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Intrafamilial transmission of *Helicobacter pylori*: genotyping of faecal samples

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ABSTRACT

Background: After more than 20 years of research, there is a little information about the detailed routes of *Helicobacter pylori* transmission. The aim of this study was to explore intrafamilial transmission of *H. pylori* in children who had indication for upper gastrointestinal endoscopy and their parents.

Methods: Children (aged up to 15 years) were studied during September 2012 to October 2013. The parents of those with positive urea breath test results were asked to provide faecal and blood samples after informed consent. Non-invasive tests such as immunoassay for serological antibodies against *H. pylori* and detection of its antigen in faeces were measured. The genetic similarity of the family strains was investigated by the random amplification of polymorphic DNA (RAPD-PCR) genotyping method.

Results: According to the genotyping results of 30 families, in 10 (33.3%) children related *H. pylori* genotypes to their mothers were found, while only 2 children (6.7%) had similar genotypes to their fathers. Interestingly, children with similar *H. pylori* genotype with their mothers had higher IgA (35.7 ± 10.8) and IgM antibody titres (87.23 ± 19.15) than other children. In addition, in these children, lower titres of IgG antibodies (9.93 ± 3.31) were found rather than children who had no *H. pylori* in their faeces or had no similarities with their parents (30.28 ± 6.15).

Conclusions: In conclusion, mother-to-child transmission is the main route of intrafamilial transmission of *H. pylori* in Iranian families. Molecular typing of *H. pylori* can be useful in identifying a high-risk population.

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Helicobacter pylori; intrafamilial transmission; genotyping

Introduction

In Iran, *H. pylori* infection exists in approximately 90% of adult population [1], and more than 50% of children were infected before the age of 15.[2] After more than 20 years of research, there is a little information about the detailed routes of *H. pylori* transmission.[3] However, the existence of *H. pylori* in saliva, dental plaques and faeces is compatible with both oral–oral and faecal–oral transmissions.[4] On the other hand, person-to-person transmission particularly intrafamilial has been considered as another mode of transmission among individuals.[5,6]. Having an infected mother has been suggested to be a more remarkable risk factor than an infected father,[11] while it is unlikely that the *H. pylori* is introduced into the family by other children.[7]

The array of tests that can be used for diagnosis of *H. pylori* infection is large, and they are usually classified as invasive and non-invasive.[8,9] In epidemiological studies, it appears that non-invasive techniques are probably the best option.[10] Genotyping *H. pylori* DNA obtained from faecal samples is considered as non-invasive method for epidemiological studies and can be used to determine the transmission routes of infection.[11] Rapid amplification of polymorphic DNA (RAPD) has been used for epidemiological studies for the examination of the infection route.[12] Supporting the hypothesis of intrafamilial transmission, several studies have used RAPD fingerprinting method to investigate the transmission of *H. pylori* in gastric biopsy [12–14] or in gastric aspirates [15,16] and faeces.[11]

The aim of this study was to explore intrafamilial transmission of *H. pylori* in children who had indication for upper gastrointestinal endoscopy and their parents using RAPD fingerprinting method.

Material and methods

This study was conducted in the Children Medical Center (Teaching Hospitals affiliated to Tehran University of

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Medical Sciences), Tehran, Iran, during September 2012 to October 2013.

Children (aged up to 15 years) attending CMC Hospital for upper gastrointestinal endoscopy of suspected *H. pylori* infection were studied. Then, the parents of those with positive urea breath test (UBT) results [17] were recruited for this study and were asked to provide faecal and blood samples after giving informed consent. This study was approved by the Ethics Committee of Tehran University of Medical Sciences.

Stool antigen test

An enzyme immunoassay kit (ASTRA, Italy) was performed in order to detect the presence or absence of *H. pylori* antigen in the stool according to the manufacturer's instructions. Briefly, after addition of diluted faecal samples and a peroxidase conjugated polyclonal antibody to the wells and incubation for one hour at 24 °C, the wells were washed to remove any unbound material. The procedure was followed by the addition of a substrate before a further 10 min of incubation. Then, a stop solution was added, and finally absorbance was measured by spectrophotometer at 450 nm.[18]

DNA extraction

For DNA analysis, DNA was extracted from stool samples. [19]

