

## **A Study of Intrafamilial *H. pylori* Transmission in Epirus: A Region of North-Western Greece**

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### **Abstract**

**Aim:** To investigate the frequency of intrafamilial transmission of Helicobacter Pylori and its relation with socioeconomic factors in Epirus.

**Methods:** *H. pylori* infection was detected by (13) C-urea breath test, stool antigen assay and histological examination in members of families with at least one member infected. Information about the sex, age, somatometry, number of family members and household conditions was selected in questionnaires. Blood tests were also performed.

**Results:** In total, 171 individuals from 43 households were tested, of whom 43 (25, 1%) were mothers with mean age MA 39,7 years, 40 (23, 4%) fathers with MA 43,1 years, and 88 (51, 5%) children with MA 11,28 years. The average number of family members in the examined families was 4 (110 individuals-64, 3%) with variation from 2 to 6. One hundred subjects in 171 (58, 5%) were living in rural areas and the rest 71 (41, 5%) in urban areas. Twenty parents in 83 (25%) are university graduates. Overall, 92/171 (53, 8%) subjects were found *H. pylori* positive, of whom 46/88 (52, 3%) were children, 26/43 (60, 5%) mothers and 20/40 (50%) fathers. Of the 23 children with both parents positive to infection 14 (60, 9%) were found positive. Positive were also found 24 children, out of 51 (47, 1%) who had one parent positive, and 6, out of 10 with parents negative to infection.

**Conclusion:** The intrafamilial transmission of infection is affected by the number of family members ( $p < 0.013$ ), the educational level of parents ( $p < 0.015$ ) and, marginally, the place of residence, either rural or urban ( $p < 0.061$ ). Symptoms of iron deficiency were detected in infected adults and children.

**Keywords:** *H. pylori*; Intrafamilial transmission; Childhood

### **Introduction**

*H. pylori* is a spiral, Gram (-) bacterium with smooth surface and multiple unipolar flagella that colonizes exclusively the gastric mucosa.

The bacterium was discovered by Marshall and Warren in 1982. It is a common pathogenic microbe causing chronic gastritis and peptic and duodenal ulcer. It has been also recognized as a class I peptic carcinogen [1]. It is estimated that 50% of the world population is infected by *H. pylori* [2]. The prevalence of infection is higher in developing countries [3,4]. The prevalence increases with the increase of the age, i.e., it is higher in adults than in children. *H. pylori* infection is highly prevalent in children of families living under poor conditions, in large families, and in families with *H. pylori* seropositive adults [5]. The main risk factors for the transmission of the infection seems to be the poor socioeconomic living conditions and the number of family members [3], especially if the mother is infected [6,7]. The majority of children seem to be infected within the first 3 years of life [6] while the transmission in first year of life, even if mothers are *H. pylori* positive, is extremely rare [8]. Person-to-person, through fecal-oral and oral-oral (e.g., saliva, kisses), is suggested as the main transmis-

sion route, but *H. pylori* can be also transmitted through environmental sources such as the drinking water [9,10]. Invasive diagnostic methods are used for the diagnosis of infection requiring endoscopic biopsy with rapid urease test, histological examination and tissue culture for *H. pylori*, with their combination to be considered the most reliable diagnostic method in children [11]. The most reliable non-invasive detection methods of *H. pylori* are the 13C-urea breath test with the isotope ratio mass spectrometry (IRMS) and the detection of *H. pylori* monoclonal antigen in the stool, which both have high sensitivity 96.5-97.9% and specificity 96.7-100% [12,13]. The aim of our study is to investigate the incidence of *H. pylori* infection and the transmission among members of the same family using invasive or non-invasive methods, and determine the factors that influence the transmission in the region of Epirus.

## Materials and Methods

### Study population

The families that participated in the study came from the Epirus region (the majority), Corfu and Aitolokarnania prefectures. Family members were tested for *H. pylori* infection at the University Hospital of Ioannina after permission from the Scientific Committee and written consent of parents.

