* seropositivity was more prevalent among men (58%), as compared to women (52%) (Table 1). Most of the participant's parents in this study had a class I (both parents unemployed) (28.1%) or IV (28.7%) jobs. After adjustment for other confounders (gender, age, place of residence, and the number of siblings), the prevalence of *H. pylori* seropositivity was significantly higher among men than women (OR:1.3, 95% CI: 1.0,1.8). Moreover, we found that the prevalence of *H. pylori* sero-positivity was 30% to 40% higher in subjects who have lived most of their lives in the non-metropolitan (OR=1.3, 95% CI: 1.0, 1.9), as well as metropolitan (OR=1.4, 95% CI: 1.1, 1.9) cities, in reference to those residing in rural areas (Table 2). We also detected 40% to 60% higher prevalence of *H. pylori* sero-positivity amongst the subjects who had three siblings (OR=1.6, 95% CI: 1.1, 2.2), as well as four or more siblings (OR=1.4, 95% CI: 1.1, 1.9), as compared to those with one or no siblings (Table 2). Albeit the risk of *H. pylori* infection did not increase among those who had two siblings (OR=1.0, 95% CI: 0.7, 1.3). The education of the parents was associated with *H. pylori* serostatus in the crude model, but did not remain significant in the adjusted model (Table 2).

H. pylori acquisition rate during the follow-up was 10.9% (27/247). Amongst the 46 concordant double-negative couples, 13 out of the 92 individuals (14.1%), who were at risk of infection, seroconverted (Table 3). We did not find any couples who both seroconverted during the follow-up period. Of the discordant 155 couples, 14 (9.0%) seroconverted during the follow-up. Simultaneously, the baseline-infected partner in 8 (5.2%) of these couples cleared the infection, during the same time (Table 3).

The cohort of discordant couples (N=155), of whom only one spouse was at risk of infection, contributed 343.6 person-years , of whom13 acquired *H. pylori* infection. In contrast, the concordant couples who were both sero-negative and at risk of infection, contributed 216.5 person-years to the cohort (Table 4). Based on the multivariable Cox regression model, there was no association between

having a positive spouse and risk of *H. pylori* acquisition (HR: 1.0, 95% CI: 0.4, 2.2). The risk of infection was significantly higher among those who were 22 to 26 years old (HR: 4.5, 95% CI: 1.1, 17.6) or older (HR: 7.5, 95% CI: 1.7, 33.3), compared to those younger than 22 years, upon admission. We also found that participants with an education level of at least the high school diploma had about 75% lower risk of *H. pylori* acquisition compared to the illiterate individuals (high school diploma HR: 0.2, 95% CI 0.1, 0.9, BSc degrees, HR: 0.3, 95% CI: 0.1, 0.9 and MSc and higher degrees, HR: 0.2, 95% CI: 0.1, 0.9). There were no statistically significant associations between *H. pylori* acquisition and gender or parent's socioeconomic status.

Our sensitivity analysis showed that given 100% sensitivity and 75% specificity of the Kit, the acquisition rate would be ~8%, instead of the ~10%, which was observed during the follow-up. However, the clearance rate during the follow-up was not altered.

Discussion

The question of the possible transmission of *H. pylori* infection, from one partner to another, remains a serious concern, particularly when taking into account the potential development of subsequent gastrointestinal complications. Here, we have addressed this question in a cohort of young population of engaged to be married couples and did not find any evidence of interspousal transmission of *H. pylori* infection. On the contrary, the rate of adult acquisition was slightly higher in the double-negative concordant couples, than the discordant couples, though it did not reach statistical significance. The adult *H. pylori* acquisition rate among this adult population was ~11 percent.

