ORIGINAL ARTICLE

Relationship between Small Intestinal Bacterial Overgrowth and Peripheral Blood ET, TLR2 and TLR4 in Ulcerative Colitis

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Abstract

Objective: To investigate the relationship between small intestinal bacterial overgrowth (SIBO) and Endotoxin (ET) concentration in peripheral blood, and levels of toll-like receptors (TLR) 2 and TLR4 expression on surface of peripheral blood mononuclear cells (PBMCs) in patients with ulcerative colitis.
Study Design: An experimental study.
Place and Duration of Study: The First Hospital of Hebei Medical University, from July 2018 to October 2019.
Methodology: The 130 patients with ulcerative colitis were included in case group. Another 72 healthy cases were selected as control group. SIBO, ET, TLR2, and TLR4, were determined, and compared.
Results: Positive rate of SIBO in case group was higher than that in control group ($p < 0.001$). Lactulose hydrogen breath test (LHBT) intestine set value, peripheral blood ET concentration, and TLR2 and TLR4 expression levels on surface of PBMCs in case group were higher than those in control group (all $p < 0.001$); the above indexes in SIBO-positive patients in case group were higher than those in SIBO-negative patients in case group (all $p < 0.001$). Pearson's correlation analysis showed that LHBT intestine set value of SIBO-positive patients in case group was positively correlated with ET concentration, and TLR2 and TLR4 expression levels on surface of PBMCs ($r = 0.910$, $p < 0.001$; $r = 0.970$, $p < 0.001$; and $r = 0.965$, $p < 0.001$ respectively). ET concentration of SIBO-positive patients in case group was positively correlated with expression levels of TLR2 and TLR4 on surface of PBMCs ($r = 0.962$, $p < 0.001$; and $r = 0.829$ $p < 0.001$ respectively).
Conclusion: Patients with ulcerative colitis are easy to occur SIBO, and SIBO increases blood endotoxin, TLR2 and TLR4 levels. Synergistic effects of endotoxin and endotoxin receptors TLR2 and TLR4 overexpression mediate body inflammation and may be involved in progression of ulcerative colitis.
Patients with ulcerative colitis with excessive growth of small intestinal bacteria are more likely to have hypertoxemia.

Key Words: *Ulcerative colitis, Small intestinal bacterial overgrowth (SIBO), Endotoxin (ET),Toll-like receptors (TLR) 2, TLR4.*

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INTRODUCTION

Ulcerative colitis, a common inflammatory bowel disease, is often chronic and recurrent.[1] The pathogenesis of ulcerative colitis is currently unknown. A study have suggested that ulcerative colitis is the result of interactions between intestinal microorganisms and the intestinal immune system.[2] The composition of the intestinal microbiota may affect the susceptibility to ulcerative colitis.[3]

Small intestinal bacterial overgrowth (SIBO) refers to the displacement of the microflora in the distal intestine into the small intestine due to various reasons, causing inflammation of the small intestinal mucosa, disruption of permeability, and damage to villus, and it mainly manifests as abdominal pain, abdominal distention, diarrhea, small bowel motility disorders, and other symptoms.[4,5] Studies have confirmed that SIBO is closely related to diseases such as non-alcoholic fatty liver disease, cirrhosis, diabetes, irritable bowel syndrome, and chronic pancreatitis.[6-8] Lactulose hydrogen breath test (LHBT) has the advantages of being cheap, simple, and non-invasive.[9] In this experiment, LHBT was used to measure SIBO. At present, there have not been many reports on the correlation between ulcerative colitis and SIBO. The toll-like receptors (TLRs), as important bridges connecting innate and adaptive immune systems, are abundantly expressed on the surface of peripheral blood mononuclear cells (PBMCs).[10] Endotoxin receptors TLR2 and TLR4 are important receptor molecules in the host's immune response against pathogenic microorganisms, exerting an important role in the initiation of the endotoxin-induced inflammatory response.[11]

Previous studies have revealed that the incidence of SIBO is higher in patients with non-alcoholic fatty liver, and the endotoxin level and TLR4 expression in patients

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with SIBO were significantly increased.[12] However, currently there has been little literature on the role of SIBO and toll-like receptor signaling such as TLR2 and TLR4 in ulcerative colitis.

