

THE VALUE OF ELISA METHOD FOR MEASURING ANTI-VINCULIN ANTIBODIES IN THE DIAGNOSIS OF BLEEDING ULCERATIVE COLITIS AND DIARRHEA-PREDOMINANT IRRITABLE BOWEL SYNDROME AT THANH NHAN HOSPITAL IN 2023

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Objectives: To evaluate the diagnostic value of the ELISA method for measuring anti-vinculin antibodies in the diagnosis of bleeding ulcerative colitis (UC) and diarrhea-predominant irritable bowel syndrome (IBS-D) at Thanh Nhan Hospital in 2023.

Subjects and methods: This was a cross-sectional study conducted with 150 participants, including 30 healthy individuals, 50 patients with UC, and 70 patients with IBS-D. The patients were examined and treated at Thanh Nhan Hospital from January 2023 to June 2024.

Results: The average duration of disease for patients with UC and IBS-D was 2.8 ± 2.0 years and 2.9 ± 4.7 years, respectively. The average age of UC and IBS-D patients was 46.8 ± 13.7 years and 48.4 ± 12.0 years, respectively. The average level of anti-vinculin antibodies in the UC and IBS-D groups was 372.0 ± 196.1 ($\mu\text{g/g}$) and 587.7 ± 315.7 ($\mu\text{g/g}$), respectively. With an anti-vinculin antibody threshold in serum of 535.8 ng/mL (1.8 OD), the diagnostic value for distinguishing IBS-D from healthy individuals had a sensitivity of 50% and a specificity of 87.5%.

Conclusions: The level of anti-vinculin antibodies in the IBS-D group (539.2 ± 299.3 ng/mL) was significantly higher than in the healthy control group (345.8 ± 126.2 ng/mL) and the UC group (372.0 ± 196.1 ng/mL) with $p < 0.001$. With a serum anti-vinculin antibody cutoff value greater than 535.8 ng/mL (1.8 OD), there is significant diagnostic value in distinguishing between healthy individuals and IBS-D patients, with sensitivity and specificity of 50.0% and 87.5%, respectively.

Keywords: Anti-vinculin antibodies, Ulcerative colitis, Diarrhea-predominant irritable bowel syndrome, Thanh Nhan Hospital.

INTRODUCTION

Ulcerative colitis (UC) is a chronic inflammatory bowel disease that often presents with cyclical remission and relapse, and it is frequently associated with complications outside the gastrointestinal

tract. The disease progresses over a long period, increasing the risk of developing colorectal cancer. The worldwide incidence of UC has been reported to be between 156 to 291 cases per 100,000 people per year, with this number increasing by 2% annually¹.

Irritable bowel syndrome (IBS) is a chronic functional disorder of the digestive system. IBS affects approximately 20% of the general population and about 5% to 15% of the Western population, predominantly in women aged 50 years (prevalence rate of 11.2%), but it can occur at any age. IBS is characterized by symptoms such as abdominal pain, bloating, a sensation of incomplete bowel movements, and changes in bowel habits². According

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to the Rome IV criteria, IBS is classified into four different entities: constipation-predominant IBS (IBS-C), diarrhea-predominant IBS (IBS-D), mixed IBS (IBS-M), and unclassified IBS³.

Both UC and IBS-D are gastrointestinal disorders that cause significant discomfort for patients with similar clinical signs and symptoms, leading to confusion between the two conditions. Currently, the gold standard for diagnosing and differentiating UC from IBS-D is colonoscopy.

