Prevalence of *Helicobacter pylori* infection among children with primary nephrotic syndrome: a cross-sectional study

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Abstract

**Background:** Limited data are available about the prevalence of *Helicobacter pylori* (*H.pylori*) infection among primary NS children.

**Objectives:** To assess the frequency and risk factors of *H.pylori* infection among children with primary NS.

**Methods:** A cross-sectional study was carried out in Mansoura University Children's Hospital, Egypt during the period from 2017 to 2019 including 100 NS children (NS group) and 100 healthy controls. NS group included 88 steroid sensitive (SSNS) and 12 steroid resistant (SRNS) cases. All patients were assessed for *H.pylori* infection using *H.pylori* stool antigen (HpSA) test. Statistical analysis was done using chi-square, Fisher exact and Mann-Whitney tests.

**Results:** With regard to HpSA test results, no significant differences were detected between control and NS groups (p = 0.193) and between SSNS and SRNS groups (p = 0.286). Concerning total biopsied cases and MCD (proven plus presumed) cases, no significant differences were found between those with positive and negative HpSA test (p = 0.648 and 0.126, respectively). The high dose of steroid therapy was associated with a higher risk of *H.pylori* infection among NS group (Odds ratio = 3.8; 95% confidence interval = 1.3-11.3).

**Conclusion:** The current study negates the increased risk of *H.pylori* infection in children with primary NS.

**Keywords:** Children; *H.pylori*; primary nephrotic syndrome.

**DOI:** https://dx.doi.org/10.4314/ahs.v20i4.15.

**Cite as:** Mahmoud A, Bakr A, Elsaid A, Wahba Y. Prevalence of Helicobacter pylori infection among children with primary nephrotic syndrome: a cross-sectional study. Afri Health Sci. 2020;20(4):1624-31. https://dx.doi.org/10.4314/ahs.v20i4.15.

Introduction

Nephrotic syndrome (NS) is a relatively common pediatric disease¹ with an immunological background proved by the successful therapeutic effects of steroids and immune-modulating drugs²,³.

*Helicobacter pylori* (*H.pylori*) are gram-negative bacteria that selectively colonize the gastric mucosa. The organism is spiral shaped with three to five polar flagella, and characterized by being urease, oxidase and catalase positive⁴. A recent meta-analysis revealed that about four billion persons were colonized with *H.pylori* worldwide in 2015⁵. In developing countries, *H.pylori* infection is highly prevalent and represents a great challenge⁶,⁷. In Egypt, this infection is very common reaching up to 72% among school children⁸.

Now, there is agreement that *H.pylori* is not only associated with gastric diseases but also with immune-mediated extra-gastrointestinal disorders⁹. It is associated with atopic dermatitis, Henoch-Schönlein purpura, idiopathic thrombocytopenic purpura, systemic sclerosis, recurrent aphthous stomatitis, alopecia areata and Sjogren’s syndrome. In addition, *H.pylori* eradication could help in management of Behcet’s disease. These effects could be attributed to the chronic systemic inflammatory state induced by the organism¹⁰,¹¹.

Limited trials were conducted in the pediatric age group to detect the association between *H.pylori* infection and NS with contradictory results⁶,¹²,¹³. The current study assessed the frequency of *H.pylori* infection among children with primary NS and the related risk factors.

Materials and methods

**Study design and participants**

A cross-sectional comparative study was carried out from January 2017 to January 2019 including 100 primary NS children (NS group) and 100 healthy controls. NS patients were recruited from both inpatient wards and outpatient nephrology clinics of Mansoura University Children's Hospital, Mansoura University Children's Hospital, Biochemistry Section.
sity Children’s Hospital, Egypt. NS group included 88 steroid sensitive (SSNS) and 12 steroid resistant (SRNS) cases. Among SSNS cases, 43 patients were infrequent relapsers, 30 patients were steroid dependent, 12 patients were first attack and 3 patients were frequent relapsers. The controls were recruited from children attending the same hospital with minor complaints e.g. mild gastroenteritis and pharyngitis.

Our study was accepted by Institutional Research Board of Medical Faculty of Mansoura University, Egypt (Code No: MS/16.08.35) and followed the Helsinki Declaration of 1975, as revised in 2000. Informed written consents were obtained from legal guardians of all study participants.

