Prevalence of CagA and VacA genotype of *Helicobacter pylori* in Thai Children

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Seroprevalence of Helicobacter pylori infection in children is variable according to geographical location and family sanitation. A previous study in Bangkok showed an incidence of 25.5% in 1998.The higher incidence in the urban and rural area is predicted in lower economic classes and poor sanitation.
Objective : *To study the occcurance of CagA and VacA genotype in Thai children using the Western blot technique.*
Material and Method : *Sera of 159 Thai native children aged 0-15 year without associated abdominal pain from different provinces in 4 parts of the Kingdom of Thailand were tested with the rapid screening test for H. pylori. The positive specimen was further tested with the Western blot technique for determination of Urea A (p37), CagA (p116) and VacA (p89).*
Result : *Fifty five and fifty two (34.6%) were tested positive by the rapid test while 32.7% were positive for the band of current infection marker (CIM).The 28 selected positive sera with complete history of housing and water supply were analysed. Thai children living in urban areas have a higher prevalence and the CagA +,VacA + are found in 96.43% of infected patients. The transmission may be through the water supply.*
Conclusion : *A high prevalence of Helicobacter pylori infection was found in childhood period in urban areas and may be associated with the local water supply.*

Keywords : *CagA, VacA, Helicobacter pylori, Water supply, Children, Thai*

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Infection with Helicobacter pylori acquired in early childhood may proceed to gastritis, peptic ulcer disease and even MALT lymphoma and adenocarcinoma of stomach in later adulthood[1,2]. The flagellated microaerophilic spiral bacteria colonizing in gastric mucosa of gerbils, crab-eater monkeys, domestic pets and human beings, can cause inflammatory processes in the gastric epithelium with metaplastic changes. The World Health Organization (WHO) has classified Helicobacter pylori as a class I carcinogen the same as tobacco, Hepatitis B virus, sunlight and others. The prevalence of the infection differs significantly within countries with higher rates of infection found in low socioeconomic status and high density of living. In Japan, the incidence of H. pylori associated gastric cancer is high in correlation with high gastrin level, and also associated with a low social class in which the incidence of intra-familial transmission[3] has been recognized. Worldwide studies annotate higher prevalence of infection in urban citizens than in cities and suggesting the transmission routes by fecal-oral contamination[4-7] and intrafamial transmission. In developing countries 80% of adults are infected, the acquistion rate is about 10% in children between 2-8 years per annum.

The genes of H. pylori eg. CagA (Cytotoxin associated gene A, VacA, (Vacuolating gene A) with S_1,S_2,M_1,M_2 have been identified as being virulence associated. These genes can be either detected from

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blood[19] with Western blot technique or from gastric biopsy[8-10], gastric juices and dental plaques by polymerase chain reaction (PCR)[6,11-13].

In Thailand, the prevalence of H. pylori in adults is as high as 74%. A previous study in Bangkok and rural provinces[14-17] showed an infection rate of about 50-80% in adults and 25.4% in pediatric groups[18]. Serological studies in children using IgG, IgM and IgA ELISA based techniques have been validated and get the sensitivity range of 50-96% with specificity range of 80-100%. One study from Brazil[19] reveals the linear relationship between the age and sensitivity of the test techniques, whereas in children between 2-6 years the sensitivity dropped to 44%. (There should be some false negative with the serological test.)

The authors used the IgG-based ELISA technique to detect the seroprevalence of H.pylori in Thai children in Bangkok during 1998, and found the mean in all ages = 25.43%, whereas the result from cord blood showed 7.12%, from 0-1 year = 4%, 1-6 year = 19%, 6-10 year = 22%, and group of 10-15 year = 34%. The higher incidence may indicate the higher sensitivity of the test technique[19].

Objective

1. To study the occurrence of CagA and VacA genotype in Thai children

2. To study the geographical variation of the prevalence of H.pylori infection in children.

Study Design

Cross-sectoinal descriptive study

Statistical analysis

Descriptive statistic, Chi-square test and t-test were applied by using SPSS. P-value of < 0.05 was considered statistically significant.

Material and Method

From January 2000 the December 2001, 159 sera of Thai chidren who visited the Out Patient Clinics of the Department of Pediatrics in King Chulalongkorn Memorial Hospital, the Chiang-Mai University Hospital, the Maharat Korat Hospital, the Chon Buri provincial Hospital and Chantaburi provincial Hospital which are located respectively in the Central, Northern, North-eastern and Eastern parts of Thailand were collected and frozen at -20°C until analysis. The basic data of these infants and children such as sex, age, birth weight, actual weight, type of nursing, duration of breast feeding, family numbers, house location and environmental background especially type of water supply and food sources were recorded.

