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Helicobacter pylori Infection is a Risk Factor for Iron-Deficiency Anemia in Korean High School Girls

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Abstract

This study investigated the relationship of *Helicobacter pylori (H. pylori)* infection to iron-deficiency anemia in high school girls. Four hundred and fourty-five subjects resided in Ulsan City in Korea were evaluated by questionnaire or blood analysis for serum indicators of iron status, daily nutrient intakes, symptoms of anemia, and *H. pylori* IgG antibody status. In *H. pylori* infected subjects, total energy intake of was 1534.14±350.81 kcal (73.0% of the Korean RDA), the iron intake of subjects was 11.38±3.90 mg (71.1% of Korean RDA) and calcium intake was 467.63±175.96 mg (58.3% of the Korean RDA). Carbohydrate ($p<0.05$), vitamin A ($p<0.05$), vitamin C ($p<0.01$) intakes were significantly lower in infected subjects than in non-infected subjects. The prevalence of anemia (hemoglobin (Hb) < 12 g/dL) in *H. pylori* infected subjects was 22.7% as compared to 14.7% in non-infected subjects. The mean serum ferritin of infected subjects (21.71±21.50 ng/ mL) was significantly lower than in non-infected subjects ($p<0.05$) (as was the mean Hb concentration (12.54±1.51 g/dL) and hematocrit (37.99±3.64%)). Total iron binding capacity (TIBC) of *H. pylori* infected subjects (449.09±78.23 μg/dL) was significantly higher than that of non-infected subjects (432.99±76.15 μg/dL) ($p<0.05$). The levels of iron, red blood cell (RBC) and transferrin saturation were similar in infected and non-infected subjects. Two symptoms of anemia, 'pale face ($p<0.01$)' and 'decreased ability to concentrate ($p<0.005$)', were significantly higher in *H. pylori* infected subjects than in non-infected subjects. High school girls are known to be more vulnerable to iron deficiency than other age groups, and this research demonstrates that infection with *H. pylori* increases their risk of iron-deficiency anemia.

Key words: iron deficiency, anemia, *H. pylori* infection, iron parameter

INTRODUCTION

Iron deficiency anemia is considered the most prevalent nutritional deficiency disease in the world. Small children and women of reproductive age are most commonly affected, and it is especially serious among adolescent girls because of rapid growth and menstruation (1,2). It has been reported that iron deficiency in developing countries may be due to both the amount of iron in the diet and utilization of iron (3). According to the 1998 Korean National Health and Nutrition Survey (4), daily iron intake of Korean women between 13 and 19 years of age was 10.8 mg, just 67.2% of the Recommended Dietary Allowance (RDA), and 69.7% received less than 75% of the RDA for iron. Therefore, it can be concluded that iron deficiency is ubiquitous among young Korean women. Iron deficiency anemia reduces physical work capacity and impairs immune function, including the capacity of leucocytes to kill microorganisms (5). Verbal learning, memory, and physical performance may also be impaired in iron-deficient adolescent girls (6,7).

H. pylori is a widely distributed bacteria throughout the world. It has been reported that *H. pylori* causes a type B gastritis, peptic ulcers, and is highly correlated with the occurrence of gastric cancer (8,9). Several recent studies reported that *H. pylori* infection presented as iron deficiency (10,11). It has been demonstrated that *H. pylori* gastritis is related to a reversible reduction of ascorbic acid concentrations in gastric juices (10). Ascorbic acid in gastric juice is important for the reduction of iron which, in turn, is important for non-heme iron absorption. Thus, *H. pylori* related chronic inflammation could exert a negative effect on iron absorption by altering the physiological secretion of ascorbic acid (11). *H. pylori* infection is associated subnormal growth at puberty (12,13); suggesting that *H. pylori* may be an environmental factor capable of impairing normal growth, especially height.

Therefore, the purpose of this study was to investigate

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whether there is an association between *H. pylori* infection and iron deficiency among Korean high school girls.

METHODS

Subjects

Among the 445 high school girls in Ulsan, Korea selected for the study, 129 (26.7%) were determined to have *H. pylori* infection by a blood test.

Nutrient intake and food frequency of subjects

Nutrient intakes were determined by the method of Moon et al. (14). Nutrient intakes were compared with the Korean Recommended Dietary Allowances (2000, Korean nutrition society, 7th ed.). Food frequencies were estimated using an 8 point Likert scale questionnaire (8: 3 times a day, 7: 2 times a day, 6: 1 time a day, 5: 5~6 days/week, 4: 3~4 days/week, 3: 1~2 days/week, 2: a little bit, 1: no)

Physical characteristics

The height and weight of the subjects were measured with an automatic instrument (Fanocs model: Fa-95). Body mass index (BMI) was calculated as the weight (kg) divided by the square of the height (m^2), (BMI, kg/m^2). PIBW (percent ideal body weight) was calculated as a percentage of ideal body weight which was measured by the method of Broca [height (cm) - 100 × 0.9].

