Brush border enzymes and absorptive capacity in extrahepatic portal venous obstruction in children

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Abstract

Background and objective Portal hypertension may affect intestinal functions, brush border enzymes and absorption parameters. Information about these in extrahepatic portal venous obstruction (EHPVO) in children is limited and poorly reported. We therefore studied the brush border enzymes and absorptive capacity in EHPVO in children.

Methods The study was conducted on 52 children of EHPVO. The diagnosis of EHPVO was made on the basis of clinical presentation and ultrasound examination. All patients underwent upper gastrointestinal endoscopy. Endoscopic tissue biopsies from duodenum were taken on aluminum foils and kept immediately at -20°C for estimation of brush border enzymes. Tissue biopsies were homogenized in sodium maleate buffer, 0.1 M pH 6.0, by a homogenizer and processed for the enzymes: lactase, maltase, and sucrase. Enzyme levels were compared to normal healthy controls ($n = 20$). D-Xylose test, stool acid steatocrit for fat excretion in stools and stool alpha-1 antitrypsin were done to know about the absorptive parameters.

Results Enzyme levels of lactase (6.21 ± 5.67 IU/mg) and sucrase (37.07 ± 21.06 IU/mg) in EHPVO group were significantly lower as compared to lactase (23.32 ± 10.48 IU/mg) and sucrase (95.96 ± 46.55 IU/mg) in normal healthy controls. Maltase levels were lower, but difference was not statistically significant in EHPVO group

(56.90 ± 28.65 IU/mg) as compared to normal controls (63.28 ± 22.88 IU/mg). There was no significant difference of urinary D-xylose and stool fat in patients with normal or short stature EHPVO patients.

Conclusion EHPVO leads to decrease in levels of brush border enzymes in small bowel but their absorption capacity remains normal.

Keywords EHPVO · Brush border enzymes · Absorption capacity

Introduction

Portal hypertension (PH) is an important cause of upper gastrointestinal bleeding in children. In developing country such as India, PH in children is caused by extrahepatic portal venous obstruction (EHPVO) in 68–70%, cirrhosis (24–28%) and infrequently due to congenital hepatic fibrosis (CHF) (3%), non-cirrhotic portal fibrosis (NCPF) (4%) and Budd–Chiari syndrome (BCS) (3%) [1–4].

Common clinical manifestations of PH are presence of splenomegaly, variceal bleeding and ascites; however, these may vary according to underlying etiology. Children with EHPVO usually present with recurrent upper GI bleeding and splenomegaly in contrast to patients with cirrhosis who present with splenomegaly, ascites, engorged abdominal veins and features of chronic liver disease (CLD). Rarely ascites may be seen in EHPVO immediately after acute episode of bleeding. A subset of patients with PH may have splenomegaly and remain asymptomatic.

Intestinal mucosa has paradoxical function of acting as a barrier to permeation of antigens and at the same time facilitating absorption of electrolytes and nutrients. Intestinal absorption and permeability are often impaired in

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adult patients with liver disease. Patients with cirrhosis, in particular, have high prevalence (35–80%) of D-xylose and fat malabsorption [5–7], although some studies have shown no evidence of monosaccharide malabsorption [8, 9]. Children with CLD frequently have evidence of malnutrition [10, 11], which when severe may be an independent risk factor for adverse outcome of liver transplantation.

The contribution of PH toward malnutrition has not been properly studied in EHPVO. In theory, PH may itself lead to mucosal edema, with reduction in villus/crypt ratio and malabsorption [12], which might contribute to malnutrition. There are varying reports of growth retardation in EHPVO in children from our country. This has been related to abnormal growth hormones also.

There is paucity of data on functional alteration in small bowel in EHPVO especially in children. As various functions performed by small intestinal mucosa are mediated by a large number of enzymes, we undertook this study to evaluate absorption capacity and brush border enzymes in EHPVO in children.

Patients and methods

The study was conducted on 52 children of EHPVO. The patients were enrolled for study from Division of Pediatric Gastroenterology and Hepatology, Post Graduate Institute of Medical Education and Research (PGIMER), Chandigarh. Both outdoor and indoor patients were included in the study. They were divided into two groups based on growth: Group 1 (normal stature) and Group 2 (short stature). Height less than 3rd percentile (NCHS) was taken as short stature.

