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Investigation of the Effect of Leaky Gut on COVID-19 Clinic

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ABSTRACT

Aim: In the course of the COVID-19 pandemic, in millions of cases were observed those of some patients do not exhibit any symptoms whereas some others are hospitalized with having fatal outcomes. One of the most significant findings is that patients with existing comorbidities are extremely exposed to severe clinical conditions developed due to excessive inflammatory response. That is to say, the indicative cause of chronic inflammation may be bacterial translocation derived from the impaired intestinal mucosal barriers.

This study is aimed to investigate the probable relations between the impaired intestinal barrier integrity and which would be associated with severity of COVID-19 clinical conditions.

Material and Methods: According to the clinical and laboratory findings, the patients were classified into three groups as mild, moderate, and severe clinical conditions. All patients' blood samples were collected on the first admission to the hospital. Serum concentrations of lipopolysaccharide-binding protein (LBP), were analyzed to evaluate the intestinal barrier function and bacterial translocation.

Results: The proportions of those with high LBP levels among all the groups were significantly different ($p<0.001$). The proportion of the patients with high LBP levels in the mild patient group (65.4%) was significantly lower than those with moderate (100%) and with severe clinical conditions (95.2%) ($p<0.05$ for each).

Conclusion: In recent years, it has been clearly demonstrated that the functions of the intestines are much more than the digestive function, and that the intestinal microbiota and mucosal barrier integrity have a great impact on the immune system. These results would indicate that the impaired intestinal barrier integrity and bacterial translocation might be effective in severe COVID-19 development.

Keywords: Bacterial translocation; COVID-19; innate immunity; leaky gut; SARS-CoV-2.

Geçirgen Bağırsağın COVID-19 Kliniği Üzerinde Etkisinin Araştırılması

ÖZ

Amaç: COVID-19 pandemisi sürecinde milyonlarca vakada bazı hastaların herhangi bir semptom göstermediği, bazılarının ise ölümcül sonuçla hastaneye kaldırıldığı gözlemlenmiştir. En önemli bulgulardan biri, komorbiditesi olan hastaların aşırı inflamatuvar yanıta bağlı gelişen ciddi klinik durumlara aşırı derecede maruz kalmasıdır. Yani kronik inflamasyonun belirleyici nedeni, bozulmuş bağırsak mukozal bariyerlerinden türetilen bakteriyel translokasyon olabilir.

Bu çalışma, bozulmuş bağırsak bariyeri bütünlüğü ile COVID-19 klinik durumlarının şiddeti ile ilişkilendirilebilecek olası ilişkileri araştırmayı amaçlamaktadır.

Gereç ve Yöntemler: Klinik ve laboratuvar bulgularına göre hastalar hafif, orta ve şiddetli klinik durumlar olarak üç gruba ayrılmıştır. Tüm hastalardan kan örnekleri hastaneye ilk başvurularında alınmıştır. Lipopolisakkarit bağlayıcı proteinin (LBP) serum konsantrasyonları, bağırsak bariyer fonksiyonunu ve bakteriyel translokasyonu değerlendirmek için analiz edilmiştir.

Bulgular: Tüm gruplar arasında yüksek LBP düzeyine sahip olanların oranları önemli ölçüde farklıydı ($p <0,001$) (Tablo 1). Hafif hasta grubunda (%65,4) yüksek LBP düzeyleri olan hastaların oranı, orta (%100) ve ciddi klinik durumları (%95,2) olanlara göre anlamlı derecede düşük bulunmuştur (her biri için $p <0,05$).

Sonuç: Son yıllarda bağırsakların fonksiyonlarının sindirim fonksiyonundan çok daha fazla olduğu ve ayrıca bağırsak mikrobiyotası ve mukozal bariyer bütünlüğünün bağışıklık sistemi üzerinde çok büyük etkisi olduğu açıkça ortaya konmuştur. Bu sonuçlar, bozulmuş bağırsak bariyeri bütünlüğünün ve bakteriyel translokasyonun şiddetli COVID-19 gelişiminde etkili olabileceğini düşündürmektedir.