All primary NS children with persistent dyspeptic symptoms were included. NS was defined as the presence of edema, serum albumin < 2.5 gm/dl, nephrotic range proteinuria (≥240 mg/m²/h or protein/creatinine ratio >2 mg/mg) and elevated serum cholesterol and triglycerides levels. Patients were labeled as SSNS if in remission, infrequent relapse, frequent relapse or steroid dependent. Steroid resistance means no remission despite daily prednisolone therapy at a dose of 2 mg/kg/d for four weeks. Patients were in remission if urinary albumin was trace or nil for three consecutive early morning specimens. Frequent relapse was defined as two or more relapses in the initial six months or more than three relapses in any 12 months of treatment. Steroid dependence means occurrence of two consecutive relapses within two weeks of steroid discontinuation or when on alternate day steroids.

Patients were excluded if secondary NS, received H. pylori treatment within the last three months before participation in the study, received proton pump inhibitors within two weeks prior to sampling, or on an antibiotic treatment at the time of the testing procedure.

History and clinical examination
Patients were subjected to detailed history taking with special emphasis on the response to steroids and the dose and duration of steroids therapy. Treatment of an initial episode of NS included oral prednisolone as a single daily dose starting at 2 mg/kg/d to a maximum 60 mg/d for 4 weeks followed by alternate-day dosing as a single daily dose starting at 1.5 mg/kg (maximum 40 mg/d) and continued for 2.5 months with gradual dose tapering. The steroid therapy should be given for at least 12 weeks. Low-dose steroid was initiated for relapse of SSNS using oral prednisolone 1 mg/kg/d (maximum 40 mg/d) for a minimum of 7 days. Once the patient was in remission and/or had completed 7 days of steroid therapy, gradual tapering of prednisolone was done over a month. If the patient developed progressive edema or failed to get remission within 7 days of therapy initiation, the prednisolone dose was increased to the standard high-dose regime (2 mg/kg/d). All patients were assessed for gastritis symptoms as dyspepsia, vomiting, recurrent abdominal pain and epigastric tenderness.

Laboratory evaluation
All participants were subjected to urine analysis, 24-h urine protein assessment/protein-creatinine ratio and serum levels of creatinine, albumin, cholesterol and triglycerides. Data about renal histopathology were retrieved from patients’ files. Renal biopsy was done for only 38 cases (29 SSNS and 9 SRNS cases). Four SSNS cases (steroid dependent) and three SRNS cases were not biopsied due to patient guardians’ refusal or current contraindications for renal biopsy (bleeding tendency).

H. pylori infection was assessed using H. pylori stool antigen test (HpSA test; ImmunoCard STAT!®; Meridian Bioscience Europe, European Union; Catalog Number: 750220). HpSA test is simple, rapid, non-invasive, and can detect the active current infection. It is considered as a 2nd generation rapid lateral flow immunoassay test using monoclonal anti-H. pylori antibodies. The specificity and sensitivity of the test are 99.2% and 94.3%, respectively as reported in the manufacturer’s product information. The test value for H. pylori diagnosis was well recognized in previous reports.

Stool samples were collected from all study participants, transported in airtight containers and tested as soon as possible but might have been held at 2°–8°C for 72 hours before testing. If testing could not be done with-
in this time frame, specimens were frozen immediately upon receipt and stored at -20° to -80°C until tested. Each stool sample (5-6 ml) was mixed thoroughly with 1 ml diluent in a test tube. Then, we dipped the reaction strip in the test tube for 10 seconds, and read the results after 5 minutes. We considered the test negative if only a blue colored band (the control line) appeared across the white central area of the reaction strip, and positive if an additional pink red band (the test line) also appeared. Any pink red line, even very weak, was considered positive. The band intensity varied according to the concentration of the antigen in the specimen. Any color or line appearing after 10 minutes had no diagnostic value. The test was considered invalid if the control band was absent.

**Statistical analysis**

SPSS version 21 was used to analyze data. Kolmogorov-Smirnov test was used to assess the data for normality. Qualitative data were described using numbers and percent. Chi-square and Fisher exact tests were used to describe the associations between the categorical variables. Non-parametric continuous variables were expressed as median (min-max), and analyzed using Mann-Whitney test. Logistic regression analysis was done for the risk factors associated with positive HpSA test among NS group. P ≤ 0.05 was considered significant.

**Results**

Both NS and control groups were matched for the gender and age. The median age of NS group was 9 (1-17) years, while that of the control group was 8.5 (2-14) years (p = 0.083). Males constituted the majority of the study participants (67 children in NS group and 57 children in control group, p = 0.145).

