Helicobacter pylori in familial clusters based on antibody profile

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Abstract

Studies have shown a high prevalence of Helicobacter pylori infection in close communities and that intrafamilial spread during early childhood may be a route of transmission. A total of 72 household members from 21 families were enrolled in this study. Sera from individuals showed 50/72 (69.4%) seropositive for IgG against H. pylori by ELISA. Western blots showed diversity in the protein profiles with molecular masses ranging from ~8 to 130 kDa. Cohen’s x statistical analysis of the blot patterns showed that nine families demonstrated similar profiles (100%), while 4 other families showed varying similarities (17–50%). The results support the hypothesis of intrafamilial transmission of H. pylori. Furthermore, serological studies can be used as an effective approach to determine the familial status in relation to H. pylori infection. © 2001 Federation of European Microbiological Societies. Published by Elsevier Science B.V. All rights reserved.

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1. Introduction

Helicobacter pylori, a Gram-negative spiral bacterium, has been identified as a pathogen, infecting approximately half the world’s population. It is implicated as the main etiological factor for the pathogenesis of peptic ulcer and type B gastritis [1]. H. pylori has not only been shown to play an active role in gastroduodenal pathology in the adult population but infection has been reported to be acquired during childhood [2,3]. Since the first isolation of H. pylori in 1983 [4], the mode of transmission has remained a mystery. However, studies have shown that the acquisition of H. pylori infection was linked to socioeconomic background and the density of living conditions [5,6]. These data support the hypothesis of person-to-person transmission. Studies suggesting person-to-person transmission are mainly evidence from the clustering of H. pylori infection in families [7,8]. On the basis of molecular typing, the study by Georgopoulos et al. [9] demonstrated similar rRNA gene patterns in eight out of 18 cohabiting married couples studied. Reports have also shown transfer from parent to child [10] and among siblings [11].

The aim of this study is to determine the possibility of intrafamilial transfer of H. pylori by enzyme-linked immunosorbent assay (ELISA) and Western blotting.

2. Subjects and methods

Twenty-one families from a cohort of 2626 healthy subjects who participated in a national serological survey conducted by the Ministry of the Environment, Singapore, formed the basis for this study. There were 72 household members aged 1–73 years. Of these 21 families, 15 were Chinese, three Malay, two Indian, and one Caucasian. Six families had three-tier relationships, 13 were of two tiers (parents and children) and the remaining two families were made up of siblings. These 21 families were selected for the present study because at least one of the family members residing in the same household was seropositive by ELISA, except for two families, 18 and 21 (Table 1). Members in these two families were seronegative and they were regarded as negative controls for the Western blotting.

2.1. H. pylori antibody determination

Serum samples were collected with consent from the 72 subjects and IgG antibodies against H. pylori were determined using an in-house ELISA with acid glycine-ex-
tracted antigen prepared using an isolate from a local patient with healed duodenal ulcer [12]. Briefly, flat-bottomed microtiter plates (Nunc) were coated with the acid glycine extract of *H. pylori*. Test serum was diluted 1:100 and tested in triplicate. Peroxidase-labelled rabbit anti-human IgG (Dako) was used as the conjugate and the substrate was 0.04% *O*-phenylenediamine dihydrochloride (Sigma). The enzymatic reaction was stopped by the addition of 2.5 M sulfuric acid. OD was read at 490 nm with an ELISA reader (Ceres 900 BioTek). The cut-off value for ELISA was based on a pre-evaluated comparative study of patients with culture-positive *H. pylori* [12].

### 2.2. Western blotting

Western blotting was carried out to assess the protein profiles for each household member. SDS-PAGE was performed according to the method of Laemmli [13]. Proteins were separated in a 10% separating gel and 6% stacking gel. The electrophoretically resolved proteins were electroblotted onto a 0.45-μm Immobilon P membrane (Millipore). The non-specific sites were blocked with phosphate-buffered saline (PBS) containing 0.05% Tween 20 and 5% skimmed milk. After blocking, the membrane was treated with 1:100 diluted individual serum. The membrane was incubated overnight at room temperature under gentle agitation and washed with PBS-Tween 20. After washing, the membrane was further incubated with horseradish peroxidase-labelled rabbit anti-human IgG (1:800) (Dako) for 1 h at room temperature. It was then washed with PBS-Tween 20 before the membrane was placed in 4-chloro-1-naphthol (Sigma). The reaction was stopped by washing with distilled water.