The purpose of this study was to investigate the relationship between SIBO and ET concentration in peripheral blood, and the levels of TLR2 and TLR4 expression on the surface of PBMCs in patients with ulcerative colitis.

METHODOLOGY

After the approval of the hospital ethics committee, this study was carried out at Department of Gastroenterology, the first hospital of Hebei Medical University, from July 2018 to October 2019. A total of 130 patients with ulcerative colitis admitted to the hospital from July 2018 to October 2019 were included in the case group. Inclusion criteria were that patients who met the diagnostic criteria for ulcerative colitis and were confirmed by colonoscopy and histopathology; and patients aged over 18 and with the first onset. Exclusion criteria were that patients with mental disorders who were unable to cooperate; severe cardiac dysfunction; unwilling to participate in the study; pregnant and lactating women; use of antibiotics and probiotics within 1 month before the experiment; diabetes; atrophic gastritis; and obese patients.

Another 72 age- and gender-matched healthy cases were selected as the control group. Inclusion criteria were that 18 years old or more; no chronic diseases in the past or no acute diseases in the recent past; and no occult diseases from laboratory tests, ultrasounds, gastroscopy and colonoscopy. Exclusion criteria were that Diabetes, coronary atherosclerotic heart disease, chronic obstructive pulmonary disease, chronic liver and kidney disease; mental illness; and pregnant and lactating women.

LHBT was used to measure the subjects' SIBO. On the day before the test, milk and soy products, crude fiber food, and spicy food were prohibited, and starchy food was mainly taken for dinner. The participants fasted on the test day. From the time point of oral administration of lactulose, the hydrogen concentration shall be detected once every 15 minutes, and the continuous detection shall not be less than 2 hours. The measured basic value exceeding 20 ppm or the exhaled hydrogen concen-tration rising more than 10 ppm after the test meal was considered as SIBO positive.[13] Otherwise, it was negative. LHBT intestinal set value, defined as the sum of 7 measured values before the second peak of the double peak or within 90 minutes.[14] It could indirectly reflect the degree of bacterial growth in the small intestine.

Five mL fasting peripheral venous blood was collected from the subjects in the morning. Serum endotoxin (ET) concentration was measured using a chromogenic endpoint tachypleus amebocyte lysate. Flow cytometry was used to detect the expression levels of TLR2 and TLR4 on the surface of PBMCs, which were expressed as the geometric mean fluorescence (GMF).[15]

SPSS version 25 was used to analyse data. Qualitative variables were expressed as frequencies and percentages and tested by chi-square. Quantitative variables like LHBT intestine set value, ET, TLR2 and TLR4 indicators were expressed as means and standard deviations and tested by independent-sample t-test. Pearson's correlation approach was used to analyze the correlation. Original 9.0 software was used to draw the relevant figures. The p-value <0.05 was taken as significant.

RESULTS

Among the 130 patients with ulcerative colitis in the case group, 70 were male (53.85%) and 60 were female (46.15%); aged 20-64 (46.32 ±3.15) years. The disease duration was 5-34 (16.52 ±2.07) months. As for the lesion site, 55 cases (42.31%) were in the rectum-rectosigmoid colon, 48 cases (36.92%) the left half colon, and 27 cases (20.77%) the whole colon. Forty five cases (34.62%) had mild, 65 cases (50.00%) had moderate, and 20 cases (15.38%) had severe disease. Among the 72 patients in the control group, 38 were male (52.78%) and 34 were female (47.22%); aged 21-63 (45.84 ±2.65) years.

The 57 cases (43.85%) were SIBO positive in the case group. The 9 cases (12.5%) were SIBO positive in the control group. The positive rate of SIBO in the case group was significantly higher than that in the control group (p <0.001). The LHBT intestine set value, peripheral blood ET concentration, and TLR2 and TLR4 expression levels on the surface of PBMCs in the case group were higher than those in the control group (all p <0.001, Table I).