With regard to HpSA test results, no significant difference was found between control and NS groups (Table 1, p = 0.193). Also, table 2 shows no significant difference between SSNS and SRNS subgroups (p = 0.286).

No significant difference was detected between total biopsied NS cases (n = 38) with positive HpSA test (20, 52.63%) and negative HpSA test (18, 47.37%) (p = 0.648). Mesangioproliferative glomerulonephritis and minimal change disease (MCD) were more frequent among biopsied NS group {16 patients (42.1%) and 10 patients (26.3%), respectively} followed by focal segmental glomerulosclerosis (5 patients, 13.2%), membranoproliferative glomerulonephritis (4 patients, 10.5%) and three patients (7.9%) had IgA nephropathy. MCD was the most frequent renal histopathology among biopsied NS patients with positive HpSA test (9 patients) with a significant difference when compared to those with negative HpSA test (1 patient) (p = 0.013). With regard to non-biopsied SSNS patients mostly presumed to be MCD (n = 59), 21 cases had positive HpSA test and 38 cases had negative HpSA test. Concerning the total MCD cases (proven plus presumed cases, n = 69), no significant difference was reported between those with positive HpSA test (n = 30; 43.48%) and negative HpSA test (n = 39; 56.52%) (p = 0.126) (Table 3).

| HpSA test | NS group (n=100) | Control group (n=100) | p value |
|-----------|----------------|----------------------|---------|
| Positive  | 44 (44%)       | 35 (35%)             | 0.193   |
| Negative  | 56 (56%)       | 65 (65%)             |         |

HpSA: Helicobacter pylori stool antigen, NS: Nephrotic syndrome.

Data are shown as numbers and percent and analyzed by chi-square test.

**Table 1** Distribution of results of *helicobacter pylori* stool antigen test among children with primary nephrotic syndrome (NS) and control groups

African Health Sciences, Vol 20 Issue 4, December, 2020 1626
The high dose of steroid therapy was associated with a 3.8-fold higher risk of *H. pylori* infection among NS group (Odds ratio = 3.8; 95% confidence interval = 1.3-11.3) (Table 4).

Table 2 Distribution of results of *helicobacter pylori* stool antigen test among children with steroid sensitive NS and steroid resistant NS

| HpSA test | Steroid sensitive NS (n=88) | Steroid resistant NS (n=12) | p value |
|-----------|-----------------------------|-----------------------------|---------|
| Positive  | 37 (42%)                    | 7 (58.3%)                   |         |
| Negative  | 51 (58%)                    | 5 (41.7%)                   | 0.286   |

HpSA: *Helicobacter pylori* stool antigen, NS: Nephrotic syndrome.
Data are shown as numbers and percent and analyzed by chi-square test.

Table 3 Distribution of renal histopathology among nephrotic syndrome children with positive and negative *helicobacter pylori* stool antigen (HpSA) tests

| Renal histopathology | Positive HpSA test | Negative HpSA test | p value |
|----------------------|--------------------|--------------------|---------|
| Biopsied NS cases (total number =38) | 20(52.63%) | 18(47.37%) | 0.648   |
| -Proven MCD | 9 (45%) | 1 (5.55%) | 0.013   |
| -Mesangiproliferative GN | 6 (30%) | 10 (55.55%) | 0.047   |
| -FSGS | 4 (20%) | 1 (5.55%) | 0.363   |
| -Membranoproliferative GN | 1 (5%) | 3 (16.66%) | 0.3     |
| -IgA nephropathy | 0 | 3 (16.66%) | -       |
| Presumed MCD (non-biopsied SSNS, n=59) | 21(35.59%) | 38(64.41%) | 0.0018  |
| Total MCD (proven plus presumed MCD) | 30(43.48%) | 39(56.52%) | 0.126   |

FSGS: Focal segmental glomerulosclerosis, GN: Glomerulonephritis, HpSA: *Helicobacter pylori* stool antigen, MCD: Minimal change disease, N: Number, SSNS: Steroid sensitive nephrotic syndrome. Data are shown as numbers and percent and analyzed by chi-square and fisher exact tests.
Discussion

