The prevalence of Helicobacter pylori and its effect on the prognosis of patients with COVID-19

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ABSTRACT

Objective: SARS-CoV-2 RNA positivity in stool in COVID-19 infection has been reported at rates varying between 6-83%. The purpose of this study was to determine the prevalence of H.pylori and investigate whether it determines the disease prognosis in COVID-19 patients.

Methods: This study was conducted on 117 confirmed COVID-19 patients who were hospitalized due to symptomatic pneumonia and tested for stool H.pylori antigen. Stool H. pylori test outcomes, demographic parameters, laboratory findings, and prognostic predictors of disease were recorded. The effect of the presence of H.pylori in patients with COVID-19 was analyzed.

Results: The mean age of 117 included patients was 49.68 ± 14.62 years, 78 (66.7%) had COVID PCR positivity and 32 (27.35%) had H.pylori positivity. There was no statistical difference in demographic data, prognosis, and laboratory parameters between those with and without H.pylori.

Conclusion: H.pylori positivity was detected as 27.35% in patients with COVID-19 infection. However, we could not find the positive or negative effect of H.pylori on the prognosis of COVID-19 disease. In conclusion, according to the results of this study, H. pylori positivity or negativity neither determined the severity of the COVID-19 disease nor the poor prognostic indicators of the disease.

Keywords: H. pylori, COVID-19, prognosis

INTRODUCTION

New coronavirus (COVID-19, SARS-CoV-2) has become a pandemic that has spread all over the world, starting in China in 2019, causing severe acute respiratory failure (1). New information is being added every day about the characteristics and treatment of COVID-19 disease. However, our knowledge of COVID-19 infection and its treatment is still limited.

SARS-CoV-2 RNA positivity in stool in COVID-19 infection has been reported at rates varying between 6-83% (2-5). Detection of viral genetic material in the stool does not mean that viable infectious virions are present in the stool, but a long-term positive gastrointestinal specimen is interpreted as the virus can replicate actively in the patient's gastrointestinal system (6). ACE-2 receptors used by SARS-CoV-2 to enter the cell are highly expressed in the small intestine and the binding affinity of ACE affects the infectivity of the virus. A number of viruses, such as coronavirus, rotavirus, and noroviruses, can invade absorptive enterocytes through the ACE-2 receptors on absorptive enterocytes in the ileum and colon, causing gastroenteritis.
There have been studies showing that intestinal epithelial cells expressing ACE-2 may be at increased risk of attack by SARS-CoV-2 and that ACE2 is highly expressed in the small intestine, especially in proximal and distal enterocytes. Therefore, the digestive system can be invaded by SARS-CoV-2 and used as an entrance of infection. It has been shown that approximately 12% of patients with SARS-CoV-2 infection have gastrointestinal symptoms including diarrhea, nausea and vomiting (7). In the meta-analysis examining the studies investigating whether the gastrointestinal system symptoms are associated with mortality in COVID-19 disease; no significant difference was shown between groups with and without gastrointestinal symptoms (8).

H. pylori is one of the most common infections affecting the human race with a high prevalence in developing countries (9). With respect to the distribution map of COVID-19 cases and mortality rates in different countries, mortality rates per million population are low in Russia, Portugal (https://www.worldometers.info/coronavirus/) whereas higher in these regions (Russia 78.5%, Portugal 86.4%). Therefore, it is assumed that H. pylori may play a role in preventing serious infections in COVID-19 infection (9,10).

Conflicting results have been reported between non-COVID viral infections and H. pylori positivity in the literature (11-13).

In our literature searches, we did not find any studies investigating COVID-19 and H. pylori infection together. To our knowledge, we have investigated for the first time the determination of the prevalence of H. pylori in patients with confirmed COVID-19 disease and whether it determines the prognosis of COVID-19 disease.

**MATERIAL and METHODS**

**Study population and sample collection**

A total of 117 patients, whose diagnosis of COVID-19 pneumonia was confirmed by nasopharyngeal (NP) PCR-RT swab and thorax computed tomography between 15 March 2020 and July 2020, were included in the study. Stool H. pylori antigen test was investigated in all patients. Patients with a history of H. pylori eradication therapy, a history of gastric malignancy and NP PCR-RT negative patients were excluded from the study. PCR results of the cases, H. pylori test results, demographic parameters, other laboratory parameters and prognosis indicators were recorded. The effect of the presence of H. pylori in patients followed-up for COVID-19 infection was statistically analyzed.

