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Original Article

Duodenal histologic findings in patients with history of COVID-19 infection

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Abstract:

Background:

While the SARS-COV2 infection affects mainly the respiratory system, there is more evidence on the gastrointestinal (GI) involvement. However, data on the pathophysiology of infection and the secondary immune response on intestinal level are still scarce. Our study aims at describing duodenal histologic findings in patients with history of COVID-19 infection.

Methods:

Between January 2021 and May 2021, we included patients with history of SARS-COV2 infection over the last 6 months and who underwent an upper GI endoscopy and duodenal biopsies (Group 1). We also selected a control group of uninfected patients (Group 2) who had an upper endoscopy during the same period. Standard anatomopathological analysis was done followed by immunohistochemistry to identify the lymphocytic phenotype notably the CD8+ T cells. We then tried to correlate histologic abnormalities to patients' characteristics.

Results:

Twenty-five patients were included in the Group 1 with a mean age of 46 years and female predominance (52%). The indication of endoscopy was mainly related to abdominal pain and diarrhea (64 and 25% of cases, respectively). The average time from the disease onset was 74.5 days. 45% of patients experienced GI symptoms during their infection mostly diarrhea and abdominal pain. On duodenal biopsies, intra-epithelial lymphocytes (IEL) count was elevated (>20/100 enterocytes) in 60% of cases and CD8+ cells were found in 48% of patients in group 1. We did not find any correlation between these abnormalities and the presence of GI symptoms. Ten patients were included in Group 2, only one had increased IEL count (20/100EC) and negative for CD8+ T cells on duodenal biopsies.

Conclusion:

The duodenal biopsy in patients with history of COVID-19 infection shows an increased IEL count as well as CD8+ T cells even few months after the disease onset.

Key words: Coronavirus; Duodenal biopsy; Intra-epithelial lymphocytes; CD8+ T cells; Gastrointestinal symptoms; Endoscopy.

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INTRODUCTION

Coronavirus is a large family of single stranded RNA viruses that affect many organs and cause a wide spectrum of symptoms (1). Coronavirus disease 2019 (COVID-19) is caused by the severe acute respiratory syndrome coronavirus 2 (SARS-COV2). This virus infects mostly the lungs and respiratory symptoms are the most common at presentation (2). Gastrointestinal (GI) manifestations including abdominal pain, diarrhea, vomiting, anorexia and loss of taste have been also

reported (3)(4). The virus RNA has been detected in the stool samples of COVID-19 infected patient (5)(6)(7). SARS-COV2 cell entry is mediated by the angiotensin-converting enzyme-2 (ACE 2) receptor and employs the cellular serine protease TMPRSS2 for S protein priming (8). ACE2 receptors are present not only in the pulmonary system but also in many other organs including the digestive tract (9). Most studies done on the respiratory system suggested that activated CD8+T cells have a critical role in the defensive mechanism and may contribute to the eradication of SARS-COV2 in the lungs (10). The pathogenesis of the viral infection in the GI tract is still unclear. Few small sized studies suggested that intestinal infection by SARS-COV2 can induce an immune response similar to the respiratory tract with a mucosal infiltration by activated T CD8+ cells (11). Our study aims at describing the intestinal histologic findings in patients with a history of COVID-19 with a highlight on the role of intestinal CD8+T cells in the pathogenesis and its correlation to clinical features.

METHODS:

We conducted an observational study by randomly including patients aged more than 18 years with a history of COVID-19 infection in the last 6 months who underwent upper gastrointestinal endoscopy in our endoscopy unit at Geitaoui University Hospital in Beirut, Lebanon, between January 2021 and May 2021. This group was defined as "Group 1". To improve the pertinence of our results, we enrolled in our study an age and sex matched control group of 10 patients without a known history of COVID infection and who had a clinically indicated endoscopy during the same study period. This group was defined as "Group 2".

We collected patients' data including age, gender, past medical and surgical history and current medications. We also noted the time since SARS-COV2 infection as well as the severity of the initial disease defined by the need for hospitalization. The presence of GI symptoms during the acute onset of infection and upon endoscopy was recorded.

We excluded patients with a history of medical conditions that could alter the duodenal histology such as celiac disease, inflammatory bowel diseases (IBD) or other autoimmune diseases. The study design was approved by the local institutional review board (IRB) at our university medical center.