Many studies suggest that the main reservoir of *H. pylori* infection is the human gastric mucosa, which may also extend to the rest of the gastrointestinal tract, including oral secretions. .. Therefore, close human contact may lead to the transmission of *H. pylori* from one person to another. Hence, *H. pylori* transmission between spouses is considered a probable mode of transmission. Several studies have evaluated the risk of infection among *H. pylori* discordant couples, yielding conflicting results (reviewed in Table 5). Early studies are mostly cross-sectional and based merely on serological findings [29, 32]. In a case-only study in England, 8 (21%) spouses of the 38 *H. pylori posi*tive subjects examined, were also colonized with *H. pylori.* Considering the 40% prevalence rate of seropositivity in their community, the authors concluded that interspousal transmission of infection is unlikely (Table 5, row 1) [29]. Similarly, of the 277 Caucasian couples who had referred to an infertility clinic in the

US, 17.3% (96/554) individuals were *H. pylori* seropositive, among whom only 6.5% (18/277) of the spouses were also *H. pylori* positive, which is too low a proportion to support the role of the spouse as a source of infection (Table 5, row 3) [32].

Several molecular studies, in line with the mentioned serological results, provide evidence for external rather than internal sources for *H. pylori* infection amongst couples [28, 30, 31, 33]. For instance, in a study from Greece, 54 (84.4%) of the 64 dyspeptic patients were *H. pylori posi*tive. Of these subjects, 42 (77.8%) had *H. pylori* positive spouses, but only 8 (44.4%) out of the 18 tested double-positive couples, shared the same infecting strain (Table 5, row 5) [34]. Amongst the 70 endoscopied subjects in Japan, 21 (30%) were not only *H. pylori* positive, but also had *H. pylori* positive spouses. But performing PCR-RFLP on the *ureB/ureC* genes of the infecting strains, revealed that of these 21 couples, only one was colonized with a similar *H. pylori* strain (Table 5, row 9) [33], again voting against interspousal transmission. In a similar study from Taiwan, although the rate of infected partners amongst infected subjects (40/55, 72.7%) was quite considerable, having used the same typing method, only one out of the 25 concordant couples tested, shared the infecting strain (Table 5, row 10) [30]. Consistent with these studies, in a cohort study following 120 previously infected/eradicated subjects, for a year, 8 re-infection cases were detected, whose spouses were also infected, but having genotyped the infecting strains, only one couple (12.5%) was infected with the same *H. pylori* strain (Table 5, row 11) [28]. Finally, a study in Southeast Asia on 31 patients found that 5 out of 13 concordant positive couples, were colonized with the same *H. pylori* strain (Table 5, row 12) [31]. Taken together, these studies found no or very little evidence for spouses, as sources of infection or re-infection.

In contrast, other studies having used serology and/or UBT, found some evidence supporting inter-spousal transmission of *H. pylori* infection [20-22, 24, 25, 36]. For instance, in a cross-sectional study in the US, 41 healthy volunteers underwent serology and UBT, which identified 19 (46.3%) positive for *H. pylori* infection. Of these, 13 (68.4%) were cohabitating an infected spouse, as compared to 2 (9%) of the 22 *H. pylori* negative subjects. Since this rate was higher than the rate of *H. pylori* prevalence at that time, the authors vouch for interspousal spread as a mode of *H. pylori* transmission (Table 5, row 2) [36]. In a study in Austria, amongst 18 dyspeptic patients, suffering from active duodenal ulcers, who were found *H. pylori* positive and their infection was eradicated, 2 became re-infected (at 14 and 43 months post-eradication) and DNA typing revealed that they both shared the infecting strains with their spouses (Table 5, row 4) [23]. Similarly, studies carried out in Italy (Table 5, rows 6 and 14) [22, 24] and Germany (Table 5, row 8) [20], detected more than 65% *H. pylori*

double-positive concordant spouses, the rate of which was well above the rate of *H. pylori* prevalence for that population at the time. According to a cohort study carried out in India, which followed 5 discordant couples for a year, 3 became concordant double-positives (Table 5, row 7) [25]. Another study on 670 women in the maternity ward and their spouses in Germany, found that a total of 34.9% of couples were both positive, with a higher prevalence (79.2%) in non-German (mostly Turkish), as compared to German (10.3%) couples (Table 5, row 13) [21].

