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Short Communication

Serum levels of iron parameters and IL-17 in children with *Helicobacter pylori* infection compared to healthy group

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ABSTRACT

Helicobacter pylori is related to iron deficiency anemia (IDA) and inflammatory responses causing gastric and duodenal ulcer and carcinoma. Moreover, it leads to deficiency of factors associated with iron adsorption and transfer. In the present study, we recruited 100 children (50 infected with *H. pylori* and 50 controls) aged 3-14 years old (40% male and 60% female) to evaluate the effect of *H. pylori* on anemia and some of its related factors (i.e., total iron binding capacity [TIBC], ferritin, and transferrin) and also the amount of IL-17 expression. For the assessment of *H. pylori*, Euroimmune (Germany) kit was used for the ELISA test according to the instructions of manufacturer. Furthermore, for the measurement of IL-17 level, ELISA test (IBL commercial specific kit, Germany) was employed. The mean iron levels in the control and infected groups were 81.5 mg/dl and 43 mg/dl, respectively, which showed a significant difference between the two groups (P=0.007). The mean levels of transferrin in the control and infected children were 291 mg/dl and 249 mg/dl respectively, demonstrating a significant difference (P=0.008). Moreover, the ferritin levels were 64.5 ng/dl and 14 ng/dl in the control and infected groups, respectively (P=0.001). The TIBC levels were 329 mg/dl and 301 mg/dl, respectively (P=0.86). The mean levels of IL-17 in the healthy and infected children were 3.93±0.93 pg/ml and 8.887±1.46 pg/ml, respectively (P=0.002). Our findings revealed that *H. pylori* can play a role in anemia and induction of inflammatory responses through reducing iron-related parameters and significantly enhancing IL-17 level among the infected children compared to the healthy group.

Keywords: *Helicobacter pylori*, Anemia, Inflammation, IL-17

Comparaison des paramètres du bilan martial et du taux d'IL-17 entre les sérums d'enfants infectés et non-infectés par l'*Helicobacterpylori*

Résumé: L'*Helicobacter pylori*est responsable d'anémies ferriprives et de réponses inflammatoires à l'origine d'ulcères gastriques ou duodénales et de carcinomes. De plus, cette infection peut également être associée à des déficiences dans l'absorption et le transfert ferriques. Dans cette étude, 100 enfants (50 infectés et 50 non-infectés par l' *H. pylori*) âgés de 3 à 14 ans ont été sélectionnés (40% de garçons pour 60%de filles) afin d'évaluer l'effet de l'*H. pylori*sur l'anémie et ses facteurs associés (total iron binding capacity [TIBC], ferritine et transferrine) ainsi que sur le taux d'expression de l'IL-17. La présence d'*H. pylori* a été évaluée par le kit ELISA Euroimmune (Allemagne) selon les instructions du fabricant. Un test ELISA commercial (IBL commercial specific kit, Allemagne) a été employé pour l'évaluation du taux d'IL-17. Le niveau moyen de fer sérique variait de façon significative entre les deux groupes témoin et infecté (P=0,007) et tombait de 81.5 mg/dl à 43 mg/dl. Le niveau moyen de transferrine des groupes témoins et infectés s'élevait respectivement à 291

mg/dl et 249 mg/dl et montrait une différence significative (P=0.008). De plus, le taux de ferritine diminuait également dans le groupe infecté (14 ng/dl) comparé au groupe témoin (64.5 ng/dl). Le taux de TIBC des groupes témoin et infecté était respectivement de 329 mg/dl et 301 mg/dl (P=0.86). Une augmentation significative (P=0.002) du taux moyen d'IL-17 a été observée dans le groupe infecté (8.887±1.46 pg/ml) comparé au groupe témoin (3.93±0.93 pg/ml). Nos résulats ont donc révélé que l' *H. pylori* peut être à l'origine d'anémie et de réponses inflammatoires en réduisant paramètres ferriques et en augmentant de façon significative le taux d'expression d'IL-17 chez les enfants infectés.

