Bangladesh J Microbiol, Volume 28, Number 1, June 2011, pp 25-31

Original Article

Serum *Helicobacter pylori* Specific Antibodies Among Children With Undernourishment: A Case Control Study

Warda Haque[1], Chowdhury Rafiqul Ahsan[1], Jamalun Nessa[1]*, and Mahmuda Yasmin[1]

[1] *Department of Microbiology, University of Dhaka, Dhaka 1000, Bangladesh*

(Received 5 February 2011; Accepted 20 August 2011)

Infection with *Helicobacter pylori* has been implicated in the development of acute and chronic gastritis, peptic ulcer disease, non-ulcer dyspepsia, and gastric MALT (mucosa-associated lymphoid tissue) lymphoma and extragastroduodenal disorders. *H. pylori* infection may lead to development of hypochlorhydria and subsequent malnourishment. This study examined the affiliation between *H. pylori* infection and some sociodemographic and nutritional variables including breast feeding practice, poverty, and availability of safe drinking water. Three hundred children with age ranging from zero to twelve years were enrolled in the study. Their nutritional status was assessed following standard protocol. A precoded questionnaire was filled up for obtaining data. Serostatus of anti *H. pylori* antibody was determined by in-house ELISA using formalin fixed *H. pylori* whole cell antigen. The cut-off value of the ELISA was further validated by Immunoblot technique. Among the 149 case (malnourished) children, 107 (72%) contained anti-*H. pylori* antibodies and among the control (nonmalnourished) population (n=151), 91 (60%) possessed anti-*H. pylori* antibodies, with an overall seroprevalence of 66%. Malnourishment was found to be more widespread among female (56%) than male children (44%). Highly significant association (p = 0.000) was reported between poor socioeconomic status and development of malnourishment. The degree of severity of malnourishment increased inversely with the practice of exclusive breast-feeding up to 5 months of age (p = 0.010). The odds ratio relating breast-feeding to malnourishment reflected 52% reduction in the risk of development of malnourishment among breast-fed children. The use of unsafe drinking water was associated with 5.33 times higher odds of developing malnourishment. Poverty reduction strategies associated with improvement of hygiene condition and promotion of breast feeding can actively contribute to the improvement of the nutritional status of the children of Bangladesh.

Key words: Malnourishment, *H. pylori* infection, Breast feeding,

Introduction

The human stomach has been considered as an inhospitable environment for bacterial growth because of its acidic pH. However, Warren and Marshall, in 1983 successfully cultured a corkscrew-shaped, microaerophilic Gram-negative bacterium from human gastric mucosa, termed as *Helicobacter pylori* and showed an association between the presence of this organism and gastric inflammation[1].

Children in the developing countries, such as Peru, Bangladesh, and Gambia start to become infected in the first few months of life, in some communities as many as 50% of the children become infected. *H. pylori* is a slow-growing, highly motile, Gram-negative spiral organism whose most striking biochemical characteristic is the abundant production of urease[1]. *H. pylori* has been identified as the underlying cause of acute and chronic gastritis, peptic ulcer disease, non-ulcer dyspepsia, gastric MALT lymphoma and extragastroduodenal disorders[2]. It may be acquired at any age but once acquired, the infection persists for years and often for a lifetime [3]. The gastric acid barrier, an important host defense against small bowel infection, may be compromised by infection with *Helicobacter pylori*. In developing countries, prevalence of hypochlorhydria is high particularly in the malnourished population, which may predispose a child to repeated gastrointestinal infection and diarrhea[4]. *H. pylori* infection has been implicated in malabsorption of vitamins and development of iron deficiency anaemia. The combined impact of these factors could contribute to the severity of malnutrition[5-6].