The strengths of our study include the unique setting, which allowed us to carry out a cohort, comparing the serostatus of one to two year-married couples to their prewed conditions. Another highlight is the study sample size, which is greater than most of the other herein referenced studies. The limitations include the assessment of *H. pylori* serostatus, as the sole method of detection of *H.* Pylori infection, which may have created false-positive subjects, who may have undergone eradication treatment, 6 to 12 months prior to their recruitment. Another limitation is the absence of molecular profiling of the infecting strains, for strain type tracking. This, however, was not feasible, as our studied subjects were healthy couples, with no indication for gastroscopy or biopsy collection. We were also concerned about potential misclassification of *H. pylori* serologic results due to the high sensitivity (100%) and relatively low specificity (75%) of the Kit. Our sensitiicty analysis, however, alleviated this cocern and showed that the transmission rate decreased from ~10% to ~8%.

Our findings indicate that in a high-prevalent countries such as Iran, *H. pylori* infection was associated with age, the male gender, residing in an urban area, and living with multiple siblings. The low level of parent's education was also strongly associated with the rate of *H. pylori* infection. We also found that *H. pylori* acquisition was not exclusive to childhood, and adult infection does occur. However, an infected spouse was not found as a risk factor for *H. pylori* acquisition in our population. To our knowledge, this was the first study evaluating adult *H. pylori* acquisition/transmission in Iran. Surely, the next steps include 1) re-evaluation of these findings in a larger multi-centered study across the country, 2) analyzing the roles of risk factors in association with the rate of acquisition, and focusing on the variables of age and gender. Only then, can we make evidence-based declarations in regards to *H. pylori* acquisiton in our population, where gastric cancer incidence and mortality rates, as well as the prevalence of *H. pylori* infection, as the main risk factor, are relatively high.

Declarations

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Conflicts of interest: Authors declare no conflicts of interest.

Availability of data and materials: All the data and biological samples are available for assessment. In addition, programming codes (i.e. do files of the Stata statistical software) are available.

Authors' contributions: M.H., R.D., F.S., R.A. managed the fieldwork and collected baseline and follow-up data of the cohort. M.H. M.Mort. and N.A. conducted data cleaning and statistical analysis. H.Kh. provided clinical service to those who were positive and asked for clinical consultation during the follow-up, M.E. and S.S. performed laboratory tests, E.P. provided advice for data analyses and preparation of the manuscript. M.H. and S.S prepared the draft of the manuscript. K.Z and M.M as the principal investigators, supervised the study, extensively revised the manuscript and approved the final version.

Ethics approval: This study was approved by the Ethics Committee of Tehran University of Medical Sciences (Code: 88-03-51-8627).

Consent to participate: All participants signed a written informs consent.

Consent for publication: Al authors consented to the publication of this report.

