Intestinal permeability in cirrhotic patients with and without ascites

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Introduction
Patients with liver cirrhosis are at high risk of developing complications. Vulnerability to the bacterial infections in those patients is owing to numerous anomalies in defense mechanisms. Increased permeability of intestine may cause translocation of bacteria, endotoxins, and pathogen-associated molecular patterns into the portal tract and other sites.

Objective
The purpose of this study was assessment and evaluation of permeability of intestine in patients with liver cirrhosis with and without ascites.

Patients and methods
This study involved 50 patients with liver cirrhosis, comprising 25 with ascites and 25 without ascites. Permeability of intestine was detected by lactulose and mannitol ratio. The lactulose and mannitol were given orally in 100 ml of water and then patient's urine was obtained for the next 5 h. Determination of lactulose and mannitol in urine was entirely done by enzymatic methods.

Results
The study showed significant impairment of permeability of intestine in cirrhotic patients compared with controls. From 50 cirrhotic patients, permeability of intestine was impaired in 32 (64%) patients, representing one (3%) patient with Child A, 10 (31%) patients with Child B, and 21 (65%) patients with Child C, and permeability was normal in 18 (36%) cirrhotic patients.

Conclusion
Permeability of intestine was impaired in cirrhotic patients and the degree of impairment is related to the severity of disease, which increased the risk of complications.

Keywords:
ascites, intestinal permeability, lactulose–mannitol ratio, liver cirrhosis

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Introduction

Patients with liver cirrhosis are at high risk of developing complications such as spontaneous bacterial peritonitis and hepatic encephalopathy [1]. There is a relationship between the liver and the gastrointestinal tract; the liver is liable to microbial products, toxins, and gastrointestinal tract pathogen [2]. Vulnerability to the bacterial infections in those patients is owing to many anomalies in defense mechanisms, causing bacterial overgrowth, and an increase in permeability of intestine, causing translocation of bacteria [3].

Translocation of bacteria causes a systemic inflammatory response with subsequent increases in portal hypertension, exacerbating the characteristic hyperdynamic circulation in these patients, all negatively affecting liver function [4].

Schematically, the complex processes of the mucous membrane of intestine can be divided into transcellular and paracellular fluxes. It follows that mucosal intestinal permeability can be evaluated based on the quality of paracellular structures of the mucous membrane of intestine. The mucosal intestinal integrity of intestine (permeability) is evaluated by urinary excretion of orally administered unmetabolizable sugars. The main idea of the test is oral administration of two sugars of a different molecular size and with a different mechanism of absorption. Monosaccharides, such as mannitol, are absorbed through the transcellular pathway and represent absorption of small molecules. Disaccharides, such as lactulose, are absorbed through the paracellular junction complex, which corresponds to the permeability of larger molecules [5].

Patients and methods

This case–control study has been performed at Internal Medicine Department at Assiut University Hospital to evaluate permeability of intestine in patients with liver cirrhosis with and without ascites.

It was performed during the period between May 2018 and May 2019. The study included 50 patients with

liver cirrhosis divided into 25 cirrhotic patients with ascites and 25 cirrhotic patients without ascites. The diagnosis of liver cirrhosis was carried out clinically in the form of spider naevi, palmar erythema, and jaundice; laboratory, in form of serum bilirubin, serum albumin, and prothrombin time; and by imaging studies, in form of transabdominal ultrasound or computed tomography. However, ascites was diagnosed clinically by examination and radiologically by either abdominal ultrasound or computed tomography.

The severity of liver disease was evaluated according to the score of Child–Pugh [6]. Control group included 26 healthy volunteers.

Exclusion criteria

The following were the exclusion criteria:

(1) Patients with malignancy.
(2) Patients with gastrointestinal or renal disease.
(3) Patients with evidence of hepatorenal syndrome.
(4) Active bleeding of gastrointestinal tract.
(5) History of NSAIDs intake in the last 2 weeks, as it is known to affect permeability of intestine.

Procedure

(1) After an overnight fasting, the patient emptied the urinary bladder, and then, drank on an empty stomach, a cup consisting of 10 g lactulose and 5 g mannitol in 100 ml of water.
(2) The patient's urine was obtained for the next 5 h. The urine of each patient was stored in a container containing chlorhexidine. Then it was stirred, the volume detected, and sample was sent to the laboratory. The samples were kept at −20°C until laboratory analysis.
(3) An enzymatic method was used to identify lactulose and mannitol in urine. Lactulose detection was entirely by enzymatic method. First, *b*-galactosidase converted lactulose into fructose and galactose. Then, the fructose formed was converted into gluconate-6-phosphate and NADPH by action of hexokinase, glucose phosphate isomerase, and glucose 6-phosphate dehydrogenase, whereas mannitol was converted into fructose and NADH by the action of mannitol dehydrogenase. Finally, the NADPH and NADH obtained from previous reactions were detected by measuring the change in absorbance at 340 nm. A Cobas Mira analyzer was used to perform (Cobas Mira Plus CC, Roche Diagnostics 9115 Hague Road Indianapolis, IN 46250-0457 1-800-428-5074, USA.) these methods.
(4) The values acquired allowed the calculation of the lactulose/mannitol ratio (LMR) and the percentage of urinary lactulose excretion of the dose given.
(5) LMR was computed for each case for each timed collection:

LMR = excretion lactulose (mg/h)/excretion mannitol (mg/h).