Gene capture was performed using an oligonucleotide *H. pylori*-specific biotinylated capture probe capC [5-GGG GAG TAC GGT CGC AAG ATT AAA ACT CAA AGG AAT A-3]) which targeted the 16S rRNA gene of *H. pylori* (Metabion, Munich, Germany).[20] Mixture of 300 µl of 6 M guanidine thiocyanate (Sigma-Aldrich) and 20 nmol of the capture probe were added to total faecal DNA suspensions and incubated overnight at 25 °C. *H. pylori* DNA sequences were harvested using 10 µl of paramagnetic polystyrene beads coated with streptavidin (Biolab Inc. New England) to allow biotin–streptavidin interaction and washed three times in wash buffer (0.1 M Tris-HCl, 0.01 M EDTA, 1 M sodium chloride, 0.1% [vol/vol] Tween 20; Sigma-Aldrich). *H. pylori* DNA was separated from the faecal flora by electromagnetic separation.[19] Final harvesting was performed at 85 °C (for 6 min). *H. pylori* DNA was transferred to a clean tube and stored at –20 °C. [11,19]

RAPD fingerprinting method

After DNA extraction from stool samples, RAPD-PCR was performed in a final volume of 25 µl containing 40 ng template DNA, 20 pmol primer, 5 mM MgCl2, 1U of Long PCR Taq DNA polymerase (Yekta Tajhiz, Iran), dNTP 250 µM each of dATP, dTTP, dGTP and dCTP and 1 × buffer. A single arbitrary primed PCR primer (1254) with sequence [5-CCGCAGCCAA-3] [10] was used to amplify isolated DNA.[11] Amplification of gene fragments was performed under the following conditions: 94 °C for 4 min, 94 °C for 30 s, 36 °C for 30 s 72 °C for 3 min repeated 30 times, followed by a final incubation at 72 °C for 10 min. Then, the PCR product were electrophoresed in 2% agarose gels.[6]

RAPD analysis

Comparison of RAPD-PCR banding patterns was performed using Gelcompar II software, version 6.5 (Applied Maths, Sint-Matens-latem, Belgium). Each gel photograph was inverted as TIFF images and then normalised using the reference marker. Similarity analysis of results was calculated using the Dice coefficient/unweighted pair-group method with arithmetic mean (UPGMA).

For the analysis of RAPD-PCR, only the reproducible bands detected by UV fluorescence were included in the software. RAPD patterns of isolates that differ in one or more of the main bands were considered different. The criteria for related clones were taken as profiles with 70% or more similar bands. Isolates with 100% similarity were considered as the same RAPD-PCR type.

Serology

Serum titres of IgG, IgM and IgA antibodies for *H. pylori* were measured using a commercial enzyme immunoassay (EIA) kit (Monobind Inc., USA). In short, diluted serum samples were added to the coated wells with biotinylated conjugate solution followed by incubation before addition of a peroxidase-bound secondary immunoglobulin, incubation, and lastly addition of a substrate showing *H. pylori* status.[8] Presence of *H. pylori* antibodies was confirmed by values of >20 U/ml for IgG and IgA and 40 U/ml for IgM.

Statistical analysis

Data were analysed using SPSS version 16.0 (SPSS Inc. Wacker Drive, Chicago, USA). Comparisons of categorical variables were undertaken by the application of chi-square test or Fisher's exact test. Student t-tests for independent samples were used for comparisons of quantitative variables, and t-test for paired samples was used to compare the differences between groups. A p value < 0.05 was taken to be significant.

Results

In this study, 90 children with the symptoms of digestive diseases who had indications of endoscopy were enrolled. Among these patients, 60 children had negative UBT and were excluded from the study. Blood and faecal samples of these 30 children with their parents

Table 1. Antibody titres in infected children with *H. pylori* (*N* = 30) and their parents.

		Serum IgG	Serum IgM	Serum IgA
	Age (mean ± SD)	(median ± IQR)		
Children	9.6 ± 3.6	25.8 ± 24	78.8 ± 63.2	23.9 ± 23.3
Younger than 7 years	6.16 ± 3.7	23.3 (CI 95%: 3.4–43.2)	58.9 (CI 95%: 27.8–90)	26.4 (CI 95%: 5.8–47)
Older than 7 years	12.57 ± 3.6	20.6 (CI 95%: 11.9- 29.3)	84.6 (CI 95%: 40.8–128.8)	27.7 (CI 95%: 14.4–41)
Mothers	35 ± 5.3	88.7 ± 86.2	45 ± 31	34 ± 27.7
Fathers	41.7 ± 6	80 ± 74.2	52.9 ± 50.6	27.2 ± 16.8

SD = Standard Deviation, IQR = inter-quartile range, CI = confidence interval.

Table 2. Results of stool antigen test and serology of infected children with *H. pylori* (*N* = 30) and their parents.

