ORIGINAL ARTICLE

Effect of *Helicobacter Pylori* Infection on Red Blood Cell Fragility

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ABSTRACT

Background: *Helicobacter pylori* produce gastric inflammation, interfering with iron and vitamin B12 metabolism. The deficiency of both these micronutrients disturbs the normal morphological characteristics of red blood cells rendering them fragile. When such red blood cells passes through tight spots in the circulation like the red pulp of the spleen, they rupture.
Objective: The objective of this study was to elucidate the effects of *Helicobacter pylori* infection on red blood cell fragility.
Subjects and methods: A total number of 90 subjects were included in the study. They were divided into group A (30 subjects with gastric symptoms and *H. pylori* infection), group B (30 subjects with gastric symptoms but without *H. pylori* infection), and group C (30 normal healthy age and sex matched subjects). *H. pylori* infection was considered positive on the basis of positive serology, rapid urease test and histopathological examination. Red blood cell fragility was determined by osmotic fragility method using different concentrations of saline.
Results: Red blood cell fragility did not show significant difference (p value > 0.05) within the individual groups nor when compared with each other.
Conclusion: *H. pylori* infection did not affect the red blood cell fragility.
Key words: Red blood cell fragility, *Helicobacter pylori*

INTRODUCTION

Human stomach is reported to be the primary reservoir for *Helicobacter pylori* where these organisms are usually found in the gastric antrum. *Helicobacter pylori* produce gastric inflammation, gastric and duodenal ulcers[1,2]. Risk factors involved in the pathogenesis of *Helicobacter pylori* are low socioeconomic status, crowding, poor hygiene, diet, alcohol consumption, occupational exposure, smoking, family history of gastric diseases and poor water condition[3]. The prevalence of *Helicobacter pylori* infection is 30% in the developed countries and 50-70% in the developing countries[4]. Many studies documented strong association of *Helicobacter pylori* infection with the iron deficiency [5-7] and vitamin B_{12} deficiency [8-10] while other studies have shown weak or no relationship with iron and vitamin B_{12} deficiency[11-14].

Iron and vitamin B_{12} are necessary for the maturation of red blood cells; the deficiency of both these micronutrients disturbs the normal morphological characteristics of red blood cells. The deficiency of iron results in the formation of small red blood cells and such type of cells are called microcytic cells. The deficiency of vitamin B_{12} leads to the development of large, immature red blood cells called macrocytes. The age of such red blood cells decreases because their membranes become fragile. When such red blood cells passes through tight spots in the circulation like the red pulp of the spleen, they rupture.[15-17] This study is planned to elucidate the effects of *Helicobacter pylori* infection on red blood cell fragility if we assume that *Helicobacter pylori* affects both iron and vitamin B_{12} levels.

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MATERIAL AND METHODS

It was a cross sectional analytical study conducted at the University of Health Sciences, Lahore. Subjects with *Helicobacter pylori* infection and subjects having gastric symptoms only were selected from the Services Hospital, Lahore. Ninety subjects including both male and female were selected for the study. The subjects were between 15-60 years of age. Subjects were divided into three groups. Group 1 was comprised of thirty subjects with *Helicobacter pylori* infection. Group 2 was composed of thirty subjects with history of gastric symptoms without *Helicobacter pylori* infection. In the group 3, age and sex matched healthy subjects without gastric symptoms and *Helicobacter pylori* infection were included.

An informed consent was taken from all the subjects after explaining the study purpose and procedure. Detailed clinical history was obtained from all the subjects. Diagnosis of *Helicobacter pylori* infection was made by performing ELISA for *H. pylori* IgG antibodies, rapid urease test and

histopathological examination. Three milliliters of blood was drawn from antecubital vein under aseptic measures in heparanized tube for osmotic fragility.

Different concentrations of saline like 0.9 %, 0.75 %, 0.65 %, 0.6 %, 0.55 %, 0.5 %, 0.4 %, 0.35 %, 0.3 %, 0.2 %, and 0.1 % were prepared from a stock solution of buffered sodium chloride (100 g/l). Five ml of each of the 11 saline solutions were delivered in 11 test tubes and 5 ml of water was delivered into the 12^{th} tube. 50 µl of well mixed blood was added to each tube, and mixed by inverting the tube several times. Incubation was given for 30 minutes at room temperature, and the contents were mixed again after 30 minutes. All the tubes were centrifuged for 5 minutes. At each saline concentration, the fraction of red cells lysis was determined calorimetrically[18].

Statistical Analysis: Paired student t test was applied to observe the difference between male and females within the groups. One way ANOVA was applied to determine the significance of difference of variables between groups. p value of less than 0.05 was considered statistically significant.

RESULTS

Hemolysis started at 0.48% ± 0.01% in males and at 0.47% ± 0.01% in females in group A. There was complete hemolysis of red blood cells at 0.30% in males and females. The difference among males and females in groups A was statistically non significant ($p > 0.05$; Table 1).