Table I: Comparison of LHBT intestine set value, ET, TLR2 and TLR4 ndicators between case group and control group.

Indexes	Control group (n=72)	Case group (n=130)	p-value
LHBT intestine set value (ppm)	37.87 ±1.66	123.66 ±50.79	<0.001
ET (EU/mL)	0.05 ±0.01	0.19 ±0.08	<0.001
TLR2 (GMF)	29.43 ±1.67	54.15 ±17.03	<0.001
TLR4 (GMF)	9.03 ±0.32	16.47 ±3.19	<0.001

Table II: Comparison of LHBT intestine set value, ET, TLR2 and TLR4 indicators between SIBO-positive patients and SIBO-negative patients in case group.

Indexes	SIBO-positive patients in case group (n=57)	SIBO-negative patients in case group (n=73)	p-value
LHBT intestine set value (ppm)	180.80 ±3.27	79.04 ±3.08	<0.001
ET (EU/mL)	0.28 ±0.05	0.12 ±0.02	<0.001
TLR2 (GMF)	73.13 ±3.03	39.32 ±1.99	<0.001
TLR4 (GMF)	19.69 ±1.81	13.95 ±1.01	<0.001

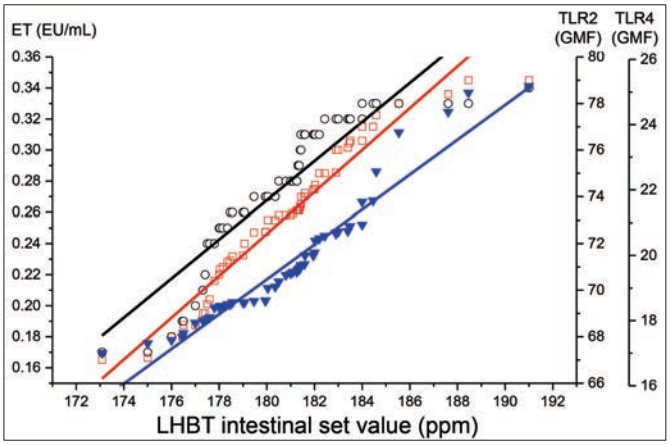

Figure 1: Correlation analysis of LHBT intestine set value with ET (black line, TLR2 (red line) and TLR4 (blue line in case group (n=130).

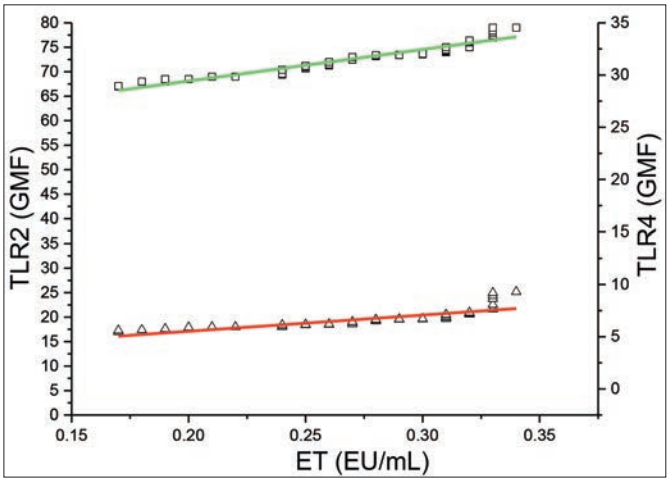

Figure 2: Correlation analysis of ET concentration with TLR2 and TLR4 in SIBO-positive patients in the case group (n=57). The green line represented the linear correlation between ET and TLR2 (r = 0.962, p <0.001); and the orange line represented the linear correlation between ET and TLR4 (r = 0.829, p <0.001).

The LHBT intestine set value, peripheral blood ET concentration, and TLR2 and TLR4 expression levels on the surface of PBMCs in SIBO-positive patients in the case group were higher than those in SIBO-negative patients in the case group (all p <0.001, Table II).