**H. pylori antigen test in faeces**

Stool samples from patients with confirmed diagnosis of COVID-19 disease were taken into special stool containers and transferred to the laboratory. H. pylori antigen test [H.pylori Antigen Rapid Test Device (faeces), China] was studied in stool samples by immunochromatographic method. After all the reagents and stool samples reached room temperature, the applicator stick coming out of the kit was dipped into three different areas of each stool sample and approximately 50mg sample was collected. After placing the applicator stick in the diluent tube and closing its cap, it was shook. The results were interpreted after 10 minutes of dropping 3 drops of the immunochromatographic cassette from the liquid sample in the tube to the sample section of the test. The formation of a colored line, even faint, was considered positive in patients with a control line showing the validity of the test. The whole procedure has been done in line with the manufacturer's recommendations.

It has been reported that the use of H. pylori antigen test in stool is reliable as a non-invasive test (14-16).

**Nucleic acid isolation and SARS-CoV 2 RT-PCR**

Combined nasopharynx and oropharynx swab samples were collected by dacron swab and placed in Viral transport medium immediately, and delivered to the laboratory by keeping them at 2-8 °C. The samples were delivered to the laboratory in accordance with the rules of cold chain with the triple transport system, complying the infection prevention and control procedures.

After taking the samples in microbiology laboratory, samples were taken to a negative pressure chamber with 3rd level bio-safety. Bio-Speedy® Viral Nucleic Acid Isolation Kit (Bioeksen, Turkey) was used for total nucleic acid isolation from the specimens. The isolation procedure was carried out according to the recommendations of the manufacturer.

Bio-Speedy® COVID-19 RT-qPCR Detection Kit (Bioeksen, Turkey) was used for the RT-PCR assays. The PCR amplification and evaluation of the results were carried out according to the recommendations of the manufacturer.

**Statistical Analysis**

Data analysis was performed by using IBM SPSS Statistics version 21.0 software (IBM Corporation, Armonk, NY, USA). Whether the distributions of continuous variables were normally or not being determined by visual (histograms, probability plots) and analytical methods (Kolmogorov-Smirnov and Shapiro Wilk tests). Descriptive analyses were presented using means and standard deviations for normally distributed variables; using medians and and 1st-3rd quarter for the non-normally distributed variables. Categorical variables are specified as numbers and percentages. Independent-Samples T test, Mann-Whitney U test, Chi Square test and Fisher’s Exact test were used to analyze the data. The significance level for all of the statistical tests was set at p<0.05.

**RESULTS**

The mean age of 117 patients with COVID-19 pneumonia was 49.68 ± 14.62 years and 48.7% were male. H. pylori was detected in 32 (27.35%) of the patients. In terms of COVID-19 PCR-RT positivity, there was no significant difference between the H.pylori positive group (Group 1) and negative group (Group 2) (p = 0.769).

Demographic characteristics of both groups were similar (Table 1). During the follow-up period, no patient died in Group-1, while 4 (4.7%) patients in group-2 died, but there was no significant difference between the two groups (p = 0.574). Concerning prognostic markers levels such as white blood count (WBC), neutrophil, lymphocyte, thrombocyte, D-Dimer, ferritin, albumin, lactate dehydrogenase (LDH), procalcitonin and CRP were similar in both groups (P> 0.05) (Table 2).
Table 1: Distribution of demographic and clinical characteristics according to H. pylori status in patients with COVID-19

