Does maternal *Helicobacter pylori* infection increase the risk of occurrence of neural tube defects in newborns in Northern Iran?

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ABSTRACT

الأهداف: تحديد العلاقة بين عدوى جرثومة الملوية البوابية المنقولة من الأم وظهور عيوب الأنبوب العصبي لدى حديثي الولادة.

الطريقة: أُجريت هذه الدراسة للحالات المراقبة في مستشفى ديزياني التعليمي، غورغان، إيران وذلك خلال الفترة من أبريل 2007م إلى مارس 2009م. شملت الدراسة 35 مولود مصاب بعيوب الأنبوب العصبي (مجموعة الدراسة)، و53 مولود معافى من مثل هذه العيوب (مجموعة الشاهد). لقد قمنا بسحب عينات الدم المحيطي من أجل تحليلها، بالإضافة إلى التحري عن الأجسام المضادة لجرثومة الملوية البوابية. وقمنا بعمل التحليل المختبري لمستويات حامض الفوليك، وفيتامين ب 12، والفيريتين، والهوموسيستين. بعد ذلك قمنا بتحليل البيانات بواسطة حساب نسبة الأرجحية، وتحليل الانحدار اللوجستي.

النتائج: لقد ثبت ظهور الأجسام المضادة IgG للجرثومة الملوية البوابية لدى 43% من المشاركين في مجموعة الدراسة، و26% من المشاركين في مجموعة الشاهد، وكان الاختلاف بين المجموعتين غير كبير من الناحية الإحصائية. ولم يؤدي ظهور الجرثومة الملوية البوابية في مصل الدم إلى زيادة خطر الإصابة بعيوب الأنبوب العصبي وذلك من الناحية الإحصائية (OR: 2.08, 95% CI: 0.84–5.17, p=0.11). ولقد قمنا باكتشاف نقص فيتامين ب 12 لدى 17% من مجموعة الدراسة، و13% من الشاهد، وكذلك نقص حامض الفوليك لدى 17% من مجموعة الدراسة، و13% من الشاهد (p=0.61). ولم يرتبط ظهور جرثومة الملوية البوابية بصورة واضحة من الناحية الإحصائية بنقص الفولات في مصل الدم (OR: 1.93, 0.58–6.4, p=0.34)، ونقص الفيريتين (OR: 1.24, CI: 0.42–3.60, p=0.68).

خاتمة: أظهرت الدراسة بأن بإمكان عدوى جرثومة الملوية البوابية زيادة خطر الإصابة بعيوب الأنبوب العصبي لدى حديثي الولادة عند انتقالها من الأم.

Objective: To determine the relation between maternal *Helicobacter pylori (H. pylori)* infection and the occurrence of neural tube defects (NTDs) in newborns.

Methods: This hospital-based case-control study was carried out in Dezyani Teaching Hospital, Gorgan, Northern Iran from April 2007 to March 2009. Thirty-five mothers with NTD-affected newborns, and 53 mothers with healthy newborns were considered the cases and controls. A peripheral blood sample was obtained from all subjects, and *H. pylori* infections were tested by *H. pylori* serum antibody. The serum folic acid, vitamin B12, ferritin, and homocysteine concentrations were measured by laboratory tests. Data were analyzed using odds ratio (OR) and logistic regression.

Results: Forty-three percent of cases, and 26% of controls were positive for *H. pylori* IgG antibody, and this difference was not significant. The *H. pylori* seropositivity non significantly increased the risk of NTD-affected pregnancies (OR: 2.08; 95% confidence interval [CI]: 0.84-5.17, p=0.11). Serum vitamin B12 deficiency was detected in 17% of cases and 13% of controls, and folic acid deficiency in 17% of cases and 13% of controls (p=0.61). The *H. pylori* seropositivity was non significantly associated with low serum folate (OR 1.93 CI: 0.58-6.4, p=0.34) and ferritin (OR 1.24; CI: 0.42-3.60, p=0.68).

Conclusions: Maternal *H. pylori* infection can increase the risk of occurrence of NTDs in newborns.