(6) Lactulose is a larger sugar, so it is not normally wholly absorbed. Lactulose is excreted in the urine, it is assumed to have passed between the cells, indicating a 'leaky' gut.
(7) In contrast, mannitol is a smaller sugar that can be absorbed through enterocytes, particularly via small pores at the top of the villi of the small intestine.
(8) The purpose of including mannitol in the drink is two-folds:
    (a) It controls for any extraneous factors that might affect absorption or excretion (like gut motility or kidney function), as those factors would affect both types of sugars equally.
    (b) It can detect villous atrophy in the small intestine, as mannitol is primarily absorbed there. Poor mannitol absorption and excretion (but normal or high lactulose) suggests probable damage to the small intestinal villi.

Statistical analysis

Data entry and data analysis were done using SPSS, version 19 (statistical package for social science). Data were presented as number, percentage, mean, and SD. Independent samples *t* test was used to compare quantitative variables between groups. Paired samples *t* test was done to compare quantitative data between patients and controls. *P* value was considered statistically significant when *P* value less than 0.05.

Ethical considerations

The protocol of our study was accepted by the Medical Ethical Committee of Faculty of Medicine, Assuit University No. 17100983. Moreover, written consents from all participants were received after description of aim of the study and methods before participation in the study. Confidentiality was assured, and the patient was given the right to leave the study at any point without consequences.

Results

This is a case–control study involving 50 cirrhotic patients (25 with ascites and 25 without ascites. Approximately half (56%) of the cases were males (n = 28) and 22 (44%) were females. The mean patients' age was 60 ± 8.4 years (range, 46–75 years). The study also included 26 controls, comprising 14 (54%) males and 12 (46%) females, with mean age of 46.9 ± 15.1 years (range, 26–70 years). The patient's group was subdivided according to Child–Pugh score into three groups: A, B, and C (Table 1).

Table 1 Clinical and biochemical characteristics of the patients and control

	Patients Child score [*n* (%)]		Control [*n* (%)]	
	A (*n*=16)	B (*n*=13)	C (*n*=21)	*n* (%)
Sex				
Male	7 (43.8)	10 (76.9)	11 (52.4)	14 (53.8)
Female	9 (56.3)	3 (23.1)	10 (47.6)	12 (46.2)
Age	56.13±7.59	59.85±8.84	63.67±7.52	46.85±15.04
BMI	27.93±5.89	26.92±5.484	27.33±5.833	28.23±3.511
HCV positivity	16 (100.0)	13 (100.0)	21 (100.0)	0
Encephalopathy				
None	16 (100.0)	13 (100.0)	21 (100.0)	26 (100.0)
Ascities				
None	16 (100.0)	9 (69.2)	0	26 (100.0)
Moderate	0	4 (30.8)	9 (42.9)	0
Severe	0	0	12 (57.1)	0
Bilirubin (mg/dl)	1.65±0.3	2.09±0.33	2.85±0.59	0.83±0.32
Albumin (g/dl)	2.98±0.11	2.73±0.43	1.74±0.60	4.25±0.4
Prothrombin time (s)	15.46±1.13	16.22±1.36	20.18±2.78	12.82±0.83

The study showed significant impairment of intestinal permeability in cirrhotic patients compared with controls (P < 0.05). Lactulose recovery was higher in cirrhotic patients (0.6 ± 0.4) compared with controls (0.0 ± 0.0) (P < 0.001). Mannitol recovery was higher in cirrhotic patients (0.7 ± 0.1) compared with controls (1.0 ± 0.0) (P < 0.001). LMR was higher (1.3 ± 0.8) in cirrhotic patients compared with controls (0.1 ± 0.01) (Table 2).

Moreover, there is a prominent impairment of permeability of intestine in cirrhotic patients according to Child score. For lactulose recovery %, those with Child score C had higher mean% (1.0 ± 0.04) compared with those with Child scores A (0.1 ± 0.09) and B (0.6 ± 0.2) (P < 0.001). Furthermore, those with Child score C had lower mean lactulose recovery % (0.97 ± 0.04) compared with those with Child score A (1.0 ± 0.0) and higher than B (0.94 ± 0.08) (P = 0.003). Regarding LMR, those with Child score C had higher mean ratio (2.0 ± 0.07) compared with those with Child score A (0.3 ± 0.05) and B (1.3 ± 0.4) (P < 0.001) (Table 3).