Mots clés: *Helicobacter pylori*, Anémie, Inflammation, IL-17

INTRODUCTION

Helicobacter pylori (*H. pylori*) is associated with gastritis and peptic ulcers in all age groups, and children are no exception. Approximately 50% of the world population are infected with *H. pylori* (Plummer et al., 2015). *H. pylori* infection leads to malignant gastro-intestinal diseases such as gastric or duodenal ulcers and cancers, lymphoma, dermatological diseases, cardiovascular diseases, hepatic and liver diseases, diabetes mellitus, iron deficiency anemia, and several other problems in pediatric population (Baker and Greer, 2010; Akcam and Aslan, 2015). Several studies have shown a relationship between *H. pylori* infection and iron deficiency anemia and some iron-related parameters among children and adults (Monzón et al., 2013; Noto et al., 2013). Several studies have shown the alteration in serum level of iron, ferritin, and transferrin among patients infected with *H. pylori* (Beydoun et al., 2015; Lopez et al., 2015; Sapmaz et al., 2016). Moreover, gastric consistent inflammation occurs in all of these patients, although *H. pylori* is non-virulent and does not enter the epithelial cells (Turkina et al., 2015). The host responses occur following the attachment of *H. pylori* to the epithelial cells. The antigenic materials of bacteria are adsorbed by epithelial cells passing lamina propria interacting and activating B and T (mostly TH17) lymphocytes (Carbo et al., 2014). Afterwards, IgG, IgA and to a lower extent, IgM antibodies are produced in response to the infection (Arnason et al., 2012; Pandya et al., 2014). Furthermore, interleukins will be produced by these cells and macrophages activating other leukocytes. IL-6, IL-12, INFγ, IL-23, and IL-17 have been shown to increase due to *H. pylori* infection (Horvath Jr et al., 2012; Pandya et al., 2014). IL-17 increases more severely in inflammatory responses and gastro-intestinal diseases due to *H. pylori* (Bagheri et al., 2013). The role of these cytokines and antibodies have been documented by several former studies in *H. pylori* infections in children. In this study, the serum levels of IgA, IgG, IL-17, ferritin, transferrin, and total iron binding capacity (TIBC) are compared between two groups of *H. pylori* infected and healthy children.

MATERIALS AND METHODS

Sera samples. One-hundred blood samples from 100 children (50 *H. pylori* infected and 50 healthy) aged 3-14 years old were obtained during a 6-months period. Sera were prepared and stored at -20°C until used for the tests.

Measurement of iron, TBIC, ferritin, and transferring. Iron and transferrin measurements were performed with AutoAnalyzer (Hitachi, 917) and enzymatic method using Bionic kit (Janulczyk et al., 1999). The AutoAnalyzer worked in a chemi-luminescence manner for detecting the level of iron-related serum parameters. For TIBC, the Biochemical AutoAnalyzer (Hitachi 917) was used according to the manufacturer's instructions. For the assessment of *H. pylori*, Euroimmune kit (Germany) was employed for the ELISA test according to the instructions of manufacturer (Chan et al., 2006). The kit contains two reagents of R1 and R2 and acts in two stages. Stage 1 determines the iron in a chromogenic reaction and an

acidic pH due to R1 addition for 5 min, and stage 2 determines TIBC in optimal condition with addition of R2 reagent for further 5 min at $OD_{600-660}$. The sera samples were stored at -20 °C up to 2 months for this test. The quantitative iron measurement reaction is based on the concentration of color in ELISA that indicates the iron level. For controlling and calibration of the test, the control serum of Zitrol TIBC and a calibrator were prepared from Biochemistry Corporation. For ferritin measurement, the AutoAnalyzer (LIAISON, Diasurin, Italy) with chemi-luminessence method was employed.

Evaluation serum level of IgG. Serum level of IgG was measured by ELISA kit and Vitro method. Briefly, the first step was coating of sera samples in the wells. Next, the conjugated enzyme (labeled anti human) was incubated for separation of bounding antibody in the samples. The kit was stored at 2-8 °C. The ELISA reader Stat Fax was used at OD_{420} and OD_{650}.

Evaluation of IL-17 level in sera. For the measurement of IL-17 level, ELISA test (IBL commercial specific kit, Germany) was employed according to the manufacturer's instructions. The kit solutions were stored at 2-8 °C and lyophilized controls were preserved at -20°C. In short, in each well, the anti-IL-17 was coated, and the serum sample was added. The conjugated monoclonal antibody-antigen and biotin remained after washing. By adding streptavidin horseradish peroxidase (HRP) to the compound, the specific reaction was stable against next washing and was colored by adding dye (Hu et al., 2010).

Data analysis. Mann Whitney U test and t-test were performed to analyze the data. P-value less than 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Iron, transferrin, ferritin, and TIBC serum levels. The mean iron levels in the control and infected groups were 81.5 mg/dl and 43 mg/dl, respectively, indicating iron deficiency in the infected group (P=0.007). Transferrin mean levels in the control and infected children were 291 mg/dl and 249 mg/dl, respectively, showing a significant reduction in the infected group (P=0.008). Moreover, the ferritin levels were 64.5 ng/dl and 14 ng/dl, respectively (P=0.001), showing a significant reduction in the infected group. The TIBC levels were 329 mg/dl and 301 mg/dl, respectively (P=0.86). The mentioned results revealed that the mean level of iron associated parameters, except for TIBC, in the infected children were significantly lower compared to the healthy group.