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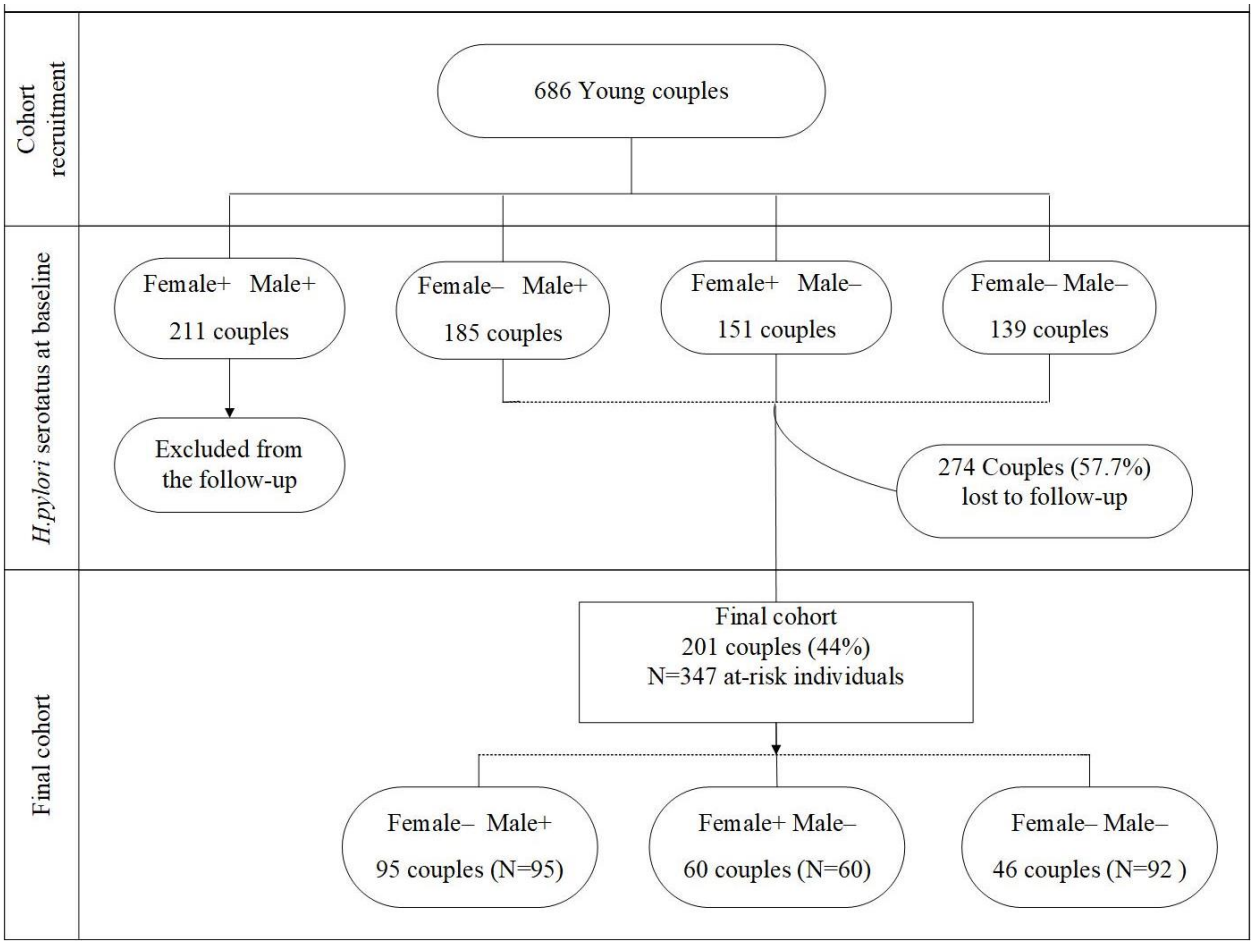
Figure 1. The schematic study design for the recruitment of the cohort members to study *H pylori* transmission among the Iranian young couple cohort



- *The plus/minus signs indicate H. pylori seropositive and seronegative subjects, respectively*

Table 1: Characteristics of the study population and *H. pylori* serostatus at baseline of the Iranian young couple cohort

Category	**All** (N = 1372) N (% column)	***H. pylori* seropositive** (N = 758) N (% row)
Gender		
Female	686 (50.0)	362 (52.8)
Male	686 (50.0)	396 (57.7)
Age (yrs)		
≤ 22	381 (27.8)	210 (55.1)
>22-24	403 (29.4)	214 (53.1)
>24-27	324 (23.6)	200 (61.7)
≥28	264 (19.2)	134 (50.8)
Place of residence (max)		
Rural	268 (19.5)	127 (47.4)
Urban, non-metropolitan	292 (21.3)	168 (57.5)
Urban Metropolitan	812 (59.2)	463 (57.0)
Number of siblings		
≤ 1	270 (19.7)	134 (49.6)
2	347 (25.3)	175 (50.4)
3	272 (19.8)	168 (61.8)
≥ 4	483 (35.2)	281 (58.2)
Parents education[1]		
Neither	846 (61.7)	482 (57.0)
One	242 (17.6)	132 (54.6)
Both	284 (20.7)	144 (50.7)
Parents occupation[2]		
Job class I	386 (28.1)	220 (57.0)
Job class II	191 (13.9)	96 (50.3)
Job class III	357 (26.0)	197 (55.2)
Job class IV	393 (28.7)	216 (55.0)
Job class V	31 (2.3)	22 (71.0)
Unknown	14 (1.0)	7 (50.0)
Parents assets[3]		
Low	141 (10.3)	71 (50.4)
Medium	435 (31.7)	233 (53.6)
High	796 (58.0)	454 (57.0)

