Metabolomic Study of a Diagnostic Model for the Metabolites of Stool Fat

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Background/Aims: Metabolomics is a powerful tool for measuring low-molecular-weight metabolites in an organism at a specified time under specific environmental conditions. The aim of this study was to determine the usefulness of metabolomics in identifying the metabolites in stool-fat-positive specimens, and to establish whether the results could be used to predict the long-term prognosis.

Methods: Fecal specimens were collected from 52 subjects with bowel habit change. The subjects were accessed using Rome III questionnaires and Bristol stool scale form, and followed after three years. The feces samples were centrifuged and the resulting extracts reconstituted for liquid chromatography/mass spectrometry analysis. The datasets were autoscaled, log-transformed, and mean-centered in a column-wise fashion prior to principal-components analysis and partial least-squares-discrimination analysis modeling.

Results: Fecal samples from 10 of the 52 patients gave a positive stool-fat result of 30-100 μm; those of the remaining 42 contained neither fatty acids nor neutral fats. The peak intensities of lithocholic acid (p=0.001), lysophosphatidyl ethanolamine (lysoPE) 16:0 (p=0.015), and lysoPE 18:1/0:0 (p=0.014) were correlated with the size of the fatty acid. Subjects with positive stool-fat result showed higher score in Bristol stool scale form than those with negative stool-fat result at initial (p=0.040) and after three years (p=0.012).

Conclusions: The metabolomic assay of stool fatty acid revealed mainly lysoPEs and lithocholic acid. The size of the fatty acid was correlated with higher concentrations of lysoPEs and lithocholic acid in stool-fat-test-positive specimens and related to loose stool even after three years of follow-up period. (Korean J Gastroenterol 2013;61:9-16)

Key Words: Bristol stool scale form; Fatty acid; Metabolomics; Stool fat

INTRODUCTION

Fecal matter can produce a positive result in the stool-fat test when the excretion of fat in the stool increases. Such stools may also float due to the excess lipid, have an oily appearance, and be especially foul smelling. Increased fat excretion can be measured by determining the fecal fat level, and the sizes of fatty acids and neutral fats can be measured by the stool-fat test. When a malabsorption disorder or other cause disrupts the process, emulsified dietary fats are not completely absorbed in the small intestine, resulting in a positive stool-fat test result.

Genomics has proven itself invaluable for providing vast amounts of data regarding the expression of genes and pro-
teins, whereas metabolomics provides data regarding all metabolic processes of a cell or organism. Metabolomics is defined as the quantitative measurement of low-molecular-weight metabolites in an organism at a specified time under specific environmental conditions. Metabolomics can provide a broader insight into the biochemical composition of living organisms and how this composition changes with time and processing. Recent developments in plant metabolomics have enabled the simultaneous detection of several hundred metabolites which makes reliable comparisons between nonspecific samples possible in an untargeted manner. Metabolomics is a powerful tool that can be used to differentiate between the phytochemical composition of different origins, varieties, or products, and can be used for quality assessment.

Metabolomics is a powerful platform for studies of the metabolism of a variety of biological samples but has not been systematically evaluated for stool-fat levels in subjects who do not have definite gastrointestinal diseases. In addition, the collection of stools is invariably difficult and often incomplete in clinical practice, since three sequential days of collection are required. It would be helpful to develop an innovative test that does not require this sequential stool-collection procedure. The aim of the present study was thus to determine whether indigenous fecal metabolomic analysis was useful for discriminating stool-fat-positive and stool-fat-negative cases.

SUBJECTS AND METHODS

1. Patient recruitment and sample collection

Consecutive patients who visited the Digestive Disease Center of Konkuk University Medical Center from December 2008 to February 2009 because of bowel habit change were asked to complete the stool tests and questionnaires on Rome criteria. Subjects with infectious disease or other causes that might induce diarrhea were excluded from the beginning by (i) history taking, (ii) stool cultures including *Salmonella*, *Shigella*, *Campylobacter*, and *Vibrio* cultures, and (iii) stool occult blood test. Subjects with cholecystectomy, small bowel resection, or specific cause that may cause abnormal finding on stool fat test were also excluded from the study. This includes malabsorption (inflammatory bowel disease, celiac disease, and abetalipoproteinemia), exocrine pancreatic insufficiency, pancreatitis, choledocholithiasis (obstruction of the bile duct by a gallstone), pancreatic cancer (if it obstructed biliary outflow), primary sclerosing cholangitis, bacterial overgrowth, short bowel syndrome, cystic fibrosis, Zollinger-Ellison syndrome, protozoan parasite infection, or a intake of certain prescribed slimming pills such as Orlistat.

Because most of the patients refused stool tests at the outpatient department, only a cohort of 52 subjects were finally included in the study. The fecal samples were collected for stool-fat quantification test in all of the subjects. Of 52 subjects, 15 subjects revealed irritable bowel syndrome (IBS) based on the Rome III criteria. Fifteen IBS patients were consisted of 11 diarrhea-dominant type, 2 constipation-dominant type, and 2 mixed type IBS. Main complaints of bowel habit change in 37 non-IBS subjects were; (i) loose stool in 34 subjects and (ii) hard stool in 3 subjects. After three years of study enrollment, the subjects were followed up again using Rome III questionnaires and Bristol stool scale form. All patients provided informed consent, and this study was approved by the Institutional Review Board of Konkuk University Medical Center.

2. Stool-fat test

Presence of stool fat in fecal material was evaluated by Sudan III stain as described in a previous study. Neutral fat appeared in orange color upon ethyl alcohol, and fatty acid was stained after managing with acetic acid and heating. Of lipid droplets, largest size was reported as result in micrometer (μm). Neutral fat which can be detected in malabsorption disorders and Vitamin B12 deficiency, was reported positive if more than 60/ high power field (HPF). Fatty acid which can be detected in obstructive jaundice due to bile juice deficiency, was reported negative if less 100 (1-4 μm)/HPF. Fatty acid was reported positive if more than 100 (6-75 μm)/HPF.

3. Specimen processing

High performance liquid chromatography (HPLC) grade Methanol for extraction was purchased from Burdick & Jackson (Muskegon, MI, USA) as described in previous studies. Acetonitrile and water used in liquid chromatography/mass spectrometry (LC/MS) were HPLC grade from Fisher Scientific (Pittsburgh, PA, USA). Formic acid was ob-