Anti-vinculin is a self-antibody against vinculin, which is an IgG protein that acts as a cell adhesion molecule in the gastrointestinal mucosa and is essential for normal intestinal motility. Anti-vinculin has been studied and applied in the diagnosis of various diseases; one of the most notable applications in the gastrointestinal system is the differentiation between ulcerative colitis (UC) and irritable bowel syndrome (IBS-D)⁴. Identifying a marker with diagnostic value for both UC and IBS-D is essential. Thanh Nhan Hospital is equipped with modern facilities, has good specialization, and serves many patients, making it necessary to find new tests that can support clinical practice. We conduct this study in order to evaluate the diagnostic value of the ELISA method for measuring anti-vinculin antibodies in the diagnosis of bleeding ulcerative colitis (UC) and diarrhea-predominant irritable bowel syndrome (IBS-D) at Thanh Nhan Hospital in 2023.

SUBJECTS AND METHODS

Subjects: Included 150 cases (consisting of 30 healthy individuals, 50 patients with ulcerative colitis (UC), and 70 patients with diarrhea-predominant irritable bowel syndrome (IBS-D), who visited and received treatment at Thanh Nhan Hospital from January 2023 to April 2024.

Inclusion criteria: Patients diagnosed with UC and IBS-D at Thanh Nhan Hospital. Complete information on age, gender, and the type of samples collected for testing must be available, with test results obtained between January 2023 and April 2024.

Exclusion criteria: Patients under 15 years old or pregnant. Patients taking certain medications such as aspirin, corticosteroids, and amino-salicylat. Patients with cancer or a history of colorectal surgery. Patients without complete information in their medical records.

Study duration and location: The study was conducted at Thanh Nhan Hospital from January 2023 to October 2024.

Study method: A cross-sectional, retrospective study design was employed.

Sampling method: The sampling method used was total sampling.

Data processing and analysis: Data were entered into Excel and analyzed using SPSS version 20.0.

RESULTS

Table 1. The frequency of isolated bacterial species and the level of antibiotic resistance

Study group	n	Mean \pm SD (Year)	p
UC	50	2.8 \pm 2.0	0.3
IBS-D	70	2.9 \pm 4.7	

Remarks: The average duration of the disease for patients with UC and IBS-D was 2.8 \pm 2.0 years and 2.9 \pm 4.7 years, respectively. There was no significant difference in disease duration between the UC and IBS-D groups ($p > 0.05$).

Table 2. Characteristics of average age

Study group	n	Mean ± SD (Year)	p
Healthy control group (1)	30	45.5 ± 10.5	(1) with (2): 0.1 (2) with (3): 0.2 (1) with (3): 0.08
UC	50	46.8 ± 13.7	
IBS-D	70	48.4 ± 12.0	

Remarks: The average age of the healthy control group, UC patients, and IBS-D patients was 45.5 ± 10.5, 46.8 ± 13.7, and 48.4 ± 12.0 years, respectively. There was no significant difference in age among the healthy control group, UC, and IBS-D groups (with $p > 0.05$). The age of the healthy control group was lower compared to the UC and IBS-D groups.

Table 3. Anti-Vinculin antibody levels in UC and IBS-D patients

Study group	n	Anti-Vinculin antibody(µg/g) Mean ± SD	p
UC	50	372.0 ± 196.1	< 0.001
IBS	70	587.7 ± 315.7	

Remarks: The average level of anti-vinculin antibodies in the UC and IBS groups was 372.0 ± 196.1 (µg/g) and 587.7 ± 315.7 (µg/g), respectively. There was a significant difference in anti-vinculin antibody levels between the UC group and the IBS group ($p < 0.05$).

Table 4. Anti-Vinculin antibody levels in healthy Individuals and IBS-D patients

Study group	n	Anti-Vinculin antibody (µg/g) Mean ± SD	p
Healthy control group	30	340.2 ± 114.5	<0.001
IBS	70	587.7 ± 315.7	

Remarks: The average level of anti-vinculin antibodies in the healthy control group and the IBS-D group was 340.2 ± 114.5 (µg/g) and 587.7 ± 315.7 (µg/g), respectively. There was a significant difference in anti-vinculin antibody levels between the healthy control group and the IBS-D group ($p < 0.05$).