Several studies were conducted to assess the possible role of \textit{H. pylori} infection in NS pathogenesis\textsuperscript{16,19–21}. The current study reported an insignificant difference between healthy controls and primary NS children with regard to HpSA test. This observation was in contrast to Zajaczkowska et al\textsuperscript{12}, Moriyama et al\textsuperscript{16} and Nagashima et al\textsuperscript{20} (Table 5). The only report that agrees with our results was that of Nutpho and Ukarapol\textsuperscript{13} (Table 5). This discrepancy could be explained by variability of the screening methods for \textit{H. pylori}, different sample size and study design. This observation may negate the increased risk of \textit{H. pylori} infection in children with primary NS.
On reviewing the literature, some authors reported presence of *H. pylori* antigens in glomeruli of renal biopsy specimens, but failed to confirm the link between the organism and NS pathogenesis. They depended upon the presumed hypothesis that *H. pylori* could increase the risk of NS through immune-mediated reactions induced by direct antigenic effects or auto-antibodies formation. The effects of *H. pylori* eradication on the treatment response was also tested in few cohort studies. Some authors found insignificant improvement in proteinuria after *H. pylori* eradication, while others failed to prove that the improvement in proteinuria was due to *H. pylori* treatment only and not due to spontaneous remission. Even for studies that proved improvement in proteinuria due to eradication of *H. pylori*, the small sample size limits generalization of their results. Thus further randomized controlled trials (RCTs) with larger sample sizes are needed to provide more details about the exact pathogenic role of *H. pylori* in NS.

Moreover, the current study was the first to describe an insignificant difference between SSNS and SRNS chil-

| Study | Studied groups | Methods for *H. pylori* screening | Study outcome |
|-------|---------------|----------------------------------|--------------|
| Nagashima et al<sup>20</sup> | 16 Adults with MN | Serology and immuno-staining for *H. pylori* in glomeruli | They found specific antigens in glomeruli and suggested *H. pylori* role in MN pathogenesis. |
| Zajaczkowska et al<sup>22</sup> | 20 children with NS | Urea breath test | NS children were more prone to *H. pylori* than controls. The longer steroid therapy was associated with a higher *H. pylori* risk. |
| Moriyama et al<sup>20</sup> | 32 Adults with primary MN & 243 controls | Serology | *H. pylori* was higher in MN than controls (p < 0.05). Its eradication reduced proteinuria in 3 of 4 MN cases who received steroids. |
| Nutpho & Ukarapol<sup>13</sup> | 60 immunocompromised Thai children including 9 NS patients | Rapid HpSA test | One out of 9 NS children was positive for *H. pylori* antigens. |
| Sugimoto et al<sup>21</sup> | A 34-years old Japanese woman with stage I MN | Histopathology and immuno-staining for *H. pylori* antigens in renal glomeruli | No *H. pylori* antigens deposited in the glomeruli. *H. pylori* eradication reduced proteinuria. |
| El-Latif et al<sup>27</sup> | 60 immunocompromised Egyptian children including 15 NS patients & 30 healthy controls | Serology | Seroprevalence of *H. pylori* was higher in secondary immunodeficiency than controls (p = 0.02) and insignificantly higher in malignancy than NS (p = 0.51). |
| Caliskan et al<sup>28</sup> | 59 adults (35 MN, 12 IgA nephropathy & 12 FSGS) | Rapid HpSA test & immuno-staining for *H. pylori* antigens in renal glomeruli | Negative staining for *H. pylori* antigens in renal glomeruli. *H. pylori* infection was higher in IgA nephropathy (83%) than MN (54%) (p = 0.045). *H. pylori* eradication decreased proteinuria in MN patients; however spontaneous remission could not be excluded. |
| Dede et al<sup>24</sup> | 33 adults (10 IgA nephropathy, 7 MN, 6 mesangioproliferative GN, 5 FSGS and 5 membranoproliferative GN) | Urea breath test & histopathology | *H. pylori* eradication failed to improve proteinuria (p = 0.99). |

**Table 5** Various studies about *helicobacter pylori* infection and nephrotic syndrome

FSGS: Focal segmental glomerulosclerosis, GN: Glomerulonephritis, HpSA: *Helicobacter pylori* stool antigen, MN: Membranous nephropathy, NS: Nephrotic syndrome

1629 African Health Sciences, Vol 20 Issue 4, December, 2020
dren with regard to H. pylori infection. However, the
high dose of steroid therapy was associated with a high-
er H. pylori risk among NS group, possibly due to the
immuno-suppressant effect of the high-dose steroid9.

In the current study, the age of patients was not con-
sidered a risk factor for H. pylori among NS group. This
observation copes with a previous national report9. Other international studies with larger sample sizes
proved that H. pylori infection rates increase with ad-
vancing age, mostly due to cumulative frequencies25-27.
Also, the gender of patients was not a risk factor for
H. pylori infection that agrees with many international
reports25,26,28,29. However, other studies reported male
predominance27,30. This discrepancy could be explained
by differences in study design and sample size.