Measurement of iron status parameter and serum *H. pylori* IgG antibody

Hemoglobin (Hb), serum iron, total iron binding capacity, serum ferritin, and serum immunoglobulin antibody to *H. pylori* were determined. The Hb, red blood cell hematocrit, RDW (red cell distribution width) were measured with an Automatic Blood Cell Counter (Sysmex NE 8000, Toa Medical Elactronics Co., Japan). Serum iron and TIBC (total iron binding capacity) were measured with an Automatic Chemistry Analyzer (Hitachi 747, Hitachi Co., Japan). Serum ferritin concentration were measured Chemiluminescence Immunoassay (CLIA) Analyzer (ACS 180, Bayer Diagnostics Co., USA). Transferrin saturation TS (%)] was calculated by dividing the concentration of serum iron by TIBC. Serum IgG antibodies to *H. pylori* were measured using an indirect two-step enzyme immunoassay (EIA), the COBAS CORE Anti-*H. pylori* EIA Quant (Roche Diagnostic Corporation, Indianapolis, IN, USA).

Questionnaire survey

Sixteen symptoms of iron-deficiency anemia were selected for a questionnaire based on the results of the previous study (15). Each question had four variables; 'did not feel at all', 'feel sometimes', 'feel often', and 'feel very often'. The variables were scored as 0-, 1-, 2-, and 3-points on a Likert type scale.

Statistical analysis

All data were statistically analyzed, using SPSS PC$^+$. The physical characteristics, nutrient intakes, iron parameters and clinical symptoms of the subjects were compared by a t-test between the *H. pylori* infected and non-infected groups. The correlation between *H. pylori* infection and blood indicators were evaluated for significance by Pearson's correlation coefficient.

RESULTS AND DISCUSSION

Physical characteristics of subjects

Table 1 shows the physical characteristics of the subjects; there were no significant differences between groups in height, weight, BMI or PIBW. The *H. pylori* infected subjects had mean heights and weights of 161.00±4.89 cm, 52.00±8.76 kg, respectively; mean BMI (kg/m^2) was 20.30±3.10 and PIBW (percent ideal body weight) was 93.52±9.84%. The mean heights and weights the subjects not infected with *H. pylori* were 161.27±4.92 cm, 51.98 ±7.12 kg which were not significantly different from the infected group. The previous study (16) of high school girls reported that the average height was 161.62 cm, which is similar to the heights of subjects in this study, but the average weight in the previous study was 53.87 kg which was slightly higher than in this study.

Nutrient intake and food frequency of subjects

Table 2 shows the nutrient intakes of the subjects. The average protein intake of *H. pylori* infected subjects was 53.03±17.13 g (81.5% of RDA) and vitamin B_1 intake was 0.74±0.24 mg (67.2% of RDA). Vitamin B_2 intake was 1.10±0.37 mg (84.6% of RDA), total energy intake was 1534.14±350.81 kcal (73.0% of RDA) and iron intake was 11.38±3.90 mg (71.1% of RDA). Dietary calcium intake was the lowest compared with the RDA (58.3%) at 467.63±175.96 mg. The low calcium intake in this study is consistent with the results of Hyun & Lee

Table 1. Characteristics of subjects

Variables	*H. pylori* infected (n = 119)	*H. pylori* non-infected (n = 326)	Significance
Height (cm)	161.00±4.89	161.27±4.92	NS[3]
Body weight (kg)	52.00±8.76	51.98±7.12	NS
BMI (kg/m^2)[1]	20.30±3.10	20.03±2.50	NS
PIBW (%)[2]	93.52±9.84	94.99±12.38	NS

[1]BMI (kg/m^2): Body mass index.
[2]PIBW: Percent ideal body weight, ideal body weight={height (cm)-100} × 0.9.
[3]Not significant.