The diagnostic potential of direct microscopy, culture, rapid urease test (RUT) of endoscopic antral biopsy tissues, urea-breath test and enzyme-linked immunosorbent assay (ELISA) of anti-*Helicobacter pylori* antibody in serum for the detection of *H. pylori* has been demonstrated[7-8]. ELISA can provide a simple, rapid and inexpensive test, with the high sensitivity (85-95%) and specificity (95%). However, a combination of immunblotting

Corresponding author:
Dr. Jamalun Nessa, Professor, Department of Microbiology, University of Dhaka, Dhaka 1000, Bangladesh, Tel (Office): (02) 9661900-73, Extn. 7744, Fax: + 880 (02) 8615583, E-mail: jamalun_nessa@hotmail.com

and ELISA was found to be the most efficient means of detecting serum antibodies to *H. pylori* antigens and could be applied to screening of human sera for *H. pylori* specific antibodies[9].

H. pylori infection is most likely acquired by ingesting contaminated food and water and through person to person contact. The epidemiology of *H. pylori* infections is clearly distinct between the developed and the developing world. The prevalence of *H. pylori* infections in individuals depends on the country where the individuals live, his or her socio-economic status and age. The prevalence of infection in asymptomatic cases increases with age in developed countries[10].

The clinical outcome of *H. pylori* infection is most likely of complex interactions among host, bacteria and environmental factors; being clustered in families and institutions in the developed world, suggesting transmission due to close contact among the infected individuals[11].

The present study was performed to determine the seroprevalence of *H. pylori* infection among malnourished and nonmalnourished children (n = 300) in Bangladesh. Probable role of relevant sociodemographic and hygiene associated risk factors for the acquisition of *H. pylori* infection and subsequent malnourishment development have also been investigated. The research was furthermore aimed to explore the effects of breastfeeding in preventing attainment of malnourishment.

Materials and Methods

Children (0 – 12 years) from various socioeconomic backgrounds attending a children-based hospital in Dhaka city for treatment or vaccination or blood grouping was included in this study. Three millilites of blood was drawn from each participant from whom serum was separated using standard method[12]. Nutritional status of each participant children was analyzed by comparing their weight, height with the medians of the NCHS[13] (National Center for Health Statistics) reference population of the same age and sex group. Children whose weight-for-age were >90% of median of the NCHS, weight-for-height were >90% of median of the NCHS, and mid arm circumference were >14 cm[14] were classified as properly nourished or control. But whose weight-for-age were <90% of median of the NCHS, weight-for-height were <90% of median of the NCHS, and mid arm circumference were <14 cm were graded as undernourished or case children. The degree of malnourishment was further classified by ranking into first (mild), second (moderate) and third (severe) degree of malnutrition [15].

To obtain relevant demographic, anthropometric, socioeconomic, clinical and nutritional data, a precoded questionnaire was filled up for each enrolled child. The whole study population was stratified into seven age groups. Socioeconomic condition was measured by using the international poverty line (monetary cut-off points separating the poor from the non-poor) proposed by the World Bank set at US$1.00 per day per capita in purchasing power parity (PPP) terms [16].

The cells of *H. pylori* (collected from ICDDR, B) were suspended in phosphate buffered saline (PBS) containing 1% (v/v) formalin and kept at 4°C for 1 hour. Then they were centrifuged (12500 g, 5 minutes) and the pellet was resuspended in 1 ml of phosphate buffer saline (PBS). Washing with PBS was repeated 4 times to remove the formalin. Finally, a suspension of 1mg ml^{-1} cells in PBS was made. In-house ELISA was performed with formalin-fixed *H. pylori* whole cell antigen (diluted as 1 µg per 100 ìl of coating buffer) and 100 µl of antigen suspension was added to each well[17]. The plate was covered with plate sealer and incubated overnight at 4°C. On the following day, the plate was washed 3 times with PBS containing Tween-20 (0.05%). The wells were blocked with 200 µl of 1% (w/v) bovine serum albumin (BSA) in PBS, and then plate was incubated at 37°C for 30 minutes. The PBS-BSA was discarded and the plate was washed 3 times with PBS – Tween20. Then, 100 µl of diluted serum sample (1:500 in PBS) was added to all wells except wells A1 and B1, which were used to calibrate the ELISA reader. The plate was covered and incubated at room temperature for 2 hours. The plate was then washed three times as described above and 100 µl of diluted antibody conjugate [1:30,000 in PBS; Rabbit antihuman polyvalent immunoglobulin (whole molecule – Peroxidase containing IgG fraction only); SIGMA, A 8794] was added, and incubated at room temperature for an additional 2 hours. The plate was again washed three times with PBS – Tween-20 and 200 ìl of enzyme substrate (H_2O_2 – OPD in 0.1M sodium citrate buffer) was added to each well[17]. The plate was placed in the dark and optical density (OD_{490}) was measured by an ELISA reader (BIORAD, USA, model No.680) exactly after 30 minutes.

