

# The pattern of CagA and VacA proteins in *Helicobacter pylori* seropositive asymptomatic children in western Saudi Arabia

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## ABSTRACT

**Objectives:** This study aimed to determine antibody prevalence to *Helicobacter pylori* (*H. pylori*) virulence factors CagA and VacA in asymptomatic *H. pylori* seropositive children in Jeddah, Kingdom of Saudi Arabia (KSA). The possibility of differences in antibodies response patterns was also investigated in relation to gender, nationality and age.

**Methods:** Two hundred and twenty-four asymptomatic *H. pylori* seropositive children (mean age  $9.3 \pm 3.9$  years; range, 1-14 years) were enrolled in this study from King Abdul-Aziz University and Maternity and Children's Hospitals, Jeddah, KSA, during the periods 2002-2003. The 35 kDa, CagA or VacA *H. pylori* antibodies were measured in the serum by immunoblot (Helico Blot 2) method.

**Results:** Immunoblot assay yielded positive results in 215/224 seropositive asymptomatic children (96%). In

those children, the prevalence of 35 kDa was 63.3%, VacA was 60%, CagA was 56.7% and for combined VacA and CagA antibodies was 45.6%. Prevalence of these bands did not show any difference between Saudi and non-Saudi children. Meanwhile, prevalence of 35 kDa, VacA, CagA, combined VacA and CagA antibodies were significantly elevated in males versus females ( $p < 0.0001$ ) and in children 10 years versus those in age groups 1-5 years and 6-9 years ( $p < 0.0001$ ).

**Conclusion:** This study showed high prevalence of *H. pylori* antibodies among asymptomatic children in Jeddah, KSA. The prevalence of antibodies against 35 kDa, CagA and VacA *H. pylori* antigens is higher in males and older children. The ELISA and immunoblot are non-invasive methods that were found to have adequate performance in pediatric population.

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*Helicobacter pylori* (*H. pylori*) is associated with different digestive diseases, such as gastritis, gastric and duodenal ulcer, and mucosa-associated changes to lymphoma and gastric cancer in adults.<sup>1</sup> The reasons for developing one or another disease are not well understood and several factors are possibly involved.<sup>2</sup> Some virulence genes of *H. pylori* infection, CagA and VacA, have been shown to influence the clinical outcome of infection in adults.<sup>3,4</sup>

Vacuolating cytotoxin, which induces vacuolation in cultured eukaryotic cells, is secreted by approximately 50% of *H. pylori* isolates in western countries. Infection with vacuolating cytotoxin positive strains is reported to be associated with particular gastroduodenal diseases.<sup>5</sup> For instance, Figura et al<sup>6</sup> showed that strains with vacuolating cytotoxin activity were found in 16 of 24 (67%) patients with peptic ulcers but in only 16 of 53 (30%) patients without it. The cytotoxin is encoded

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**Table 1** - Antibody response against each antigen in *H. pylori* seropositive asymptomatic children obtained by ELISA and immunoblot techniques.

Different antibody response	ELISA number of immunoblot positive samples	ELISA positive samples (%)	Immunoblot number of immunoblot positive samples	Immunoblot positive samples (%)
19.5 (urease E)	123	(54.9)	117	(54.4)
26.5 (urease A)	171	(76.3)	169	(78.6)
30 (urease H)	130	(58)	129	(60)
35	136	(60.7)	136	(63.3)
89 (VacA)	129	(57.6)	129	(60)
116 (CagA)	122	(54.5)	122	(56.7)
89 and 116 (VacA and CagA)	98	(43.8)	98	(45.6)
Positive	215	(96)		
<b>Total</b>	<b>224</b>	<b>(100)</b>	<b>215</b>	<b>(100)</b>
ELISA - enzyme-linked immunosorbent assay.				

by the VacA gene,<sup>7</sup> and thus, is also called VacA protein.<sup>4</sup> The CagA protein is another putative virulence factor, encoded by the *CagA* gene (cytotoxin associated gene A).<sup>8</sup> It has been reported that this gene is present in approximately 60% of *H. pylori* isolates, and nearly all *CagA* gene positive strains express the protein.<sup>9</sup> Although the function of *CagA* protein is not known, the protein has been reported to be associated with particular gastro-duodenal diseases.<sup>10</sup> Ching et al<sup>11</sup> found serum anti-CagA antibody in 165 of 197 (84%) of adult patients with duodenal ulcer, but in only 25 of 45 (56%) patients with non-ulcer dyspepsia. Recently, *CagA* positive *H. pylori* strains have been shown to be associated with interleukin-8 (IL-8) induction in gastric epithelium. Neutrophilic infiltration into the gastric epithelium, which is characteristic of *H. pylori* infection, may be due to increased production of IL-8. The CagA protein may thus be related to gastric inflammation and gastroduodenal diseases.<sup>12,4</sup>