	Stool antigen test		Serum IgG		Serum IgM		Serum IgA		Total
	*N**	%	*N**	%	*N**	%	*N**	%	*N*
Children	20	67	16	53	18	60	10	33	30
Mothers	20	67	19	63	19	63	19	63	30
Fathers	24	80	19	63	11	37	19	63	30

*The number of positive cases according to cut-off of the kits.

Table 3. The genotyping results of 30 families and similarities between children and their parents.

Group	*N*	%
No relation (similarity <70%)	14	46.7
Relation between mother and child (similarity≥70%)	10	33.3
Relation between father and child (similarity≥70%)	2	6.7
Had no *H. pylori* in stool	4	13.3
Total	30	100

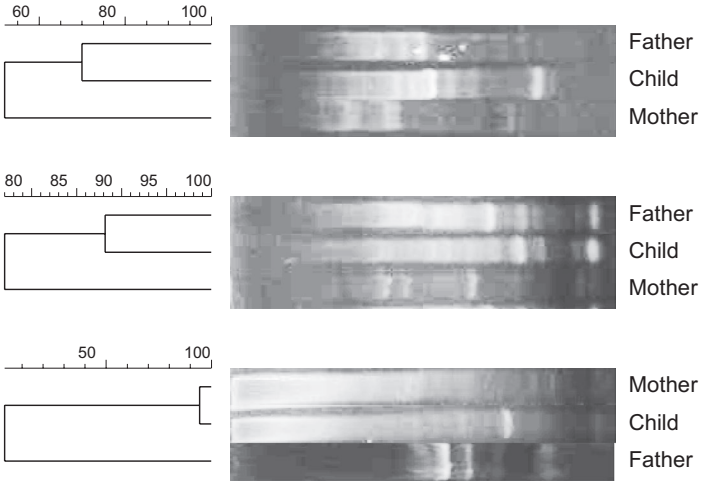

Figure 1. RAPD fingerprinting patterns of *H. pylori* obtained from the children and family members (3 families were demonstrated). The PCR products were separated with 1.5% agarose electrophoresis. Similarity analysis of results was calculated using the Dice coefficient/unweighted pair-group method with arithmetic mean (UPGMA).

were measured by ELISA test and the stool antigen test. Demographic data and serology results of 30 children with positive *H. pylori* and their parents are indicated in Table 1. Among 30 children, 12 (40%) of them were girls and 18 (60%) were boys. Thirteen children (43%) were younger than 7 years, while 17 (57%) were older. According to gastric endoscopy results, 20 children (67%) had mild nodularity, whereas in others severe nodularity was shown.

The results of stool antigen test and serology of infected children with *H. pylori* (*N* = 30) and their parents are shown in Table 2.

Among 30 children, 20 (67%) had positive stool antigen test. In this study, 33.3% of girls (10 patients) and 55.6% of boys (20 patients) with *H. pylori* infection had positive stool antigen. Antibody titres of IgM in children older than 7 years were more in children younger than 7 years (Table 1). The positivity of both serum IgM and stool antigen test in children was 44.4% (*n* = 8), and negative results in both tests were seen in 6 children (50%) (*p* = 0.7). The positivity of IgG and stool antigen test were 62.5% (*n* = 10), and in 71.4% (*n* = 10) of children, these two tests were negative (*p* = 0.63). In addition, in 20% (*n* = 2) and 40% of the patients (*n* = 8), both IgA and stool

Table 4. Summary.

What is known about this subject:
There is limited evidence of intrafamilial transmission of *H. pylori* infection because past epidemiologic studies have been based mainly on results of serology, urea breath test or *H. pylori* stool antigen.
RAPD fingerprinting provides easily producing typable data based on familial groupings over a short time span.
What this article adds:
Mother-to-child transmission is the main route of intrafamilial transmission of H. pylori in Iranian families.
Majority of children younger than 7 years (60%) had similar genotypes to their parents, particularly with their mothers.
Children with similar *H. pylori* genotype with their mothers had higher IgA and IgM antibody titres than other children.

antigen tests were positive and negative, respectively ($p = 0.58$). Serum antibody levels in children with positive and negative stool antigen tests were different. The level of serum IgA (mean = 36.57, CI 95%: 19.54–53.60) and IgM antibody (mean = 84.93, CI 95%: 39.7–130.1)] in children with negative was more than the children with positive stool antigen test [(mean IgA = 14.04, CL95%: 10.89–17.19 and mean IgM = 58.82, CI 95%: 32.06–85.58)]. The serum IgG antibody levels in children with negative stool antigen test were lower (mean = 12.73, CI95%: 6.41–19.06) than the children with positive stool antigen test (mean = 34.39, CI95%: 16.46–52.32); while in children with negative stool antigen test, IgA antibody titres were more (mean = 75.86, CI 95%: 38.58–113.13) than those with positive stool antigen test (mean = 50.04, CI 95%: 31.56–68.52).