Pearson's correlation analysis showed that the LHBT intestine set value of SIBO-positive patients in the case group was positively correlated with ET concentration, and TLR2 and TLR4 expression levels on the surface of PBMCs (r = 0.910, p <0.001; r = 0.970, p <0.001; and r = 0.965, p <0.001 respectively, Figure 1).

The ET concentration of SIBO-positive patients in the case group was positively correlated with the expression levels of TLR2 and TLR4 on the surface of PBMCs (r = 0.962, p <0.001; and r = 0.829, p <0.001 respectively, Figure 2).

DISCUSSION

The best diagnostic method for SIBO is considered to be aspiration of intestinal juice from the proximal jejunum for quantitative bacterial counts. The presence of colony forming units (CFU) ≥105 per milliliter of jejunal aspirate can be diagnosed as SIBO positive.[16] However, being invasive, time-consuming and high-cost, the method of jejunal aspirate is limited in clinical application, which may reduce the detection rate of SIBO. Therefore, in recent years, breath tests (such as LHBT) have been widely used for SIBO detection due to its easy operation and non-invasiveness.[17] This study used LBHT to detect the incidence rate of patients with ulcerative colitis. The results showed that the positive rate of SIBO in patients with ulcerative colitis was 43.85%.In this study, the authors found that the detection of SIBO using LHBT was fast, simple, noninvasive, accurate and agreeable to patients; but it is easy to be affected by some factors, including the change of intestinal bacteria (the application of antibiotics, enema, *etc.* would inhibit the generation of hydrogen); diet and smoking (eating high fiber food, smoking, *etc.* would increase the amount of hydrogen) and other factors. Combined with fluoroscopy, radioactive prism scintigraphy, gastrointestinal pressure and other methods could improve the sensitivity and specificity of the detection of SIBO using LHBT.

It was also found that the LHBT intestine set value of patients with ulcerative colitis was significantly higher than that of normal people; the LHBT intestine set value of patients with ulcerative colitis combined with SIBO positive was significantly higher than that with SIBO negative. It suggested that bacterial contamination of the small intestine was common in patients with ulcerative colitis, and it may be related to the severity of the disease. The reason may be that SIBO causes intestinal flora disturbance and produces inflammatory mediators to trigger intestinal mucosal immune response, thereby promoting mucosal inflammatory damage and further aggravating ulcerative colitis.[18,19]

One study suggested that TLR2 mainly recognizes gram-positive bacteria.[20] TLR4 mainly recognizes lipopoly-saccharide (LPS) of gram-negative bacteria, which is an important protein for transducing endotoxin signals into cells. LPS binds to lipopolysaccharide binding protein (LBP) in the serum, and then forms a complex with CDl4. With the participation of TLR4, the activation signal is transmitted into the cell, inducing the activation of monocyte-phagocytic cells, exerting the functions of phagocytosis and antigen presentation, and secreting a large number of inflammatory factors.[21] This study found that compared with healthy people, patients with ulcerative colitis had higher levels of TLR4 in peripheral blood, which was basically consistent with other literature reports.[22]

The authors further found that the levels of ET, TLR2 and TLR4 in peripheral blood of patients with ulcerative colitis and SIBO positive were higher than those of patients with ulcerative colitis and SIBO negative. Pearson correlation analysis showed that the LHBT

intestine set value of SIBO positive patients in the case group was positively correlated with ET concentration, TLR2 and TLR4 expression levels on the surface of PBMCs; and ET concentration was positively correlated with TLR2 and TLR4 expression levels on the surface of PBMCs, suggesting that SIBO may increase blood endotoxin, TLR2 and TLR4 levels. The reason may lie in the increase in intestinal endotoxin production and absorption in patients with ulcerative colitis combined with SIBO, while the reduced clearance of endotoxin in the body results in the increased endotoxin levels in peripheral blood in patients with ulcerative colitis; and hyperendotoxemia in turn aggravates the process of ulcerative colitis.[23] In addition, a large amount of endotoxin enters the blood and comes into contact with mononuclear cells, causing increased stress and sensitivity of mononuclear cells to endotoxin, and increased TLR expression. TLRs recognize endotoxin, initiate transmembrane transduction of inflammatory signals, activate the transduction pathway, and induce cells to produce a large number of inflammatory cytokines.[24] Inflammatory factors can cause disorders in the immune system of the body, further aggravating ulcerative colitis and forming a vicious circle.