Unexpectedly, the total duration of steroids therapy did
not increase the risk for H. pylori, possibly due to inter-
rupted courses of therapy with variable intervals that
increased the therapy duration among patients. This
finding was in contrast to Zajaczkowska et al12.

Concerning total biopsied NS cases and MCD (prov-
en plus presumed cases), the insignificant differences
found between those with positive and negative HpSA
tests suggest lack of association between H. pylori infec-
tion and renal histopathology in NS group. Our find-
ings were in variance with Nagashima et al20.

Conclusion
The current study provides useful information about
H. pylori prevalence among primary NS children in
Egypt. Our study negates the increased risk of H. pylori infection in children with primary NS. The high dose of
steroid therapy was associated with a higher H. pylori risk
among those patients. Large scale RCTs multicenter
studies are still needed.

Limitations of the study
A single center association study with limited studied
H. pylori risk factors, lack of long term follow-up and a
potential selection bias of patients.

Funding section
This research received no grant from any funding agen-
cy in the public, commercial or not-for-profit sectors.

Declaration of interest
The authors declare no conflicts of interest.

List of abbreviations
CI: Confidence interval
FSGS: Focal segmental glomerulosclerosis
GN: Glomerulonephritis
H. pylori: Helicobacter pylori
HpSA: Helicobacter pylori stool antigen
MCD: Minimal change disease
MN: Membranous nephropathy
NS: Nephrotic syndrome
OR: Odds ratio
RCTs: Randomized controlled trials
SPSS: Statistical Package For The Social Sciences
SRNS: Steroid resistant nephrotic syndrome
SSNS: Steroid sensitive nephrotic syndrome