For the Alkaline Phosphatase (AP) substrate system, the similar procedure of the HRP system was followed with a suitable secondary antibody (Goat anti-human polyvalent antibody conjugated with alkaline phosphatase; diluted 1:30000 times in PBS; SIGMA, A - 3313), and substrate (p-nitrophenyl phosphate in diethanolamine buffer). The colour reaction was measured at 405 nm[18].

Each test serum was analyzed in duplicate wells to minimize the handling error. For each ELISA plate, the average of blank values was subtracted from the absorbance value for each test serum.

Preparation of whole cell extracts for SDS-PAGE

H. pylori cells were harvested after confluent growth, and taken into pre-weighed screw-capped Eppendorf tubes. Bacterial cells were sedimented by centrifugation (12,5000x g for 2 min) and suspended in sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) solubilizing buffer[18] to a concentration of 500 µg /5 µl. The suspension was incubated at 100°C for 5 min to denature bacterial proteins and DNA was disrupted by brief sonication (30 sec). The suspension was reheated at 100°C for 2 min and diluted in SDS-PAGE solubilizing buffer to produce a final concentration of 70 µg whole cell extract per 5 µl. The aliquotes were stored at -20 °C until required.

SDS-PAGE

H. pylori whole cell extract for the SDS-PAGE profile was prepared using the method of Laemmli[19] as described previously[19]. Gels comprised of a 4.5% (w/v) acrylamide stacking gel and 12.5% (w/v) acryamide separation gel. Samples were applied to the gel alongside protein molecular weight standard (1610305, Bio-Rad, UK). Electrophoresis was performed using a mini-gel system (Consort, UK) with a constant current of 35 mA for 60 min. Subsequently theses gels were used for immunoblotting.

Immunoblotting

Immunoblotting technique as described previously[18] was used to determine the serostatus of *H. pylori* in the serum samples. The SDS-PAGE protein profiles were transferred onto nitrocellulose sheets[20] using a semi-dry electrotransfer apparatus (Bio-Rad, UK). Individual protein profile was prepared by cutting nitrocellulose sheets into strips. The strips were incubated separately with human serum (1: 200 dilution) samples (primary antibody). Antibody-antigen complexes were detected using an antihuman polyvalent antibody conjugated with alkaline phosphatase (A-5034, Sigma, UK), diluted 1:1000 in PBS. Colour development was carried out in a polyethylene bag at 37^{o} C in the dark for 10 min. Serum samples containing at least five types of anti-*H. pylori* antibodies were considered as seropositive for *H. pylori* infection[18].

Statistical method used

Analysis of data was carried out using SPSS (statistical package for social services) version 11.5. Proportions were compared by two tailed χ^2 – test. A '*p*' value <0.05 was taken as significant. '*p*' value <0.001 was taken as highly significant. Frequency was analysed and seroprevalence was calculated. Proportions were compared by two tailed χ^2 – test, and odds ratios (ORs) with 95% confidence intervals (95% CIs) were calculated.

Results and Discussion

The acquisition of *H. pylori* infection occurs mostly in early childhood and results in hypochlorhydria which may facilitate the subsequent attainment of other enteropathogens. *H. pylori* has been implicated as the underlying cause of peptic ulcer disease, non-ulcer dyspepsia, gastric MALT lymphoma, acute and chronic gastritis and extragastroduodenal disorders[2-3]. Infection with *H. pylori* has also been shown to be involved in micronutrient deficiencies and malabsorption of vitamins. These impacts may play a role in childhood malnourishment[2]. So, it is crucial to survey the seroprevalence of *H. pylori* infection amongst children living in developing countries and to diagnose the risk factors related to the acquisition of *H. pylori* infection and subsequent malnourishment.