The limitation of this research lies in the small sample size, the lack of sensitivity and specificity analysis of LHBT detection, and the absence of data analysis of continuous variables like ET, TLR2, TLR4 levels amongst the three groups (Control group, SIBO-positive patients in case group, SIBO-negative patients in case group) of ulcerative colitis severity, and sub group analysis of controls with positive SIBO. There is need for further research on this subject using larger sample size.

CONCLUSION

Patients with ulcerative colitis are likely to develop SIBO, which increases blood endotoxin, TLR2 and TLR4 levels. Synergistic effects of endotoxin and endotoxin receptors TLR2 and TLR4 overexpression mediate body inflammation and may be involved in the progression of ulcerative colitis. Attaching importance to the diagnosis and treatment of S1BO in patients with ulcerative colitis will likely improve the disease or delay the disease process.

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ETHICAL APPROVAL:
The study had prior formal approval of hospital's Ethical Review Committee of the first hospital of Hebei Medical University.

PATIENTS' CONSENT:
Informed consent was obtained from subjects.

CONFLICT OF INTEREST:
Authors declared no conflict of interest.

AUTHORS' CONTRIBUTION:
CY: Clinical evaluation, acquired data, contribution towards results preparation and writing.
XG: Data analysis and contribution to result analysis and discussion.
JW: Data analysis and contribution to result analysis and discussion.
HF: Overall guidance, data analysis.
XH: Acquired data, contributed substantially to its revision.
LD: Acquired data, contributed substantially to its revision.
ZD: Data analysis, read and approved the final manuscript.

REFERENCES

1. Eshelman MA, Jeganathan NA, Schieffer KM, Kline BP, Mendenhall M, Deiling S, *et al.* Elevated colonic mucin expression correlates with extended time to surgery for ulcerative colitis patients. *J Gastrointestin Liver Dis* 2019; **28**:405-13.
2. Cucchiara S, Stronati L, Aloi M. Interactions between intestinal microbiota and innate immune system in pediatric inflammatory bowel disease. *J Clin Gastroenterol* 2012; **46**(Suppl): S64-6.
3. Gophna U, Sommerfeld K, Gophna S, Doolittle WF, Veldhuyzen van Zanten SJ. Differences between tissue-associated intestinal microfloras of patients with Crohn's disease and ulcerative colitis. *J Clin Microbiol* 2006; **44**:4136-41.
4. Maslennikov R, Pavlov C, Ivashkin V. Is small intestinal bacterial *overgrowth a cause of hyperdynamic circulation in cirrhosis? Turk J Gastroenterol* 2019; **30**:964-75.
5. Pilipenko VI, Isakov VA, Vlasova AV, Naidenova MA. Features of nutrition pattern of patients with small intestinal bacterial overgrowth resistant to therapy. *Vopr Pitan* 2019; **88**:31-8.
6. Cuoco L, Montalto M, Jorizzo RA, Santarelli L, Arancio F, Cammarota G, *et al.* Eradication of small intestinal bacterial overgrowth and oro-cecal transit in diabetics. *Hepatogastroenterology* 2002; **49**:1582-6.
7. Moraru IG, Moraru AG, Andrei M, Iordache T, Drug V, Diculescu M, *et al.* Small intestinal bacterial overgrowth is associated to symptoms in irritable bowel syndrome. Evidence from a multicentre study in Romania. *Rom J Intern Med* 2014; **52**:143-50.
8. DiMagno MJ, Forsmark CE. Chronic pancreatitis and small intestinal bacterial overgrowth. *Pancreatology* 2018; **18**:360-2.
9. Gemignani L, Savarino V, Ghio M, Parodi A, Zentilin P, de Bortoli N, *et al.* Lactulose breath test to assess oro-cecal transit delay and estimate esophageal dysmotility in scleroderma patients. *Semin Arthritis Rheum* 2013; **42**:522-9.
10. Johnson RH, Kho DT, O'Carroll SJ, Angel CE, Scott Graham E. The functional and inflammatory response of brain endothelial cells to Toll-Like Receptor agonists. *Sci Rep* 2018; **8**:10102.
11. Saturnino SF, Prado RO, Cunha-Melo JR, Andrade MV. Endotoxin tolerance and cross-tolerance in mast cells involves TLR4, TLR2 and fcepsilonR1 interactions and SOCS expression: Perspectives on immunomodulation in infectious and allergic diseases. *BMC Infect Dis* 2010; **10**:240.
12. Kapil S, Duseja A, Sharma BK, Singla B, Chakraborti A, Das A, *et al.* Small intestinal bacterial overgrowth and toll-like receptor