[1] With high school diploma or higher

[2] Parents' occupation was categorized as follows: both parents unemployed (Class I); both manual workers or one manual worker, the other unemployed (Class II); both self-employed, or one self-employed the other of class I or II (Class III); both office employees, or one office employee the other of Classes I-III (Class IV); and both managers, or one manager the other of Classes I-IV (Class V).

[3] Low (no assets reported), Medium (house or car ownership), High (house and car ownership)

Table 2: Risk factors associated with *H. pylori* seropositivity at baseline of the Iranian young couple cohort

Category	Crude OR (95% CI)	*P* value	Adjusted OR* (95% CI)	*P* values
Gender				
Female	Ref.	-	Ref.	-
Male	1.2 (0.97, 1.5)	0.07	1.3 (1.0, 1.6)	0.04
Age				
≤ 22	Ref.	-	Ref.	-
22-24	0.9 (0.7, 1.2)	0.6	0.9 (0.7, 1.2)	0.5
24-27	1.3 (0.97, 1.8)	0.07	1.1 (0.8, 1.6)	0.4
≥ 28	0.8 (0.6, 1.1)	0.3	0.7 (0.5, 1.0)	0.06
P values for trend			0.20	
Place of residence (maximum)				
Rural	Ref.	-	Ref.	-
Urban, non-metropolitan	1.5 (1.1, 2.1)	0.02	1.3 (0.96, 1.9)	0.09
Urban metropolitan	1.5 (1.1, 1.9)	0.006	1.4 (1.1, 1.9)	0.02
Number of siblings				
≤ 1	Ref.		Ref.	
2	1.0 (0.8, 1.4)	0.8	1.0 (0.7, 1.3)	0.8
3	1.6 (1.2, 2.3)	0.005	1.6 (1.1, 2.2)	0.01
≥ 4	1.4 (1.0, 1.9)	0.02	1.4 (1.0, 1.9)	0.04
Parents education[1]				
Both < high school	Ref.			
One ≥ high school	1.0 (0.7, 1.2)	0.5	--	--
Both ≥ high school	0.8 (0.6, 1.0)	0.06	--	--
		P-trend 0.05	--	--
Parents occupation[2]				
Job class I	Ref.			
Job class II	0.8 (0.5, 1.1)	0.1	--	--
Job class III	0.9 (0.7, 1.2)	0.6	--	--
Job class IV	0.9 (0.7, 1.2)	0.6	--	--
Job class V	1.8 (0.8, 4.1)	0.1	--	--
Parents assets[3]				
Low	Ref.			--
Medium	1.1 (0.8, 1.7)	0.5	--	--
High	1.3 (0.9, 1.9)	0.1	--	--

[1] With high school diploma

[2] Parents' occupation was categorized as follows: both parents unemployed (Class I); both manual workers or one manual worker, the other unemployed (Class II); both self-employed, or one self-employed the other of class I or II (Class III); both office employees, or one office employee the other of Classes I-III (Class IV); and both managers, or one manager the other of Classes I-IV (Class V).

[3] Low (no assets reported), Medium (house or car ownership), High (house and car ownership)

*Adjusted for gender, age, place of residence, and the number of siblings. Note: Since parents' education, occupation, and assets did not remain the final model, we did not provide HR and 95% CI for these variables in the adjusted model.