Table 2. Average daily nutrient intakes and % RDA of *H. pylori* infected and non-infected subjects

Nutrient	RDA[1]	*H. pylori* infected (n = 119)		*H. pylori* non-infected (n = 326)	
		Mean ± SD	%RDA	Mean ± SD	%RDA
Protein (g)	65	53.03 ± 17.13	81.5	54.73 ± 16.23	84.2
Fat (g)		34.57 ± 9.12		36.05 ± 10.37	
Carbohydrate (g)		251.38 ± 58.31		264.20 ± 58.32*	
Fe (mg)	16	11.38 ± 3.90	71.1	11.99 ± 3.99	74.9
P (mg)	800	774.57 ± 240.39	96.8	809.03 ± 240.66	101.1
Ca (mg)	800	467.63 ± 175.96	58.3	500.49 ± 178.28	62.5
Vitamin A (RE)	700	774.80 ± 373.93	110.6	856.65 ± 376.09*	122.3
Vitamin B_1 (mg)	1.1	0.74 ± 0.24	67.2	0.78 ± 0.25	70.9
Vitamin B_2 (mg)	1.3	1.10 ± 0.37	84.6	1.17 ± 0.37	90.0
Niacin (mg)	14	12.23 ± 3.99	87.3	12.65 ± 4.05	90.3
Vitamin C (mg)	70	92.80 ± 43.16	132.5	105.04 ± 45.46**	150.1
Energy (kcal)	2100	1534.14 ± 350.81	73.0	1601.08 ± 355.98	76.2

[1]RDA: Recommended Dietary Allowance (2000).
*$p < 0.05$, **$p < 0.005$.

(17) who also found that calcium intakes of Korean adolescent girls is significantly less than the RDA. We previously found that high school girls had calcium intakes at 77.76% of the RDA (16).

Non-infected subjects had an average protein intake of 54.73 ± 16.23 g (84.2% of RDA), iron intake of 11.99 ± 3.99 mg (74.9% of RDA), calcium intake of 500.49 ± 178.28 mg (62.5% of RDA), vitamin B_1 of 0.78 ± 0.25 mg (70.9% of RDA), and total energy intake of 1601.08 ± 355.98 kcal (76.2% of RDA). Only carbohydrate ($p < 0.05$), vitamin A ($p < 0.05$), vitamin C ($p < 0.005$) intakes were significantly lower in infected subjects than in non-infected subjects.

It has been demonstrated that the most powerful promoter of non-heme iron absorption is ascorbic acid (18). Ascorbate helps to reduce ferric iron to ferrous iron and forms a chelate with ferric chloride, which is stable at the alkaline pH of the duodenum (19). Therefore, the higher intake of vitamin C in non-infected subjects could be an important factor in the different iron status between the two groups.

Table 3 shows the food frequency of the subjects. The frequency of eating '*kimchi*' was the highest at 4.92 ± 2.19, followed by vegetables (4.10 ± 2.13), milk (3.92 ± 1.86), fruit (3.71 ± 1.69), greasy foods (3.47 ± 1.78), eggs 3.23 ± 1.42), green leafy vegetables such as carrots and spinach (3.21 ± 1.90), bean or tofu 3.32 ± 1.42), seaweed (2.86 ± 1.31), pork (2.55 ± 1.00), fish (2.48 ± 0.99), meat (2.37 ± 1.04), and chicken (2.35 ± 0.83). Food frequencies were higher for all foods among the *H. pylori* non-infected than infected subjects, except for egg. However, only the frequencies for *kimchi* ($p < 0.05$) and beef ($p < 0.05$) were significant higher in the non-infected subjects. Since beef is a rich source of highly bioavailable heme iron, the decreased frequency of eating beef could also contribute to iron deficiency in the infected subjects.

Table 3. Food Frequencies of *H. pylori* infected and non- infected subjects

Food	*H. pylori* infected (n = 119)	*H. pylori* non-infected (n = 326)
kimchi	4.92 ± 2.19[1)2)]	5.40 ± 2.17*
Vegetables	4.10 ± 2.13	4.49 ± 2.09
Milk everyday	3.92 ± 1.86	3.97 ± 2.02
Fruit	3.71 ± 1.69	3.99 ± 1.77
Greasy food	3.47 ± 1.78	3.81 ± 1.70
Eggs	3.23 ± 1.42	3.07 ± 1.47
Green leafy vegetables such as carrot or spinach	3.21 ± 1.90	3.58 ± 1.86
Bean or tofu	3.13 ± 1.28	3.32 ± 1.51
Seaweed	2.86 ± 1.31	2.92 ± 1.46
Pork	2.55 ± 1.00	2.76 ± 1.17
Fish	2.48 ± 0.99	2.56 ± 1.21
Beef	2.37 ± 1.04	2.63 ± 1.18*
Chicken	2.35 ± 0.83	2.47 ± 1.07

[1)]Mean ± SD.
[2)]1: no, 2: a little bit, 3: 1~2 days/week, 4: 3~4 days/week, 5: 5~6 days/week, 6: 1 time a day, 7: 2 times a day, 8: 3 times a day.
*$p < 0.05$.