The exclusion criteria were the children who had either blood or plasma transfusion and history of recurrent abdominal pain. All 159 sera were handled in multiple sample alliquots and kept at -20°C during transportation to Bangkok where they were further kept in a -70°C refrigerator before performing ELISA and Western blot technique. These sera were analysed for anti H. pylori IgG (by ASSURE RT, H. pylori Genelabs Diagnostics, Singapore). The test gives the positive result by a pink band seen at the control line, the test line and/or with the pink band at the current infection line (Fig. 1). The sera of the children with positive CI band from the Maharat Korat Hospital (North-eastern province) were selected to perform the Western blot technique (Helicoblot 2.1, Genelabs Diagnostics, Singapore) to detect the urea A (p37), CagA (p116) and VacA (p89).

The basic data of these children were classified according to age, type of nursing, water supply and house distance from the city center.

Results

Sera from 159 Thai children aged 0-16 years of age revealed positivity of Helicobacter pylori infection by the positive test line = 55 (34.6%) and by the positive current infection band = 52 (32.7%) (Fig. 1).

Fig. 2 demonstrates the age distribution of the prevalence of H. pylori infection in Thai children. For infants under 3 years old the H. pylori infection rate was 20%, 3-6 years old 33.3%, 6-12 years old 30%, and 12-16 years old 75% Fig.3 shows the comparison of the seroprevalence by the geographical distribu-

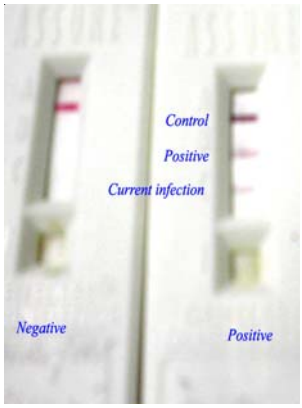


Fig. 1 Positive and negative results by Rapid test

Fig. 2 Age distribution of *Helicobacter pylori* infection in Thailand

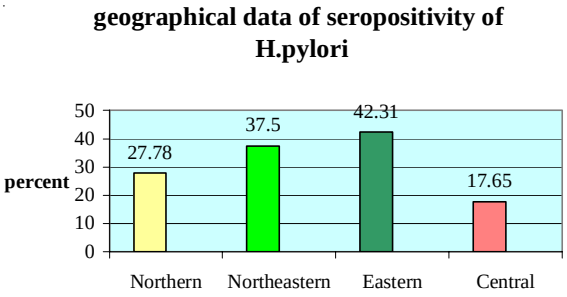
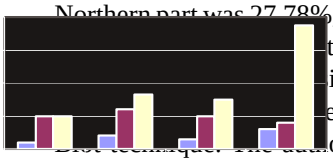


Fig. 3 Geographical distribution of seroprevalence of H. pylori infection

tion in Thailand, the prevalence of H. pylori infection in the Bangkok Metropolitan area was 17.65%, the Northern part was 27.78%, the North eastern part was [illegible] was 42.31%.



[illegible] H. pylori from the children were analysed with Western Blot technique. The authors found the positivity of urea A genotype = 22/28 (78.57%); the CagA genotype = 27/28 (96.43%) VacA genotype 27/28 (96.43%). The percentage of $CagA^+$, $VacA^-$ = 1/28 (3.57%) and the urea A = 78.57%

The basic data of these children: Mean weight ± SD = 22.86 ± 12.82 gm, Mean family size of the H. pylori negative = 5.01 ± 2.18 and with H. pylori positive is 4.93 ± 1.67. Percentage of breast feeding in the population in Korat was 61.25%.The selected group of seropositive children had 51% of exclusive breast feeding for more than 6 months and in only 21.5% the seropositive children had a history of breast feeding less than 6 months Which is statistically significant by chi-suare test (p value ≤ 0.05) the mode of water supply (Fig. 4) contribute in rate of seroprevalences as found that 7% in the seropositive group used water mainly from a municipal pipe water, whereas 27% of the seropositive group used water from village water or well water. Exploring the house location, the authors also found that 36 families with positive serology in one child living in the rural area when only 6 families from 100 studied families were living in the central or urban area of the province.

Discussion

Helicobacter pylori are associated with 95% duodenal ulcer and 80% of gastric ulcer. Gastric cancer is one of the common cancers in Asian countries and many studies have suggested the association of H. pylori and gastric cancer. In Thailand the annual incidence rate of gastric cancer in males and females was 4.9 and 3.0 per 100,000 population in 1992-1994[20]. In Japan, the incidence of H. pylori associated gastric cancer is high. This comes to the concept of no H. pylori, no gastric cancer. Prevalence of H. pylori infection correlates with socio-economic status rather than race[21]. From a global aspect, 30-40% of people in the United States and Western Enrope are infected with H. pylori, compared with 70-80% of people in China[22] and Indochina. In developing countries most adults are infected and the acquistion rate occurs in about 2% of children per annum between the ages of 2-8 years. (In Bangkok the incidence in 1998 was 25.5%, but from the present study at the end of the year 2000 found the incidence had declined to 17.65%. There should be some correlation between the lower incidence and sanitation).