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Neural tube defects (NTDs) are comprised of a group of congenital malformations that include spina bifida, anencephaly, and encephalocele, which arise during the process of neurulation between the third and fourth weeks of human pregnancy.[1,2] The incidence of NTDs varies in different parts of the world, and the average rate of NTDs is 1/1000 live births.[3,4] The incidence rate of NTDs is reported as 2.8 per 1000 in Northern Iran.[5] The NTD as a multifactorial disease is affected by genetic, environmental, and nutritional factors.[6,7] Genes encode proteins relating to the metabolism of folic acids and methionine, which affect the induction of NTDs.[8] The 5,10-methylenetetrahydrohydrofolate reductase (MTHFR) gene mutation is known as a risk factor for the occurrence of NTDs.[7] The NTDs are related to diabetes mellitus in pregnant mothers,[9] anti-epileptic drugs such as valproic acid,[10] mother's obesity,[11] hyperthermia during pregnancy,[12] folate and B12 deficiency.[13,14] Recently, a study in a Mexican-American population along the Texas-Mexico border in the USA reported that *Helicobacter pylori (H. pylori)* could can play a role in NTD causation by reducing folate and vitamin B12 concentrations.[15] *Helicobacter pylori* infections in Iranian women were reported as 46.6-66%,[16,17] and this rate was 34.7% in women of childbearing age.[18] This rate was reported as 56.6% in Turkish,[19] and 50% in American pregnant women.[20]

In view of the adverse effect of *H. pylori* infections on micronutrients, particularly folic acid and vitamin B12 absorption,[15] and the high *H. pylori* infection in pregnant women in Northern Iran, this study was conducted to look for an association between maternal *H. pylori* seropositivity and NTD-affected pregnancies in Northern Iran. Also, we evaluated whether individual characteristics including age, ethnicity, education, family income, habitat, folic acid consumption, and nutrition status act as potential effect modifiers.

Methods.

Setting. This hospital-based case-control study was conducted from April 2007 to March 2009 at the Dezyani Teaching Hospital in Gorgan, Northern Iran. The Human Research Review Committee at Golestan University of Medical Sciences approved the study, and it was carried out according to the Principles of the Helsinki Declaration. The mothers' consent was obtained for the study, along with a clearance from the Institutional ethical committee.

Dezyani Hospital in Gorgan, the capital city of Golestan province in Northern Iran is a referral hospital with an annual rate of more than 6000 deliveries, accounting for the largest portion of deliveries (20%) in Golestan province, with a 1.5 million population. Native Fars, Turkman, and Sistani are the 3 main ethnic groups in Gorgan. Natives Fars is the predominant inhabitants, with the highest population. Turkman is an ethnic group that emigrated from central Asia more than 3 centuries ago, and the Sistani group emigrated from southeastern Iran half a century ago. All babies delivered in this hospital during the investigation period (2007-2009) were examined after delivery for NTDs by a gynecologist, and a pediatrician later confirmed the diagnosis. The health of newborns in the control group was assessed clinically by a pediatrician before the mothers entered the study. Mothers with non-syndromic affected NTD newborns were included in the study. Mothers with syndromic affected NTD infants were excluded from the study. The cases consisted of 35 mothers with non-syndromic affected NTD newborns, and 53 mothers with healthy newborns were considered the control group. The controls were chosen from the hospital delivery list so to be immediately before and after delivery of cases. A checklist covering all relevant clinical and demographic factors was completed for each mother in the case and control groups by a nurse during an interview with the mothers. The checklist consisted of 4 parts of questions: the first part comprised variables of socio-demographic status including maternal age (<20, 20-24, 25-29, >29), level of education (illiterate, under diploma, diploma, upper diploma), family income (poor: lower than $300, good: >$300 per month), ethnicity, folic acid intake, and residency in an urban or rural area. The second part of the checklist included questions about dietary intake during pregnancy. It was based on the consumption of meat, cheese, milk, yoghurt, eggs, vegetables, fruit, bread, grain, and dry beans. Scoring was designed so that cases with poor nutrition received a score less than 40.[21] The third part was composed of infant characteristics including gender, gestational age, and type of anomaly. The fourth section included laboratory results.