Among the children attending a women and children based hospital in Dhaka for check up, vaccination or treatment programme during July 2007 to April 2008, 300 were enrolled in this work. The research included a total of 149 children (0 -12 years) with malnourishment (case) and 151 children (0 -12 years) without malnourishment (control).

Very limited serological cross-reactions of *H. pylori* with other bacteria[21] have encouraged the use of serodiagnostic approaches for the identification of this bacterial population. Our study diagnosed the presence of anti-*H. pylori* antibodies among collected serum samples by in house ELISA employing formalin fixed *H. pylori* whole cell antigen. Early studies employed crude antigens such as whole cells or whole cell sonicates with acceptable results[22]. Improved performance values were reported later using partially purified antigens such as acid glycine extracts or ultracentrifused supernatant from whole cell sonicate[23]. However, the importance of using highly purified antigens for the serodiagnosis *of H. pylori* infection is still a matter of controversy. Wulffen (1992)[24] reported the similar level of performance of crude antigens as measured by sensitivity analysis while compared that with more purified antigens.

For the diagnosis of serum samples by in house ELISA using formalin fixed *H. pylori* whole cell antigen and AP substrate system, absorbance >1 at 405nm were interpreted as positive for *H. pylori* infection by previous researchers [12]. Ninety-six serum samples were evaluated by both HRP and AP substrate system in this study to establish the cut-off value for HRP substrate system for the serodiagnosis of *H. pylori* infection. Accordingly, absorbance >0.2 at 490nm in HRP substrate system were considered as indicative of the presence for significant levels of anti *H. pylori* antibodies while the test serum samples were being examined by HRP substrate system. The immunoblot method has a very high sensitivity, one reason for this being the fact that any particular immunogenic protein concentrates in a very thin line on the nitrocellulose membrane. Hence, the cut-off value marked in ELISA was further authenticated (p = 0.0000) by immunoblotting technique, employing *H. pylori* (the same strain used to make whole cell antigen for ELISA) whole cell sonicated extract as antigen mixture. Crude antigens such as whole cell sonicates have been used by researchers previously with acceptable results[22].

Correlation of the two techniques (in house ELISA and immunoblotting) was determined by $\chi 2$ test (Table 1). For the preparation of antigen for ELISA (formalin fixed whole cell) and immunoblotting (sonicated whole cell extract), same strain of *H. pylori* was employed. Among the total samples (n = 300), 22 samples with absorbance values from each category (0.0 - 0.1; 0.1 – 0.2; 0.2 – 0.3; 0.3 – 0.4; 0.4 – 0.5; 0.5 – 0.6, 0.6 – 1; 1.0 – 2.0) obtained by ELISA were selected for performing immunoblotting (Table 1). Immunoblotting has been demonstrated to have high validity in this pediatric population and has been particularly useful in cases with doubtful ELISA results[25]. Highly significant correlation (p = 0.000) was found between ELISA and Immunoblotting.

The study population was divided into different age groups and seven groups were constructed accordingly. The number of children with malnourishment (case) and without malnourishment (control) in different age categories is shown in Table 2.

Table 1. *Correlation between ELISA and immunoblotting in determining seroprevalence of H. pylori*

Technique employed	No. of Samples tested	Sera with anti-*H pylori* antibodies	Sera without anti-*H. pylori* antibodies	χ^2 (*p*-value)
Immunoblotting	22	16¥	6¥	0.000
ELISA	*22*	*16**	*6**	

¥ In immunoblotting 5 or more than 5 antibody bands indicated positive results
*Absorbance values of e"0.2 at 490nm were considered as indicative of positive results by HRP substrate system

Table 2. *Age strata of 149 children with malnourishment (case) and 151 children without malnourishment (control)*

