World Journal of Gastrointestinal Oncology

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DOI: 10.4251/wjgo.v8.i5.459

World J Gastrointest Oncol 2016 May 15; 8(5): 459-465
ISSN 1948-5204 (online)

MINIREVIEWS

Urinary metabolites as noninvasive biomarkers of gastrointestinal diseases: A clinical review

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Author contributions: All authors were equally involved in drafting, reviewing and finalizing the manuscript.

Conflict-of-interest statement: There is no conflict of interest associated with any of the authors of this manuscript.

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Received: September 30, 2015
Peer-review started: October 1, 2015
First decision: November 13, 2015
Revised: January 12, 2016
Accepted: March 7, 2016
Article in press: March 9, 2016
Published online: May 15, 2016

Abstract

The diagnosis of gastrointestinal (GI) disorders is usually based on invasive techniques such as endoscopy. A key important factor in GI cancer is early diagnosis which warrants development of non- or less-invasive diagnostic techniques. In addition, monitoring and surveillance are other important parts in the management of GI diseases. Metabolomics studies with nuclear magnetic resonance and mass spectrometry can measure the concentration of more than 3000 chemical compounds in the urine providing possible chemical signature in different diseases and during health. In this review, we discuss the urinary metabolomics signature of different GI diseases including GI cancer and elaborate on how these biomarkers could be used for the classification, early diagnosis and the monitoring of the patients. Moreover, we discuss future directions of this still evolving field of research.

Key words: Metabolomics; Gastrointestinal diseases; Cancer; Inflammatory bowel disease; Metabolome; Urine

Core tip: Scientists are always searching for new disease biomarkers. An acceptable biomarker could help us in early diagnosis and classification of the diseases as well as the prediction of disease outcome. The diagnosis of gastrointestinal (GI) diseases is usually based on techniques such as upper or lower GI endoscopy, while highly sensitive and specific non-invasive diagnostic or screening tools are usually lacking. In this review, we have discussed the potentials of urinary metabolomics study as a future tool for the screening, diagnosis, classification and surveillance of GI diseases including inflammatory bowel disease and cancer.

Sarosiek I, Schicho R, Blandon P, Bashashati M. Urinary metabolites as noninvasive biomarkers of gastrointestinal diseases: A clinical review. *World J Gastrointest Oncol* 2016; 8(5): 459-465 Available from: URL: http://www.wjgnet.com/1948-5204/full/v8/i5/459.htm DOI: http://dx.doi.org/10.4251/wjgo.v8.i5.459

INTRODUCTION

The rapid growth of high-quantity technologies and computational contexts allows the analysis of organic systems in distinctive details. New technologies such as DNA sequencing and mass spectrometry have permitted observing thousands of molecules concurrently instead of a few components that have been analyzed in old-fashioned research[1].

By considering epigenetic ruling and posttranslational alterations, metabolites serve as direct signatures of biochemical activity in biological systems. Moreover, beyond genes and proteins, they are usually in direct association with disease phenotypes[2]. Metabolomics or metabolic profiling is based on comprehensive and rapid analysis of thousands of metabolites simultaneously in biological samples including plasma and urine and is a feasible strategy for biomarker discovery[3].

The routine urine analysis is often used for the diagnosis of diseases in the urinary tract. However, more than 3000 metabolites are detectable in the urine and their levels may be used as the signature of systemic diseases[4]. These signatures are affected by energy and nutrient intake, body and cellular metabolisms and the environmental factors such as microbiota which have close cross-talk with the gastrointestinal (GI) system. Therefore, any disease in the GI tract may change the metabolic profile of the body that can be reflected in the bodily fluids including blood and urine.

This review provides an insight to the urinary metabolic profile of the GI diseases and its potential application in the clinical diagnosis and predicting their clinical as well as treatment outcome.

TECHNIQUES AND EVALUATION OF URINARY METABOLOMES

Assessment of some GI diseases requires the use of endoscopic methods which are not without risks. Determination of disease biomarkers in easily obtainable biofluids like urine, therefore, would be a valuable adjunct or even an alternative to conventional methods. Many serological markers for inflammatory bowel diseases (IBD) already exist, however, they are less helpful in determining disease subtypes (*i.e.*, Crohn's disease and ulcerative colitis) or forms of indeterminate colitis[5]. Biomarkers or biomarker profiles that can predict and discriminate these subtypes with high probability are therefore desirable. Various studies pursuing this goal have been performed in the past couple of years and have increased the list of metabolites found in higher or lower concentrations in body fluids, including urine, during IBD[6]. These metabolites have been measured in IBD patients by highly sensitive techniques, for instance, by 1H nuclear magnetic resonance (NMR) spectroscopy[7-9], ion cyclotron resonance-Fourier transform mass spectrometry[10] and by ultra-performance liquid chromatography coupled with electrospray ionization quadruple time-of-flight mass spectrometry (MS) in rodents with experimental colitis[11]. While the latter techniques are characterized as extremely sensitive, 1H NMR spectroscopy is maybe less sensitive but known to produce highly reproducible results. A recent study compared different techniques for the detection of urine metabolites in humans and concluded that the NMR technique is the best method for identifying and quantifying urinary compounds[4].

