SciMedCentral

Annals of
Pediatrics & Child Health

Special Issue on
Pediatric Gastroenterology Disorders

Edited by:
Hillel Naon, M.D.
Acting Division Head, Pediatric Gastroenterology, Hepatology and Nutrition, Children's Hospital Los Angeles, University Southern California, Keck School of Medicine, Los Angeles, USA

Research Article

Is Disaccharidase Deficiency in Pediatric Patients with Graft- Versus-Host Disease (Gvhd) a Contributing Cause for Diarrhea?

Brynie Slome Collins, Tanaz Farzan Danialifar*, Neena Kapoor and Hillel Naon

Division of Gastroenterology and Nutrition, University of Southern California, USA

***Corresponding author**
Tanaz Farzan Danialifar, Childrens Hospital Los Angeles, Division of Gastroenterology and Nutrition MS #78, 4650 Sunset Blvd. Los Angeles, CA 90027, USA, Tel: 323-361-6083; Fax: 323-361-3718; Email: tdanialifar@chla.usc.edu

Submitted: 19 December 2014

Accepted: 08 January 2015

Published: 04 February 2015

Keywords
- Graft versus host disease (GVHD); Hematopoietic progenitor cell transplant (HPCT)

Abstract

Objectives: Graft versus host disease (GVHD) is the most common cause of diarrhea in allogeneic hematopoietic progenitor cell transplant (HPCT) recipients. Diarrhea may be caused by infection or epithelial cell damage secondary to chemotherapy or radiation, which in turn can decrease brush border enzyme activity. Disaccharidase levels and the potential for lactose malabsorption as a contributing factor for diarrhea have not been studied in patients with GVHD. The aim of this study is to evaluate the prevalence of disaccharidase deficiency in pediatric HPCT patients with GVHD and its potential role in causing diarrhea.

Methods: A retrospective review of 21 HPCT patients with protracted diarrhea referred for gastrointestinal endoscopy and biopsy to rule out GVHD. Biopsies were evaluated for infection, GVHD, or other abnormalities. Duodenal samples were frozen and sent for disaccharidase analysis.

Results: One patient was excluded from the study because disaccharidase analysis was not sent. Grade 1 GVHD was found in 11 patients (55%), grade 2 in 1 patient (.05%), and no GVHD in the remaining 8 patients (40%). Ten patients with GVHD (83%) and 6 without (75%) had an isolated lactase deficiency ($p = 0.27$). Six patients had virus present on intestinal biopsies and 1 patient had C. Difficile infection.

Conclusions: There is not a greater prevalence of either isolated lactase or global disaccharidase deficiency in pediatric HPCT patients with Grade 1 or 2 GVHD. However, regardless of presence of GVHD, the majorities of HPCT patients with protracted diarrhea have isolated lactase deficiency and may benefit from a lactose-free diet.

Cite this article: *Collins BS, Danialifar TF, Kapoor N, Naon H (2015) Is Disaccharidase Deficiency in Pediatric Patients with Graft- Versus-Host Disease (Gvhd) a Contributing Cause for Diarrhea? Ann Pediatr Child Health 3(2): 1038.*

INTRODUCTION

Acute graft-versus-host-disease (GVHD) is the leading cause of morbidity and mortality in patients who have had an allogeneic hematopietic progenitor cell transplant (HPCT) [1], with approximately 18-70% of these patients developing acute GVHD [2-4]. The main organs targeted by acute GVHD are the skin, liver and gastrointestinal (GI) tract. Profuse diarrhea is the most frequent GI symptom associated with GVHD, and is commonly accompanied by abdominal pain, anorexia, nausea, vomiting and GI bleeding [5]. The diarrhea is often green, mucoid and watery, and may contain exfoliated cells.

The basic pathophysiology of GVHD involves three phases: activation of antigen-presenting cells in the target tissue; donor t-cell activation, proliferation, differentiation, and migration; and finally destruction of the target tissues [6]. Within the gastrointestinal tract this manifests as diarrhea as a result of epithelial cell damage, surface area loss with subsequent impaired absorption, and increased vascular permeability due to cytokine release [7]. Damage to the GI tract also increases the translocation of inflammatory stimuli such as endotoxin, which promotes further inflammation and additional GI tract damage [8]. The main sites of GVHD associated intestinal injury are the distal ileum and proximal colon, followed by the remainder of the small intestine, distal colon and rectum [8].