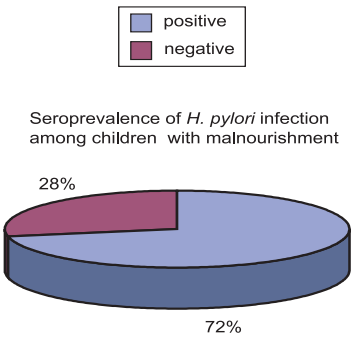
Age of children	Number (%) of children without malnourishment (Control)	Number (%) of children with malnourishment (Case)	Total Number (%) of children
0 – 6m	30(60%)	20(40%)	50(17%)
6 – 12m	11 (31%)	24 (69%)	35(12%)
1 – 2y	19 (35%)	35 (65%)	54(18%)
2 – 4y	20(43%)	26 (57%)	46 (15%)
4 – 6y	26 (53%)	23 (47%)	49 (16%)
6 – 8y	22 (67%)	11 (33%)	33(11%)
8 – 12y	23(70%)	10(30%)	33 (11%)
Grand total	151	149	300

Amongst the 149 case children, 107 (72%) contained anti-*H. pylori* antibodies and among the control population (n=151), 91 (60%) children possessed anti-*H. pylori* antibodies, with an overall seroprevalence of 66%.

Malnourishment was found to be more common in female children (56%) than in male children (44%) in this work. A male child in this society is considered more precious than a female child. This cultural behavior and subsequent discrimination may explicate the difference in the nutritional status observed among these two sex categories. However, no statistical correlation was observed between gender of the child and malnourishment in case and control group.

The degree of hygiene, nourishment intake and standard of living condition of a population is directly or indirectly regulated by Socio-economic status. All these factors are interrelated to the possibility of exposure to infectious agents such as *H. pylori* [3]. In this study, parental education and per capita per day income was used as measures of socioeconomic stratification. The per capita per day income of the studied population had been demonstrated to be the best indicator of socioeconomic status for this work. In a investigation performed among children in Turkey, it was demonstrated that seroprevalence of *H. pylori* infection was inversely related to economic status[26]. Highly significant relationship between socio-economic status and malnutrition was pronounced (p=0.000; p=0.000) from the tables (Table 3 and Table 4). It was seen that properly nourished children were primarily from relatively upper socio-economic situation whereas malnourished children were mostly from poor economic condition. Per day per capita income[16] (Table 3) was used as the indicator of socioeconomic status for the family of the children. Other socioeconomic status deciding indicators like father's education (Table 4) were also considered in this study and had revealed similar conclusion.

H. pylori infection among children population might be prevented by maternal antibodies. The defense may also have been due to predominant breast-feeding during early period of life that could have resulted in provision not only of specific antibodies but also of other possible protective components in breast milk as well as



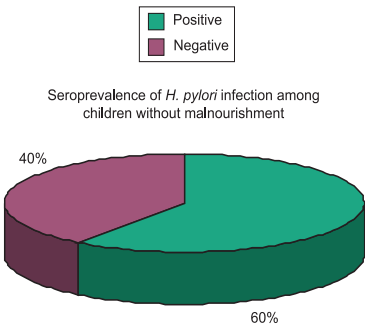


Fig. 1 and 2. *Seroprevalence of H. pylori infection among the children with malnourishment (case; n = 149), Fig. 2. Seroprevalence of H. pylori infection among the children without malnourishment (control; n = 151)*

Table 3. *Socio-economic status among malnourished (case) and nonmalnourished (control) children*

Socioeconomic status	Nonmalnourished children (Control)	Malnourished children (Case)	Total	χ^2 (p – value)
Poor	72(39%)	113(61%)	185	0.000
Medium	47(68%)	22 (32%)	69	
Upper (non poor)	32(70%)	14 (30%)	46	
Total	151	149	300	

Table 4. *Relationship between nutritional status and socio-economic condition among case and control children population*

Father's education status	Control	Degree of malnourishment			Total	χ^2 (p – value)
		Mild	Moderate	Severe		
Illiterate	10 (21%)	11 (22%)	9 (18%)	19 (39%)	49	0.000
Primary	18(35%)	16 (31%)	5(9%)	13 (25%)	52	
Secondary and higher secondary	58 (53%)	30 (27%)	14 (13%)	7 (6.4%)	109	
Graduate	65 (72%)	20 (22%)	4 (5%)	1 (1.1%)	90	
Total	151	77	32	40	300	

exposure to pathogenic microorganisms[27]. Researchers have shown that human milk inhibits adherence of *H. pylori* to certain receptor cells and thus might reduce the ability of colonization of these microorganisms to host epithelial cells[28]. Furthermore, mothers with higher titers of specific antibodies against *H. pylori* urease antigen had been reported recently to have lower frequency of *H. pylori* -colonized children than mothers secreting breast milk with lower titers of these antibodies[29].