Anahtar Kelimeler: Bakteriyel translokasyon; COVID-19; doğal bağışıklık; geçirgen bağırsak; SARS-CoV-2.

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INTRODUCTION

Since the declaration of the Coronavirus pandemic by World Health Organization (WHO), in the course of COVID-19 pandemic, millions of cases were observed to those of some patients do not exhibit any symptoms whereas some others are hospitalized with having fatal outcomes. One of the most significant findings is that patients, who exist comorbidities also exhibit low-grade systemic inflammation, are extremely exposed to severe clinical conditions developed due to the excessive inflammatory response (1). One of the indicative cause of chronic inflammation would be bacterial translocation derived from the impaired intestinal mucosal barriers.

This study is aimed to raise awareness about the probable relations between the impaired intestinal barrier integrity and COVID-19 which would be associated with the severity of the clinical conditions.

Approximately 70% of the immune system is located in the intestinal mucosa-associated lymphoid tissue so intestinal health is definitely related to immunity. The deterioration of this epithelial structure is closely related to the nutrition, dysbiosis, drug use, toxic substances and being elderly. As the barrier is impaired the intestinal content translocation occurs then chronic systemic inflammation develops (2-4). Lipopolysaccharide (LPS) is a well-characterized pathogen-associated molecular pattern found in the outer leaflet of the outer membrane of most of the gram negative bacteria, and the circulating endotoxins are derived from dead bacteria or the LPS shed from the cell wall of viable bacteria. LPS originating from bacteria passing into the bloodstream through impaired intestinal permeability can evoke an inflammatory response by activation of monocytes and endothelial cells. The serum LBP concentration is a stable indicator of the exposure to the lipopolysaccharide (5,6).

We investigated whether the impaired intestinal barrier integrity has an effect in the course of COVID-19 clinical conditions.

MATERIAL AND METHODS

Study design

This study was conducted at Duzce University Research and Application Center between November 01, 2020-December 21, 2020. Patients who tested SARS CoV-2 positive through PCR were included in the study. According to the clinical and laboratory findings, the patients were divided into three groups as mild, moderate and severe. All patients' blood samples were collected on the first admission to the hospital.

In the patients having mild symptoms; normal lymphocyte count and C reactive protein (CRP) levels and also their oxygen saturation levels were mentioned as in the mild clinical group. The patients having moderate clinical symptoms were demonstrated among those of whom were hospitalized. In this group, the measurement of lymphocyte count and O2 saturation levels were lower than normal. Besides their CRP levels were higher than normal. As in the final group of the patients' having severe clinical findings were presented as being hospitalized in the intensive care unit.

Measurements

In our laboratory, as abnormal lymphocyte count ≤ 0.82 ($\times 10^9$/L), mean oxygen saturation (SaO2) <94, CRP >0.5 mg/L and LBP >10ng/ml were accepted. The patients' serum samples were stored at -20 ° C till they were analyzed. Human Lipopolysaccharide Binding Protein levels by Human Lipopolysaccharide Binding Protein ELISA Kit (Bioassay Technology Laboratory, China) were analyzed.

Statistical Analysis

Appropriate descriptive statistics were calculated according to the type of the data and the analysis. Descriptive statistics were presented as numbers and percentages. Numerical variables were summarized as the mean ± standard deviation (min-max). Normality assumption of continuous quantitative variables was checked through Shapiro Wilk test and the homogeneity of group variances was checked via Levene test. Welch (post hoc: Fisher LSD test) and Pearson Chi-square (post hoc: Bonferroni test) tests were used for comparisons among groups in terms of age and gender. Generalized Linear Modeling (post hoc: Fisher LSD test) approach was used in the group comparisons in terms of biomarker and biochemistry results, taking into account the determined covariate effect. Relationship between categorical variables was examined with Fisher-Freeman-Halton (post hoc: Bonferroni test) tests. SPSS 22 program and special macros were used for statistical evaluations. A $p < 0.05$ was considered statistically significant.