Of the totally 171 subjects who participated, the mothers were 43 (25.1%) with MA 39.69 years (28.98 -53.39), the fathers 40 (23.4) with MA 43,10 years (30.44 - 54.63) and children 88 (51.5%) MA 11.28 years (1.5 - 21). Of the 88 children, 6 (6.8%) were up to 5 years of age, 29 (33%) were of age 5-10 years, 47 (53.4%) of age 10-16 years and 6 (6,8%) over 16 years. The average number of family members was 4 (110 people - 64.3%) (variation 2 - 6 members). Males were 91 subjects (53.2%) and females 80 (46.8%).

For the purpose of the study, as family is considered the group with at least one parent with at least one child living in the same house and with one member diagnosed with *H. pylori* infection. This member is defined as the initially-infected family member. All family members were tested for *H. pylori* infection. Each family member completed a questionnaire containing information on various reference parameters such as age, sex, weight and body height. The educational level of the family parents, the place of residence and the living conditions were also taken into consideration. Any symptoms that could be attributed to infection by *H. pylori*, such as dyspepsia, nausea-vomiting, belching, abdominal pain, and chest pain, have been recorded.

### Materials

Non-invasive methods were used initially to investigate the infection:

Helicobacter Test INFAI 75mg powder for oral solution 13C-urea: Participants had not received antibiotics and bismuth compounds for at least two months, neither H2 receptor antagonists, proton pump inhibitors and antacids for at least 2 weeks. The breath test (13C-urea) was performed by qualified personnel in an appointment at the outpatient clinic of Pediatrics and Hepatogastroenterology following detailed instructions for proper receipt of the sample. Analysis of breath samples were done by the Isotope Ratio Mass Spectrometry method (IRMS). The critical point to distinguish negative and positive result concerning the presence of the bacterium *H. pylori*, was set the 4 ‰ of the difference  $\Delta\delta$  (between the values for "00 minutes" and "30 minutes"), which means that the increase in the  $\Delta\delta$  value by more than 4 ‰ indicates an infection (sensitivity 96.5 to 97.9% and specificity from 96.1 to 100%) [14,15].

One step *H. pylori* Antigen Rapid Test in Human fecal specimen:

The detection of antigen in feces was done with the use of SD Bioline *H. pylori* Ag kit which has 100% sensitivity and 97.8% specificity. The stool sample (approximately 50mg) was collected in sterile containers and examined directly.

Endoscopic and Histological tests: Endoscopies in adults were performed in the Hepatogastroenterology Unit of the University Hospital of Ioannina with their written consent. For the children, endoscopy was performed if *H. pylori* infection was detected with non-invasive methods and severe symptoms of infection of the digestive system coexisted.

Endoscopy in children was performed by a pediatric and an adult gastroenterologist in the Hepatogastroenterology Unit or in the operating room when sedation was needed in young children. The parent's consent was always preceding of tests. Two samples were taken dur-

ing endoscopy from the antrum and one from the body of the stomach. The samples were sent to the Pathology Department where Giemsa staining to determine the presence of *H. pylori* infection was performed. CLO test was also used for the rapid detection of the activity of urease of *H. pylori* in gastric biopsy by the color change 24 hours after immersion of the sample to determine its positivity to infection. Samples were taken for blood tests with the consent of the patient (complete blood count, ESR, CRP, biochemical tests: Fe, Ferritin, B12, folic acid) to estimate the iron deficiency.