Table 3: *H. pylori* serostatus amongst concordant and discordant couples at baseline and follow-up the Iranian young couple cohort

At baseline	**At follow-up**	**N**	**Percentage (95% CI)**
F- M- (both non-infected) (n = 46)	**F- M- (**unchanged)	33	71.7 (56.5, 84.0)
	F- M+ (female-unchanged, male-infected)	8	17.4 (7.1, 31.4)
	F+ M- (female-infected, male-unchanged)	5	10.9 (3.6, 23.6)
F- M+ (male-infected) (n = 95)	**F- M+ (**unchanged)	84	88.4 (8.02, 94.1)
	F+ M+ (female-infected, male-unchanged)	6	6.3 (2.4, 13.2)
	F- M- (female-unchanged, male-cleared)	5	5.3 (1.7, 11.9)
F+ M- (female-infected) (n = 60)	**F+ M-** (unchanged)	51	85.0 (73.4, 92.9)
	F+ M+ (female-unchanged, male-infected)	6	10.0 (37.6, 20.5)
	F- M+ (female-cleared, male-infected)	2	3.3 (0.04, 11.5)
	F- M- (female-cleared, male-unchanged)	1	1.7 (0.0, 8.9)

F: female,
M: male

Table 4: *H. pylori* acquisition during the follow-up period and the associated risk factors in the Iranian young couple cohort

Variable	**Person time** (yrs)	**Incidence of *H. pylori* infection** (n)	**Crude HR**	**Adjusted HR (95% CI)**
H. pylori serostatus (baseline)				
Positive	343.6	14	Ref.	Ref.
Negative	216.5	13	1.4 (0.6, 2.9)	1.0 (0.4, 2.2)
Age (yrs)				
≤21	197.1	3	Ref.	Ref.
22-26	188.4	13	4.5 (1.3, 16.1)	4.5 (1.1, 17.6)
>26	174.6	11	5.3 (1.4, 19.6)	7.5 (1.7, 33.3)
Sex				
Female	321.2	11	Ref.	Ref.
Male	238.9	16	1.9 (0.9, 4.0)	1.2 (0.5, 2.8)
Participants Education				
Illiterate to < high school diploma	81.2	7	Ref.	Ref.
High school diploma	168.9	5	0.3 (0.1, 1.1)	0.2 (0.1, 0.9)
BSc degree	149.1	7	0.5 (0.2, 1.5)	0.3 (0.1, 1.1)
≥ MSc degree	160.9	8	0.7 (0.3, 1.9)	0.3 (0.8, 1.0)
Parents assets[1]				
Low	192.2	9	Ref.	Ref.
Medium	188.3	8	0.9 (0.3, 2.3)	1.9 (0.7, 5.6)
High	179.6	10	1.2 (0.5, 2.9)	2.4 (0.8, 7.0)

[1] Low (no assets reported), Medium (house or car ownership), High (house and car ownership)