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With the decision number: 2020/172, Clinical Research Ethics Committee at Duzce University Faculty of Medicine approved this study. The appropriate participants' informed consent in compliance with the Helsinki Declaration were taken.

RESULTS

Out of the 79 patients included in the study; 46.8% (n = 37) were female, 53.2% were male, and the total mean age was 54.5 ± 14.7 years (25-82). 32.9% (n = 26) of the patients were in mild clinical condition, 40.5% (n = 32) were in moderate and 26.6% (n = 21) were in severe clinical condition. The sociodemographic and clinical characteristics of the patients are shown in Table 1 based on their clinical conditions.

As to the patients' clinical conditions, considering their gender distributions there was no significant difference in all the patients (p=0.057); however, age distributions were different (p<0.001). The mean age of the patients with severe COVID-19 clinical condition was significantly higher, whereas the ages of the patients in the mild group were comparatively demonstrated as the lowest (p <0.001).

The proportions of those with high LBP levels among all the groups were significantly different (p <0.001) (Table 1). The proportion of the patients with high LBP levels in the mild patient group (65.4%) was significantly lower than those with moderate (100%) and with the severe clinical conditions (95.2%) (p <0.05 for each).

Table 1. The Sociodemographic and Clinical Characteristics of The Patients According to The Clinical Condition

	Clinical condition								
	Mild (n=26)		Moderate (n=32)		Severe (n=21)		Total (n=79)		p
	n	%	n	%	n	%	n	%	
Sex									
Female	17	65.4	11	34.4	9	42.9	37	46.8	0.057
Male	9	34.6	21	65.6	12	57.1	42	53.2	
Age(Years)*									
	41.3±7.3 (25-54)		56.9±10.5 (28-71)		67.3±14.3 (25-82)		54.5±14.7 (25-82)		**<0.001**
LBP(ng/ml)									
≤10	9	34.6	0	0.0	1	4.8	10	12.7	**<0.001**
>10	17	65.4	32	100.0	20	95.2	69	87.3	

LBP: Lipolysaccharide binding protein
*mean±standard deviation (minimum-maximum)

Regarding ages, the groups were not homogeneous. So the term of "age" was considered as the covariate in order to eliminate the factor of age in relation to the levels of LBP. Namely, descriptive values and comparative results of the age-adjusted LBP results are demonstrated and detailed, in Table 2.

There was a significant difference among the groups in terms of the mean level of LBP with the age adjusted patients ($p < 0.05$). The mean level of LBP measured in patients with severe COVID-19 clinical conditions was significantly higher than those of patients with mild and moderate clinical conditions ($p = 0.031$ $p = 0.020$, respectively).

Table 2. Descriptive Statistics and Comparative Result of the Age-Adjusted LBP

	Clinical condition						
	Mild		Moderate		Severe		
	Mean±SE*	95% Wald CI	Mean±SE*	95% Wald CI	Mean±SE*	95% Wald CI	p
LBP	29.3±5.7	18.2-40.4	33.4±4.1	25.4-41.4	54.8±8.5	38.1-71.5	0.048

* The age-adjusted estimated levels (for age ≅54.5), SE: Standard error, CI: Confidence interval, LBP: Lipolysaccharide binding protein

The mean ± SE levels of LBP levels are demonstrated in clinical conditions of COVID-19 patients in Figure 1.