Our research revealed the protective effect of exclusive breast-feeding (Table 5) up to 5 months of age against the development of malnourishment. Among the malnourished children, exclusive breast-feeding was found to be less practiced than the nonmalnourished or control children. The degree of severity of malnourishment increased inversely (Table 6) with the practice of exclusive breast-feeding up to 5 months of age ($p = 0.010$). The odds ratio (Table 6) relating breast-feeding to undernourishment (OR = 0.473; 95% CI = 0.275 to 0.812); p=0.006) reflected 52% reduction in the risk of development of malnourishment among breast-fed children.

The highly significant relationship of exclusive breast-feeding with nutritional status observed in this study echoed the importance of breast milk upon child health. Breast milk fully meets the nutritional requirements of the infant in the first few months of life, is easily digested and promotes "bonding" between the mother and the child. Moreover, breast milk contains maternal antibodies, which may protect the infants from various diseases and thus contributes to enhance their nutritional status[30]. Anti *H. pylori* IgG antibodies derived from the mothers and the specific IgA antibodies in breast milk has been speculated to play role in protection against *H. pylori* during infancy[31].

Factors for developing malnourishment might include unsafe and insufficient water supply, poor hygiene, and inadequate sanitation[32-33]. Water from tube well, groundwater and boiled pipeline water have been considered as safe for drinking for this research. Highly significant association (p=0.000) between drinking unsafe water and development of malnourishment was detected (Table 6) in our work. Unsafe water could amplify the risk of acquiring infectious diseases like diarrhea, shigellosis, enteric fever, cholera etc. The use of unsafe drinking water was associated with 5.33 (95% CI = 1.969 – 14.432) times higher odds of developing malnourishment than the children consuming safe water (Table 7) in this investigation.

Table 5. *Exclusive breast-feeding practice among malnourished (case) and nonmalnourished (control) children*

Exclusive breast feeding upto 5 months	Control	Degree of malnourishment			Total	χ^2 (p – value)
		Mild	Moderate	Severe		
Yes	124 (55%)	57 (25%)	122(9.7%)	23 (10.3%)	226	0.010
No	27 (36.5%)	20 (27%)	10 (13.5%)	17(23%)	74	
Total	151	77	32	40	300	

Table 6. *Association between exclusive breast-feeding and the risk of exposure to malnourishment among case and control children population*

Exclusive breast feeding upto 5 months	Children with malnourishment (Case)	Children without malnourishment (Control)	Total	χ^2 (p-value)	Odds Ratio (OR) (95% confidence interval)
Yes	102(45%)	124(55%)	226		0.473
No	47(64%)	27(36%)	74	0.006	(0.275 - 0.812)
Total	151	149	300		

Table 7. *Relationship between drinking unsafe water and malnourishment*

Status of drinking water	Children with malnourishment (Case)	Children without malnourishment (Control)	Total	χ^2 (*p*-value)	Odds Ratio (OR) (95% confidence interval)
Unsafe water	23(82%)	5(18%)	272	0.000	5.330 (1.969 - 14.432)
Safe water	126(46%)	146(54%)	28		
Total	151	149	300		

The present study indicated that Seroprevalence of *H. pylori* infection amongst malnourished children was significantly higher than the properly nourished children population in Bangladesh. *H. pylori* infection and related undernourishment was highly prevalent among young children belonging to poor socioeconomic conditions. The beneficial effects of breast feeding for preventing acquisition of malnourishment implicates that breast feeding practice should be encouraged throughout the young childhood age. Malnourishment was higher among female children, so this group should be monitored and taken care of. Poverty reduction and hygiene improvement strategies would play a prominent role in prevention of *H. pylori* infection and following development of malnourishment.