The IL-17 serum level. The levels of IL-17 in the healthy and infected children were 3.93±0.93 pg/ml and 8.887±1.46 pg/ml, respectively (P=0.002), showing a significant increase in the infected children that indicates induction of inflammation in this group.

In the present study, the serum levels of ferritin, transferrin, and iron were significantly lower in *H. pylori*-infected children compared to the control group. Moreover, the prevalence of anemia was significantly higher in infected children. Several previous studies have similarly revealed that frequency of *H. pylori* infection is significantly higher among anemia patients (Rahman et al., 2013; Yamanouchi et al., 2014). Sato identified *H. pylori* infection among 121 iron deficiency patients, and ferritin and pro-hepcidin levels were lower and higher than healthy individuals, respectively (Sato et al., 2015). In a study by Chuan Xie, the serum levels of hemoglobin and red and white blood cells in gerbils infected with *H. pylori* were higher compared to control group; however, the mean corpuscular volume was significantly lower in the infected group. Therefore, it was suggested that *H. pylori* alone could not lead to anemia and other agents such as host polymorphisms could be at play (Xie et al., 2014). Nonetheless, a study by Bazmamoun did not exhibit any association between *H. pylori* infection and iron deficiency anemia or ferritin level among infected children (Bazmamoun et al., 2014). Darvishi demonstrated a significant relationship between *H. pylori* infection and iron deficiency anemia and ferritin serum level in children under six years of age (Darvishi et al., 2015). Another study by Mubarak conducted on 179 pregnant women infected with *H. pylori* illustrated

that 69% were specific IgG positive, 24% had anemia, 28% had iron deficiency, and 11% had iron deficiency anemia, whereas no relationship was observed between *H. pylori* infection and iron deficiency or thrombocytopenia (Mubarak et al., 2014). In a study by Quiroz performed in Latin America, endoscopy of proximal gastrointestinal part exhibited that the number of patients infected with *H. pylori* was higher compared to England; moreover, it was found that *H. pylori* infection was significantly associated with hemoglobin and ferritin deficiency among children (Queiroz et al., 2013). In another study, it was found that anemia was significantly more prevalent in patients infected with *H. pylori* without cancer compared to healthy individuals (El-Omar, 2013). In a study by Miernyk, the geometric means of ferritin and anemia were significantly different between infected and healthy groups. The ferritin deficiency has also been demonstrated among infected pregnant women in comparison with healthy ones (Miernyk et al., 2013). In a study by Milman, the serum level of hemoglobin was not related to *H. pylori* infection, but ferritin level in both infected men and women was significantly lower in infected subjects (Milman et al., 1998). Herein, the serum level of IL17 was significantly higher among infected children (P=0.002), showing a significant increase in infected children that exhibits induction of inflammation in this group. In a study by Amedei, IL17 and IL23 levels were increased against HP0175 of *H. pylori* during 48 h. Furthermore, IL17 enhanced expression of vascular endothelial growth factor, and it was noted that IL17 activates neutrophils and T cells (Amedei et al., 2014). Another study carried out on children infected with *H. pylori* showed that the Th-17 (producing IL17) cells level was significantly higher in infected children (Gil et al., 2014). On the other hand, in mice infected with *H. pylori*, it was demonstrated that Th17 CD4+ cells were increased in gastric tissue. Co-culture of these cells with *H. pylori* increased mRNA level of IL17 and IFN-γ (Zhuang et al., 2011). Another study by Shi yielded similar results, that is, Th17 cells increased with enhanced expression of IL17 and IFN-γ in gastric tissue of mice. The expression levels of IL12 and IL13 increased in macrophages. In fact, the response pattern was in a Th17/Th1 complex manner (Shi et al., 2010). Similarly, Bhuiyan showed that both Th1 and Th17 responses were essential for *H. pylori* infection, and expression levels of IL17 and IFN-γ in gastric mucosa of infected adults were significantly higher than healthy control; furthermore, this level was significantly higher among infected children in comparison with infected adults (Bhuiyan et al., 2014). The results of the present study showed that iron deficiency anemia was significantly more prevalent among *H. pylori* infected children compared to the healthy group. In addition, the serum level of IL17 was significantly higher in the infected children.