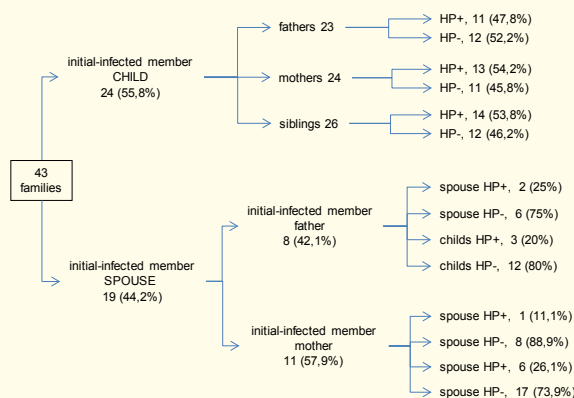
## Statistical Analysis

The mean and standard deviation were used for the analysis of data of continuous variables. The two independent samples t-test was used for the differences between interest groups after the Shapiro Wilk test for the normality of the samples values. For categorical variables numbers and percentages were used while X-squared test was used for the correlations between them and alternatively the Fisher's exact test, depending on the completeness of the assumptions. The sensitivity and specificity of diagnostic tests evaluated by ROC curve analysis. In all cases the level of significance was set equal to 0.05. Results were generated using the Statistical Package for the Social Sciences SPSS v21.0 statistical software.

## Results

Forty-three families were examined that included 171 people (83 parents and 88 children). Overall, 92/171 (53.8%) subjects were found *H. pylori* infected. Of these 46/88 (52.3%) were children, 26/43 (60, 5%) mothers and 20/40 (50%) fathers.

In 24 of the 43 families (55.8%) the initially-infected member was a child while in 19 (44.2%) was a parent, in 8 (42, 1%) cases the father and in 11 (57.9%) the mother. When the initially-infected member was a parent, the children and the other parent of the family were less likely to be positive while in families with initially infected member a child higher probability of infection in other family members was found. A detailed relationship of the presence of infection between the initially-infected member and the other members of their families is seen in (Figure 1).



**Figure 1:** The relationship of the presence of infection between the initially-infected member and the other members of their families.

The clinical symptomatology mentioned in the medical history of the 46 positive to the *H. pylori* infection children included abdominal pain in 24 (52.2%), belching in 6 (13%), nausea and vomiting in 6 (13%), anorexia and weight loss in 5 (10.9%), retrosternal pain-burning in 5 (10.9%), dyspepsia in 4 (8.7%) cases, and 2 cases of halitosis.

Table 1 shows the children's infection in relation with the infection of parents. The probability of a child to be infected is higher when one of the parents, irrespectively if the mother or father, is infected. It seems that this probability increases further if both parents are infected.

	Families (%)	Children's Disease			
		number	positive (%)	negative (%)	p
Total	43 (100%)				
Families with 2 parents involved	40 (93%)	84	44 (52,4%)	40 (47,6%)	
Healthy parents	6 (15%)	10	6	4	
One parent infected	24 (60%)	51	24 (47,1%)	27 (52,9%)	
(infected mother)	14 (58%)	29	12 (41,4%)	17 (58,6%)	
(infected father)	10 (41,7%)	22	12 (54,5%)	10(45,5%)	
Both parents infected	10 (25%)	23	14 (60,9%)	9 (39,1%)	0,4125
families with one parent involved					
healthy mothers/ fathers' unknown	1 (2,3%)	1	1		
infected mothers/ fathers' unknown	2 (4,7%)	3	1	2	

**Table 1:** The children's infection in relation with the infection of parents.

One hundred of the 171 subjects (58.5%) lived in rural areas while the remaining 71 (41.5%) lived in urban areas. Frequent contact with animals and presence of domestic pets in the house has been reported in 73/171 (43%) cases and no pets in the remaining 97 (57%) (no information was provided for one case). Concerning the education of parents, 63 (75%) were graduates of primary and secondary education while 20 (25%) were university graduates. We found that the educational level plays significant role ( $p < 0.015$ ) in the transmission of infection. Forty-four out of the 98 infected subjects belonged to a family with primary or secondary education while 6/29 (20.7%) belonged to a family with a university education. The effect of residence in an urban or rural area is marginally significant ( $p < 0.061$ ). Apart from the initially-infected subjects, transmission to members of the same family took place in 35/75 (46.7%) subjects of families living in an urban environment. Families in which transmission was observed had an average number of 4.5 members while those with no transmission had fewer members ( $p < 0.013$ ). We have also found a statistically significant difference in age ( $p < 0.001$ ) with infected adults (31/72) having an age of  $32,96 \pm 14,53$  years, while the non-infected ones an age of  $21,13 \pm 14,68$  years.

