TOTAL LEUKOCYTE COUNTS AND NEUTROPHIL-LYMPHOCYTE COUNT RATIOS AMONG *HELICOBACTER PYLORI*-INFECTED PATIENTS WITH PEPTIC ULCERS: INDEPENDENT OF BACTERIAL CagA STATUS

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Abstract. Elevated leukocyte counts can be a marker of inflammation and infection. The aim of this study was to determine the total leukocyte count and neutrophil-lymphocyte count ratio (NLCR) among *Helicobacter pylori*-infected patients with peptic ulcer disease (PU) and among asymptomatic subjects (AS) and to evaluate if there is an association between these lab values and the presence of the *H. pylori* virulence factor cytotoxin-associated gene A (*CagA*). Sixty *H. pylori*-infected PU patients, 63 AS carriers and 32 healthy *H. pylori*-negative subjects (controls) were included in the study. The total white blood cell (WBC) counts and differentials were determined using standard hematological methods. The mean total WBC count, mean neutrophil count and NLCR were significantly higher among PU patients than in controls ($p<0.001$, $p<0.001$ and $p<0.001$, respectively). Similarly, the mean WBC count, mean neutrophil count and NLCR were significantly higher among AS patients than in controls ($p<0.005$, $p<0.001$ and $p<0.02$, respectively). The differences of mean WBC counts mean neutrophil counts and NLCR were also significantly different ($p<0.005$, $p<0.001$ and $p<0.001$, respectively) between the PU and AS patients. There were no differences in the PU and AS patients in regard to anti-CagA positivity. These results show the CagA factor was not associated with the presence or absence of symptoms in *H. pylori* infected patients.

Keywords: *Helicobacter pylori*, peptic ulcer, leukocyte count, neutrophils-lymphocyte count ratio, anti-CagA

INTRODUCTION

Gastric colonization with *Helicobacter pylori* leads to peptic ulcer in 10-20% and gastric cancer in less than 3% of those infected (Sanchez-Zauco *et al*, 2010). The reciprocal interactions between bacterial pathogenic factors and host genetic parameters determine the outcome of infection (Snaith and El-Omar, 2008). Genetically, *H. pylori* is divided into *cagA*-positive and *cagA*-negative strains; in some studies, a powerful association has been observed between PU disease and *cagA*+ strains (Jafarzadeh and Salari, 2006). The seroprevalences of *H. pylori* among healthy Iranian adults and children is 67.5% and 46.6%, respectively, and the prevalence of anti-CagA antibodies

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among Iranian adults and children is 72.8% and 67.4% among infected subjects, respectively (Jafarzadeh *et al*, 2007).

Some epidemiological studies have suggested a positive association between coronary heart disease and *H. pylori* infection (Jha *et al*, 2008; Jafarzadeh *et al*, 2010). The possible mechanisms by which *H. pylori* infection may increase the risk of ischemic heart disease have been proposed as induction of dyslipidemic alterations, elevated levels of fibrinogen, induction of C-reactive protein (CRP), increases in blood leukocyte counts and homocysteine, induction of hypercoagulability and causation of impaired endothelial function (Manolakis *et al,* 2007; Huang *et al,* 2011). CRP is a marker of inflammation and infection; in our previous study higher concentrations of CRP were observed in *H. pylori*-infected subjects (Jafarzadeh *et al,* 2009a).

White blood cells (WBC) are found in various parts of the body, including the blood, lymphoid organs (including lymph nodes, the spleen, Peyer's patches, adenoids and tonsils, and mucosal associated lymphoid tissue) and most other organs (Randall *et al,* 2008; Ruddle and Akirav, 2009). Among those compartments, the blood is the easiest site to obtain WBC.

Alteration of blood leukocyts, specifically and neutrophils has commonly been attributed to inflammation (Nagatomi, 2006). Blood leukocyte counts are known to change in response to pathologic situations such as infections (de Jager *et al,* 2010). Numerous epidemiological and clinical studies have found an association between leukocyte counts and ischemic heart disease (Huang *et al,* 2009; Madjid *et al,* 2004). Recently, the neutrophil to lymphocyte count ratio (NLCR) has been proposed as an independent predictors of various clinical problems ranging from cardiovascular events to cancer (Muhmmed Suliman *et al,* 2010; Ubukata *et al,* 2010). This suggests peripheral leukocyte counts and NLCR may be diagnostically useful. However, there are no published studies of NLCR among *H. pylori*-infected patients. We studied the total leukocyte counts and NLCR among *H. pylori*-infected PU and AS patients and their association with CagA[+].