Table 5. Anti-Vinculin antibody levels in healthy individuals and UC patients

Study group	n	Anti-Vinculin antibody(µg/g) Mean ± SD	p
Healthy control group	30	340.2 ± 114.5	0.2
UC	50	372.0 ± 196.1	

Remarks: The average level of anti-vinculin antibodies in the healthy control group and the UC group was 340.2 ± 114.5 (µg/g) and 372.0 ± 196.1 (µg/g), respectively. There was a significant difference in anti-vinculin antibody levels between the healthy control group and the UC group ($p > 0.05$).

**Table 1.** The frequency of isolated bacterial species and the level of antibiotic resistance

AUC	95% CI	p	Cut-off Point Anti-Vinculin	Sensitivity Sens (%)	Specificity Spec (%)
0.70	0.61 - 0.8	0.000	535.8 (ng/mL) 1.8 (OD)	50.0	87.5

Remarks: With a serum anti-vinculin antibody threshold of 535.8 ng/mL (1.8 OD), it has diagnostic value in distinguishing between IBS-D and healthy individuals, with sensitivity and specificity of 50% and 87.5%, respectively.

DISCUSSIONS

In this study, the results from 150 participants, including 30 healthy individuals, 50 patients with ulcerative colitis (UC), and 70 patients with diarrhea-predominant irritable bowel syndrome (IBS-D), who visited and received treatment at Thanh Nhan Hospital in 2023, showed that the average age of UC and IBS-D patients was 46.7 ± 14.5 and 49.1 ± 13.0 years, respectively (this difference was not statistically significant with $p > 0.05$). According to the study by Vicente-Steijn, R. and colleagues, the median age of UC patients is 47 years⁵, and IBS patients is 36 years^{6,7}. Most studies worldwide agree that most patients with inflammatory bowel disease are first diagnosed between the ages of 30 and 50⁸. The study aimed to determine the level of anti-vinculin antibodies, and the results showed significant differences in anti-vinculin antibody levels among groups: IBS-D patients and healthy individuals; and between UC and IBS-D patients, with $p < 0.05$. These results are consistent with other studies worldwide. Maysaa El Sayed Zaki, in a 2021 study involving blood samples from 100 IBS patients and 100 healthy controls, found a significant increase in optical density (OD) of anti-vinculin antibodies in IBS patients (1.58 ± 0.496 OD, 2.47 ± 0.60 OD) compared to controls (1.13 ± 0.249 OD, 2.1 ± 0.24 OD), with $p < 0.001$

for both¹⁰. The level of anti-vinculin antibodies in the IBS-D subgroup was significantly higher than in other subgroups ($p < 0.001$). Several other studies also support the use of anti-vinculin antibodies as a first-line tool for diagnosing the presence of IBS-D/IBS-M according to Rome criteria¹¹. The results of this study indicate that the sensitivity and specificity for diagnosing IBS were 50.0% and 87.5%, respectively, with an AUC of 0.7, which is considered valuable for clinical consideration. Research seeking specific blood tests for IBS has led to the approval of anti-vinculin antibodies as the first blood test in the United States for diagnosing IBS, with a specificity of 90% and sensitivity of 40%. This test has since been permitted for use in diagnosing the condition in other countries, such as Mexico, where it is used to differentiate between IBS-D and IBS-M patients¹². Bayoume S. and colleagues used the ELISA method to determine the levels of anti-vinculin antibodies from blood samples of 70 patients with diarrhea-predominant irritable bowel syndrome (IBS-D) and 20 healthy volunteers used as a control group. The results indicated that the levels of anti-vinculin antibodies were significantly higher in IBS-D patients (1.3 ± 0.4 OD) compared to healthy controls (0.6 ± 0.1 OD)³. These findings are also consistent with my research, where anti-vinculin antibodies serve as a valuable marker

to differentiate IBS-D from healthy individuals, with an area under the ROC curve of 0.7 and sensitivity and specificity of 50% and 87.5%, respectively.