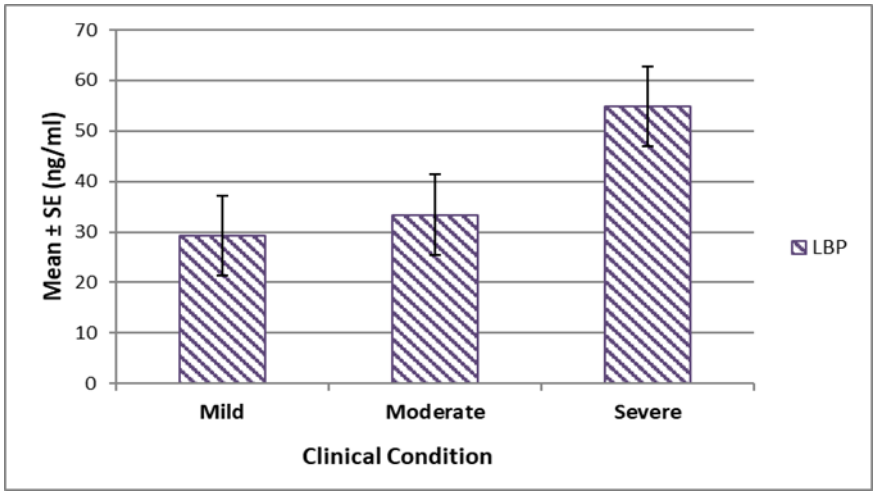

Figure 2. LBP Levels in Different Clinical Conditions of COVID-19 Patients

DISCUSSION

During the COVID-19 pandemic, millions of people have been infected. At the beginning of the pandemic, everyone was too vulnerable to this infection; some of those had a mild infection whereas some were hospitalized with fatal outcomes. This outcome makes us think that individual immunity has an impact in the clinical course of the disease. The innate immune response to viruses occurs in the first hours of the infection by the natural defense mechanisms at the entry site and limits infections immediately. Barrier integrity and eubiotic microbiota are essential for innate immunity to work properly (5,6).

At the end of the second year of the COVID-19 pandemic, we have observed immune-escape infections in those who are positive for SARS CoV-2 IgG (7,8). Currently, variants of SARS CoV-2 are widespread throughout the world (9,10). In the clinical course of these variant infections, as in wild virus infections, individual health conditions seem to be the determining factor rather than the variant type itself (11).

When the blood-intestinal barrier integrity is impaired, bacterial translocation in systemic blood circulation occurs. When bacterial cell product is detected in the blood circulation even at subclinical levels, LBP occurs as an acute phase protein synthesized mainly in the liver. LBP concentrations have been reported to peak in 12 hours after the exposure to the small amounts of LPS and have a long half-life. Serum LBP concentration is a stable indicator of LPS exposure (12,13). In our study, LBP levels were evaluated to be higher than normal levels in all groups, and these results were significantly higher in the severe clinical group compared to the other two groups (Figure 1).

Consistent with our study, Giron et al. reported in their study that systematic induction of LBP in patients with severe COVID-19 compared to the mild COVID-19 groups were demonstrated (14). In the studies conducted among the patients with multiple sclerosis, as a neuroinflammatory disease, LBP which serum concentrations were highlighted to be higher (15,16).

Giron et al., found that the severity of COVID-19 is strongly associated with the disruption of the blood-gut barrier integrity and microbial translocation (14). Similarly, in our study, we found the LBP blood levels at the highest levels in the patient group with severe COVID-19.

Furthermore, these data support our hypothesis that the microbial translocation linked with excessive immune activation in severe COVID-19 is resulted by the impaired intestinal barrier integrity. Developing chronic systemic inflammation depends on impaired mucosal integrity and microbial translocation. The patients having chronic inflammation earlier are extremely prone to having excessive immune activation. Besides that, those patients are also sensitive to develop the severity of COVID-19 clinical conditions.

In recent years, it has been clearly demonstrated that the functions of the intestines are shown out of much more than digestive function and also the intestinal microbiota and mucosal barrier integrity have an enormous effect on the immune system.

Dysbiosis (reduction in microbial diversity and the loss of beneficial bacteria) causes the impairment of the integrity of the intestinal barrier (17). Dysbiosis causes leaky gut and consequently bacterial translocation.

CONCLUSIONS

LBP was found to be higher in patients who required hospitalization. These results indicated that the impaired intestinal barrier integrity and bacterial translocation have an effect in the course of COVID-19 clinical conditions.

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Conflicts of interest

The authors have no conflicts of interest to declare.

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REFERENCES

1. Chiappetta S, Sharma AM, Bottino V, Stier C. COVID-19 and the role of chronic inflammation in patients with obesity. Int J Obes. 2020; 44: 1790-2.