| Parameters                                      | All patient (n=117) | Stool H. pylori + (n=32) (%27.35) | Stool H. pylori – (n=85) (%72.65) | p       |
|------------------------------------------------|---------------------|-----------------------------------|-----------------------------------|---------|
| PCR + n(%)                                      | 78 (66.7)           | 22 (68.8)                         | 56 (65.9)                         | 0.769*  |
| Age (Years) Mean ± SD                          | 49.68±14.62         | 46.78±13.80                       | 50.78±14.85                       | 0.189   |
| Median(1st-3rd quarter)                        | 47.00 (40.00-60.00) | 45.50 (37.00-57.00)               | 48.00 (40.50-60.50)               |         |
| Sex n (%)                                       |                     |                                   |                                   |         |
| Male                                            | 57 (48.7)           | 13 (40.6)                         | 47 (55.3)                         | 0.157*  |
| Female                                          | 60 (51.3)           | 19 (59.4)                         | 38 (44.7)                         |         |
| Having a Chronic illness + n (%)                | 64 (54.7)           | 15 (46.9)                         | 49 (57.6)                         | 0.297*  |
| No chronic disease n(%)                        | 53 (45.3)           | 17 (53.1)                         | 36 (42.4)                         |         |
| One chronic disease n(%)                       | 45 (38.5)           | 11 (34.4)                         | 34 (40.0)                         |         |
| Two chronic disease n(%)                       | 16 (13.7)           | 4 (12.5)                          | 12 (14.1)                         |         |
| Three or more chronic disease n(%)             | 3 (2.6)             | -                                 | 3 (3.5)                           |         |
| Diabetes Mellitus n (%)                        | 17 (14.5)           | 4 (12.5)                          | 13 (15.3)                         | 1.000** |
| Hypertension n (%)                              | 46 (39.3)           | 9 (28.1)                          | 37 (43.5)                         | 0.128*  |
| Coronary artery disease n (%)                  | 4 (3.4)             | 1 (3.1)                           | 3 (3.5)                           | 1.000** |
| Chronic obstructive Pulmonary disease n(%)     | 3 (2.6)             | -                                 | 3 (3.5)                           | 0.561** |
| Asthma n (%)                                    | 7 (6.0)             | 4 (12.5)                          | 3 (3.5)                           | 0.088** |
| Blood groups                                    |                     |                                   |                                   |         |
| A Rh(+)                                         | 22 (38.6)           | 7 (38.9)                          | 15 (38.5)                         |         |
| B Rh (+)                                        | 8 (14.0)            | 2 (11.1)                          | 6 (15.4)                          | -       |
| O Rh (+)                                        | 20 (35.1)           | 7 (38.9)                          | 13 (33.3)                         |         |
| AB Rh (+)                                       | 3 (5.3)             | -                                 | 3 (7.7)                           |         |
| A Rh (-)                                        | 3 (5.3)             | 2 (11.1)                          | 1 (2.6)                           |         |
| O Rh (-)                                        | 1 (1.8)             | -                                 | 1 (2.6)                           |         |
| Oncset of complaints n (%)                     | 90 (76.9)           | 23 (71.9)                         | 67 (78.8)                         | 0.427*  |
| Cough n (%)                                     | 39 (33.3)           | 8 (25.0)                          | 31 (36.5)                         | 0.241*  |
| Fever n (%)                                     | 36 (30.8)           | 9 (28.1)                          | 27 (31.8)                         | 0.704*  |
| Shortness of breath n (%)                      | 31 (26.5)           | 8 (25.0)                          | 23 (27.1)                         | 0.822*  |
| Throat ache n (%)                               | 10 (8.5)            | 5 (15.6)                          | 5 (5.9)                           | 0.134** |
| Diarrhea n (%)                                  | 3 (2.6)             | 3 (9.4)                           | -                                 | 0.019** |
| Vomiting n (%)                                  | 2 (1.7)             | 1 (3.1)                           | 1 (1.2)                           | 0.474** |
| Headache n (%)                                  | 2 (1.7)             | 1 (3.1)                           | 1 (1.2)                           | 0.474** |
| Support in/Antiviral treatments n (%)          |                     |                                   |                                   |         |
| Hydroxychloroquine                              | 117 (100)           | 32 (100)                          | 85 (100)                          | -       |
| Oseltamivir                                     | 117 (100)           | 32 (100)                          | 85 (100)                          | -       |
| Favipiravir                                      | 19 (16.2)           | 2 (6.3)                           | 17 (20.0)                         | 0.072*  |
| Azithromycin                                    | 110 (94.0)          | 29 (90.6)                         | 81 (95.3)                         | 0.390** |
| Antibacterials                                  | 25 (21.4)           | 6 (18.8)                          | 19 (22.4)                         | 0.672*  |
| Radiologic involvement n (%)                   |                     |                                   |                                   |         |
| Bilateral                                       | 97 (83.6)           | 25 (78.1)                         | 72 (84.7)                         | 0.601*  |
| Unilateral                                      | 19 (16.4)           | 6 (18.8)                          | 13 (15.3)                         |         |
| Mortality n(%)                                  | 4 (3.4)             | -                                 | 4 (4.7)                           | 0.574** |

* Chi Square test, ** Fisher’s Exact test, IQR = Interquartile range
Table 2. Distribution of laboratory values according to H. pylori status in Patients with COVID-19

| Parameters                  | All patient | H. pylori + | H. pylori - | p        |
|-----------------------------|-------------|-------------|-------------|----------|
| WBC (10^9/µl) Median (1st-3rd quarter) | 6.64 (4.47-8.59) | 6.99 (4.71-8.57) | 6.32 (4.44-8.68) | 0.523*   |
| Lymphocyte (10^3/µl) Mean±SD | 1.68±0.79 | 1.71±0.88 | 1.67±0.75 | 0.808** |
| Neutrophil (10^9/µl) Median (1st-3rd quarter) | 3.89 (2.69-5.69) | 4.00 (2.80-6.46) | 3.89 (2.64-5.69) | 0.709*   |
| Platelet (10^9/µl) Median (1st-3rd quarter) | 172.00 (142.00-225.50) | 172.00 (139.25-222.25) | 172.00 (145.00-225.50) | 0.976*   |
| D-Dimer (ng/mL) Median (1st-3rd quarter) | 429.00 (283.50-987.75) | 378.00 (280.00-714.00) | 482.00 (280.00-1047.00) | 0.315*   |
| Ferritin (10^9/µl) Median (1st-3rd quarter) | 221.00 (100.50-543.50) | 128.50 (66.70-542.00) | 237.00 (125.00-543.50) | 0.082*   |
| Albumin (g/L) Median (1st-3rd quarter) | 34.25 (32.68-37.65) | 35.50 (32.20-37.83) | 34.05 (32.68-37.50) | 0.551*   |
| LDH (U/L) Median (1st-3rd quarter) | 269.00 (212.50-354.50) | 249.00 (195.50-332.25) | 274.00 (219.50-359.00) | 0.253*   |
| Procalcitonine (ng/mL) Median (1st-3rd quarter) | 0.05 (0.02-0.26) | 0.06 (0.02-0.47) | 0.05 (0.02-0.20) | 0.762*   |
| CRP (mg/L) Median (1st-3rd quarter) | 23.30 (4.70-76.90) | 20.25 (3.12-76.38) | 25.80 (5.31-76.90) | 0.446*   |