Exclusion criteria:

- Age > 18 years
- Liver dysfunction
- History of non-steroidal anti-inflammatory drugs or proton pump inhibitors intake in preceding 2 weeks
- History of antibiotics intake in preceding 3 weeks
- Refusal to participate in study or refusal to undergo any of listed diagnostic test
- Any co-morbid illness known to affect brush border enzymes and growth.

Study protocol

Informed consent was obtained from all parents. Each patient had choice to withdraw from study at any time during study period. Result of any investigation carried out for the study purpose was made available to the patients. Detailed history, examination and growth parameters of the

patients were recorded in the pretested proforma. Each patient was subjected to hemogram, liver function tests, renal function tests, coagulogram, D-xylose test, acid steatocrit, stool alpha-1 antitrypsin, ultrasonography (USG) abdomen and esophagogastroduodenoscopy. Results of other investigations carried out during routine patient care were recorded in the proforma.

- D-Xylose test in urine was done after overnight fast.
- Stool fat estimation was done by standard acid steatocrit [13].
- Alpha-1 antitrypsin was estimated in stool by diffuse-plate method [14].
- USG and Doppler ultrasound were done in all patients after overnight fast by a single experienced radiologist within the department.
- Upper GI endoscopy was done by Olympus GIF endoscope.

Endoscopy was done after overnight fast, using video endoscope, after using intravenous ketamine in dose of 1 mg/kg. Details of mucosal findings in esophagus, stomach and duodenum (1st and 2nd part) were recorded. In the same sitting, endoscopic biopsies from the duodenum were taken for assay of brush border enzymes and for histopathological examination. Brush border enzymes were compared to age matched healthy control population ($n = 20$) who had endoscopy done for gastro-esophageal reflux disease (GERD) symptoms.

Endoscopic tissue biopsies were taken on aluminum foils and kept immediately at -20°C for estimation of brush border enzymes. Tissue biopsies were homogenized in sodium maleate buffer, 0.1 M pH 6.0, by a homogenizer and processed for the enzymes: lactase, maltase, and sucrase. Total protein present in the tissue was also estimated and the level of enzymes was expressed as IU/mg of protein.

The activities of sucrase, maltase and lactase were determined in biopsy homogenate by measuring D-glucose liberated from sucrose, maltose and lactose using glucose-peroxidase system of Dalquist [15]. Total protein was estimated by the method of Lowry et al. [16].

Statistical analysis

Statistical analysis was performed with Statistical Package for the Social Sciences Software (SPSS version 13.0, Chicago, IL, USA). In total study group, for continuous data mean, standard deviation and range were calculated. For comparing categorical data, Chi-square test and Fisher's exact test were applied. For comparing non-parametric data, Mann-Whitney was used. P value less than 0.05 was taken significant.

Results

This prospective study included total 52 patients of EHPVO with 41 patients in Group 1 (normal stature) and 11 patients in Group 2 (short stature) as per NCHS standards. Weight being a labile parameter was not considered. Brush border enzymes (lactase, sucrase and maltase), absorption parameters (urine D-xylose, stool fat and stool alpha 1 antitrypsin) were compared in both groups as well as to healthy controls. There was no significant difference in gender ratio between Groups 1 and 2. Mean age of onset in Group 1 was 6.5 years versus 6.45 years in Group 2, difference was statistically not significant. There was no significant difference in mean age of presentation and duration of disease between Groups 1 and 2 (Table 1).

History of UGI bleed was present in 82.9% patients of Group 1 and 90.9% in Group 2. There was slight higher trend in Group 2, but difference was statistically not significant. Hemoglobin, spleen size and bleeding episodes were also not significantly different. There was no significant difference of portal hypertensive gastropathy and duodenopathy between Groups 1 and 2 (Table 2). On ultrasound, portal

vein block with cavernoma was seen in all patients but superior mesenteric vein block was present in five patients.

Mucosal enzyme levels

Levels of three brush border enzymes (lactase, sucrase and maltase) were compared in Groups 1 and 2 (Table 3). There was no significant difference between both groups. Levels of brush border enzymes of all patients were compared to brush border enzymes in GERD patients. There was significant difference between levels of lactase and sucrase between patients and controls, but not significant with respect to maltase (Table 4). The levels of lactase and sucrase were significantly different between when Groups 1 and 2 separately compared to controls. There was trend toward lower enzyme levels in relation to the presence of duodenopathy, but difference was not statistically significant (Table 5). There was no significant difference of urinary D-xylose and stool fat in Groups 1 and 2 (Table 6).