The aim of this study was to evaluate the performance of an immunoblot test, Helico Blot 2, in the diagnosis of *H. pylori* infection and to investigate the prevalence of serum CagA or VacA *H. pylori* antibodies in asymptomatic seropositive children diagnosed by ELISA test in Jeddah, Kingdom of Saudi Arabia (KSA). The possibility of differences in antibodies response patterns was investigated in relation to gender, nationality and age.

**Methods.** In our previous study,<sup>13</sup> serum samples from 1024 asymptomatic children who attended the outpatient department at both King

Abdul-Aziz University and Maternity and Children's Hospitals, Jeddah, KSA during the period December 2002 - November 2003 were evaluated for *H. pylori* IgG antibody using the commercially available high-molecular-weight-cell-associated protein test (HM-CAP) enzyme immuno-assay method. These children were considered asymptomatic after they denied any gastrointestinal symptoms. Previous reports showed that (HM-CAP) kit has an adequate accuracy rate in the pediatric population (sensitivity and specificity >90%).<sup>14</sup> The sera of 224 asymptomatic children (145 boys and 79 girls; mean age  $9.3 \pm 3.9$  years; range, 1-14 years) were positive for *H. pylori* antibody. These sera were used in this study for determination of CagA or VacA *H. pylori* antibodies. All serum were kept at -20°C for future *H. pylori* antibodies analysis. In addition, dermatographic data, including gender, nationality and age were collected from all participants. The study was approved by the Medical Ethical Committee of King Abdul-Aziz University and informed consent was obtained from the parents of the participants.

The *H. pylori* virulence was determined by a commercially available immunoblot kit (Helico Blot 2.0; GeneLabs Diagnostic, Singapore). Previous studies in adults and children showed that this test is comparable to other diagnostic tests for *H. pylori* infection, including serology, or polymerase chain reaction technique, with a sensitivity and specificity exceeding 90%.<sup>10</sup> The *H. pylori* virulence was determined according to the manufacturer's kit instructions. Briefly, diluted serum samples [1:100] were incubated with Helico Blot strips for one hour.

**Table 2** - Seropositivity against CagA and VacA, 35kDa and CagA and VacA antigen, according to nationality, gender and age in immunoblot positive children (n=215).

Factors	Wave length of Helico Blot 2.1							
	35 kDa	(%)	89 (VacA)	(%)	116 (CagA)	(%)	89 and 116 (VacA and CagA)	(%)
<b>Nationality</b>								
Saudi (n=99)	61	(28.4)	60	(27.9)	56	(26)	44	(20.5)
Non-Saudi (n=116)	75	(34.9)	69	(32.1)	66	(30.7)	54	(25.1)
Significance	$p>0.05$		$p>0.05$		$p>0.05$		$p>0.05$	
<b>Gender</b>								
Male (n=140)	90	(41.9)	91	(42.3)	84	(39.1)	68	(31.6)
Female (n=75)	46	(21.4)	38	(17.7)	38	(17.7)	30	(14)
Significance	$p<0.0001$		$p<0.0001$		$p<0.0001$		$p<0.0001$	
<b>Age group</b>								
(1-5) years (n=42)	26	(12.1)	24	(11.2)	23	(1.7)	20	(9.3)
(6-9) years (n=63)	40	(18.6)	36	(16.7)	33	(15.3)	25	(11.6)
( 10) years (n=110)	70	(32.6)	69	(32.1)	66	(30.7)	9	(45.6)
Significance	$p<0.0001$		$p<0.0001$		$p<0.0001$		$p<0.0001$	

The strips were then incubated and washed consecutively with conjugated antibody (IgG) followed by substrate before drying. Results were read according to the standard control protein bands provided by the company. The standard *H. pylori* antigens available in the strip included: 116 kDa (CagA), 89 kDa (VacA), 35 kDa, 30 kDa (urease H), 26.5 kDa (urease A), and 19.5 kDa (urease E). The presence of any one of the first 3 largest antigens or the presence of 2 of the 3 smallest antigens are considered a positive test for the diagnosis of *H. pylori* infection. Sera that does not fulfill the criteria were classified as negative. CagA (116 kDa protein) and VacA (89 kDa protein) are associated with isolates, which are more pathogenic. Patients having antibodies to either of these proteins on the blot would indicate that the patient has probably been infected with a more pathogenic isolate of *H. pylori*.