Males in group B showed start of hemolysis at 0.49% ± 0.01% while in females, hemolysis started at 0.48% ± 0.02%. Complete hemolysis of red blood cells occurred at 0.30% in both males and females. The difference between males and females in groups B was not significant ($p > 0.05$; Table 2).

Start of hemolysis among males (0.48% ± 0.02%) and females (0.47% ± 0.01%) in group C was not statistically significant ($p > 0.05$). There was complete hemolysis of red blood cells in males and females at 0.30% in group C (Table 3).

Red blood cell osmotic fragility in groups A, B and C is shown in Table 4. Hemolysis started at 0.472, 0.478 and 0.475% NaCl concentrations in groups A, B and C respectively. There was complete hemolysis of red blood cells at 0.30% in all the three groups. The difference among groups A, B and C was not statistically significant ($p > 0.05$; Table 4).

Table 1 Comparison of red blood cell osmotic fragility between males and females in group A

Parameter	**Male** (n = 18)	**Female** (n = 12)	**p value**
NaCl Concentration at which hemolysis started	0.48%±0.01%	0.47%±0.01%	0.50*
NaCl Concentration at which hemolysis completed	0.30%	0.30%	1.00*

*The values are statistically non significant

Table 2 Comparison of red blood cell osmotic fragility between males and females in group B

Parameter	**Male** (n = 16)	**Female** (n = 14)	**p value**
NaCl concentration at which hemolysis started	0.49% ± 0.01%	0.48% ± 0.02%	0.79*
NaCl concentration at which hemolysis completed	0.30%	0.30%	1.00*

* The values are statistically non significant

Table 3 Comparison of red blood cell osmotic fragility between males and females in group C

Parameter	**Male** (n = 14)	**Female** (n = 16)	**p value**
NaCl Concentration at which hemolysis started	0.48 ± 0.01	0.47 ± 0.01	0.68*
NaCl Concentration at which hemolysis completed	0.30%	0.30%	1.00*

* The values are statistically non significant

Table 4 Comparison of red blood cell osmotic fragility between groups A, B and C

Parameter	**Group A** (n=30) (*H.pylori* +ve patients)	**Group B** (n=30) (*H.pylori* -ve patients)	**Group C** (n= 30) (Healthy control)	**P value**
NaCl Concentration at which hemolysis started	0.47%± 0.01%	0.48%± 0.01%	0.48±0.01%	0.49*
NaCl Concentration at which hemolysis completed	0.30%	0.30%	0.30%	1.00*

* The values are statistically non significant

DISCUSSION

Red blood cells are biconcave discs with very elastic and highly deforming properties. Red blood cell membrane is composed of three very important proteins which include ankyrin, spectrin, and band-3. These proteins maintain the integrity and shape of red blood cells[19].

Red blood cells do not contain usual cell organelles and are without nucleus, so they can not divide and synthesize structural proteins and enzymes. The energy demands are also very low. The red blood cells derive energy from anaerobic glycolysis and hexose monophosphate pathway. Due

to these features, the life span of red blood is relatively short and is only 120 days[17].

In the presence of iron and vitamin B12 deficiency, the membranes of red blood cells become fragile and red blood cells rupture before time when they pass through tight spots in the pulp of spleen. Many studies in the past reported that *Helicobacter pylori* results in the deficiency of iron and vitamin B_{12}, while other studies mentioned no relationship[5,6,8,9,12,13]. *Helicobacter pylori* results in iron deficiency by neutralizing and decreasing gastric acidity; thus leading to decreased absorption of iron[20]. Secondly; *Helicobacter pylori* also results in iron deficiency anemia by decreasing the vitamin C content of the gastric juice that is necessary for the reduction of ferric to ferrous form.[21] *Helicobacter pylori* infection results in decreased absorption of vitamin B_{12} by decreasing the secretion of gastric acid and intrinsic factor which are necessary for absorption[22].

Due to the above mentioned extragastric complications of *Helicobacter pylori* infection, we planned to see the effect of *Helicobacter pylori infection* on red blood cell fragility. The average life spans of red blood cells are 120 days after which the cells become more and more fragile. The fragile cells then rupture by passing through tight pores in the spleen especially the red pulp of the spleen[15]. When the osmotic fragility is normal, red blood cells begin to hemolyze when suspended in 0.5% saline and complete lysis occurs at 0.35% saline concentration[19].

The present study was the first ever study in which red blood cell fragility was carried out in *Helicobacter pylori* positive and negative subjects. In the present study, no significant change in red blood cell fragility was noted between *Helicobacter pylori* positive and negative subjects. Regarding red blood cell fragility very scarce data is present in literature. Regarding association of *Helicobacter pylori* infection with red blood cell fragility, very little data is present in literature. When different isolates of *Helicobacter pylori* were treated *in vitro* with red blood cells of different blood groups, only blood group A red blood cells showed hemolysis[23]. In another study, it was proposed that anti *Helicobacter pylori* antibodies can cross react with some human red cell membrane antigens and can result in hemolysis[24].

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