Stool alpha 1 antitrypsin levels were done in all patients and that was undetectable in both Groups 1 and 2.

Table 1 Demographic profile of the two groups of patients

Variables	Group 1 (<i>n</i> = 41)	Group 2 (<i>n</i> = 11)	<i>P</i> value
Total number	41	11	NS
Male (%)	29 (70.7%)	8 (72.7%)	NS
Female (%)	12 (29.3%)	3 (27.3%)	NS
Age of onset (years)			
Mean $\pm$ SD (range)	6.512 $\pm$ 2.96 (1–15)	6.455 $\pm$ 2.97 (3–12)	NS
Age of presentation (years)			
Mean $\pm$ SD (range)	10.33 $\pm$ 2.89 (5–15)	10.82 $\pm$ 3.02 (5–16)	NS
Duration of disease (years)			
Mean $\pm$ SD (range)	3.75 $\pm$ 3.10 (0.08–12)	3.83 $\pm$ 2.61 (0.17–10)	NS

NS not significant

Table 2 Clinical characteristics of two groups of patients

Variables	Group 1 (<i>n</i> = 41)	Group 2 (<i>n</i> = 11)	<i>P</i> value
Bleeder (%)	34 (82.9)	10 (90.9)	NS
Bleeding episodes (%)			
Mean $\pm$ SD (range)	1.39 $\pm$ 0.58 (1–3)	1.72 $\pm$ 0.78 (1–3)	NS
Hemoglobin (g/dl)			
Mean $\pm$ SD (range)	9.08 $\pm$ 1.74 (4.4–12.9)	8.77 $\pm$ 1.83 (5.4–10.8)	NS
Spleen size (cm)			
Mean $\pm$ SD (range)	5.22 $\pm$ 2.29 (1–12)	6.41 $\pm$ 4.54 (3–20)	NS
PHG (%)			
Nil	4 (9.8)	0 (0)	NS
Mild	31 (75.6)	8 (72.7)	
Severe	6 (14.6)	3 (27.3)	
Duodenopathy (%)			
Nil	37 (90.2)	10 (90.9)	NS
Present	4 (9.8)	1 (9.1)	

PHG portal hypertensive gastropathy, NS not significant

Table 3 Comparison of brush border enzymes in Groups 1 and 2

Enzymes (IU/mg protein)	Group 1 (<i>n</i> = 41)	Group 2 (<i>n</i> = 11)	<i>P</i> value
Lactase			
Mean $\pm$ SD (range)	6.41 $\pm$ 5.62 (1.29–26.22)	5.46 $\pm$ 6.09 (1.54–22.42)	NS
Sucrase			
Mean $\pm$ SD (range)	37.03 $\pm$ 21.38 (6.72–86.62)	37.23 $\pm$ 20.80 (9.25–78.83)	NS
Maltase			
Mean $\pm$ SD (range)	56.27 $\pm$ 26.05 (15.71–164.8)	59.24 $\pm$ 38.28 (12.43–143.63)	NS

NS not significant

Table 4 Comparison of brush border enzymes in patients and controls

Enzymes (IU/mg protein)	Patients (<i>n</i> = 52)	Controls (<i>n</i> = 20)	<i>P</i> value
Lactase			
Mean $\pm$ SD (range)	6.21 $\pm$ 5.67 (1.29–26.22)	23.32 $\pm$ 10.48 (4.60–39.90)	<0.005
Sucrase			
Mean $\pm$ SD (range)	37.07 $\pm$ 21.06 (6.72–86.62)	95.96 $\pm$ 46.55 (23.10–254.00)	<0.005
Maltase			
Mean $\pm$ SD (range)	56.90 $\pm$ 28.65 (12.43–164.83)	63.28 $\pm$ 22.88 (29.20–116.80)	NS

NS not significant

Table 5 Comparison of brush border enzymes with respect to portal hypertension duodenopathy