For the discrimination of IBD subtypes and the determination of severity and progress of GI diseases, many metabolites need to be identified. To organize and correctly interpret the large number of data, statistical methods, like multivariate analysis (*e.g.*, principal component analysis and orthogonal partial least squares projections) are applied. In the case of NMR, a method called "targeted or quantitative metabolic profiling" has been used to detect new possible sets of biomarkers[12]. Here, the spectra of already characterized metabolites are stored in a database, and spectra measured in a new biofluid sample are compared with those from the database and thus identified and quantified. The determined metabolites not only may have importance as potential biomarkers but they can, at the same time, provide a link to the pathophysiology of the disease. In this respect, knowledge on the role of the determined molecule within the metabolic pathway is important. A urine metabolome database that allows researchers access to the types, structures and concentrations of urinary metabolites in different diseases has been therefore introduced by the Metabolomics Innovation Centre (http://www.metabolomicscentre.ca/; a platform hosted by the University of Alberta, Canada)[4].

Taken together, urinary metabolites can be evaluated in GI diseases by different experimental methods of high or low sensitivity. Irrespective of the method used, detection of a unique metabolic fingerprint either for diagnosis, treatment, or detection of disease mechanisms is the primary goal.

URINARY METABOLOMICS IN IBD

IBD affecting over 1 million individuals in the United States and 2.5 million in Europe is a common chronic gastrointestinal disease with substantial costs for healthcare. Moreover, it is estimated that the absolute number of IBD patients in newly industrialized countries may approximate that in the Western world until 2025[13]. Despite this increase in the burden of IBD, gold standard tests for its diagnosis, monitoring and management are

usually invasive and sometimes inconclusive. Therefore, biomarkers including noninvasive methods such as urinary metabolomics studies might be useful for the management of patients with IBD[14,15].

Urinary metabolomics has been studied in different mouse models of IBD. Interleukin-10 (*IL-10*) gene-deficient mice which are genetically susceptible to inflammation and colitis have shown different urinary metabolomics compared to non-inflamed animals. For instance, Murdoch *et al*[16] showed that several urinary metabolites such as trimethylamine (TMA) and fucose are changed dramatically in the *IL-10* gene-deficient mice after 8 wk of age which is the timeline for development of severe histological injury and colitis. These alterations in the metabolomics are majorly mediated by commensal microflora which play a key role in the disease process.

In another study on *IL-10* gene deficient mice, Lin *et al*[17] showed an association between 15 metabolites including fucose, xanthurenic acid, and 5-aminovaleric acid with intestinal inflammation. Elevated urinary xanthurenic acid in gene deficient mice was linked to increased plasma levels of kynurenine[17]. In a further study, the same group validated these findings by showing that feeding *IL-10* gene-deficient and wild-type mice with Kiwifruit increases Kiwifruit-derived urinary metabolites more significantly in *IL-10* gene-deficient mice compared to wild-type mice without affecting urinary metabolites levels previously associated with inflammation[18].

In another study, Otter *et al*[19] showed association between the concentrations of xanthurenic acid, α-CEHC glucuronide, and an unidentified metabolite m/z 495(-)/497(+) with inflammation in *IL-10* gene deficient mice.

Overall, studies on *IL-10* gene deficient mice generally agree with changes in urinary xanthurenic acid, a product of tryptophan catabolism through the kynurenine pathway. Bacterial lipopolysaccharides and pro-inflammatory cytokines are the activators of this pathway and its metabolites act as the moderators of T-cell tolerance to intestinal microbiota. As colitis does not usually develop in germ-free *IL-10* gene deficient mice, the role of intestinal microbiota looks considerable in the induction of urinary metabolomics alterations during colitis[17,19-21].

Although, overall studies indicated that *IL-10* gene deficient mice have different urinary metabolomics profile compared to wild-type mice, Tso *et al*[22] showed that these differences are gender and age specific.

Schicho *et al*[23] expanded metabolomics study to an acquired model of chemical colitis induced by dextran sodium sulfate (DSS). After studying 69 urinary metabolites, they showed that urinary creatine, carnitine, and methylamines (including TMA and TMAO) were increased whereas antioxidant metabolites were decreased in DSS mice.