The body-mass index (BMI) of adult participants in the study was within the normal range in the majority of the cases, 40 (49.4%), with second the overweight (35.4%) and only few obese 11 (12.7%) or underweight 2 (2.5%). The BMI for children up to 16 years, according to their ranking in the growth charts, was normal in 53 (64.6%), overweight in 13 (15.9%), and obese in 10 (12.2%) cases, while only 6 children (7.3%) were underweight. In the present study, transmissibility seems not to be affected neither by the BMI nor by the age (continuous and categorical scale).

Blood tests in adults showed that the difference in the hematocrit is statistically significant ( $p < 0.036$ ),  $39,83 \pm 3,27$  % for infected subjects compared to  $41,7 \pm 3,3327$ mg/dl in non-infected ones. The difference in hemoglobin was also found statistically significant ( $p < 0.010$ ) with the infected subjects (27/58) to have lower hemoglobin,  $13,34 \pm 1,32$  g/dl, than the non-infected ones,  $14,22 \pm 1,2$  g/dl. The serum iron ( $p < 0.025$ ) is lower in the infected subjects (21/50),  $66 \pm 40$   $\mu$ g/dl, compared to non-infected ones,  $89,66 \pm 32,17$   $\mu$ g/dl. Folic acid with  $p < 0.040$  was lower in infected subjects (18/41) with a value of  $7,62 \pm 2,62$  ng/ml compared to  $11,26 \pm 7,53$  ng/ml in non-infected ones. Interestingly, serum albumin with  $p < 0.044$  appears to be reduced in infected subjects (11/24), with a value of  $4,23 \pm 0,27$  g/dl, compared to  $4,47 \pm 0,27$  g/dl in non-infected ones [16].

The blood tests in children showed statistically significant differences in the following: hematocrit ( $p < 0.004$ ) with the infected subjects (41/73) to have  $38,75 \pm 2,97$  % while the non-infected ones had  $40,90 \pm 3,11$  %, in hemoglobin ( $p < 0.002$ ) with infected subjects (41/73) to have  $13,15 \pm 1,08$  g/dl while the non-infected ones had  $13,98 \pm 1,12$  g/dl, in iron ( $p < 0.019$ ) with the infected subjects (35/63) to have  $74,11 \pm 35,87$   $\mu$ g/dl while the non-infected ones had  $95,36 \pm 33,13$   $\mu$ g/dl.

Gastroscopy was performed in 28 adults and 22 children of the 92 infected subjects with their written consent. The endoscopic observations showed that erythema was diffused in 13/28 (46.4%), gastritis in 9/28 (32.1%), duodenal ulcer in 3/28 (10.7%), mucosal nodularity in 2/28 (7.1%) cases, and only in one case the mucosa was found normal. In children, mucosal nodularity was found in 13/22 (59.1%), diffuse gastritis in 6/22 (27.3%), erythema in 4/22 (18.2%) and ulcer in 3/22 (13, 6%) cases.

Active gastritis was found in 19/25 (76%) of adult biopsies, gland atrophy in 9/25 (36%) and inactive chronic gastritis in 7 (28%) cases. Abundant *H. pylori* were detected by Giemsa staining in 24 (96%) cases. Active gastritis was found in biopsies of 13/21 (61.9%) children, chronic inactive gastritis in 4/21 (19%) and mild gland atrophy in 2/21 (9, 5%). In one case each were observed: a sizable follicular formation (11.5 years), a slight increase of lymphocyte infiltrate (7.2 years), rare lymphocytes to infiltrate the glandular epithelium (11.5 years) and lymphocyte aggregates on mucosal layer (5 years). Abundant *H. pylori* were detected by Giemsa staining in 20 (95.2%) cases.