MATERIALS AND METHODS

Subjects

Sixty patients with PU (aged 40.4 ± 11.3 years) from Ali-ebne Abitaleb Hospital in Rafsanjan (Kerman Province, southeastern Iran), were included in this case-controlled study conducted from March to December 2010. Inclusion criteria for subjects were symptoms suggestive of peptic disease (burning abdominal pain, chronic vomiting or hematemesis) with endoscopy proven PU disease who were not taking medications, including non-steroidal anti-inflammatory drugs at the time of the study. In the PU patients, *H. pylori* status was determined by rapid urease test and serological assessments of anti-*H. pylori* IgG antibodies using an enzyme-linked immunosorbent assay. The rapid urease test was performed on biopsy specimens from the gastric antrum collected during endoscopy. A patient was considered positive for *H. pylori* infection if the rapid urease test and the serology for anti-*H. pylori* IgG were positive.

Sixty-three *H. pylori*-infected AS subjects (aged 41.4 ± 11.6 years) were also included in the study. The inclusion criterion for AS cerriers was a positive serology for anti- *H. pylori* IgG. The control group consisted of 32 asymptomatic patients (aged 39.7 ± 11.3 years) with negative serology for anti-*H. pylori* IgG. None of the AS or control subjects had a history

of gastrointestinal disease. Individuals with a history of pulmonary disease, cardiovascular disease, diabetes mellitus, hypertension, renal failure, anemia, asthma or neoplasia were excluded from the study. Subjects in the non-infected and AS groups were without illness and did not undergo endoscopy. Serum samples were collected from all subjects and stored at -70ºC until used. The Ethics Committee of Rafsanjan University of Medical Sciences approved this study. All subjects gave informed consent before being enrolled in the study.

Determination of *H. pylori* antibodies

Serum immunoglobulin G (IgG) antibody levels against *H. pylori* were measured using a commercial enzyme-linked immunosorbent assay (ELISA) kit (Trinity Biotech, Jamestown, NY). The Immune Status Ratio (ISR) was computed for each sample and an ISR level >1.0 was considered positive for *H. pylori*. Serum anti-CagA IgG levels were determined by ELISA using a commercial kit (Diagnostic Bioprobes, Milano, Italy). The serum anti-CagA concentration was measured as arbitrary units per milliliter (Uarb/ml) since no international standard unit is available. Based on the kit guidelines, a cutoff value of 5 Uarb/ml was used to distinguish negative from positive samples.

Leukocyte counts

Total and differential leukocyte counts were determined on samples obtained from the pheripheral blood. The total cell count was determined with a hemacytometer (T-890, Culter, USA). Giemsa-stained blood films were used to determine the differential count. Absolute counts were calculated by multiplying the percentage of the cell by the number of white blood cells (WBC). The WBC counts and NLCR were expressed as means± SD.

Statistical analysis

The differences in variables were analyzed using a *t*-test, ANOVA, Mann-Whitney *U*, Kruskal-Wallis or chi-square test where as appropriate. A *p*-value< 0.05 was considered significant.

RESULTS

The laboratory values among the various study groups are summarized in Table 1. The mean total WBC count, neutrophil count and NLCR among the PU subjects were significantly higher than among the control group ($p<0.001$, $p<0.001$ and $p<0.001$, respectively). The mean total WBC count, neutrophil count and the NLCR in the AS group were also significantly higher than among the control group ($p<0.005$, $p<0.001$ and $p<0.02$, respectively). The mean WBC count, neutrophil count and NLCR were significantly different between the PU and AS groups ($p<0.005$, $p<0.001$ and $p<0.001$, respectively). No significant differences in lymphocyte counts were seen among the 3 study groups.