Another study on trinitrobenzene sulphonic acid-induced acute colitis in rats indicated that urinary tryptophan metabolites [4-(2-aminophenyl)-2,4-dioxobutanoic acid and 4,6-cihydroxyquinoline], gut microbial metabolites (phenyl-acetylglycine and p-cresol glucuronide), and the bile acid 12α-hydroxy-3-oxocholadienic acid which are associated with damage of the intestinal barrier function, microbiota homeostasis, immune modulation and the inflammatory response are altered during experimental colitis[11].

Moreover, in a naïve T cell adoptive transfer experimental model of colitis, Martin *et al*[24] showed decrease in Krebs cycle intermediates in urine (succinate, α-ketoglutarate) indicating reduction in the glutaminolytic pathway related to overall loss of energy homeostasis during colitis.

Besides studies on animal models of colitis, studies on IBD patients have confirmed the diagnostic potentials of urinary metabolomics. By studying 206 Caucasian subjects [86 Crohn's disease (CD) patients, 60 ulcerative colitis (UC) patients, and 60 healthy controls], Williams *et al*[9] showed that urinary metabolites, which were in correlation with intestinal microbiota, were different in IBD patients compared to controls. In brief, urinary hippurate differed significantly between the three groups with the lowest level in CD patients. Moreover, 4-cresol sulfate levels were lower and formate levels were higher in CD patients compared to UC patients or controls. This study could significantly differentiate CD from UC[9].

Another study compared the urinary metabolomic signature of patients with active UC, quiescent UC, and controls. In this study no significant difference in the urinary metabolomics profile of these 3 groups was observed[8]. On the other hand, based on a recent study, a significant partial least squares discriminant analysis model was obtained through measuring urinary metabolomics in patients with active IBD *vs* a group with IBD in remission. Based on this study, glycine was increased in urine and acetoacetate decreased in urine during active IBD. Moreover, in active IBD, urinary citrate, hippurate, trigonelline, taurine, succinate and 2-hydroxyisobutyrate were decreased compared to the controls. Despite mentioned observations, this study could not clearly differentiate CD and UC patients based on the analysis of urine samples. Interestingly, contrary to the serum samples, up-regulation of acetoacetate and down-regulation of citrate, hippurate, taurine, succinate, glycine, alanine and formate in the urine samples of patients with IBD in remission could distinguish them from healthy controls[25].

Another study showed that urinary metabolomics including tricarboxylic acid (TCA) cycle intermediates, amino acids, and gut microflora metabolites are different in patients with IBD compared to healthy controls. Comparison of CD and UC patients revealed different metabolomics fingerprints, but removal of patients with the surgical intervention revealed that CD could not be differentiated from UC[26].

Schicho *et al*[23] expanded their findings in DSS mice by studying human subjects with IBD. Their study showed an increase in mannitol, allantoin, xylose, and carnitine in the urine and a decrease in urinary betaine and hippurate during IBD. However, the same as above mentioned studies[25,26], they could not differentiate CD

and UC based on their metabolomics profile[7].

Putting together, based on the metabolomics studies in IBD, the absolute urinary metabolomics signature of IBD is not yet clear. However, the body of literature supports the diagnostic role of urinary metabolites in IBD. More specifically, it seems that microbiota derivative metabolites are altered in IBD and are involved in the pathophysiology of this chronic inflammatory condition. Future multicenter studies on larger sample sizes and with considering confounders such as age, gender and medications should clarify whether urinary metabolomics could be used to: (1) Differentiate UC from CD; (2) predict outcome of the treatments; and (3) define the stage and severity of inflammation.

URINARY METABOLOMICS IN GI CANCERS

GI cancers are common and their burden is huge. Based on a global study in 2013, colorectal, stomach and esophageal cancer are ranked third, fifth and ninth for cancer incidence and fourth, second and sixth for cancer deaths, respectively[27].

Despite available screening method for colorectal cancer which are usually costly and invasive, screening tests for upper GI cancers have not been well developed. Early detection of cancer or pre-cancerous lesions is always desirable. This could benefit from a urine-based cost-effective diagnosis and noninvasive screening assay whereby patients with undiagnosed cancer could be screened.