Iron status parameter in serum of subjects and prevalence of anemia

Table 4 shows the iron status parameters in serum and prevalence of anemia among the subjects. Hb and hematocrit levels of *H. pylori* infected subjects were 12.54 ± 1.51 g/dL, 37.99 ± 3.64%, respectively, which were significantly lower than in non-infected subjects (Hb: 12.79 ± 1.07 g/dL, hematocrit: 38.95 ± 2.92%) ($p < 0.005$). Serum ferritin concentration is a reliable indicator of iron storage *in vivo*. It is known that a serum ferritin concentration of less than 20 ng/mL is indicative of iron deficiency (20). Serum ferritin concentrations were significantly lower ($p < 0.05$) in *H. pylori* infected (21.71 ± 21.50 ng/mL) than non-infected subjects (27.30 ± 21.87 ng/mL). TIBC is a

Table 4. Iron status parameter in serum of *H. pylori* infected subjects and non-infected subjects

Iron status parameter	*H. pylori* infected (n = 119)	*H. pylori* non-infected (n = 326)
Hb (g/dL)[1]	12.54 ± 1.51	12.97 ± 1.07**
Hematicrit (%)	37.99 ± 3.64	38.95 ± 2.92**
Ferritin (ng/mL)	21.71 ± 21.50	27.30 ± 21.87*
Fe (μg/dL)	64.25 ± 42.39	70.98 ± 38.75
TIBC (μg/dL)[2]	449.09 ± 78.23	432.99 ± 76.15*
TS (%)[3]	15.23 ± 10.92	17.07 ± 9.74
RBC (10^6/mm^3)[4]	4.45 ± 0.25	4.47 ± 0.27
RDW (%)[5]	14.98 ± 2.16	14.71 ± 1.83
Prevalence rates of anemia (Hb concentrations < 12 g/dL)	22.7%	14.7%

[1]Hb: Hemoglobin. [2]TIBC: Total iron binding capacity.
[3]TS: Transferrin saturation. [4]RBC: Red blood cell.
[5]RDW: Red cell distribution width.
*$p < 0.05$, **$p < 0.005$.

measure of the amount of iron than can bind to transferrin. TIBC rapidly increases when iron is deficient (21). The TIBC of *H. pylori* infected subjects was 449.09 ± 78.23 μg/dL, which was significantly ($p < 0.05$) higher than in non-infected subjects (432.99 ± 76.15 μg/dL).

TS (%) is calculated by dividing the concentration serum iron by TIBC. It is considered to be a more reliable criterion for iron deficiency anemia than TIBC, since the value of serum iron decreases but TIBC increases when there is an iron deficiency. TS (%) of *H. pylori* infected subjects (15.23 ± 10.92%) was lower than that of non-infected subjects (17.07 ± 9.74%), but the difference was not statistically significant between the two groups.

RDW (Red Cell Distribution Width) is an index of the variation in the cell volume of red cells. A high RDW is a highly sensitive indicator of the early stages of iron deficiency (22). The RDW of *H. pylori* infected subjects (14.98 ± 2.16%) was not different than non-infected subjects (14.71 ± 1.83%). Anemia was observed in 22.7% of the *H. pylori* infected subjects, which was significantly higher than in non-infected subjects (14.7%) ($p < 0.05$).

Anemia symptoms of subjects

Table 5 shows the prevalence of anemia symptoms among the subjects. The *H. pylori* infected subjects experienced 'decreased ability to concentrate (2.95 ± 0.89)' most often, followed by 'tired out easily (2.83 ± 0.94)', 'shortness of breath when going upstairs (2.69 ± 0.95)', 'get a cold easily (2.58 ± 1.02)', 'poor memory (2.52 ± 0.96)', 'feel dizzy when standing up (2.42 ± 1.02)', 'have headache (2.42 ± 1.04)', 'cold hands & feet (2.41 ± 1.19)', 'usually dizzy (2.34 ± 1.06)', 'difficult digestion (2.24 ± 0.99)', 'no appetite (2.19 ± 0.79)', 'feeling blue (2.12 ± 1.00)', 'easily bruised (1.93 ± 0.97)', 'suffering from constipation (1.80 ± 0.98)', 'inflamed inner mouth (1.78 ± 0.86)', and 'pale face (1.63 ± 0.96)'. *H. pylori* non-infected subjects exhibited a reduced frequency of most clinical symptoms compared to *H. pylori* infected subjects. 'Decreased ability to concentrate ($p < 0.05$)' and 'pale face ($p < 0.005$)' symptoms were significantly more frequent in *H. pylori* infected than non-infected subjects. We previously reported (15) that 'cold hands & feet', 'tired out easily', 'shortness breath when going upstairs', and 'fatigue' were frequent symptoms in female college students with impaired iron status.