The CagA strain of H. pylori is noted as the strain related with carcinoma of the stomach[2,23]. The strain type in Thai adults has been proposed as mostly CagA + ve and VacA + ve. In the present study when selected in one area of Northeastern province, the strain type of $CagA^+$, $VacA^+$ confirmed 96.43%. By far, there may be wide transmission of this bacteria through the same type of water supply because the

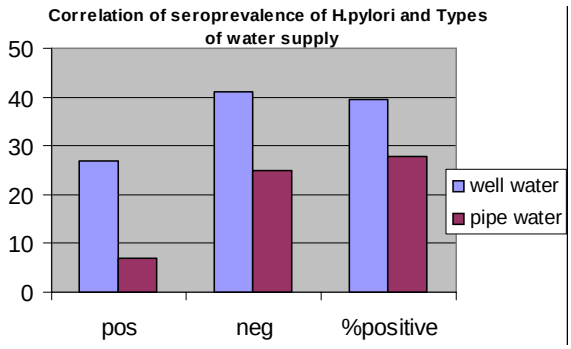


Fig. 4 Correlation of seroprevalence of H. pylori and Types of water supply

seroprevalence of the rural people using a village water well and a house well is higher than people using the treated municipal water supply. Lu Y[24] et al isolated VacA classes Heliobacter pylori in untreated waste water which may be the potential source of H. pylori transmission. Baker[25] et al found that H. pylori is resistant to chlorine and ozone but not monochloramine. This supports the idea of a water borne route of transmission[26].

Intrafamilial transmissions either by mother to child or father to child have been reported, in the present study the duration of breast feeding which reveals the correlation between longer breast feeding time and higher seropositive prevalence. The overall infection rate at 0-3 years of age was 20% with 3-12 years 33% but uphigh to 75% after 12 years. Thus, the authors propose the evidence that environmental contamination plays a more significant role in H. pylori transmission.

Further epidemiological study should be done with the role of $CagA^+$ and $VacA^+$ genotype of H. pylori and clinical manifestation of peptic ulcer disease, non ulcer dyspepsia and stomach cancer. The present study provides the basic data of Helicobacter pylori infection in a Thai population especially in the North-eastern part of Thailand where more investment for public health promotion is needed.

Conclusion

The seroprevalence of Helicobacter pylori detemined with Rapid test correlates well with the Western blot technique and is also an effective screening method in children. The $CagA^+$, $VacA^+$ genotype of H. pylori is the predominant strain (96.43%) in local Thai people. The transmission of Helibacter pylori may be through the untreated local water supply.

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ความชุกของ Helicobacter Pylori CagA, VacA ในเด็กไทย

บุษบา วิวัฒน์เวคิน, อภิรดี เทียมบุญเลศ, นิพัทธ์ สีมาขจร, ลำดวน วงศ์สวัสดิ์

วัตถุประสงค์ *: เพื่อศึกษาอุบัติการณ์ของ CagA และ VacA genotype ของ Helicobacter pylori ใน1เด็กไทย*
วิธีการศึกษา *: ตรวจซีรั่มเด็กจำนวน 159 ราย อายุ 0-15 ปี ที่ไม่มีอาการปวดท้องจาก 4 จังหวัดใน 4 ภาคของประเทศไทยสำหรับโรค Helicobacter pylori น้ำเหลืองที่ให้ผลบวกนำมาตรวจซ้ำด้วยเทคนิค Western Blot เพื่อหา Urea A, CagA และ VacA*
ผลการศึกษา *: เด็ก 55 ราย (34.6%) พบเป็นโรค และ 52 ราย (32.7%) ยืนยันว่าเป็นโรคด้วยวิธีตรวจ CIM เด็กเหล่านี้จำนวน 28 ราย ได้ประวัติอย่างละเอียดเกี่ยวกับที่อยู่อาศัยและน้ำดื่มเพื่อนำมาวิเคราะห์ พบว่าเด็กในแถบชานเมืองมีโอกาสเป็นโรคสูงพบ CagA และ VacA ร้อยละ 96.3 น้ำดื่มน่าจะเป็นแหล่งติดเชื้อ*
สรุป *: เด็กที่อยู่ในแถบชานเมืองมีความชุกสูงในการติดเชื้อ Helicobacter Pylori และน่าจะได้เชื้อมาจากน้ำดื่ม*