Table 5: Literature review of *H. pylori* transmission studies among couples

Row	Year Author	Study type	Study population		*H. pylori* detection method	Results			Couple transmission (Y/N)
			Ethnicity/ Country	Number of couples		Index	Partner		
1	1987 Jones DM et al. [29]	Case-only	England	38	Index: Culture Spouses: Serology	8 *H. pylori* +	8 *H. pylori* + (21%)		N
						30 *H. pylori* +	30 *H. pylori* -		
2	1991 Malaty HM. et al. [36]	Cross-sectional	USA	41 Healthy volunteers	Serology UBT (*H. pylori* +: Sero+ or UBT+)	13 *H. pylori* +	13 *H. pylori* + (68%)		Y
						6 *H. pylori* +	6 *H. pylori* -		
						2 *H. pylori* -	2 *H. pylori* + (9%)		
						20 *H. pylori* -	20 *H. pylori* -		
3	1991 Perez-Perez GI et al. [32]	Cross-sectional	Caucasian USA/Other countries	227 Infertility clinic referred	Serology	18 *H. pylori* +	18 *H. pylori* + (6.5%)		N
						26 *H. pylori* +	26 *H. pylori* -	(21.7%)	
						34 *H. pylori* -	34 *H. pylori* +		
						199 *H. pylori* -	199 *H. pylori* - (71.8%)		
4	1995 Schütze K et al. [23]	Cohort (14 & 43 months)	Austria	18	Culture RUT Histology Serology DNA typing (Rapid PCR)	16 *H. pylori* - (*H. pylori* - eradicated)	-		Y
						2 *H. pylori* - (*H. pylori* - eradicated)	2 *H. pylori* +	2/2 with the same *H. pylori* strain which identical to pre-treatment strain	
5	1996 Georgopoulos SD et al. [34]	Cross-sectional	Greece	64	Histology Culture DNA typing	42 *H. pylori* +	42 *H. pylori* + (18 Endo)	8/18 same *H. pylori* strain (44%)	Y/N
						12 *H. pylori* +	12 *H. pylori* -	-	
						10 *H. pylori* -	2 *H. pylori* +	-	
6	1996 Parente F. et al. [22]	Case-control	Italy	124 + 248 control (age+gender match)	Histology/RUT Serum IgG anti-*H. pylori*	88 *H. pylori* +	88 *H. pylori* + (71%)		Y
						36 *H. pylori* +	36 *H. pylori* -		
						248 Population Controls	145 *H. pylori* + (58%) Rate of prevalence		
7	1999 Singh V et al. [25]	Cohort (12 months)	India	25	RUT Serology	15 *H. pylori* +	15 *H. pylori* + (83%)		Y
						3 *H. pylori* +	3 *H. pylori* -	3 *H. pylori* + (60%) (1 y later)	
						2 *H. pylori* -	2 *H. pylori* +		

						(28%)		
					5*H. pylori* -	5*H. pylori* -		
8	1999 Brenne H et al. [20]	Cross-sectional	Germany	110	UBT	10*H. pylori* +	10*H. pylori* + (62.5%)		Y
					6*H. pylori* +	6*H. pylori* -		
					14*H. pylori* -	14*H. pylori* +(14.9%)		
					80*H. pylori* -	80*H. pylori* -		
9	1999 Suzuki J et al. [33]	Cross-sectional	Japan	70	Endoscopy PCR-RFLP (ureB/C)	21*H. pylori* +/+	1/21 with same *H. pylori* strain		N
					35*H. pylori* +/-	-		
					14*H. pylori* -/-	-		
10	1999 Kuo CH et al. [30]	Case-only	Taiwan	55	Serology PCR-RFLP (ureB/C, vacA)	40*H. pylori* +/+	1/25 with same *H. pylori* strain		N
					15*H. pylori* -/+	-		
11	2002 Gisbert JP et al. [28]	Cohort (6-12 months)	Spain	120	UBT Histology PCR-RFLP (ureC)	8*H. pylori* - (*H. pylori* - eradicated)	8 *H. pylori* +	7/8 *H. pylori* reinfection (87%) different patterns	N
					112*H. pylori* - (*H. pylori* - eradicated)	112*H. pylori* -	85 *H. pylori* reinfection (76%)		
12	2002 Luman W et al. [31]	Case-only	Chinese/ Indian/ Malay	31	Serology Histology PCR-RFLP	16*H. pylori* +	16*H. pylori* + (13 Endo)	5/13 same *H. pylori* (38%)	N
					15*H. pylori* +	15*H. pylori* -	-		
13	2006 Brenner H et al. [21]	Cross-sectional	German Turkish Italian	670	UBT Stool Ag	51*H. pylori* +	51*H. pylori* + (40.2%)		Y
					76*H. pylori* +	76 *H. pylori* -		
					95*H. pylori* -	95*H. pylori* + (17.5%)		
					448 *H. pylori* -	448*H. pylori* -		
14	2018 Sgambato. D et al. [24]	Cross-sectional	Caucasian Italians	161	UBT	120 *H. pylori* +	120 *H. pylori* + (74.5%)		Y
					41*H. pylori* +	41 *H. pylori* -		
					161 population control	52*H. pylori* + (32.3%) Rate of prevalence			
						109*H. pylori* -		