By analyzing urine samples from esophageal cancer patients and a control healthy group, Hasim *et al*[28] showed that mannitol, glutamate, γ-propalanine, phenylalanine, acetate, allantoin, pyruvate, tyrosine, β-glucose and guinolinate were higher in the urine of patients with esophageal cancer; however, N-acetylcysteine, valine, dihydrothymine, hippurate, methylguanidine, 1-methylnicotin- amide and citric acid were lower. Based on this study, urinary metabolomics could differentiate cancer and control groups. In addition, different pattern of metabolites were positively correlated with the rate of lymph node metastasis and clinical stages. Moreover, unsaturated lipids were a unique marker in differentiating late stages (> 1b2) and early stage (≤ 1b2) diseases[28].

Based on another study, urinary metabolomics signatures clearly distinguished both Barrett's esophagus and esophageal cancer from controls. Although some overlaps were detected, the metabolomics profile of esophageal cancer was different than Barrett's esophagus[29].

Metabolomics studies in gastric cancer are also promising. In a model of gastric adenocarcinoma-bearing mice, the urinary levels of TMAO and hippurate were significantly decreased, although the levels of 3-indoxylsulfate, 2-oxoglutarate, and citrate were significantly increased[30].

Another animal study of implanted human gastric cancer detected significant metabolic differences among normal, non-metastatic and metastatic groups. Based on this study, 10 selected metabolites were different between cancer and control groups. Briefly, the level of lactic acid, butanedioic acid, malic acid, citric acid and uric acid were higher in cancer indicating increase in aerobic glycolysis, respiration (mainly TCA cycle) and the impairment of mitochondrial enzymes. Moreover, glycerol and hexadecanoic acid as indicators of adipocyte lipolysis were higher in cancerous animals. Seven metabolites were also different between non-metastasis and metastasis groups. Alanine and glycerol (as substrates for glycolytic pathway) and L-proline were lower in cancerous animals with metastasis possibly due to a higher level of consumption. On the other hand, the level of myo-inositol in the urine of metastasis group was higher[31].

In a recently published article, the urinary metabolomics of gastric cancer patients was compared to healthy individuals. Based on this study, urinary metabolomics related to amino acids and lipid metabolism was significantly different in cancer *vs* control and could successfully discriminate both groups. Interestingly, the metabolomics signature of cancer showed much higher sensitivity compared to carbohydrate antigen 19-9 and carcinoembryonic antigen. 4-hydroxyphenylacetate, alanine, phenylacetylglycine, mannitol, glycolate, and arginine levels were significantly correlated with cancer T stage. Together with hypoxanthine level, the above mentioned metabolites were tended toward control after surgical treatment[32].

In a study by Chen *et al*[33], urinary lactic acid, arginine, leucine, isoleucine and valine were significantly higher, while citric acid, histidine, methionine, serine, aspartate, malic acid, and succinate were remarkably lower in the gastric cancer patients *vs* controls. In addition, the urinary valine and isoleucine levels were lower in advanced stages compared to early-stages of cancer[33].

Another study also showed that urinary metabolomics could effectively differentiate gastric cancer patients from controls[34]; however, the metabolites which were distinctive, were different than previously mentioned studies[32,33], suggesting complexity in interpreting metabolomics results.

A study on urine metabolites of a colorectal cancer group of patients and their age-matched healthy controls as well as a rat model of chemically induced precancerous colorectal lesion revealed good separations between cancer patients or rats with pre-cancerous lesions and their healthy equivalents. Moreover, altered TCA cycle as well as gut microflora metabolisms were detected in cancer patients and the rat disease model. After surgery, the urinary metabolomic profile of cancer patients altered significantly compared to the preoperative stage since gut microflora metabolism and TCA cycle were down-regulated. In addition, 5-hydroxytryptophan significantly decreased after surgery suggesting an improvement of the tryptophan metabolism[35].

The findings of the above mentioned study in colorectal

cancer were confirmed in a further study which also showed that a panel of urinary metabolite markers composed of citrate, hippurate, p-cresol, 2-aminobutyrate, myristate, putrescine, and kynurenate was able to discriminate colorectal cancer subjects from their healthy counterparts[36].

Studies on the urinary metabolomics of GI cancers reveal alterations in microbiota, proteins and lipid mediated metabolites which are involved in the initiation and dissemination of cancer as well as the cellular overgrowth and proliferation, although no unique signature has been yet recognized. As a huge amount of variability is attributed to between-individual differences, future studies on larger sample sizes of GI cancer patients are required in order to detect associations with moderate effect sizes[37].

URINARY METABOLOMICS IN OTHER GI CONDITIONS

Although many of the metabolomics studies have focused on GI conditions such as cancer and IBD, a few studies have assessed the roles of urinary metabolomics in other diseases.