**Statistical analysis.** The results were expressed as number and percentage. Comparisons of percentages were assessed using chi-square test using SPSS software version 10. Pearson correlation was made between antibody responses and gender, nationality and age of the children. Statistical significance was determined by  $p<0.05$ .

**Results.** All studied samples were positive for *H. pylori* antibodies by enzyme immunoassay (HM-CAP). Immunoblotting yielded positive results in 215 (96%) of the 224 asymptomatic seropositive children. The prevalence to *H. pylori* antibodies was shown in Table 1. In immunoblot *H. pylori* positive children, the 26.5 kDa (urease A) was the most frequently recognized band (169/215, 78.6%), followed by 35 kDa (136/215, 63.3%), 30 kDa

(urease H) (129/215, 60%), 89 kDa (VacA) (129/215, 60%), 116 kDa (CagA) (122/215, 56.7%), 19 kDa (urease E) (117/215, 54.4%). Antibodies against both VacA and CagA proteins were detected in 98/215 (45.6%) (Table 1).

According to nationality, 99 of the immunoblot seropositive children who participated were Saudi whereas, 116 were non-Saudi. The percentage of seropositivity against 35 kDa showed no significant difference in Saudi compared to non-Saudi (28.4% versus 34.9%,  $p>0.05$ ), VacA (27.9% versus 32.1%,  $p>0.05$ ), CagA (26% versus 30.7%,  $p>0.05$ ) and VacA and CagA antigens (20.5% versus 25.1%,  $p>0.05$ ) (Table 2).

In immunoblot seropositive participants, 140 children were male and 75 children were female. The percentage of seropositivity against 35 kDa was significantly elevated in male compared to female (41.9% versus 21.4%,  $p<0.0001$ ), VacA (42.3% versus 17.7%,  $p<0.0001$ ), CagA (39.1% versus 17.7%,  $p<0.0001$ ) and VacA and CagA antigens was (31.6% versus 14.0%,  $p<0.0001$ ) (Table 2).

With increasing age of children, the percentage of seropositivity against *H. pylori* antigens increased significantly. In immunoblot seropositive participants, 42 children were in age group (1-5 years), 63 children were in age group (6-9 years) and 110 children were 10 years. The percentage of seropositivity against 35 kDa was significantly elevated in children 10 years compared to those in age group of 1-5 years and 6-9 years (32.6% versus 12.1% and 18.6%,  $p<0.0001$ ), VacA (32.1% versus 11.2% and 16.7%,  $p<0.0001$ ), CagA (30.7% versus 10.7% and 15.3%,  $p<0.0001$ ) and VacA and CagA antigens (45.6% versus 9.3% and 11.6%,  $p<0.0001$ ) (Table 2). No significant correlation was found

between antibody response and gender, nationality and age of the children, data not shown.

**Discussion.** Although clinical and epidemiological studies indicate that *H. pylori* are generally acquired during childhood,<sup>15</sup> high grades of gastric inflammation are rare in children.<sup>16</sup> Several reports describe completely normal histology of gastric mucosa in 27-30% of children with *H. pylori* infection.<sup>17,18</sup> In contradiction to other reports,<sup>19,20</sup> this study showed a high accuracy of ELISA test compared to immunoblot method. As 96% of seropositive children who participated determined by ELISA were positive by immunoblot method.

In this study, antibodies against 26.5 kDa, 35 kDa antigens were the most prevalent, also a high prevalence against VacA and CagA were reported. Our result of the highest performance index of 78.6% for the 26.5 kDa band is in accordance with the findings in previous publications.<sup>21-23</sup> Mitchell et al<sup>24</sup> described the 26 kDa band to be the first to appear after infection with *H. pylori* in childhood, soon followed by other low molecular weight antigens.