Biochemical analysis. A peripheral blood sample (4 milliliter) was collected from mothers in the case and control groups after a maximum 2 hours from delivery. The serum samples were analyzed for folic acid and vitamin B12 concentration by the radioimmunoassay

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method (MP Biomedical, Solon, OH, USA). Serum levels of homocysteine and ferritin were evaluated by ELISA (Axis-Shield, Dundee, Scotland, UK). The *H. pylori* IgG and IgM were determined by the luminance (CLIA) quantitative method (Monobind, Lake Forest, CA, USA). A serum folate level of less than 5 ng/ml, a vitamin B12 level of less than 60 pg/ml, a ferritin level of less than 10 µg/L, and a homocysteine level of less than 5 micromole/liter was used as the cutoff to label the individual as deficient.

Statistical analysis. Data were analyzed using the Statistical Package for Social Sciences software version 16.0 (SPSS Inc., Chicago, IL, USA). The baseline characteristics and nutrient status in the case and control groups were compared using Chi square test. Logistic regression analysis was fitted to the data to calculate odds ratio, and 95% confidence interval (95% CI) for association between *H. pylori* seropositivity and nutrient status with NTD, and *H. pylori* seropositivity with nutrient status. Stratified analysis was used to assess effect modification of the association between NTD and *H. pylori*. We considered age, ethnicity, education, income, habitat, acid folic consumption, and nutrition status as probable effect modifiers.

Results. Age, education, economic conditions, ethnicity, folic acid consumption, nutrition status, and residency of participants including 35 cases and 53 controls are depicted in **Table 1**. Poor nutrition was detected in 60% of cases and 45.3% of controls (p=0.17). Three (8.8%) subjects in the case group, and 5 (9.4%) in the control group consumed folic acid during the preconception period (p=0.89) (**Table 1**). Serum vitamin B12 deficiency was detected in 17% of cases and 13% of controls, and folic acid deficiency in 17% of cases and 13% of controls (p=0.61) (**Table 2**). Fourteen (26.4%) controls, and 15 (42.9%) cases were seropositive for *H. pylori* (IgG) tests (p=0.10) (**Table 2**). There was a non significant association between *H. pylori* seropositivity and NTD-affected pregnancies (OR 2.08; 95% CI: 0.84-5.17, p=0.11). The OR was elevated, particularly in women in the 20-24 years age range (OR 40; 95% CI: 2.96-539.64, p=0.005), also in women without education (OR 4; 95% CI: 0.329-48, p=0.52), and in women with a diploma of education (OR 12; 95% CI: 1.61-141, p=0.04) (**Table 3**). There was a non significant association between *H. pylori* seropositivity and NTD-affected pregnancies in the native Fars ethnic group (OR 3.8; 95% CI: 0.94-15.25, p=0.54) (**Table 3**). *Helicobacter pylori* seropositivity was non significantly associated with low serum folate (OR

Table 1 - Sociodemographic characteristics of the NTD-affected cases and controls from Gorgan, Northern Iran.

Variables	Cases (n=35)	Controls (n=53)	*P*-value
	n (%)		
Maternal age			0.19
<20	7 (20)	8 (15.1)	
20-24	7 (20)	17 (32.1)	
25-30	16 (43)	13 (24.5)	
>30	6 (17)	15 (28.3)	
Maternal education			0.44
Illiterate	5 (14.3)	5 (9.5)	
Under diploma	15 (42.9)	25 (47.2)	
Diploma	10 (28.5)	19 (35.8)	
Upper diploma	5 (14.3)	4 (7.5)	
Preconception folic acid			0.89
Yes	3 (8.8)	5 (9.4)	
No	31 (91.2)	48 (90.6)	
Maternal ethnicity			0.94
Sistani	14 (40)	18 (36.7)	
Turkman	5 (14.3)	7 (14.3)	
Fars	16 (45.7)	24 (49)	
Income			0.90
Poor	14 (40)	23 (43.4)	
Good	21 (60)	30 (56.4)	
Nutrition			0.17
Poor	21 (60)	24 (45.3)	
Good	14 (40)	29 (54.7)	
Residency			0.56
Rural	21 (60)	35 (66)	
Urban	14 (40)	18 (34)	

NTD - neural tube defect, poor income - <$300, good income - >$300 per month, poor nutrition - score <40

Table 2 - Distribution of normal and abnormal levels of B12, homocysteine, ferritin, folic acid, IgG, and IgA of *Helicobacter pylori* NTD-affected cases and controls from Gorgan, Northern Iran.