GVHD as the cause of diarrhea is supported by presentation with profuse, green mucoid stools as well as by a falling serum albumin secondary to protein-losing enteropathy [9]. Enteric pathogens such as adenovirus, rotavirus, HHV6, norovirus CMV, C. Difficile and ova and parasites may be a triggering event for GVHD. Liver and skin manifestations of GVHD can also be helpful clues; however, the definite diagnosis is made by endoscopy with mucosal biopsies. Endoscopic features that suggest the presence of GVHD include hyperemia, erythema, granularity, mucosal ulcerations or diffuse mucosal sloughing [5]. While involvement can be patchy, several studies have demonstrated that in limited endoscopic evaluation with esophagogastroduodenoscopy and flexible sigmoidoscopy is effective and safe [10]. Specific histologic criteria are used to establish the diagnosis of GI GVHD (Table 1). The classic finding is epithelial single-cell apoptosis, which may or may not be accompanied by increased inflammation, crypt abscesses, crypt cell degeneration, nuclear atypia or flattened abnormal surface epithelium [11-13].

Since the 1960's we have known that disaccharidases are found in the brush border of the epithelial cells along the intestinal villous [14]. Disaccharidase deficiency is associated with carbohydrate malabsorption and leads to symptoms of profuse diarrhea, abdominal distention and pain. Given the significant small intestinal mucosal damage that can occur with GVHD, we aimed to determine if among pediatric HPCT patients with diarrhea there was a greater prevalence of disaccharidase deficiency in those with GVHD compared patients without GVHD.

Table 1: Histopathologic grading of GVHD [25].

Grade 1	Individual crypt cell necrosis
Grade 2	Crypt abscess, crypt cell flattening, with or without crypt cell degeneration
Grade 3	Dropout of 1 or more whole crypts in a biopsy
Grade 4	Total denudation of epithelium

MATERIALS AND METHODS

Population and Sample Collection

The study was conducted at Children's Hospital Los Angeles (CHLA), a large tertiary care center. Approval was obtained by the institutional review board prior to initiation. A retrospective chart review was performed of all patients with protracted diarrhea and weight loss who had been referred by their primary hematopoietic progenitor cell transplant team between July 2004 and September 2005 for upper endoscopy and flexible sigmoidoscopy or colonoscopy with biopsies to rule out GVHD. None of the subjects had clinical symptoms of lactose intolerance prior to HPCT. For each patient, stool studies were performed prior to endoscopy for routine bacteria, ova and parasites, C. difficile, CMV, Adenovirus, Rotavirus, Norovirus, and HHV-6. Endoscopic biopsies were then obtained from the esophagus, stomach, duodenum and colon. In addition to standard histology, tissue was also sent for PCR and staining for HSV, HHV-6, EBV, and CMV. A duodenal biopsy was also collected at the time of endoscopy, frozen, and sent to Joli Diagnostics in Williamsville, NY, for analysis of lactase, sucrase, maltase and palatinase levels.

Analytic methods

All biopsies were read by a single pathologist for evidence of infection, GVHD or other abnormalities, and were graded according to the histologic criteria shown in Table 1. Disaccharidase analysis was performed by Joli Diagnostics using a standard technique: Duodenal tissue was homogenized and incubated with disaccharide substrate, enzyme hydrolysis resulted in glucose formation, and then a quantitative measure of glucose was analyzed [15]. Control values have been established based on children undergoing endoscopy with normal histology and correlate closely with those values found in healthy animals serving as controls [16].

Statistical analysis

Means and standard deviations were calculated. Comparison of means for each disaccharidase level was performed using a two-tailed, Student's *t*-test. In order to evaluate global disaccharidase deficiency, a total score was created by adding up each disaccharidase level. Comparison of means of the total scores as well as of the number of patients with concomitant intestinal infections was performed using the same Student's t-test. Statistical significance was defined as $P< 0.05$.