Ethics

I hereby declare all ethical standards have been respected in preparation of the submitted article.

Conflict of Interest

The authors declare that they have no conflict of interest.

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References

Akcam, M., Aslan, N., 2015. Helicobacter pylori Related Health Problems in Children. Iran J Pub Health 44, 877.

Amedei, A., Munari, F., Della Bella, C., Niccolai, E., Benagiano, M., Bencini, L., 2014. Helicobacter pylori secreted peptidyl prolyl cis, trans-isomerase drives Th17 inflammation in gastric adenocarcinoma. Int Emerg Med 9, 303-309.

Arnason, J.E., Campigotto, F., Neuberg, D., Bussel, J.B., 2012. Abnormalities in IgA and IgM are associated with treatment-resistant ITP. Blood 119, 5016-5020.

Bagheri, N., Rahimian, G., Salimzadeh, L., Azadegan, F., Rafieian-Kopaei, M., Taghikhani, A., *et al.*, 2013. Association of the virulence factors of Helicobacter pylori

and gastric mucosal interleukin-17/23 mRNA expression in dyspeptic patients. EXCLI J 12, 5-14.

Baker, R.D., Greer, F.R., 2010. Diagnosis and prevention of iron deficiency and iron-deficiency anemia in infants and young children (0–3 years of age). Pediatrics 126, 1040-1050.

Bazmamoun, H., Razavi, Z., Esfahani, H., Arefian, M.S., 2014. Evaluation of Iron deficiency anemia and BMI in children suffering from Helicobacter pylori infection. Iran J Ped Hematol Oncol 4, 167-171.

Beydoun, M.A., Dore, G.A., Canas, J.A., Beydoun, H.A., Zonderman, A.B., 2015. Helicobacter pylori Seropositivity's Association with Markers of Iron, 1-Carbon Metabolism, and Antioxidant Status among US Adults: A Structural Equations Modeling Approach. PloS one 10, e0121390.

Bhuiyan, T.R., Islam, M.M., Uddin, T., Chowdhury, M.I., Janzon, A., Adamsson, J.*, et al.*, 2014. Th1 and Th17 responses to Helicobacter pylori in Bangladeshi infants, children and adults. PLoS One 9, e93943.

Carbo, A., Olivares-Villagómez, D., Hontecillas, R., Bassaganya-Riera, J., Chaturvedi, R., Piazuelo, M.B.*, et al.*, 2014. Systems modeling of the role of interleukin-21 in the maintenance of effector CD4+ T cell responses during chronic Helicobacter pylori infection. MBio 5, e01243-01214.

Chan, J.C.W., Liu, H.S.U., Kho, B.C.S., Sim, J.P.U., Lau, T.K.H., Luk, Y.W., 2006. Pernicious anemia in Chinese: a study of 181 patients in a Hong Kong hospital. Medicine 85, 129-138.

Darvishi, M., Ziari, K., Mohebi, H., Alizadeh, K., 2015. Association between Iron Deficiency Anemia and Helicobacter Pylori Infection among Children Under Six Years in Iran. Acta Med Iran 53, 220-224.

El-Omar, E.M., 2013. Iron deficiency and Helicobacter pylori–induced gastric cancer: too little, too bad. J Clin Invest 123, 113.

Gil, J.H., Seo, J.W., Cho, M.-S., Ahn, J.-H., Sung, H.Y., 2014. Role of Treg and TH17 cells of the gastric mucosa in children with Helicobacter pylori gastritis. Journal of pediatric gastroenterology and nutrition 58, 245-251.

Horvath Jr, D.J., Washington, M.K., Cope, V.A., Algood, H.M.S., 2012. IL-23 contributes to control of chronic Helicobacter pylori infection and the development of T helper responses in a mouse model. Front Immunol 3.

Hu, Q., Akatsuka, S., Yamashita, Y., Ohara, H., Nagai, H., Okazaki, Y.*, et al.*, 2010. Homozygous deletion of CDKN2A/2B is a hallmark of iron-induced high-grade rat mesothelioma. Labo Invest 90, 360-373.

Janulczyk, R., Pallon, J., Björck, L., 1999. Identification and characterization of a Streptococcus pyogenes ABC transporter with multiple specificity for metal cations. Molecul Microbiology 34, 596-606.

Lopez, A., Cacoub, P., Macdougall, I.C., Peyrin-Biroulet, L., 2015. Iron deficiency anaemia. The Lancet.