The PU and AS groups were divided into two subgroups based on the presence or absence of anti-CagA antibodies (Table 1). Among the PU and AS groups the WBC count, neutrophil count, lymphocyte count and NLCR were not significantly differ between subjects with positive and negative tests for anti-CagA antibodies.

The subjects were classified into subgroups based on the total WBC count, neutrophil count and NLCR (Table 2). In the PU group, the number of subjects with a total WBC count >10,000 cells/mm^3 was significantly higher than in the AS and control groups ($p<0.01$). In the control group the number of subjects with a total WBC count <8,000 cells/mm^3 was significantly higher than in the PU and AS groups

Table 1

Comparison of the total WBC count, neutrophil count, lymphocyte count and the neutrophil-lymphocyte ratio among PU, AS and control groups (mean±SD).

Groups	Anti-CagA status	No. of subjects	Total leukocyte count (cells/mm^3)	Neutrophil count (cells/mm^3)	Lymphocytes count (cells/mm^3)	Neutrophil-lymphocyte ratio
PU	Anti-CagA$^+$	36	8,972 ± 2,745	5,678 ± 2,225	3,263 ± 718	1.71 ± 0.54
	Anti-CagA$^-$	24	8,216 ± 2,250	5,202 ± 1,823	3,052 ± 643	1.72 ± 0.41
	Total	60	8,670 ± 2,566	5,488 ± 2,071	3,179 ± 692	1.71 ± 0.49
AS	Anti-CagA$^+$	34	7,664 ± 1,659	4,431 ± 1,150	3,183 ± 806	1.46 ± 0.56
	Anti-CagA$^-$	29	6,872 ± 1,096	3,974 ± 888	2,885 ± 475	1.43 ± 0.39
	Total	63	7,300 ± 1,472	4,221 ± 1,055	3,046 ± 686	1.44 ± 0.48
Control		32	6,465 ± 620	3,509 ± 445	2,929 ± 247	1.20 ± 0.12

PU, subjected with peptic ulcer disease; AS, asymptomatically infected subject; WBC, white blood cells

(p<0.01). All non-infected subjects had a total WBC count <8,000 cells/mm^3 (Table 2).

In the control group the frequency of subjects with a neutrophil count <4,000 cells/mm^3 was significantly higher than in the PU and AS groups (p<0.1 and p<0.05, respectively). All non-infected subjects had a neutrophil count <5,500 cells/mm^3. In the PU group, the number of subjects with a neutrophil count >7,000 cells/mm^3 was significantly higher than in the AS and control groups (p<0.01) (Table 2).

In the PU group, the number of subjects with a NLCR >1.75 was significantly higher than in the AS and control groups (p<0.01). In the control group the number of subjects with a NLCR <1.25 was significantly higher than in the PU and AS groups (p<0.05).

DISCUSSION

Among controls the total WBC counts ranged from 5,000 to 10,000 cell/mm^3. Leukocytosis (WBC >10,000 cells/mm^3) can be attributed to infection, inflammation, tissue damage, burns, dehydration, thyroid storm, leukemia, stress or steroids (George and Panos, 2005).

In this study a higher total WBC count, neutrophil count and NLCR were seen among *H. pylori*-infected PU patients and in the AS group compared to the control group. A study from Japan found *H. pylori* eradication decreases blood neutrophil and monocyte counts (Kondo *et al*, 2004). The mechanism causing a higher total WBC count and neutrophil count in *H. pylori* infections remains to be determined. The generation of leukocytes from bone marrow stem cells is potentiated by proinflammatory cytokines, such as tumor necrosis factor (TNF), interleukin (IL)-1 and IL-6 (Hawley *et al*, 1991). An increased production of TNF, IL-1, IL-6 and IL-8 has been reported among *H. pylori*-infected individuals (Romero-Adrian *et al*, 2010). These cytokines may enhance the differentiation of WBC during *H. pylori* infection.

In the present study, a higher NLCR was observed among subjects infected with *H. pylori*; this reflects an increased neutrophil count without a change in lymphocyte count. These observations suggest

Table 2
Classification of PU, AS and control groups according to the total WBC count, neutrophil count and NLCR.