RESULTS

Between July 2004 and September 2005 there were 21 patients referred by their primary hematopoietic progenitor cell transplant team for upper and lower endoscopy to rule out GVHD. One patient was excluded from the study, as disaccharidase analysis was not performed. Of the remaining 20 patients, there were 7 females (35%) and 12 males (65%). The mean age was 6 years, 7months, with a range of 8 months -16 years.

Pathology reports confirmed Grade 1 GVHD in 11 patients (55%) and Grade 2 GVHD in 1 patient (.05%). The remaining 8 patients (40%) had no evidence of GVHD or any other significant

histological abnormalities. Table 2 summarizes the outcome measures evaluated. Ten patients with GVHD (83%) and 6 without GVHD (75%) had an isolated lactase deficiency (p =0.27). However, only 1 patient (.08%) with GVHD (either grade 1 or 2) and 2 patients (25%) without GVHD had global disaccharidase deficiency. (p = 0.19).

Infectious organisms were identified in the intestinal biopsies or stool of seven patients, 4 with GVHD and 3 without (p =0.08). Table 3 summarizes the organisms identified.

DISCUSSION

GVHD is the leading cause of diarrhea in HPCT patients [17], with roughly 50% of these patients requiring evaluation by a gastroenterologist [5,18] The aim of this study was to determine if there is a higher prevalence of disaccharidase deficiency in pediatric HPCT patients with GVHD compared to HPCT patients without GVHD which would suggest that carbohydrate malabsorption is a contributing factor in development of diarrhea. While there is strong evidence that GVHD can cause mucosal damage throughout the small intestine [3,5], our retrospective review showed no statistically significant difference in any isolated disaccharidase deficiencies or in global disaccharidase deficiency in those patients with Grade 1 or 2 GVHD compared to those without histologic evidence of GHVD (p =0.19). There was a trend toward lower disaccharidase levels in patients with GVHD but this did not reach statistical significance possibly due to small number of subjects in the study. To date, we have been unable to find previous reports in the literature about the prevalence of disaccharidase deficiency in patients with GVHD. However, our findings are consistent with studies on disaccharidase deficiency in patients with chemotherapy related diarrhea, a condition where histologic damage to the intestinal mucosa is similar to that seen in GVHD [19,20].

Table 2: Comparison of Disaccharidase levels.

Enzyme Levels	No GVHD	GVHD	P Value
	(n=8)	(Grade 1 n=1t1, Grade 2 n=1)	
Lactase	14.725 ± 15.5	8.03 ± 5.56	0.27
Sucrase	68.04 ±39.78	43.03 ± 24.24	0.14
Maltase	189.61 ± 95.02	142.58 ± 79.3	0.27
Palatinase	11.65 ± 6.17	8.63 ± 4.85	0.27
Total	284.02 ±144.47	201.82 ± 106.66	0.19

Data in mean ± SD
Enzyme levels reported in μM/min/gram protein
Normal ranges for enzymes: Lactase 24.5 ± 8.0, Sucrase 54.4 ± 25.4, Maltase 160.8 ± 62.8, Palatinase 11.1 ± 6.5.

Table 3: Summary of concomitant infections.

Infectious Organisms	No GVHD	Grade 1 GVHD	Grade 2 GVHD
Adenovirus *	2	1	0
HHV-6 **	1	1	1
CMV †	1	0	0
C. Difficile Toxins A and B ‡	0	1	0

Data in number of patients
* isolated by direct antigen assay (DAA) of tissue
** isolated by polymerase chain reaction (PCR) of tissue
† isolated from tissue culture
‡ isolated from stool

Despite no significant difference being found between those with GVHD and those without, the majority of HPCT patients (83% with GVHD and 75% without GVHD) were found to have isolated lactase deficiency. The majority of our patients were African American or Hispanic, and lactose intolerance is reported to occur in 11-58% of children with these ethnic backgrounds [21-23]. While this may, in part, explain the high prevalence of lactase deficiency seen in our patients, the percentages of HPCT patients (both with and without GVHD) with lactase deficiency are still much higher than those reported in the literature for healthy Hispanic and African American children. While no histological abnormalities were seen in a subset of patients with lactase deficiency we hypothesize that because individual disaccharidases are found in an array along the intestinal villous, with lactase being the most distal [24-26], this enzyme may be more vulnerable to the minor intestinal damage that may be caused by chemotherapeutic agents used for preconditioning and immunosuppresion or by grade 1 or 2 GVHD.