## Discussion

The study design allowed us to examine the transmission of *H. pylori* infection within families that had already one *H. pylori* infected member, adult or child. The results of the present study showed that when both parents are infected with *H. pylori* the probability of children to be infected is higher ( $p < 0,41$ ), as it was also shown in a study of intrafamilial transmission with multiple generations living in the same household [17]. In our study, there were two cases of school-age children 5 and 7.5 years old, without siblings and with parents negative in infection, who were infected. This indicates that they were perhaps infected through other transmission routes, e.g., drinking water, oral-oral, e.g., kissing, saliva, or from other environmental sources [9, 18]. In another study it was found that in a family with 4 children, one *H. pylori* infected child had *H. pylori* isolates that were not related to those of the other five family members [19]. Raymond J ., *et al.* compared the sequences of two housekeeping genes, *hspA* and *glmM*, in samples from family members and detected mixed infection with two to five strains circulated within a family [20]. The increased rate of infection in children under 6 years of age when the mother is infected can be explained by their close contact [21, 22]. However no statistically significant association was found in the present study between maternal infection and children positivity to *H. pylori* infection, 24/51 (47.1%), in the same family (Table 1), as in families where the child was initially infected, despite the fact that the mother was positive to *H. pylori* infection (13/24 (54.2%) versus 11/23 (47.8%) of the fathers).

The transmissibility of the infection seems to be influenced by the number of family members ( $p < 0.013$ ) as mentioned above in a continuous and categorical scale [23]. Families living in rural areas are more affected by the infection ( $p < 0.061$ ) than families living in urban areas. Their eating habits that include fresh food, such as vegetables and dairy products or their contact with nature and animals may increase the probability of infection since *H. pylori* antibodies were found in several domestic animals such as cows, sheep, pigs, dogs as well as in milk [24-27]. It is known that the incidence of infection is influenced by the socioeconomic status of families [21-28]. The prevalence of the *H. pylori* infection in children with well-educated parents is lower compared to this of children whose parents have not received higher education [29]. Similarly, we have found in our study that the level of education of parents affects the transmissibility in the family. Families with parents of higher education had a statistically lower probability of being infected compared to families with parents of lower education ( $p < 0.015$ ).

The body-mass-index (BMI), weight and height of infected subjects compared to non-infected ones doesn't seem to have any significant difference. The probability of infection increases significantly with age ( $p < 0.001$ ) [30].

Literature studies refer to an association of low hematocrit and iron deficiency with the *H. pylori* infection, in both, adults and children, as it is also found in our study. Studies have shown that increased gastric concentrations of IL-1 or TNF- $\alpha$ , can inhibit the secretion of gastric acid that is required for the absorption of iron leading to iron-deficiency anemia [31]. Also, in *H. pylori* infected patients with hypochlorhydria, a reduction of iron in serum has been observed [32-34].

Differences in iron, hemoglobin and hematocrit in adults and children were found in our study in accordance with previous studies that showed an association of iron deficiency and *H. pylori* infection.

According to literature, based on endoscopies of adults as well as of children, *H. pylori* colonizes and causes lesions on the mucous membrane of the stomach [35]. Similarly, it was also found in our study that diffuse erythema, mucosal nodularity, gastritis and ulcers are histologically associated with the detection of *H. pylori* in Giemsa staining [36,37].

## Conclusions

In our study, which refers to one of the poorest regions of Europe, intrafamilial transmission seems to be affected by the level of education of parents as well as the number of family members. Another factor that affects transmission is the place of residence. Infected individuals seem to suffer from iron deficiency. Children are more likely to be infected when both parents are *H. pylori* positive. It seems that infected adults have reduced serum albumin levels, but this observation needs further investigation.

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