Table 5. Differences in clinical iron-deficiency symptoms between *H. pylori* infected and non-infected subjects

Symptoms	*H. pylori* infected (n = 119)	*H. pylori* non-infected (n = 326)
Decreased ability to concentrate	2.95 ± 0.89	2.69 ± 0.89**
Tired out easily	2.83 ± 0.94	2.81 ± 0.95
Shortness of breath when going upstairs	2.69 ± 0.95	2.59 ± 0.91
Get a cold easily	2.58 ± 1.02	2.42 ± 1.02
Poor memory	2.52 ± 0.96	2.39 ± 0.90
Feel dizzy when standing up	2.42 ± 1.02	2.39 ± 0.98
Have headache	2.42 ± 1.04	2.36 ± 0.97
Cold hands & feet	2.41 ± 1.19	2.30 ± 1.16
Frequently dizzy	2.34 ± 1.06	2.36 ± 1.00
Difficult digestion	2.24 ± 0.99	2.15 ± 0.98
No appetite	2.19 ± 0.79	2.09 ± 0.79
Feeling blue	2.12 ± 1.00	2.12 ± 0.99
Easily bruised	1.93 ± 0.97	1.86 ± 0.96
Suffering from constipation	1.80 ± 0.98	1.91 ± 1.02
Inflamed inner mouth	1.78 ± 0.86	1.87 ± 0.93
Pale face	1.63 ± 0.96	1.45 ± 0.77*

Score = 1: Did not feel at all, 2: Feel sometimes, 3: Feel often, 4: Feel very often.
*$p < 0.05$, **$p < 0.005$.

The correlation between serum iron parameters and *H. pylori* infection

Table 6 shows the correlation between serum iron parameter and *H. pylori* infection. *H. pylori* infection was negatively correlated with serum Hb ($t = -0.156$, $p < 0.05$) and hematocrit ($t = -0.134$, $p < 0.005$), and was positively correlated with TIBC ($t = 0.092$, $p < 0.05$). Some epidemiological studies suggest an association between *H. pylori* infection and iron deficiency (23,24). Infection with *H. pylori* can cause iron deficiency or iron deficiency anemia by impairing iron uptake or increasing iron demand. The possible mechanisms by which *H. pylori* infection could cause iron deficiency and further lead to anemia are largely speculative. Banerjee et al. (11) reported that the bacterium can cause a decrease in the concentration of ascorbic acid in gastric juice, ascorbic acid is an important promoter of non-heme iron absorption. It has also been demonstrated that *H. pylori* infection may lead to an imbalance of body iron homeostasis by increasing iron demand since, as for many other bacteria, iron is an es-

Table 6. Correlation coefficient between *H. pylori* infection and hematological indices

Hematological indices	*H. pylori* infection
Hb[1)]	-0.156*
TIBC[2)]	0.092*
TS (%)[3)]	-0.080
Ferritin	-0.113
Fe	-0.074
Hematocrit	-0.134**
RBC[4)]	-0.038
RDW[5)]	0.061

[1)]Hb: Hemoglobin. [2)]TIBC: Total iron binding capacity.
[3)]TS: Transferrin saturation. [4)]RBC: Red blood cell.
[5)]RDW: Red blood cell distribution width.
*$p < 0.05$, **$p < 0.005$.

sential growth factor for *H. pylori* (25). *H. pylori* contains an iron-binding protein resembling ferritin with a binding activity for heme iron in erythrocytes (26). Choe et al. (27) reported that adolescent female athletes commonly have *H. pylori*-associated iron deficiency anemia, and concluded that when their anemia is refractory to iron administration, they should be evaluated for *H. pylori* infection.

This research suggests that high school girls are more vulnerable to iron deficiency than other age group, and that impaired iron status is exacerbated *H. pylori* infection. Two dietary factors may have contributed to the increased incidence of anemia among the subjects. First, the lower frequency of eating beef may have reduced the amount of heme iron ingested by infected students. Second, the decreased vitamin C may have reduced the absorption of non-heme iron in the same group. However, *H. pylori* infection may also be a causative factor for iron deficiency anemia, independent of dietary factors. Therefore, it would be prudent to have adolescents who exhibit iron deficiency anemia evaluated for *H. pylori* infection. Furthermore, when *H. pylori* infection is associated with iron deficiency anemia in adolescent girls, *H. pylori* eradication combined with iron supplementation is indicated.

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