Additionally, our data show that 3 patients with GVHD and 3 without GVHD had viruses in their intestinal biopsies, and 1 patient with GVHD had C. Difficile toxins A and B in his stool (Table 3). This difference in number of patients with concomitant intestinal infections is not significant (p=0.8), and we can therefore conclude that mild, Grade 1-2 disease does not increase the risk of intestinal infection. It remains unclear, however, if there is a greater prevalence of infections in patients with more severe GVHD and consequently more severe mucosal damage.

There are several limitations to this study. The sample size in the study was small. While there was no statistically significant difference in global disaccharidase deficiency or isolated lactase deficiency between the GVHD and non GVHD groups, the levels of each disaccharidase were lower in the GHVD group. Given the variability in levels no statistically significant changes were detected. In addition, as previously stated, the majority of our patients with GVHD had mild (Grade 1-2) disease. It would be interesting to see if there were similar findings in patients with more severe GVHD (Grade 3-4), not only with respect to disaccharidase activity but also with respect to prevalence of concomitant microbial infections.

In conclusion, our study revealed that there does not appear to be an increased prevalence of isolated disaccharidase deficiency or global disaccharidase deficiency in pediatric HPCT patients with mild Grade 1 or 2 intestinal GVHD as compared to HPCT patients without GVHD. There was however a trend toward lower disacchariase levels in patients with GVHD. It is possible that mild GVHD is not sufficient to result in significant disaccharidase loss. Additionally, GVHD is not a uniform process and may result in patchy intestinal injury and disaccharidase loss. However, regardless of whether there is biopsy evidence of GVHD or infection, the majority of HPCT patients with protracted diarrhea has isolated lactase deficiency and may therefore benefit from a specialized lactose-free diet.

REFERENCES

1. Wagner JE Jr, Vogelsang GB, Beschorner WE. Pathogenesis and

pathology of graft-vs.-host disease. Am J Pediatr Hematol Oncol. 1989; 11: 196-212.

2. Jones RJ, Vogelsang GB, Hess AD, Farmer ER, Mann RB, Geller RB, et al. Induction of graft-versus-host disease after autologous bone marrow transplantation. Lancet. 1989; 1: 754-757.

3. Hood AF, Vogelsang GB, Black LP, Farmer ER, Santos GW. Acute graft-vs-host disease. Development following autologous and syngeneic bone marrow transplantation. Arch Dermatol. 1987; 123: 745-750.

4. Sullivan KM, Parkman R. The pathophysiology and treatment of graft-versus-host disease. Clin Haematol. 1983; 12: 775-789.

5. Iqbal N, Salzman D, Lazenby AJ, Wilcox CM. Diagnosis of gastrointestinal graft-versus-host disease. Am J Gastroenterol. 2000; 95: 3034-3038.

6. Ferrara JL, Levine JE, Reddy P, Holler E. Graft- versus-host disease. Lancet. 2009; 373: 1550-1561.

7. McDonald GB, Shulman HM, Sullivan KM, Spencer GD. Intestinal and hepatic complications of human bone marrow transplantation. Part I. Gastroenterology. 1986; 90: 460-477.

8. Hill GR, Ferrara JL. The primacy of the gastrointestinal tract as a target organ of acute graft-versus-host disease: rationale for the use of cytokine shields in allogeneic bone marrow transplantation. Blood 2000; 95: 2754-9.

9. Lerner KG, Kao GF, Storb R, Buckner CD, Clift RA, Thomas ED. Histopathology of graft-vs.-host reaction (GvHR) in human recipients of marrow from HL-A-matched sibling donors. Transplant Proc. 1974; 6: 367-371.