In the genetic diversity of the *H. pylori* bacterium, 2 distinct regions, CagA and VacA, were identified. These regions encode for the cytotoxin-associated gene A (*CagA*), and the vacuolating cytotoxin (*VacA*).<sup>25</sup> The *CagA* and *VacA* genes were associated clinically with increased morbidity in symptomatic adults.<sup>25</sup> Very limited data are available on the prevalence of CagA or VacA antibodies in symptomatic and asymptomatic children. In the present study, we report the prevalence rate of CagA and VacA antibodies (60.0% and 56.7%) in asymptomatic seropositive children. Similarly, other authors found a high prevalence of infection with *CagA* positive strains (76 or 82%) in asymptomatic children,<sup>26,27</sup> although others<sup>28</sup> found a lower prevalence (54%). Kato et al<sup>28</sup> studied a group of 25 children with ulcers and compared the CagA prevalence with that of a group of asymptomatic children, finding no significant differences: 80% for patients with gastric ulcers, 93.3% for those with gastritis, 95% for those with duodenal ulcers, and 81.8% for asymptomatic children. Previous investigators have shown that the prevalence of a *CagA*-positive gene in symptomatic children was between 40%<sup>29</sup> and 80%.<sup>30</sup> Preliminary pediatric data have estimated the prevalence of *H. pylori* CagA strain or CagA serum antibody (or both) in symptomatic children to be between 33% and 80%<sup>31,32</sup> and, in asymptomatic children, to be 82.6%.<sup>33</sup> Gzyl et al<sup>30</sup> and Husson et al<sup>29</sup> reported a positive association between the *CagA* gene and the severity of gastric inflammation. In preliminary data, Queiroz et al<sup>34</sup> showed that CagA antibody correlated with increased gastric inflammation but not with the presence of histological follicles. Some

authors found that adult patients suffering from ulcers more frequently have antibodies against 3 single antigens (CagA, VacA, and 35 kDa antigen), with the anti-VacA antibody being a more powerful marker of ulcers than anti-CagA, and the anti-35 kDa antibody appears to be the best marker of ulcers. Moreover, the simultaneous presence of anti-VacA and anti-35 kDa antibodies predicts with good sensitivity a predisposition to ulcers.<sup>35</sup>

This study showed significant increase in the prevalence of antibodies against 35 kDa, CagA and VacA and combined CagA and VacA antigen in males compared to females. It was reported previously<sup>36,37,13</sup> that risk of *H. pylori* infections in male children was higher than female. In consistence with others,<sup>38-40</sup> this study showed increase antibodies response against specific antigen with increasing age of the children. Similar results were previously reported in Riyadh, KSA, in which the prevalence of *H. pylori* infection increased rapidly with age from 40% of those 5-10 years old to be 70% at 20 years old.<sup>41</sup> In Italy, the risk of *H. pylori* infection in children 4-7 years was 12%, 8-11 years was 13% and 12-15 years was 23%.<sup>42,43</sup> Another study from Arkansas showed overall *H. pylori* prevalence of 24% by 5 years of age and 45% by 20 years.<sup>44</sup> In United State children, the prevalence of *H. pylori* infection increased with age from 16.7% in 6-9 years old to 26.2% in 10-14 years old and to 29.1% in 15-19 years olds.<sup>45</sup> In healthy Japanese children, the risk of *H. pylori* infection in age groups of <1 year was approximately 3%, 1-4 years was 10%, 5-9 years was 19%, 10-14 years was 25%, and 15-19 years was 29%.<sup>46</sup> Higher risk of *H. pylori* infection in Turkey was reported for children aged 10-14 (47.3%) and 15-19 (58.4%) years old.<sup>37</sup>

Expression of *CagA* protein is closely associated with that of vacuolating cytotoxin,<sup>9</sup> although the underlying mechanism is not understood. Thus, Xiang et al<sup>47</sup> classified *H. pylori* strains into 2 groups, Type I and Type II. They reported that the Type I strains, which were positive for both vacuolating cytotoxin and *CagA*, were strongly associated with peptic ulcer diseases in the host. The clinical importance of *CagA* and *VacA* genes in children has not yet been elucidated. To our knowledge, no long-term prospective data regarding children are available to permit accurate assessment of this question. Although an association between positive CagA antibody and increased severity of gastritis in children was suggested by others<sup>28,30</sup> the true clinical implication has yet to be determined. In this study, we report many asymptomatic children who harbored antibodies to the virulent strain. These data may suggest that either the *CagA* gene locus is inactive in children or other factors are involved in the development of duodenal ulceration and in the determination of their clinical outcome.<sup>28</sup>

In conclusion, this study revealed that both ELISA and immunoblot are non-invasive methods that were found to have adequate performance in pediatric population and can be used in *H. pylori* screening in children. The prevalence of *H. pylori* antibodies among asymptomatic children in Jeddah, KSA is high. Prevalence of antibodies against 35 kDa, CagA and VacA *H. pylori* antigens are more in males than females and in older children than young for all the investigated bands. Whether these data will aid in determining the clinical outcome of these children or not remains unknown.

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