RAPD fingerprint patterns of genomic DNAs from *H. pylori* isolates from infected children and their parents were obtained. The genotyping results of 30 families (children with their parents) and similarity between children and their parents are shown in Table 3. According to RAPD-PCR analysis (Figure 1), 14 children (46.7%) had no relation with their parents, while 10 (33.3%) of them were related to their mothers and only 2 (6.7%) were similar to their fathers.

Children with similar *H. pylori* genotyping with their parents were younger (mean = 7.5 years, 95% CI: 5.17–9.82) than the other infected children (mean = 11.7 years, 95% CI:9.64–13.78, $p < 0.05$). Interestingly, children with similar *H. pylori* genotype with their mothers had higher IgA (35.7 ± 10.8) and IgM antibody titres (87.23 ± 19.15, $p < 0.05$) than other children. In addition, in these children, lower titres of IgG antibodies to *H. pylori* (9.93 ± 3.31) were found rather than the children who had no *H. pylori* in their faeces or had no similarities with their parents (mean (30.28 ± 6.15, $p < 0.05$) (see Table 4).

Discussion

There is limited evidence of intrafamilial transmission of *H. pylori* infection because past epidemiologic studies have been based mainly on results of serology, UBT, or *H. pylori* stool antigen.[16] RAPD fingerprinting provides easily producing typable data based on familial groupings over a short time span.[11] In the current study, molecular typing of *H. pylori* strains among families with an infected child demonstrated that infected parents particularly mothers may play a crucial role in transmission of *H. pylori* to the child.

Several studies have identified the same or similar strains of *H. pylori* among family members using the DNA typing technique,[11,12,14–16] This study similar to other reports [21,22] supports a person–person mode of transmission and raises the possibility of the oral–oral route, especially from mother to child.

The acquisition of *H. pylori* infection occurs by close contact with infected individuals in early childhood, especially via contact with infected mothers, common use of spoons, the sharing of the teats of feeding bottles, and even the masticating or testing of the child's food. [15,23] According to our study, in mothers who had high similarity (over 70%) with their children had higher titres of IgA than the others. This point suggests that wrong methods of feeding to children might be a possible mode of a mother-to-child *H. pylori* transmission in our country. However, lower educational level of the mother as well as socioeconomic situation and frequent diarrhoea might increase the transmission of *H. pylori*.[14]

The relationship of infection between parents and children has been examined in several studies, and in most such studies like ours, infected mother had more vital role than infected father in the transmission of the bacteria.[5,16,24–28] Of course, evidence on intrafamilial spread has been limited because the bases of previous epidemiologic studies have been mainly focused on results of serology, UBT or stool antigen test.[16]

In a study on 11 families in Greece,[29] finding of the same genome of *H. pylori* in families strengthened the hypothesis of transmission from person-to-person in the family (97% with mothers and 93% with their fathers, compared with 78% of siblings).

In a 5-year study on 44 mothers and infants up to the age of 5 years, according to RAPD-PCR, the frequency of mother-to-child transmission of *H. pylori* infection had been reported in 11%, and 5 children had similar *H. pylori* DNA to their mothers.[15] In our study, majority of children younger than 7 years (60%) had similar genotypes to their parents, particularly with their mothers.

In Osaki et al. study, mother-to-child transmission of *H. pylori* was demonstrated in four of five families, whilst transmission from father to child was seen in two families.[30] Fialho et al. did not find any association between *H. pylori* status of the fathers and infection in their children. The probable explanation of this fact

might be due the fact that, in our country, like in many other countries, the mother is the primary caretaker of children.[31] Therefore, intense relationship with the mother highlights the role of household transmission as a main route of transmission of infection.

In this study, individuals with positive stool antigen test had lower IgM and IgA antibody titres, while high titre of these two antibodies was found in those with negative faecal antigen test. In addition, high titre of IgG was found in individuals with positive stool antigen test due to bacterial shedding into stool. It is in agreement with She et al. study which found better correlation between HpSA and IgG than IgA or IgM.[32]

In conclusion, our data provide further evidence that mother-to-child transmission is the main route of intrafamilial transmission of *H. pylori* in Iranian families. Molecular typing of *H. pylori* can help to control the spread of the infection. In addition, it can be useful in identifying a high-risk population in order to reduce the cost and risk of its complications.

This work represents an advance in biomedical science because molecular typing of *H. pylori* strains among families with an infected child demonstrated that infected parents particularly mothers may play a crucial role in transmission of *H. pylori* to the child.

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