10. Cox GJ, Matsui SM, Lo RS, Hinds M, Bowden RA, Hackman RC, et al. Etiology and outcome of diarrhea after marrow transplantation: a prospective study. Gastroenterology. 1994; 107: 1398-1407.

11. Crowell KR, Patel RA, Fluchel M, Lowichik A, Bryson S, Pohl JF. Endoscopy in the diagnosis of intestinal graft-versus-host disease: is lower endoscopy with biopsy as effective in diagnosis as upper endoscopy combined with lower endoscopy? Pediatr Blood Cancer. 2013; 60: 1798-1800.

12. Bombí JA, Nadal A, Carreras E, Ramírez J, Muñoz J, Rozman C, et al. Assessment of histopathologic changes in the colonic biopsy in acute graft-versus-host disease. Am J Clin Pathol. 1995; 103: 690-695.

13. Snover DC, Weisdorf SA, Vercellotti GM, Rank B, Hutton S, McGlave P. A histopathologic study of gastric and small intestinal graft-versus-host disease following allogeneic bone marrow transplantation. Hum Pathol. 1985; 16: 387-392.

14. Epstein RJ, McDonald GB, Sale GE, Shulman HM, Thomas ED. The diagnostic accuracy of the rectal biopsy in acute graft-versus-host disease: a prospective study of thirteen patients. Gastroenterology. 1980; 78: 764-771.

15. Dahlqvist A. The intestinal disaccharidases and disaccharide intolerance. Gastroenterology. 1962; 43: 694-696.

16. Dahlqvist A. Assay of intestinal disaccharidases. Anal Biochem. 1968; 22: 99-107.

17. Gupta SK, Chong SK, Fitzgerald JF. Disaccharidase activities in children: normal values and comparison based on symptoms and histologic changes. J Pediatr Gastroenterol Nutr. 1999; 28: 246-251.

18. Barker CC, Anderson RA, Sauve RS, Butzner JD. GI complications in pediatric patients post-BMT. Bone Marrow Transplant. 2005; 36: 51-58.

19. Appleton AL, Sviland L, Pearson AD, Green MA, Eastham EJ, Malcolm AJ. The need for endoscopic biopsy in the diagnosis of upper gastrointestinal graft-versus-host disease. J Pediatr Gastroenterol Nutr. 1993; 16: 183-185.

20. Smit JM, Mulder NH, Sleijfer DT, Bouman JG, Koudstaal J, Elema JD, et al. Gastrointestinal toxicity of chemotherapy and the influence of hyperalimentation. Cancer. 1986; 58: 1990-1994.

21. Keefe DM, Brealey J, Goland GJ, Cummins AG. Chemotherapy for cancer causes apoptosis that precedes hypoplasia in crypts of the small intestine in humans. Gut. 2000; 47: 632-637.

22. Paige DM, Bayless TM, Mellitis ED, Davis L. Lactose malabsorption in preschool black children. Am J Clin Nutr 1977:30; 1018-22.

23. Paige DM, Bayless TM, Dellinger WS Jr. Relationship of milk consumption to blood glucose rise in lactose intolerant individuals. Am J Clin Nutr. 1975; 28: 677-680.

24. Woteki CE, Weser E, Young EA. Lactose malabsorption in Mexican-American children. Am J Clin Nutr. 1976; 29: 19-24.

25. Gupta SK, Chong SK, Fitzgerald JF. Disaccharidase activities in children: normal values and comparison based on symptoms and histologic changes. J Pediatr Gastroenterol Nutr. 1999; 28: 246-251.

26. Przepiorka D, Weisdorf D, Martin P, Klingemann HG, Beatty P, Hows J, et al. 1994 Consensus Conference on Acute GVHD Grading. Bone Marrow Transplant. 1995; 15: 825-828.

Cite this article

Collins BS, Danialifar TF, Kapoor N, Naon H (2015) Is Disaccharidase Deficiency in Pediatric Patients with Graft- Versus-Host Disease (Gvhd) a Contributing Cause for Diarrhea? Ann Pediatr Child Health 3(2): 1038.