Miernyk, K., Bruden, D., Zanis, C., McMahon, B., Sacco, F., Hennessy, T.*, et al.*, 2013. The effect of Helicobacter pylori infection on iron stores and iron deficiency in urban Alaska Native adults. Helicobacter 18, 222-228.

Milman, N., Rosenstock, S., Andersen, L., Jørgensen, T., Bonnevie, O., 1998. Serum ferritin, hemoglobin, and Helicobacter pylori infection: a seroepidemiologic survey comprising 2794 Danish adults. Gastroenterology 115, 268-274.

Monzón, H., Forné, M., Esteve, M., Rosinach, M., Loras, C., Espinós, J.C.*, et al.*, 2013. Helicobacter pylori infection as a cause of iron deficiency anaemia of unknown origin. World J Gastroenterol 19, 4166-4171.

Mubarak, N., Gasim, G.I., Khalafalla, K.E., Ali, N.I., Adam, I., 2014. Helicobacter pylori, anemia, iron deficiency and thrombocytopenia among pregnant women at Khartoum, Sudan. Transactions of The Royal Society of Tropical Medicine and Hygiene 108, 380-384.

Noto, J.M., Gaddy, J.A., Lee, J.Y., Piazuelo, M.B., Friedman, D.B., Colvin, D.C.*, et al.*, 2013. Iron deficiency accelerates Helicobacter pylori–induced carcinogenesis in rodents and humans. J Clin Invest 123, 479-492.

Pandya, H.B., Patel, J.S., Agravat, H.H., 2014. Non-Invasive Diagnosis of Helicobacter pylori: Evaluation of Two Enzyme Immunoassays, Testing Serum IgG and IgA Response in the Anand District of Central Gujarat, India. J Clin Diagnos Research: JCDR 8, DC12.

Plummer, M., Franceschi, S., Vignat, J., Forman, D., de Martel, C., 2015. Global burden of gastric cancer attributable to Helicobacter pylori. Int J Cancer 136, 487-490.

Queiroz, D., Harris, P.R., Sanderson, I.R., Windle, H.J., Walker, M.M., Rocha, A.*, et al.*, 2013. Iron status and Helicobacter pylori infection in symptomatic children: an international multi-centered study. PLoS One 8, e68833.

Rahman, A.S., Sarker, S.A., Ahmed, T., Islam, R., Wahed, M.A., Sack, D.A., 2013. Relationship of intestinal parasites, H. pylori infection with anemia or iron status among school age children in rural Bangladesh. J Gastroenterol Hepatol Res 2.

Sapmaz, F., Başyiğit, S., Kalkan, İ.H., Kısa, Ü., Kavak, E.E., Güliter, S., 2016. The impact of Helicobacter pylori

eradication on serum hepcidin-25 level and iron parameters in patients with iron deficiency anemia. Wiener klinische Wochenschrift, 1-6.

Sato, Y., Yoneyama, O., Azumaya, M., Takeuchi, M., Sasaki, S.y., Yokoyama, J., *et al.*, 2015. The Relationship Between Iron Deficiency in Patients with Helicobacter Pylori□Infected Nodular Gastritis and the Serum Prohepcidin Level. Helicobacter 20, 11-18.

Shi, Y., Liu, X.-F., Zhuang, Y., Zhang, J.-Y., Liu, T., Yin, Z., *et al.*, 2010. Helicobacter pylori-induced Th17 responses modulate Th1 cell responses, benefit bacterial growth, and contribute to pathology in mice. J Immunol 184, 5121-5129.

Turkina, M.V., Olofsson, A., Magnusson, K.-E., Arnqvist, A., Vikström, E., 2015. Helicobacter pylori vesicles carrying CagA localize in the vicinity of cell–cell contacts and induce histone H1 binding to ATP in epithelial cells. Microbiol Letters 362, fnv076.

Xie, C., Xu, L.-Y., Li, W., Yang, Z., Lu, N.-H., 2014. Helicobacter pylori infection in Mongolian gerbils does not initiate hematological diseases. World J Gstroenterol 20, 12308.

Yamanouchi, J., Azuma, T., Yakushijin, Y., Hato, T., Yasukawa, M., 2014. Dramatic and prompt efficacy of Helicobacter pylori eradication in the treatment of severe refractory iron deficiency anemia in adults. Annals Hematol 93, 1779.

Zhuang, Y., Shi, Y., Liu, X.-F., Zhang, J.-Y., Liu, T., Fan, X., *et al.*, 2011. Helicobacter pylori-infected macrophages induce Th17 cell differentiation. Immunobiology 216, 200-207.