All patients had their endoscopies done in the same center. At least four biopsies were obtained from the duodenum and the bulb, put in formalin and sent to the pathology department for histologic analysis. The covid-19 status in these patients was not shared with the pathologistin order to decrease the confirmation bias. First, the duodenal biopsies were evaluated by two independent pathologists with hematoxylin and eosin stain for villous atrophy, crypt hyperplasia and the presence of intraepithelial lymphocytes (IEL). Then, Immunohistochemistry (IHC) with a panel including anti-CD3, anti-CD4 and anti-CD8 was done, in order to characterize the type of intraepithelial lymphocytes. The results reported independently by both pathologists were then compared to each other. Finally, we underwent an analysis of the histologic findings with a special emphasis on the CD8+ T cells and IELs and their correlation with patients and COVID infection characteristics.

Statistical analysis was done using the SPSS statistics program. Chi-squared test for independence or Fisher's exact test was used to compare frequencies and percentages for qualitative results and independent t test for mean comparisons in quantitative results. P value of less 0.05 was considered as statistically significant. The odds ratio (OR) and the 95% confidence interval (CI) were computed for each independent factor.

We declare that this paper has not been published elsewhere.

RESULTS:

Thirty-five patients with history of COVID-19 were initially included in our study over 5 months (Group 1). Ten were then excluded for various reasons (Seven could not have a complete histological analysis including IHC studies, two had IBD and one had rheumatoid arthritis treated with rituximab). Among the 10 patients selected for control group (Group 2), only 5 were able to have full histologic analysis including IHC study.

Patients' demographics:

Twenty-five patients were finally included in the Group 1 with a mean age of 46.2 years +/- 17.9. Fifty-two percent were females (Table 1). Five patients were on Aspirin (20%) at cardiac dose, seven were on PPI (28%) and one on Angiotensin Receptor Blockers (4%). The indication of Upper GI endoscopy was mainly related to abdominal pain in 16 patients (64%), diarrhea in 5 patients (20%) and other causes (pyrosis, nausea) in 6 patients (24%).In Group 2, mean age was 43.3 years +/- 18.3. Fifty percent were females. Endoscopy was done for abdominal pain in 60% and for diarrhea in 30% of patients. There was no difference in demographic patients' characteristics between group 1 and 2.

Table 1. Patient's characteristics

	Group 1	Group 2	P value	OR	95% CI
Age (mean in years +/- SD)	46.2 +/- 17.9	43.3 +/- 18.3	0.67	-	-
Female sex	13/25 (52%)	5/10 (50%)	0.92	1.08	0.21-4.0
Interval between COVID and endoscopy (mean in days +/- SD)	74.48 +/- 37.9	-	-	-	-
• **COVID severity**	•	•	•	•	•
Mild	22/25 (88%)	-	-	-	-
Severe	3/25 (12%)	-	-	-	-
GI symptoms during COVID	11/25 (44%)				
Diarrhea	8/25 (32%)	-	-	-	-
Abdominal pain	6/25 (24%)	-	-	-	-
Nausea/vomiting	3/25 (12%)	-	-	-	-
No GI symptoms	14/25 (66%)	-	-	-	-
GI symptoms upon endoscopy					
Abdominal pain	16/25 (64%)	6/10 (60%)	0.7	1.41	0.31-6.47
Diarrhea	5/25 (20%)	3/10 (30%)	0.69	0.74	0.14-3.77
Other	6/25 (24%)	1/10 (10%)	0.64	2.8	0.29-27.20
Endoscopic findings					
Normal aspect of the duodenum	25/25 (100%)	10/10 (100%)	-	-	-
Duodenal biopsy findings					
Mean IEL per 100EC	21.36 +/- 8.5	11.7 +/- 3.9	<0.0001		
IEL≥20 per 100EC	15/25 (60%)	1/10 (10%)	0.01	13.5	1.47-123.7
Increased CD8+ T cells	12/25 (48%)	0/5 (0%)	-	-	-
Crypt hyperplasia	0/25 (0%)	0/10 (0%)	-	-	-

Group 1: Patients with history of COVID-19; Group 2: Control group; SD: standard deviation, COVID: Coronavirus disease 2019, GI: gastrointestinal, IEL: Intra-epithelial lymphocytes, EC: enterocytes.