### 2.3. Immunoblot analysis

A GS 700 densitometer (Bio-Rad) was used to determine the molecular mass of each band. The blots were scanned with GS 700 and the image profiles were analyzed using the Quantity one program (Bio-Rad).

### 2.4. Statistical analyses

Cohen’s κ statistical analysis was used to assess agreement among the seropositive members in each household. According to Fleiss [14], values greater than 40% are indicative of moderate levels of agreement above chance. Values exceeding 75% suggest strong agreement above chance.

The protein weight of each band was calibrated and the

<table>
<thead>
<tr>
<th>Family</th>
<th>Number of family members</th>
<th>Seropositive members</th>
<th>Comparisons with agreement (%)</th>
<th>Family relationship</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<tr>
<td>1</td>
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</tr>
<tr>
<td>2</td>
<td>5</td>
<td>4</td>
<td>1/6 (17%)</td>
<td>Mother and son</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3/3 (100%)</td>
<td>Father and sons; siblings</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>2</td>
<td>1/1 (100%)</td>
<td>Siblings</td>
</tr>
<tr>
<td>5</td>
<td>3</td>
<td>2</td>
<td>1/1 (100%)</td>
<td>Mother and son</td>
</tr>
<tr>
<td>6</td>
<td>3</td>
<td>2</td>
<td>0/1 (0%)</td>
<td>Grandmother and grandsons; mother and daughter; mother and sons; siblings</td>
</tr>
<tr>
<td>7</td>
<td>4</td>
<td>4</td>
<td>6/6 (100%)</td>
<td>–</td>
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<td>8</td>
<td>3</td>
<td>2</td>
<td>0/1 (0%)</td>
<td>–</td>
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<tr>
<td>9</td>
<td>5</td>
<td>4</td>
<td>3/6 (50%)</td>
<td>Father and son; husband and wife; siblings</td>
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<td>2</td>
<td>1/1 (100%)</td>
<td>Husband and wife</td>
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<td>4</td>
<td>2</td>
<td>1/1 (100%)</td>
<td>Mother and daughter</td>
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<tr>
<td>12</td>
<td>3</td>
<td>1</td>
<td>–</td>
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<td>0/1 (0%)</td>
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<td>15</td>
<td>4</td>
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<td>1/3 (33%)</td>
<td>Husband and wife</td>
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<td>3/10 (30%)</td>
<td>Father and daughter; father and son; grandfather and granddaughter</td>
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<tr>
<td>17</td>
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<td>3/3 (100%)</td>
<td>Mother and sons; siblings</td>
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<td>0</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
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<td>1/1 (100%)</td>
<td>Father and son</td>
</tr>
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<td>2</td>
<td>1/1 (100%)</td>
<td>Mother and son</td>
</tr>
<tr>
<td>21</td>
<td>2</td>
<td>0</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Total</td>
<td>72</td>
<td>50</td>
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</tr>
</tbody>
</table>

The table shows comparison of immunoblots between household members. There were no comparisons for families 12, 18, and 21. Similar antibody profiles were observed in nine families.

\[
\text{% Similarity} = \frac{\text{No. of comparisons with } \geq 40\% \text{ similarity}}{\text{No. of comparisons between family members}} \times 100
\]
degree of agreement was determined statistically by the statistical program SPSS 10. A κ value of ≥ 40% was taken to indicate similarity between the compared immunobLOTS.

3. Results

The distribution of sero-status of the 72 subjects by ELISA shows that 50/72 (69.4%) were seropositive (Table 1). Seropositivity was more prevalent in the older family members of a household. It was observed that of the 19 families with at least one seropositive member, one or more of the younger members in the same family were found to be seropositive. However, this pattern was not seen in the two remaining families, 18 and 21. In family 6 (Table 1), the child was seronegative even though both parents were seropositive. ELISA results for family 10 showed that although both grandparents were seropositive, the grandchildren were negative (Table 1).