CONCLUSIONS

The study shows the characteristics of the subject groups, the levels, and the value of anti-vinculin antibodies in differentiating between ulcerative colitis (UC) and diarrhea-predominant irritable bowel syndrome (IBS-D) at Thanh Nhan Hospital in 2023. It establishes the diagnostic value of serum anti-vinculin antibody levels in UC and IBS-D. The concentration of anti-vinculin antibodies in the IBS-D group (539.2 ± 299.3 ng/mL) was statistically significantly higher than in the healthy control group (345.8 ± 126.2 ng/mL) and the UC group (372.0 ± 196.1 ng/mL) with $p < 0.001$. With a serum anti-vinculin antibody cutoff value greater than 535.8 ng/mL (1.8 OD), there is significant diagnostic value in distinguishing between healthy individuals and IBS-D patients, with sensitivity and specificity of 50.0% and 87.5%, respectively.

REFERENCES

1. Mirsepasi-Lauridsen HC. Therapy Used to Promote Disease Remission Targeting Gut Dysbiosis, in UC Patients with Active Disease. *J Clin Med*. 2022 Dec 16;11(24):7472.
2. Di Rosa C, Altomare A, Terrigno V, Carbone F, Tack J, Cicala M, et al. Constipation-Predominant Irritable Bowel Syndrome (IBS-C): Effects of Different Nutritional Patterns on Intestinal Dysbiosis and Symptoms. *Nutrients*. 2023 Mar 28;15(7):1647.
3. Schmulson M, Balbuena R, Corona de Law C. Clinical experience with the use of anti-CdtB and anti-vinculin antibodies in patients with diarrhea in Mexico. *Rev Gastroenterol Mex*. 2016;81(4):236-9.
4. Pimentel M, Morales W, Rezaie A, Marsh E, Lembo A, Mirocha J, et al. Development and validation of a biomarker for diarrhea-predominant irritable bowel syndrome in human subjects. *PLoS One*. 2015;10(5):e0126438.
5. Pavlidis P, Chedgy FJ, Tibble JA. Diagnostic accuracy and clinical application of faecal calprotectin in adult patients presenting with gastrointestinal symptoms in primary care. *Scand J Gastroenterol*. 2013 Sep;48(9):1048-54.
6. Mindemark M, Larsson A. Ruling out IBD: estimation of the possible economic effects of pre-endoscopic screening with F-calprotectin. *Clin Biochem*. 2012 May;45(7-8):552-5.
7. Analytical and clinical performance of the fully-automated LIAISONXL calprotectin immunoassay from DiaSorin in IBD patients - PubMed [Internet]. [cited 2024 Sep 4]. Available from: <https://pubmed.ncbi.nlm.nih.gov/32637525/>
8. Cosnes J, Gower-Rousseau C, Seksik P, Cortot A. Epidemiology and natural history of inflammatory bowel diseases. *Gastroenterology*. 2011 May;140(6):1785-94.
9. Zaki MES, Elhammady D, Foda Salama M, Abdelsalam M, Osman AOB. Study of Antibodies to Cytolethal Distending Toxin B (CdtB) and Antibodies to Vinculin in Patients with Irritable Bowel Syndrome. *F1000Research*. 2021 Oct 15;10:303.
10. Zhan H, Chen H, Liu C, Cheng L, Yan S, Li H, et al. Diagnostic Value of D-Dimer in COVID-19: A Meta-Analysis and Meta-Regression. *Clin Appl Thromb Off J Int Acad Clin Appl Thromb*. 2021;27:10760296211010976.
11. Gao YD, Ding M, Dong X, Zhang JJ, Kursat Azkur A, Azkur D, et al. Risk factors for severe and critically ill COVID-19 patients: A review. *Allergy*. 2021 Feb;76(2):428-55.
12. Pimentel M. Update on Irritable Bowel Syndrome Diagnostics and Therapeutics. *Gastroenterol Hepatol*. 2016 Jul;12(7):442-5.