Variables	Cases (n=35)	Controls (n=53)	*P*-value
	n (%)		
B12 (pg/ml)			0.61
60-970	29 (82.9)	46 (86.8)	
<60	6 (17.1)	7 (13.2)	
Homocysteine (µmol/L)			0.72
5-15	28 (80)	44 (83)	
>15	7 (30)	9 (17)	
Ferritin(µg/ml)			0.81
10-124	27 (77.1)	42 (79.2)	
<10	8 (22.9)	11 (20.8)	
Folic acid (ng/ml)			0.61
5-17	29 (82.9)	46 (86.8)	
<5	6 (17.1)	7 (13.2)	
Helicobacter (IgG) U/ml			0.10
<20	20 (57.1)	39 (73.6)	
≥20	15 (42.9)	14 (26.4)	
Helicobacter (IgA)U/ml			0.68
<20	32 (91.4)	50 (94.3)	
≥20	3 (8.6)	3 (5.7)	

NTD - neural tube defect, ng/mL - nanogram/milliliter, µg/L - microgram/liter, pg/mL - picogram/milliliter, µmol/L - micromol/liter, U/mL - Unit/milliliter

Table 3 - Association between *Helicobacter pylori* seropositivity and risk of NTDs: crude and by individual characteristics (effect modifiers) among NTD-affected cases and controls from Gorgan, Northern Iran.

Variables	*H. pylori* seropositive (case/control)	*H. pylori* seronegative (case/control)	Effect of *H. pylori* seropositive OR (95% CI)	*P*-value
Crude odds ratio	15/14	20/39	2.08 (0.84-5.17)	0.11
Folic acid				
Consumption +	2/1	1/4	8 (0.3-2.6)	0.46
Consumption -	12/13	19/35	1.7 (0.65-4.45)	0.27
Nutrition				
Poor	9/8	12/16	1.5 (0.44-5.03)	0.51
Good	6/6	8/23	2.87 (0.71-11.54)	0.16
Habitat				
Rural	8/10	13/25	1.53 (0.49-4.85)	0.46
Urban	7/4	7/14	3.5 (0.76-6.11)	0.14
Income				
Poor	5/6	8/17	1.77 (0.41-7.5)	0.58
Good	9/8	10/21	2.36 (0.7-7.95)	0.62
Ethnicity				
Sistani	4/6	9/12	0.89 (0.19-4.41)	0.60
Turkman	1/2	3/5	3.83 (0.51-13.66)	0.72
Fars	8/5	8/19	3.8 (0.94-15.25)	0.54
Education				
Illiterate	2/2	2/8	4 (0.329-48)	0.52
Under diploma	6/9	8/16	1.33 (0.35-5.07)	0.67
Diploma	4/1	4/12	12 (1.61-141)	0.04
Upper diploma	2/0	2/2	3 (0.18-47.96)	0.46
Age				
<20	4/1	3/7	9.33 (0.71-122. 57)	0.09
20-24	5/1	2/16	40 (2.96-539.64)	0.005
25-29	4/6	11/7	0.42 (0.08-2.06)	0.28
>29	2/6	4/9	0.75 (0.10-5.47)	0.77

NTD - neural tube defect, *H. pylori - Helicobacter pylori,* OR - odds ratio, CI - confidence interval, poor income - <$300, good income - >$300 per month, poor nutrition - score <40

Table 4 - Association of serum folate, B12, ferritin and homocysteine concentration with *Helicobacter pylori* seropositivity among NTD-affected cases and controls from Gorgan, Northern Iran.