Western blotting shows the diversity of protein profiles with molecular masses ranging from ~8 to 130 kDa (Fig. 1). The older members in a family exhibited more reactive bands as compared to those younger members residing in the same household. Analysis of the immunoblot profiles showed a high molecular mass protein of 120–130 kDa observed in 49/50 seropositive samples (Fig. 1). As observed from the blots, medium-molecular-mass proteins (~40–60 kDa) were common to both seronegative and seropositive samples.

Comparisons of immunobLOTS between household members residing in the same family are illustrated in Table 1. The agreement between antibody profiles of seropositive samples in the same household was assessed statistically using Cohen’s κ analysis. A total of 50 blot comparisons were carried out for 18 families. There were no comparisons for the remaining three families, no seropositive member was observed in two of the families (18 and 21) and only one seropositive member in the other family (family 12). Table 1 shows that five families exhibited no similarity (0%), four families showed varying patterns ranging from 17 to 50%, while nine families demonstrated similar profiles (100%). Of those blots that were compared and analyzed, 15 from the various households showed similarities in blot patterns between parent and children, five between siblings, three between grandparents and grandchildren, and three between spouses.

4. Discussion

The current study shows that seropositivity increases with age. This result is consistent with data reported earlier in a larger local population study [15]. As at least one member of the family was seropositive, a possible reason is that the children were living with parents and/or grandparents who were seropositive. This further suggests the possibility of vertical transmission.

The high-molecular-mass protein, ~120–130 kDa, which was present in 98% of the seropositive samples is probably the CagA protein. The cagA gene has been reported to be present in 90% of the population studied by Zheng et al. [16] but it has no association with peptic ulcer disease when compared with patients with Non-ulcer dyspepsia (NUD) in our population. As H. pylori was present in the population (peptic ulcer and NUD) studied by Zheng et al. [16], it further strengthens the usefulness of this protein. As shown in this study, it does not exclude the importance of this high-molecular-mass protein as a reliable marker for H. pylori infection/colonization. As shown in Fig. 1, the medium-molecular-mass proteins (40–60 kDa) were present in both seropositive and seronegative samples, including seronegative family 18. These medium-size proteins, which parallel subunits of urease, heat shock proteins, and flagellins, were reported to be responsible for cross-activity with antigens of other bacterial species [17,18]. Thus, these groups of proteins are not useful in discriminating for H. pylori infection.

Numerous data have been collated to show familial clusterings of H. pylori infection [8–11,19]. The same strains of H. pylori have also been reported to be identified among families based on molecular techniques [9,20]. In a recent study by Dominici et al. [21] on the general population, they reported that children with both parents seropositive had double the risk of being infected with H. pylori than those from families in which both parents were seronegative. The present study reveals the possibility of intrafamilial transmission. Of the 21 families studied, based on Cohen’s κ analysis, 13 showed a certain degree (17–100%) of agreement in protein patterns among individual household members. Furthermore, among the blots compared, parent–child transfer seems to be more frequently observed as there were more immunoreactive bands from the parents as compared to the children.

As noted earlier, in families 6 and 10, the younger mem-
bers remained seronegative despite the seropositivity of the older members. Family 6 comprised a young couple and a 1-year-old child. The parents were seropositive but the child was seronegative, possibly there was no seroconversion at the time of this study. For family 10, the relationship of the members was grandparents and grandchildren and results from blot comparisons showed that only three sets illustrated similar protein profiles for this relationship.

Despite the data supporting a possible vertical familial transfer, there were five blot comparisons that showed no similar protein profiles among household members. These subjects could have acquired the infection from other sources besides the family members. Therefore, interfamilial transmission should not be ruled out as a probable mode of acquiring H. pylori infection.

Our study is in agreement with the hypothesis of intrafamilial vertical transmission. It is also suggested that ELISA and Western blotting in combination can be a useful approach in determining familial status in relation to H. pylori infection.

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References