* Mann-Whitney U test. ** Independent-Samples T test, IQR = Interquartile range, WBC: White blood cell. LDH: lactate dehydrogenase. CRP: C reactive protein.

**DISCUSSION**

To our knowledge, there is no study investigated the frequency of H. pylori and the prognostic significance of H. pylori positivity in patients with COVID-19 disease. In the present study, we determined a 27.35% of H. pylori positivity in patients with COVID-19. Our outcomes are much lower than the rates in the normal and non-COVID viral infectious population. The prevalence of H. pylori, which has lower rates in studies conducted in European countries, varies between 7-87% worldwide (17). In a recently published systematic meta-analysis, the mean positive frequency of H. pylori antigen in stool was found to be 49.4% 9. In a study conducted in a country, a prevalence rate of 25.2% was reported in stool H. pylori antigen test studies in the normal population (18).

Studies on the frequency of H. pylori in various non-COVID-19 viral infections have been identified. For instance; information from epidemiologic works suggest that the frequency of H. pylori infection is clearly lower in HIV-positive compared with the HIV-negative population and that it further declines with the progression of immunodeficiency in HIV-infected patients. In a study of 1095 HIV-positive and 107 HIV-negative patients using the stool antigen test, the prevalence of H. pylori was significantly higher in the HIV-negative group (51.5% vs 88%, respectively, p < 0.05) (11).

In a meta-analysis evaluated 29 studies; all studies, except one study, reported a higher rate of H. pylori infection in HIV negative subjects (12). In contrast, another study found a higher prevalence of H. pylori resistant strain in HIV-positive patients than in HIV-negative patients (13). In addition, a meta-analysis including 2977 chronic hepatitis B and 1668 control patients, the H. pylori prevalence was found to be higher in patients with chronic hepatitis B positive. Also, the incidence of H. pylori has been shown to be positively correlated with HBV-associated hepatocellular carcinoma (19). However, the H. pylori prevalence in 235 asymptomatic HBV carriers, 573 alcohol users and 1637 non-alcoholic individuals was similar as 38.67%, 26.98%, and 35.94%, respectively (20).

Recently, the importance of gastrointestinal microbiota as a decisive for the systemic immune response has been recognized, and a number of extraintestinal, immune-related efficacious of H. pylori positivity have been reported (21-22).

With respect to the association between the immune system hypoactivation and H. pylori coinfection, it is known that H. pylori decrease markers (HLA DR, CD38, CD4) of the immune activation system by decreasing T-cell activation in HIV-positive and in HIV-negative individuals. This finding might explain the association of H. pylori infection in the intestine with favorable parameters of HIV disease progression (23). The immune response to H. pylori infection is predominantly T-cell mediated. It has been shown that the H. pylori vacuolating toxin directly inhibits T-cell activation by interfering with the maturation of antigen present cells and dendritic cells (24). It is known that lymphopenia is frequently reported in patients with COVID-19 and is considered to be a determinant of the prognosis of the disease. However, in our study, with respect to lymphopenia, no significant relationship was shown between the H. pylori positive patient group and the negative group. Various poor prognostic markers such as WBC, neutrophil, D-Dimer, ferritin, LDH, procalcitonin, high CRP, thrombocytopenia, and hyponatremia have been demonstrated in COVID-19 infection (25). In our study, no significant difference was found in terms of these prognostic markers between groups with and without H. pylori positive. The most important limitation of the present study was that the immune T lymphocyte activation parameters were not detected in H. pylori positive COVID-19 patients.

**CONCLUSION**

In conclusion, according to the results of this study, H. pylori positivity or negativity neither determined the severity of the COVID-19 disease nor the poor prognostic indicators of the disease. However, larger and controlled studies are needed to confirm these findings.
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