Nutrient status indicator	*H. pylori* seropositive	*H. pylori* seronegative	OR (95% CI)	*P*-value
	n (%)			
Folate (ng/ml)				0.34
5-17	23 (79.3)	52 (88.1)	1.0 Referent	
<5	60 (20.7)	87 (11.9)	1.93 (0.58-6.4)	
Ferritin(µg/ml)				0.68
10-124	22 (75.9)	47 (79.7)	1.0 (Referent)	
<10	7 (24.1)	12 (20.3)	1.24 (0.42-3.60)	
B12 (pg/ml)				0.56
60-970	25 (86.2)	50 (84.7)	1.0 (Referent)	
<60	4 (13.8)	9 (15.3)	0.889 (0.24-3.17)	
Homocysteine (µmol/L)				0.87
5-15	24 (82.8)	48 (81.4)	1.0 (Referent)	
>15	4 (17.2)	11 (18.6)	0.90 (0.28-2.9)	

NTD - neural tube defect, ng/mL - nanogram/milliliter, µg/L - microgram/liter, pg/mL - picogram/milliliter, µmol/L - micromol/liter

1.93; CI: 0.58-6.4, p=0.34) and ferritin (OR 1.24; CI: 0.42-3.60, p=0.68) (**Table 4**).

Discussion. Our study indicated a possible causal link between *H. pylori* and NTDs. This study indicated that *H. pylori* seropositivity in pregnant women can non significantly increase the risk of occurrence of NTDs in newborns in Northern Iran. Our findings are similar to Felkner et al's case-control study (103 cases and 159 controls) from the USA[15] that showed that seropositivity of mothers with *H. pylori* can non significantly increase the occurrence of NTDs in offspring up to 1.4 times. They also found a strong association between low serum vitamin B12 and NTD risk. Similarly, in our study the levels of folic acid and vitamin B12 in women with an NTD-affected pregnancy were lower than in women with a healthy pregnancy, although these differences were not significant. Several studies have found folic acid and vitamin B12 deficiency in NTD-affected pregnancies compared with normal pregnancies.[2,22-24] It is reported that *H. pylori* infection is common in Iranian women particularly in the childbearing age.[16-18] Several studies reported that serum/plasma vitamin B12 and folate levels are lower in persons with *H. pylori* infections compared with uninfected persons.[25-30] Also, several investigations indicated that vitamin B12, and folate levels improve after *H. pylori* eradication.[31-34] Regarding the high prevalence of *H. pylori* in childbearing aged women in Iran, we think this infection prevents micronutrient absorption and consequently, reduced folate levels and B12 deficiency causes NTDs in offspring. Although folic acid is the micronutrient most related with NTDs, the most compelling relation between *H. pylori* and its adverse effects on micronutrients is the effect of *H. pylori* infection on impaired iron metabolism.[15] Studies reported lower levels of serum ferritin and iron deficiency anemia in subjects with current or past *H. pylori* infections in comparison with uninfected subjects.[35-42] Several studies also found that eradication of *H. pylori* infection improves iron status.[36,38-40,43-45]

Regarding the physiological condition of women, *H. pylori* has a greater impact on women's iron status than on men's, probably because of women's vulnerability to multiple iron stressors.[34,36,42,46-48] Furthermore, Carmichael et al's studies[49,50] found that poor diet quality, defined partly as low dietary iron intake as low serum ferritin, an indicator of iron stores,[51] has an effect on NTD risk. In our study, the level of ferritin in women with an NTD-affected pregnancy was lower than in women with a healthy pregnancy, but this difference was not significant. Our results are similar to Felkner et al's study,[15] which found no relation between NTDs and serum ferritin level.

Our study had some limitations: firstly, the sample size (number of case- and control-women) was small. Our non significant results regarding the association of *H. pylori* seropositivity and NTDs could have been due to the small sample size. Secondly, our samples were taken postpartum rather than the first month of gestation, the time of neural tube closure.

In conclusion, this study indicated that *H. pylori* seropositivity in pregnant women can increase the risk of the occurrence of NTDs in newborns in Northern Iran up to 2 times. Further studies with a larger sample size including all hospitals in the Golestan province of Northern Iran are required. We also suggest health programs for the control of *H. pylori* infection, particularly in childbearing age women.

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