COVID INFECTION CHARACTERISTICS:

Twenty-two patients (88%) in Group 1 had mild COVID-19 infection and three (12%) had a severe disease needing hospitalization (two to regular floor and one to intensive care unit). The mean interval between COVID infection and the endoscopy was 74.5 days +/- 37.9. During the acute onset of their disease, 11 out of the 25 patients (44%) experienced GI symptoms predominantly a diarrhea present in 8 patients (32%), abdominal pain in 6 patients (24%) and nausea/vomiting in 3 patients (12%).

ENDOSCOPIC AND HISTOLOGIC FINDINGS:

All patients in both groups had a normal aspect of the duodenal mucosa upon endoscopy. Fifteen patients (60%) in Group 1 had an increased number of IEL to more than 20/100 enterocytes (EC) on duodenal biopsies vs 10% in the control group (P=0.01). The mean IEL count in Group 1 was 21.36/100EC and it was significantly higher than the mean IEL count in Group 2 (11.7/100EC) (P<0.0001). No one in both groups had villous atrophy. Crypt's morphology was normal in all patients. We did not note any crypt hyperplasia. IHC studies in Group 1 identified an increased number of CD8+ T lymphocytes in 12 patients (48%). Three of these patients had no increased IEL count (two had 15 IEL/100EC and one had 10 IEL/100EC). Among the five patients who had their IHC studies in the control Group 2, no one had an increased CD8+ cells. Sub-analysis of patients in the Group 1 according to IEL count. We underwent an analysis of patients' characteristics in the Group 1 according to whether they had an increased number of IELs≥ 20 /100EC or not (Table 2). Age and sex distribution was similar between both groups. Mean time between COVID-19 and endoscopy was 76.8 days +/- 35.5 for patients with IEL≥20/100EC vs 71 days +/- 43 for patients with IEL<20/100EC (P value = 0.72). Only one patient with increased IEL count had history of severe SARS-COV2 infection vs two in the lower IEL count group (P value = 0.54). Abdominal pain and diarrhea were present at time of COVID-19 disease in 13.3 and 26.7% of patients with high IEL count, respectively, vs 40% in patients with lower IEL count (P value = 0.17 and 0.67 for abdominal pain and diarrhea, respectively). Upon endoscopy, 80% of patients with IEL≥20/100EC reported abdominal pain and 12% reported diarrhea (vs 50 and 30% respectively in the IEL<20/100EC group, P value >0.05).

Table 2. Patients' analysis in Group 1 according to their IEL status

IEL count (/100 EC)	≥20 (n=15)	<20 (n=10)	P value	OR	95% CI
Age (mean in years +/- SD)	47 +/- 16.8	44.9 +/-20.2	0.78	-	-
Sex (female)	9 (60%)	4 (40%)	0.43	0.44	0.09-2.3
Severe Covid	1 (6.7%)	2 (20%)	0.54	0.28	0.02-3.68
Time since COVID (mean in days +/- SD)	76.8 +/- 35.5	71 +/- 43	0.72	-	-
GI symptoms at COVID onset	5 (33.3%)	6 (60%)	0.24	0.33	0.06-1.75
Diarrhea	4 (26.7%)	4 (40%)	0.67	0.54	0.1-3
Abdominal pain	2 (13.3 %)	4 (40%)	0.17	0.23	0.03-1.63
GI symptoms upon endoscopy					
Diarrhea	3 (20%)	3 (30%)	0.65	0.58	0.09-3.71
Abdominal pain	12 (80%)	5 (50%)	0.19	4	0.68-23

IEL: Intra-epithelial lymphocytes, EC: enterocytes, SD: standard deviation, OR: odds ratio, CI: confidence interval, COVID Coronavirus disease 2019

Sub-analysis of patients in the Group 1 according to the presence of CD8+ T cells:

We underwent an analysis of patients' characteristics in the Group 1 according to the CD8+ T cells predominance on duodenal biopsies (Table 3).

There was no significant difference in age or sex distribution between the two populations. The interval between COVID infection and the endoscopy was also similar between both groups (77.6 and 71.5 days for CD8 and non CD8 predominant groups, respectively, P value = 0.69). Only one patient with CD8 predominant T cells had history of severe COVID-19 vs two in the other group (P value = 0.59).

About third of patients with CD8+ predominance had diarrhea and two thirds had abdominal pain upon endoscopy. However, these proportions were not significantly higher than those present in patients without CD8 predominance (P value = 0.38 and 0.89 for diarrhea and abdominal pain, respectively). Additionally, we did not notice a correlation between the presence of GI symptoms during the initial SARS-COV2 infection and the predominance of CD8+ cells on duodenal biopsies (P value = 0.43).