Parameter	Cell count (range)	PU % (No.)	AS % (No.)	Control % (No.)
Total WBC count (cells/mm^3)	4,000-6,000	15 (9)	15.9 (10)	12.5 (4)
	>6,000-8,000	31.7 (19)	50.8 (32)	87.5 (28)
	>8,000-10,000	18.3 (11)	31.7 (20)	0 (0)
	>10,000	35 (21)	1.6 (1)	0 (0)
	Total	100 (60)	100 (63)	100 (32)
Neutrophil count (cells/mm^3)	2,400-4,000	25 (15)	54 (34)	87.5 (28)
	>4,000-5,500	30 (18)	34.9 (22)	12.5 (4)
	>5,500-7,000	16.7 (10)	9.5 (6)	0 (0)
	>7,000	28.3 (17)	1.6 (1)	0 (0)
	Total	100 (60)	100 (63)	100 (32)
NLCR	1-1.25	15 (9)	36.5 (23)	78.1 (25)
	>1.25-1.50	16.7 (10)	31.7 (20)	18.8 (6)
	>1.50-1.75	26.7 (16)	12.7 (8)	3.1 (1)
	>1.75	41.7 (25)	19 (12)	0 (0)
	Total	100 (60)	100 (63)	100 (32)

NLCR, neutrophil-lymphtocyte count ratio; PU, subjects with peptic ulcer disease; AS, subjects with asymptomatic *H. pylori* infection; WBC, white blood cells

H. pylori-infected subjects produce greater numbers of neutrophils. The proinflammatory cytokine IL-17A, the signature cytokine of TH17 cells, has been shown to increase neutrophil counts via induction of G-CSF (von Vietinghoff and Ley, 2009). IL-17A expression is also increased in *H. pylori*-infected individuals (Jafarzadeh *et al*, 2009b; Kimang'a *et al*, 2010). Interestingly, it has been demonstrated *H. pylori*-derived neutrophil-activating protein (HP-NAP) increases the lifespan of neutrophils (Cappon *et al*, 2010).

The results of the present study show the mean total WBC count and the mean neutrophil were significantly higher in the AS group compared to the control group. These observations are consistent with our previous findings regarding differences in CRP, a sensitive inflammatory marker, among AS and *H. pylori*-negative control groups (Jafarzadeh *et al*, 2009a). The higher total WBC and neutrophil counts among the AS group may be due to the induction of subclinical microinflamatory reactions caused by *H. pylori*.

We also studied the association among CagA antibodies and total WBC counts and neutrophil counts among *H. pylori* infected subjects. The results show no differences in WBC or neutrophil counts among PU and AS groups in relationship to cagA$^+$ *H. pylori* strains. In our previous study, we found CRP levels were not influenced by the expression of bacterial CagA virulence factor (Jafarzadeh *et al*, 2009a).

It has been reported cagA$^+$ *H. pylori* strains cause more serious gastric inflammation than cagA-negative strains and are associated with a higher risk for PU disease and gastric cancer (Costa *et al*, 2009). Some studies, failed to find an asso-

ciation between inflammatory cytokines, such as IL-6 and TNF-α, and virulence factors, such as CagA (Kim *et al,* 2000). Polymorphism among genes encoding for cytokines, such as IL-1, TNF-α and IFN-γ, has been associated with *H. pylori*-induced gastric adenocarcinoma and peptic ulcers (Basso and Plebani, 2004). Major variations in *cagA⁺* genotype (Lopez-Vidal *et al,* 2008). Both host and bacterial factors should be considered in understanding *H. pylori*-associated inflammatory responses, such as elevated WBC counts.

The present study showed no significant differences between CagA-positive and CagA-negative strains regarding the total WBC counts and total neutrophil counts. Total WBC and neutrophil counts were independent of the CagA status among H. *pylori* strains. Therefore, the total WBC and neutrophil counts were not suitable markers to determine *H. pylori* strains.

Monitoring the total WBC and neutrophil counts and NLCR prior to, during and after treatment of PU disease may improve predictive or prognostic values. Our results suggest further studies are needed in this field.

In conclusion, the results of the present study show higher total WBC counts and NLCR among PU and AS groups compared to controls. However, these parameters were not impacted by bacterial CagA status.

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