References
1. Chanchlani R, Parekh RS. Ethnic differences in
childhood nephrotic syndrome. Front Pediatr 2016;4:39
2. El-Hakim IZ, Mohammed AA, Afifi HM, El-Sayed
SA. Circulating dendritic cells in pediatric patients with
nephrotic syndrome. Egypt J Pediatr Allergy Immunol
2011;9(1):41–47
3. Segarra-Medrano A, Carnicer-Cáceres C, Ar-
bós-Via MA, Quiles-Pérez MT, Agrav-Pamplona I,
Ostos-Roldán E. Biological markers of nephrotic syn-
drome: a few steps forward in the long way. Nefrologia
2012;32(5):558–572
4. Wroblewski LE, Peek RM, Wilson KT. Helicobacter
pylori and gastric cancer: factors that modulate disease
risk. Clin Microbiol Rev 2010; 23(4):713–739
5. Hooi JKY, Lai WY, Ng WK, Suen MMY, Underwood
FE, Tanyingoh D, et al. Global prevalence of Helicobac-
ter pylori infection: systematic review and meta-analysis.
Gastroenterology 2017;153(2):420–429
6. Muhsen K, Ornoy A, Akawi A, Alpert G, Cohen
D. An association between Helicobacter pylori infection
and cognitive function in children at early school age: a
community-based study. BMC Pediatr 2011;11:43
7. Zamani M, Ebrahimtabar F, Zamani V, Miller WH,
Alizadeh-Navaei R, Shoek-Shirvani J, et al. Systematic-
ic review with meta-analysis: the worldwide prevalence
of Helicobacter pylori infection. Aliment Pharmacol Ther
2018; 47(7):868–876
8. Mohammad MA, Hussein L, Coward A, Jackson SJ.
Prevalence of Helicobacter pylori infection among Egyp-
tian children: impact of social background and effect
on growth. Public Health Nutr 2008;11(3):230–236
9. El-Latif AMA, Ali ASA, Abdel-Hady M, Borai MBM.
Seroprevalence of Helicobacter pylori in secondary immu-
nocompromised children. J Am Sci 2011;7(9):592–595
10. Tan HJ, Goh KL. Extragastrointestinal manifestations of Helicobacter pylori infection: facts or myth? A critical review. *J Dig Dis* 2012;13(7):342–349
11. Fathy G, Said M, Abdel-Raheem SM, Sanad H. Helicobacter pylori Infection: A possible predisposing factor in chronic plaque-type psoriasis. *J Egypt Women Dermatol Soc* 2010;7(1):39–43
12. Zajaczkowska M, Durakiewicz T, Papierkowski A. The assessment of Helicobacter pylori eradication with the urea breathing test (13C UBT). *Pol Merkur Lek organ Pol Tow Lek* 2000;8(46):230–232
13. Nutpho P, Ukarapol N. Helicobacter pylori and immunocompromised children. *Emerg Infect Dis* 2006;12(1):171–172
14. Gipson DS, Massengill SF, Yao L, Nagaraj S, Smoyer WE, Mahan JD, et al. Management of childhood onset nephrotic syndrome. *Pediatrics* 2009;124(2):747–757
15. Bagga A. Revised guidelines for management of steroid-sensitive nephrotic syndrome. *Indian J Nephrol* 2008;18(1):31–39
16. Moriyama T, Kaneko T, Fujii M, Tsubakihara Y, Kawano S, Imai E. High prevalence of Helicobacter pylori infection in Japanese patients with membranous nephropathy. *Aliment Pharmacol Ther* 2006;24:189–193
17. Lombel RM, Gipson DS, Hodson EM. Treatment of steroid-sensitive nephrotic syndrome: new guidelines from KDIGO. *Pediatr Nephrol* 2013;28:415–426
18. Raja K, Parikh A, Webb H, Hothi D. Use of a low-dose prednisolone regimen to treat a relapse of steroid-sensitive nephrotic syndrome in children. *Pediatr Nephrol* 2017;32(1):99–105
19. Caliskan B, Yazici H, Caliskan Y, Ozeluk Y, Gulluoglu M, Kilicaklan I, et al. The effects of Helicobacter pylori eradication on proteinuria in patients with primary glomerulonephritis. *Int J Nephrol* 2014;180690
20. Nagashima R, Maeda K, Yuda F, Kudo K, Saitoh M, Takahashi T. Helicobacter pylori antigen in the glomeruli of patients with membranous nephropathy. *Virchows Arch* 1997;431(4):235–239
21. Sugimoto T, Furukawa T, Maeda T, Somura M, Uzu T, Kashiwagi A. Marked reduction of proteinuria after eradication of gastric Helicobacter pylori infection in a patient with membranous nephropathy: coincidental or associated? *Intern Med* 2007;46(17):1483–1484
22. Ma JY, Borch K, Sjöstrand SE, Janzon L, Mårdh S. Positive correlation between H, K-adenosine triphosphatase autoantibodies and Helicobacter pylori antibodies in patients with pernicious anemia. *Scand J Gastroenterol* 1994;29(11):961–965
23. Negri R, Lisato L, Zanella I, Cavazzini L, Gulini S, Villanacci V, et al. Helicobacter pylori infection induces antibodies cross-reacting with human gastric mucosa. *Gastroenterology* 1991;101(2):437–445
24. Dede F, Ayl D, Gonul I, Yüksel O, Oztürk R, Yildiz A, et al. The effect of Helicobacter pylori eradication on proteinuria in patients with primary glomerulonephritis. *Arch Med Sci* 2015;11(4):764–769
25. Soltani J, Amirzadeh J, Nahedi S, Shahsavari S. Prevalence of Helicobacter pylori infection in children, a population-based cross-sectional study in west Iran. *Iran J Pediatr* 2013;23(1):13–18
26. Oleastro M, Pelerito A, Nogueira P, Benoliel J, Santos A, Cabral J, et al. Prevalence and incidence of Helicobacter pylori Infection in a healthy pediatric population in the Lisbon area. *Helicobacter* 2011;16(5):363–372
27. Hestvik E, Tylleskar T, Kaddu-Mulindwa DH, Ndeze G, Grahnquist L, Olafsdottir E, et al. Helicobacter pylori in apparently healthy children aged 0-12 years in urban Kampala, Uganda: a community-based cross sectional survey. *BMC Gastroenterol* 2010;10:62
28. Talaiezadeh A, Borhani M, Moosavian M, Rafiee A, Kazem Neisi A, Hajiani E, et al. Prevalence of Helicobacter pylori Infection evaluated by Stool antigen test in Khuzestan Province since September to October 2009, south-west of Iran: a population based study. *Jundishapur J Microbiol* 2013;6(2):100–104
29. Nguyen BV, Nguyen KG, Phung CD, Krempe O, Kalach N, Dupont C, et al. Prevalence of and factors associated with Helicobacter pylori infection in children in the north of Vietnam. *Am J Trop Med Hyg* 2006;74(4):536–539
30. de Martel C, Parsonnet J. Helicobacter pylori infection and gender: a meta-analysis of population-based prevalence surveys. *Dig Dis Sci* 2006;51(12):2292–2301