Based on a study which compared the urinary metabolomics of 34 patients with celiac disease and 34 healthy controls, patients with celiac disease had a significantly lower levels of mannitol, glutamate, glutamine and pyrimidines, and higher levels of indoxyl sulfate, choline, glycine, acetoacetate, uracil, meta-hydroxyphenyl propionic acid, and phenylacetylglycine. This metabolomic signature is consistent with the hypothesis of small bowel dysbiosis in these patients[38]. A further study hypothesized that the metabolomic signature of patients with potential celiac disease, defined as patients with the immunological abnormalities of celiac disease who lack jejunal biopsy findings consistent with their disease, is similar to those with overt celiac disease. Surprisingly, although these patients shared similar metabolomic profile in their serum, no clear joined signature was found in their urine, suggesting that defective small intestinal histology is needed for the development of a urinary metabolomic fingerprint of celiac disease[39].

Studies on the urinary metabolomics of other GI diseases are limited. An animal study has shown the value of urinary metabolomics in the assessment of NSAIDs induced GI ulcer. Based on this study, a panel of urinary metabolites including 2-oxoglutarate, acetate, taurine and hippurate were significant biomarkers for the gastric damage induced by indomethacin in rats and could successfully predict the degree of GI damage, suggesting that NSAIDs induced gastric damage can be possibly screened in the preclinical stages by using urinary metabolomics[40].

CONCLUSION

Urinary metabolomics studies show altered signature in patients with GI disorders compared to healthy controls. The body of literature in this area has majorly focused on IBD and GI cancers. What is shared in all of these disorders is the alteration of urinary metabolites which are in association with GI microbiota and possibly dysbiosis in these chronic conditions. In addition, in cancer patients, the metabolomes which define cell proliferation and differentiation are altered. In IBD, differentiating UC and CD based on urinary metabolomic profile does not look simple at this stage, since confounders such as the clinical severity of the disease and medications may interfere with the metabolism in the body and the metabolomics profile of these patients. The most important use of urinary metabolomics in GI cancer is for early detection of pre-cancerous lesions. Whether the metabolomics signature in patients with pre-cancerous lesions such as Barret's esophagus and colon polyps can predict the future outcome, *i.e.*, the possible chance of progressing to cancer is still under debate. Predicting the outcome of the diseases in response to medical or surgical therapies is also important in this area. In conclusion, although literature supports the role of urinary metabolomics in the diagnosis of some GI conditions, the fingerprints of these diseases are not unique and usually have overlaps.

LIMITATIONS OF URINARY METABOLOMICS IN GI DISORDERS

In 2009, Scalbert *et al*[41] extensively reviewed the limitations of mass-spectrometry-based metabolomics studies. Confounding effects of the diet, large Inter- and intra-individual variations, variations induced by sample collection, handling and storage and inconsistency in data extraction, interpretation and analytical methods were proposed as the major limitations of metabolomics studies. These limitations still affect the metabolomics studies. Moreover, the technology used for the measurement of metabolomics has limitations. For example, NMR is able to measure approximately 8% and gas chromatography MS is able to measure approximately 7% of the human urine metabolomes[4]. For the urinary metabolomics, effects of the kidney function as well as the metabolic function of the body which may affect secretion and reabsorption of the circulating metabolites may confound the final results[42].

FUTURE DIRECTION

Both organic and functional GI disorders usually lack well-defined noninvasive biomarkers which can help us with the diagnosis, treatment and the prediction of their outcome. In functional disorders like irritable bowel syndrome, the diagnosis is not usually definite and is based on exclusion. Moreover, the diagnosis of organic GI disorders usually relies on invasive techniques. Although, the urinary metabolomics signature shows alterations in different GI conditions compared to healthy subjects,

no unique signature has been yet defined. IBD, GI cancers and celiac disease have all shown alterations in the urinary metabolomics which are associated with possible GI dysbiosis, but to our knowledge, no study has systematically evaluated the GI microbiota profile concurrently. Studies on the urinary metabolomics profile of GI diseases have not usually considered confounding factors and the ways of analysis which have been used in these studies are not similar and sometimes cause different results in a single disease setting. Future studies should focus on the validation of the methods and should enhance our knowledge of metabolomic profiles which are in association with different metabolic pathways. The same as breath testing for helicobacter pylori and small bowel bacterial overgrowth, future urinary metabolomics studies may focus on metabolomic profiles induced through the consumption of labeled specific agents. Metabolomics of volatile *vs* non-volatile compounds is also an important area which should be considered. In addition, the effects of urinary diseases on GI system and microbiota as what has been recently observed in patients with chronic kidney diseases[42] should be taken into account when interpreting urinary metabolomics studies.

ACKNOWLEDGMENTS

We thank Ms. Yvette Gomez for administrative support.

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P- Reviewer: Meshikhes AN, Otegbayo JA
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