Enzymes (IU/g protein)	Duodenopathy absent	Duodenopathy present	<i>P</i> value
Lactase			
Mean $\pm$ SD (range)	6.4 $\pm$ 5.89 (1.29–26.22)	3.64 $\pm$ 1.64 (1.54–6.14)	NS
Sucrase			
Mean $\pm$ SD (range)	38.83 $\pm$ 21.11 (6.72–86.62)	20.54 $\pm$ 12.36 (7.40–33.94)	NS
Maltase			
Mean $\pm$ SD (range)	58.25 $\pm$ 29.28 (15.71–164.83)	44.21 $\pm$ 19.59 (22.34–64.81)	NS

NS not significant

Table 6 Comparison of absorption parameters in Groups 1 and 2

Absorption parameters	Group 1 (<i>n</i> = 41)	Group 2 (<i>n</i> = 11)	<i>P</i> value
Urine D-xylose (g/5 g)			
Normal (%)	36 (87.8)	10 (90.9)	NS
Abnormal (%)	5 (12.2)	1 (9.1)	
Stool fat (g/24 h)			
Normal (%)	39 (95.1)	11 (100)	NS
Abnormal (%)	2 (4.9)	0 (0)	

NS not significant

On duodenal biopsy, mild villous changes in form of blunting of villi and duodenopathy were seen in both groups, the difference being non-significant.

Discussion

The purpose of this prospective study was to look for the functional changes in the small intestinal brush border in the form of derangements in the levels of brush border enzymes and absorption parameters brought about by the morphological changes in the small intestinal mucosa in patients

with EHPVO, encompassed by the term portal hypertensive intestinal vasculopathy. Several studies in the past have alluded to the fact that there are functional as well as structural alterations in the intestinal brush border as well as changes in the brush border enzymes. Most studies are in adults and have focused on alcohol, its effect on brush border and also its contribution to malnutrition [17–23].

In contrast to CLD there is no hepatocellular failure in EHPVO and all the changes are exclusively due to PH. The available evidence on the effects of PH on small intestinal morphology as well as enzyme activities is conflicting [24–26]. Brush border enzymes are important in the digestive and absorptive functions of small intestine. Changes in the status of these enzymes may reflect the physical damage to the enterocytes. The mechanism of malabsorption in PH is mixed and may involve the activity of mucosal disaccharidases and increase in the permeability which facilitates the output of water and solutes. In this study the patients of EHPVO had significantly lower levels of lactase and sucrase in their duodenal mucosa as compared to the controls. There was trend toward lower levels of maltase, though the difference did not reach the statistical significance. Differential lowering of enzymes between the EHPVO and controls may be due to variation in congestive

gastropathy and duodenopathy. Such differential lowering of the mucosal enzymes is noted in previous studies also [20–22]. When compared with presence of duodenopathy there was trend toward lower enzymes levels in duodenopathy group. Kaur et al. studied the effect of extrahepatic and intrahepatic PH on brush border membrane enzymes in rats after partial portal vein ligation. Sucrase levels were elevated in intestinal homogenate of rats [27].

Patients with cirrhosis, in particular, have a high prevalence (35–80%) of D-xylose and fat malabsorption. The contribution of PH per se to this malabsorption is not clear. PH alone without liver dysfunction (EHPVO) by itself may lead to mucosal edema with reduction in villous/crypt ratio and malabsorption which might contribute to growth retardation in children. In a study by Taylor et al. [28] in children measuring excretion of 3-O-methyl-D-glucose, D-xylose and L-rhamnose in urine, authors found significant malabsorption of monosaccharides in both hepatic and prehepatic groups and concluded that increased portal pressures reduced the absorptive capacity of small intestine. We looked upon the absorptive capacity and measured D-xylose in urine, fat in stool and alpha 1 antitrypsin stool clearance. Stool alpha 1 antitrypsin was found in undetectable range in all patients while other two parameters were found within normal range in majority of patients. In both groups the difference was not statistically significant. This indicates that malabsorption probably is not the major cause of growth retardation in EHPVO.

These findings taken together conclude that patients with EHPVO tend to have lower lactase, sucrase and maltase in their small intestinal mucosa compared to normal healthy controls but their absorption capacity remains normal as indicated by normal D-xylose, stool fat excretion and alpha 1 antitrypsin clearance. Presence of duodenopathy had no impact.

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