signaling in patients with non-alcoholic fatty liver disease. *J Gastroenterol Hepatol* 2016; **31**:213-21.

13. Saad RJ, Chey WD. Breath testing for small intestinal bacterial overgrowth: Maximizing test accuracy. *Clin Gastroenterol Hepatol* 2014; **12**:1964-e120.
14. Newberry C, Tierney A, Pickett-Blakely O. Lactulose hydrogen breath test result is associated with age and gender. *Biomed Res Int* 2016; **2016**:1064029.
15. Martínez-Moreno J, Hernandez JC, Urcuqui-Inchima S. Effect of high doses of vitamin D supplementation on dengue virus replication, Toll-like receptor expression, and cytokine profiles on dendritic cells. *Mol Cell Biochem* 2020; **464**:169-80.
16. Gabbard SL, Lacy BE, Levine GM, Crowell MD. The impact of alcohol consumption and cholecystectomy on small intestinal bacterial overgrowth. *Dig Dis Sci* 2014; **59**:638-44.
17. Ghoshal UC, Ghoshal U, Das K, Misra A. Utility of hydrogen breath tests in diagnosis of small intestinal bacterial overgrowth in malabsorption syndrome and its relationship with oro-cecal transit time. Indian *J Gastroenterol* 2006; **25**:6-10.
18. Walzer N, Buchman AL. Development of crohn's disease in patients with intestinal failure: A role for bacteria? *J Clin Gastroenterol* 2010; **44**:361-3.
19. Lichten E. Are the estrogenic hormonal effects of environmental toxins affecting small intestinal bacterial and microfilaria overgrowth? *Med Hypotheses* 2017; **109**:90-4.
20. Robichon C, Vidal-Ingigliardi D, Pugsley AP. Depletion of apoli—poprotein N-acyltransferase causes mislocalization of outer membrane lipoproteins in Escherichia coli. *J Biol Chem* 2005; **280**:974-83.
21. O'Callaghan P, Li JP, Lannfelt L, Lindahl U, Zhang X. Microglial heparan sulfate proteoglycans facilitate the cluster-of-differentiation 14 (CD14)/Toll-like receptor 4 (TLR4)-dependent inflammatory response. *J Biol Chem* 2015; **290**:14904-14.
22. de Paiva NM, Ayrizono ML, Milanski M, Coope A, Oliveira LM, Fagundes JJ, *et al.* Differential expression of TLR2, TLR4 and JNK in mucosa of ileal pouches for ulcerative colitis. Is there a role for bacterial antigen pathway in asymptomatic patients? *Int J Clin Exp Med* 2011; **4**:179-86.
23. Amati L, Caradonna L, Leandro G, Magrone T, Minenna M, Faleo G, *et al.* Immune abnormalities and endotoxemia in patients with ulcerative colitis and in their first degree relatives: Attempts at neutralizing endotoxin-mediated effects. *Curr Pharm Des* 2003; **9**:1937-45.
24. Rana SV, Sharma S, Kaur J, Prasad KK, Sinha SK, Kochhar R, *et al.* Relationship of cytokines, oxidative stress and GI motility with bacterial overgrowth in ulcerative colitis patients. *J Crohns Colitis* 2014; **8**:859-65.