2. Janda L, Mihalcin M, Stastna M. Is a healthy microbiome responsible for lower mortality in COVID-19? Biologia. 2021; 76: 819-29. https://link.springer.com/article/10.2478/s11756-020-00614-8

3. Fasano A. Zonulin and its regulation of intestinal barrier function: The biological door to inflammation, autoimmunity and cancer. Physiol Rev. 2011; 91: 151-75. https://www.tandfonline.com/doi/full/10.1080/21688370.2016.1251384

4. Turner JR. Intestinal mucosal barrier function in health and disease. Nat Rev Immunol. 2009; 9(11):799-809.

5. Ruiz GA, Casafont F, Crespo J, Cayon A, Mayorga M, Estebanez A, et al. Lipopolysaccharide-binding protein plasma levels and liver TNF-alpha gene expression in obese patients: Evidence for the potential role of endotoxin in the pathogenesis of non-alcoholic steatohepatitis. Obes Surg. 2007; 17: 1374-80.

6. Lim PS, Chang YK & Wu TK. Serum Lipopolysaccharide-binding protein is associated with chronic inflammation and metabolic syndrome in hemodialysis patients. Blood Purif. 2019; 47: 28-36.

7. Grossberg AN, Koza LA, Ledreux A, Prusmack C, Krishnamurthy HK, Jayaraman V, et al. A multiplex chemiluminescent immunoassay for serological profiling of COVID-19-positive symptomatic and asymptomatic patients. Nat Commun. 2021; 12: 740.

8. Loconsole D, Passerini F, Ostilio PV, Centrone F, Sallustio A, Pugliese S, et al. Recurrence of COVID-19 after recovery: a case report from Italy. Infection. 2020; 48: 965-7.

9. Abdool Karim SS, de Oliveira T. New SARS-CoV-2 variants - clinical, public health, and vaccine implications. N Engl J Med. 2021; 384(19): 1866-8.

10. Karim SSA. Vaccines and SARS-CoV-2 variants: the urgent need for a correlate of protection. Lancet. 2021; 3: 397.
11. Korber B, Fischer WM, Gnanakaran S, Yoon H, Theiler J, Abfalterer W, et al. Tracking changes in SARS-CoV-2 spike: Evidence that D614G increases infectivity of the COVID-19 virus. Cell. 2020; 182: 812-27.
12. Schumann, R. R. Old and new findings on lipopolysaccharide-binding protein: A soluble pattern-recognition molecule. Biochem Soc Trans. 2011; 39: 989-93.
13. Citronberg JS, Wilkens LR, Marchand LL, Lim U, Monroe KR, Hullar MAJ, et al. Plasma lipopolysaccharide-binding protein and colorectal cancer risk: a nested case-control study in the Multiethnic Cohort. Cancer Causes Control. 2018; 29: 115–23.
14. Giron LB, Dweep H, Yin X, Wang H, Damra M, Goldman AR, et al. Severe COVID-19 is fueled by disrupted gut barrier integrity. MedRxiv [Internet]. 2020 Jan 1;2020.11.13.20231209. Available from: http://medrxiv.org/content/early/2020/11/16/2020.11.13.20231209.abstract
15. Ferreira TB, Hygino J, Barros PO, Teixeira B, Kasahara TM, Linhares UC, et al. Endogenous interleukin-6 amplifies interleukin-17 production and corticoid-resistance in peripheral T cells from patients with multiple sclerosis. Immunology. 2014; 143: 560-8.
16. Escribano BM, Medina-Fernandez FJ, Aguilar-Luque M, Agüera E, Feijoo M, Garcia-Maceira FI, et al. Lipopolysaccharide binding protein and oxidative stress in a multiple sclerosis model. Neurotherapeutics. 2017; 14(1): 199-211.
17. Belizario JE, Faintuch J, Garay-Malpartida M. Gut microbiome dysbiosis and immunometabolism: New frontiers for treatment of metabolic diseases. Mediators Inflamm. 2018; 2037838.