Acknowledgements

We would like to convey our gratitude to the participants of this study, and to the management and staff of Ad-din Hospital, Dhaka for their appreciable cooperation.

References:

1. Beckwith CS, McGee DJ, Mobley HL and Riley LK. 2001. Cloning, expression, and catalytic activity of *Helicobacter hepaticus* urease. *Infect Immun*. **69:** 5914-5920.
2. Johannes GK, van Vliet AHM and Kuipers EJ. 2006. Pathogenesis of *Helicobacter pylori* Infection. *Clin Microbiol Rev*. **19**(3): 449–490.
3. Soylu OB and Ozturk Y. 2008. *Helicobacter pylori* infection: effect on malnutrition and growth failure in dyspeptic children. *Eur J Pediatr*. **167**: 557 – 562.
4. Mahalanabis D, Rahman MM, Sarker SA, Bardhan PK, Hildebrand P, Baglinger C and Gyr K. 1996. Helicobacter pylori infection in young Bangladesh: Prevalence, socioeconomic and nutritional aspects. Intl J Epidemeol. **25**:894-898.
5. Windle JH, Kelleher D and Crabtree JE. 2007. Childhood *Helicobacter pylori* infection and growth impairment in developing countries: a viscous cycle? *Pediatrics* .**119**: 754-759.
6. Yakoob J, Jafri W and Abid S. 2003. *Helicobacter pylori* infection and micronutrient deficiencies. *World J Gastroenterol.* **9**(10): 2137-2139.
7. Tinberg Y, Bengtsson C, Bergstorm M and Granstorm M. 2001. The accuracy of serologic diagnosis of *Helicobacter pylori* infection in school-aged children of mixed ethnicity. *Helicobacter*. **6** (1): 24 – 30.
8. Dhar R, Mustafa AS, Dhar PM, Khan MS, Al-Rashidi FJ, Al-Shamali AA and Ali FH. 1998. Evaluation and comparison of immunological assays for *Helicobacter pylori* antibodies with culture results. *Diagn Microbiol Infect Dis*. **30**: 1- 6.
9. Nessa J, Chart H, Owen RJ and Drasar B. 2001. Human serum antibody response to *Helicobacter pylori* whole cell antigen in an institutionalized Bangladeshi population. *J Appl Microbiol*. **90**: 68-72.
10. Dooley CPF, Cohen PL, Bauer H, Appleman M, Perej-Perej MD and Baser MJ. 1989. Prevalence of *Helicobacter pylori* infection and histological gastritis in asymptomatic persons. *N Engl J Med*. **321**: 1562-1566.
11. *Rahman MM, Mahalanabis D, Sarker SA, Bardhan PK, Hildebrand P, Baglinger C and Gyr K. 1998. Helicobacter pylori* colonization in infants and young children is not necessarily associated with diarrhea. *J Trop Ped*. **44**(2): 280 – 287.
12. Islam ABMMK, Yasmin M, Ahasn CR and Nessa J. 2005. *Helicobacter pylori* infection in Bangladeshi population: Serodiagnosis, Seroprevalence and risk factors. *Dhaka Univ J Bio Sci.* **14**(1): 1-8.
13. Hamill PV, Drizd TA, Johnson CL, Reed RB, Roche AF and Moore WM. 1979. Physical growth: National Center for Health Statistic Percentages. *Am J Clin Nutr.* **32**:607-29.
14. Islam MZ, Yesmin L, Rahman MM and Hassan F. 2005. In: Health, Nutrition and Population Sector. Human Resources Management, Planning and Development Unit, Ministry of Health and Family Welfare, Government of People Republic of Bangladesh. Pp- 283.
15. Pelletier JG. 1993. In: Severe Malnutrition: A Global Approach. Children in the Tropics. pp. 30-32.

16. Wahed T, Bhuiya A, Chowdhury AMR and Ahmed SM. 2007. Use of Household Income and Consumption Data as a Measure of Poverty in Rural Bangladesh, ICDDR, B. pp. 1-2. Scientific Report No. 99.