Table 3. Patients' analysis in Group 1 according to CD8 status

CD8 profile	Predominant (n=12)	Non-predominant (n=13)	P value	OR	95% CI
Age (mean in years +/- SD)	45.9 +/- 18	46.4 +/- 18	0.95	-	-
Sex (female)	6 (50%)	6 (46.2%)	0.85	1.16	0.24-0.61
Severe Covid	1 (8.3%)	2 (15.4%)	0.59	0.5	0.04-6.35
Time since COVID (mean in days +/- SD)	77.6 +/- 39	71.5 +/- 37	0.69	-	-
GI symptoms at COVID onset	4 (33.3%)	7 (53.8%)	0.43	0.4	0.1-2.2
Diarrhea	4 (16%)	4 (30.8%)	0.89	1.1	0.2-6
Abdominal pain	1 (8.3%)	5 (38.5%)	0.16	0.14	0.02-1.5
GI symptoms upon endoscopy					
Diarrhea	4 (33.3%)	2 (15.4%)	0.38	2.7	0.4-18
Abdominal pain	8 (66.7%)	9 (69.2%)	0.89	0.9	0.16-4.7

SD: standard deviation, OR: odds ratio, CI: confidence interval, COVID: Coronavirus disease 2019

DISCUSSIONS

SARS CoV-2 infection is known to involve the GI tract and viral RNA was detected in 66.7% in stool specimens of infected patients (5). Gaebler et al detected SARS-CoV-2 RNA in duodenal and ileal epithelial cells 3 to 5 months after a COVID-19 diagnosis without being able to identify inflammatory infiltrates (12). As in the respiratory tract, it is very likely that the infection induces an immune dysregulation in the intestinal mucosa and this hypothesis was supported by our findings.

In our study, an increased IEL count was found in 60% of patients with a history of SARS-COV2 infection and increased CD8+ T lymphocytes were identified in 48%

of their duodenal biopsies. Even if the number of patients in the control group was small especially for IHC studies, those percentages were significantly higher in the COVID-19 group. These abnormalities suggest the GI involvement during the course of the disease. Moreover, we were able to identify these histologic findings even few months after the initial infection (2.5 months in average) which can witness, as it is now known, the long effect of the immune dysregulation related to the infection. However, we did not find a correlation between COVID 19 severity and the presence of these abnormalities on duodenal biopsies provided that the number of severe cases in our study was very small to conclude on such correlation. Additionally, patients with increased CD8+ T cells had not experienced more GI symptoms during the acute SARS-COV2 infection nor upon endoscopy, raising the question of the role these cells are playing in the clinical digestive manifestations.

Lehmann et al (11) have described the immune profile of five patients with moderate COVID-19 who experienced abdominal pain or diarrhea during their hospitalization. Histologic analysis showed villous blunting and an increase in intraepithelial lymphocytes (IEL) in four out of five patients. As in our study, intra-epithelial CD8+ T cells count were particularly higher in infected patients when compared to a control group. In addition, they found an increased IEC apoptosis as well as a compensatory epithelial regeneration. However, the study by Livanos et al (13) has found somewhat contradictory results. While they were able to identify small viral particles in the epithelial intestinal cells 25 days after SARS-COV2 infection, they have not found a significantly increased intraepithelial lymphocytes count nor an increased CD8+ cell count. They have even described a downregulation of the intestinal pro-inflammatory mediators as well as the dendritic cells in patients with intestinal COVID, suggesting a potential role of the GI tract in attenuating systemic inflammation in these patients.

Our work is still a small sized study and results must be evaluated prospectively on a larger population to confirm their pertinence. However, we provided a detailed description of the duodenal histologic findings in patients with a history of COVID-19 and we tried to correlate these findings to clinical manifestations. Besides its small size, the study presents some other limitations. We did not evaluate other common causes of increased IEL count such Helicobacter pylori infection or bacterial overgrowth. Also, the control group was smaller in size in comparison with the group of COVID-19 patients especially for those who had IHC analysis. On the other hand, our study was done away from the acute infection and these histologic abnormalities might be more prominent at the time of ongoing infection rather than later on. In conclusion,COVID-19 infection is associated with an increase in IELs and CD8+ T cells count on duodenal biopsies. The presence of these cells was not associated with the appearance of GI symptoms during infection or later on. These abnormalities could be identified even few months after the onset of COVID-19. Our findings should be confirmed by a larger prospective study.

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