Moreover, there is a significant impairment of intestinal permeability in ascitic patients compared with nonascetic patients (P < 0.05). Lactulose recovery was higher in ascitic patients (91.44 ± 13.39) compared with nonascitic patients (28.24 ± 27.01) (P < 0.0001). LMR was higher (1.90 ± 0.27) in ascitic patients compared with nonascitic patients (0.64 ± 0.63) (P < 0.0001) (Table 4).

Table 2 There is statistically significant difference in intestinal permeability between cirrhotic patients and controls

	Mean±SD		*P*
	Cases (*n*=50)	Control (*n*=26)	
Lactulose recovery %	0.59±0.39	0.0±0.0	<0.001**
Mannitol recovery %	0.97±0.05	1.0±0.0	<0.000**
LMR	1.28±0.81	0.01±0.01	<0.000**

LMR, lactulose and mannitol ratio.

Table 3 Comparison of intestinal permeability in patients with liver cirrhosis according to score of Child-Pugh

	Child score (Mean±SD)			*P*
	A (*n*=16)	B (*n*=13)	C (*n*=21)	
Lactulose recovery %	0.09±0.09	0.61±0.18	0.96±0.04	<0.001**
Mannitol recovery %	1.0±0.0	0.94±0.08	0.97±0.03	0.003**
LMR	0.3±0.05	1.33±0.44	1.99±0.07	<0.001**

LMR, lactulose and mannitol ratio.

Discussion

The current study adopted a case–control design and recruited 76 respondents (50 cirrhotic cases and 26 controls) from Internal Medicine Department at Assiut University Hospital over 1-year period. Intestinal permeability parameters were analyzed and compared from 24-h urinary output.

The findings of this study showed significant impairment of permeability of intestine in cirrhotic patients. Lactulose recovery was greater in cirrhotic patients compared with controls. LMR was greater in patients with liver cirrhosis in comparison with controls. There is a prominent impairment of permeability of intestine in cirrhotic patients with Child score C compared with those of Child scores A and B.

These findings are in accordance with those of Benjamin *et al*. [7] who reported a significant high LMR in cirrhotic patients compared with controls. In Benjamin study, 80 cirrhotic patients of Child class B and C without any complications were monitored for 6 months. Intestinal permeability was evaluated by LMR in cirrhotic patients and 50 controls. Intestinal permeability was impaired in 28 (35%) patients. LMR of patients was greater than controls.

Table 4 Comparison of intestinal permeability in ascitic patients and nonascitic patients

	Ascitic patients (mean±SD)	Nonascitic patients (mean±SD)	*P*
Lactulose recovery %	91.44±13.39	28.24±27.01	<0.0001**
Mannitol recovery %	95.92±4.89	98.12±4.64	0.1093**
LMR	1.90±0.271	0.64±0.63	<0.0001**

LMR, lactulose and mannitol ratio.

These results are equal to our study despite larger sample size, which also used the same method (lactulose and mannitol excretion ratio) used in our study. Impairment of intestinal permeability in our study is 64% greater than in Benjamin study (35%).

Choi *et al*. [8] also reported a significant high LMR in cirrhotic patients compared with controls. The study by Choi included 27 cirrhotic patients and 45 controls. Permeability of intestine was detected by plasma endotoxin levels and urinary excretion level of polyethylene-glycol following oral administration. Severity of liver cirrhosis was evaluated according to the score of Child–Pugh.

Permeability of intestine was greater in cirrhotic patients compared with controls. These results are matched with our study despite the smaller sample scope of study of Choi, and the study by Choi used different methods to asses intestinal permeability.

Cariello *et al*. [9] also reported that permeability of intestine was impaired in advanced cirrhotic patients in comparison with controls. This study included 83 patients with liver cirrhosis and 134 volunteers. Permeability of intestine was detected by LMR. These results are equal to our study despite the larger sample size of the study by Cariello, and the study by Cariello used additional method to confirm data in addition to the same method (lactulose and mannitol excretion ratio) of our study.

Conclusion

Permeability of intestine is impaired in cirrhotic patients, and degree of impairment is directly related to severity of the disease, which worsens the course of the disease.

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Authors' contributions: M.A.A.: concept, design, literature search, clinical studies, experimental studies manuscript preparation, editing, and review; E.A.A.: definition of intellectual content, literature search, and manuscript review; A.R.A.M.: clinical studies, experimental studies, and data acquisition; and R.F.N.: design, data analysis, statistical analysis, manuscript preparation, editing, and review.

Approval for this study was obtained from institutional review board (IRB) of Faculty of Medicine Assiut University before study execution, with registration number: NO 17100983. In addition, all participants received a written consent form. The informed consent was clear and indicated the purpose of the study, and their freedom to participate or withdraw at any time without any obligation. Furthermore, participants' confidentiality and anonymity were assured by assigning each participant with a code number for the purpose of analysis only. The study was not based on any incentives or rewards for the participants.

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Conflicts of interest

There are no conflicts of interest.

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