17. Haque W, Ahsan CR, Nessa J and Yasmin M. 2009. Association between malnutrition and other possible risk elements with the acquisition of *H. pylo*ri infection among the children population in Bangladesh. *Bang J Microbiol*. **26**(1&2): 9-14.

18. Rahman F, Islam ABMMK, Nessa J, Ahsan CR and Yasmin M. 2005. Correlation in prevalence, risk factors and serological detection of *Helicobacter pylori* by different teqniques. *Bang J Microbiol*. **22**: 152-157.

19. Laemmli UK. 1970. Cleavage of structural proteins during the assembly of bacteriophage T_4. *Nature*. **227**: 680 – 685.

20. Towbin H and Gordon J. 1984. Immunoblotting and dot immunoblotting – Current status and outlook. *J Immunol Methods*. **72**: 313 – 340.

21. Andersen LP and Espersen F. 1992. Immunoglobulin G antibodies to *H. pylori* in patients with dyspeptic symptoms investigated by the western immunoblot technique. *J Clin Microbiol***. 30:**1743-1751.

22. Jones DM, Lessels AM and Eldridge J.1984. *Campylobcter* like organisms on the gastric mucosa: culture, histological and serological studies. *J Clin Path*.**37**: 1002 -1006.

23. Rathbone BJ, Shires SE, Towsend C, Wyatt JI, Tompkins DS, Heatly RV and Losowky MS. 1988. Serum *Campylobacter* specific IgG ELISA as a marker of colonization in prevalence studies. In Kaijser H, Falsen E (ed): *Campylobacter* IV. University of Goteborg, p-182.

24. Wulffen von H.1992. An assessment of serological test for detection of *H. pylori*. Eur J Clin.**11**: 577 -582.

25. Grantham-McGregor S, Ani C. 2001. A review of studies on the effect of iron deficiency on cognitive development in children. *J Nutr.* **131**(2S–2): 649S –666S.

26. Kaya AD, Gencay E, Ozturk CE and Yavuz T. 2008. Seroprevalence of *Helicobacter pylori* infection in children in Northwest Region of Turkey: Relationship with iron deficiency anemia. *J Trop Pediatr*. **54**(5): 353-354.

27. Bhuiyam TR, Qadri F, Saha A and Svennerholm AM. 2009. Infection by *Helicobacter pylori* in Bangladeshi children from birth to two years. *Pediatr Infect Dis J.* **28:** 79-85.

28. Clyne M, Thomas J, and Weaver L. 1997. *In vitro* evaluation of the role of antibodies against *Helicobacter pylori* in inhibiting adherence of the organism of gastric cells. *Gut.* **40**: 731– 738.

29. Thomas JE, Bunn JE, Kleanthous H, et al. 2004. Specific immunoglobulin A antibodies in maternal milk and delayed *Helicobacter* colonization in Gambian infants. *Clin Infect Dis.* **39**: 1155 – 1160.

30. Ahmed F, Clemens JD, Rao MR, Sack DA, Khan MR, Haque E. 1992. Community-based evaluation of the effect of breast-feeding on the risk of microbiologically confirmed or clinically presumptive shigellosis in Bangladeshi children. *Pediatrics.* **90**(3): 406-11.

31. Bhuiyan TR, Saha A, lundgren A, Qadri F and Svennerholm AM. 2010. Immune responses to Helicobacter pylori infection in Bangladeshi children during their first two years of life and the assciatoion between maternal antibodies and onset of infection. *The J of Inf Dis*. **202**(11): 1676-1684

32. Mahbub MM, Yasmin M, Ahsan CR and Nessa J. 2008. Influence of some sociodemographic factors on *Shigella flexneri* infection and malnourishment among the children population in Bangladesh. *Bang J Med Sci*. **14**(1): 67-74.

33. Chart H, Jenkins C, Smith HR and Rowe B. 1998. Serum antibodies to secreted proteins in patients infected with *Escherichia coli* O157 and other VTEC. *Epidemiol Infect*. **129**: 239 – 243.