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Chapter

Application of Urine Metabolomics as a Marker in Health and Disease

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Abstract

Advances in metabolomics research have yielded an avenue for utilizing this laboratory-based modality as a platform for clinical diagnosis, identification of novel biomarkers, and longitudinally monitoring the health status of individuals from normal physiological and pathophysiological perspectives. This chapter provides insight on the application of urinalysis in health and disease from the standpoint of deciphering a larger span of metabolite and biomarker identification using metabolomics, specifically focusing on infectious diseases, oncology, metabolic, and inflammatory diseases in humans.

Keywords: urine metabolome, urinalysis, pre-analytical factors, cancer, infectious disease, inflammation, metabolic disease, renal disease

1. Introduction

Urinalysis or uroscopy is the science of disease diagnosis by means of observation and examination of the urine. Urinalysis has been considered as an adjunct of all laboratory tests applied for medical diagnosis by correlating with the symptoms exhibited by patients and is historically referenced as the first body fluid to be studied scientifically [1, 2]. Ancient medical literature from India and China have referenced the accumulation of ants and insects around sites of urination of certain individuals who had obviously suffered from diabetes. The science of uroscopy was advocated in 300 BC by Hippocrates and was considered as a popular testing modality to link his observations with the doctrine of the four humors, that is, the phlegm, blood, yellow bile, and black bile. During those times, analysis of urine color, consistency, transparency, odor, and the presence or absence of froth aided to make a general assessment of the balance of the four humors and possibly location of the disease within the body and overall prognosis [2]. Throughout the medieval and post-seventeenth century periods, medical literature had cited urinalysis as an important foundation for medical practice. Although medical practice during the mid-nineteenth century had done away with the practice of uroscopy due to the advancements in human medicine, urinalysis still prevails as an important foundation and a powerful tool for clinical diagnosis [3]. Urinalysis has been used to identify genetic diseases as well as diagnose pathophysiological processes by measuring abnormal urine constituents such as
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glucose, bile pigments, white blood cells, proteins, etc. In addition, the presumptive diagnosis of diseases affecting the urogenital system can be inferred through urinalysis [3–5]. This chapter describes the applications of urine metabolomics in defining the biomarkers of health and clinical disease states.

2. Metabolomics in clinical urinalysis

Urine is an easily accessible biological fluid for noninvasive collection of large volumes with the possibility of repeat sampling at different time points, as needed for monitoring health status [6]. Traditionally, urinalysis tests conducted in diagnostic laboratories measured only one or two metabolite components (e.g., glucose, ketone bodies, etc.) at a given time [7]. Also, the abnormal urinary constituents tested via traditional methods lack specificity and are noticeable in urine samples after tissue injury. Therefore, an ideal biomarker in urine should be highly sensitive and specific and should be capable of indicating an early phase of disease progression [8]. Since the 1970s, urinary metabolome analysis has facilitated in investigation of metabolic signatures or fingerprints of urinary metabolites during the disease process in the human body [4, 8, 9]. Metabolomics is defined as the systemic identification and quantification of all metabolites in a given organism or biological sample [10]. Since metabolites generated in an organism are a result of several gene-level (host-dependent, genetic factors) and environmental-level interactions, the general metabolome, which includes the sum of all the metabolites in an organism, can serve as a critical biomarker analyte fingerprint for the health status of an individual's phenotype. Emerging technologies based on mass spectrometry (MS) and nuclear magnetic resonance spectrometry (NMR) enable the monitoring of hundreds of metabolites from tissues or body fluids. Metabolites change rapidly in response to physiological alterations, thus they act as the first-line chemical reporters of abnormal or disease phenotypes. Current advancements in high-resolution metabolomics platforms capable of detecting hundreds of low-molecular-weight metabolites in tissues and body fluids have evolved as a powerful biomarker analysis tool. The datasets generated from clinical trials and research studies curate for clinical databases that support diagnosis and therapeutic assessment of several disease states [7]. However, these datasets require standardization and validation to create a clinical reference guide that is applicable for any biofluid, as well as FDA approval for utilizing this platform for clinical diagnostic purposes [11].

3. Sample collection considerations for urine metabolomics

Urine metabolome has aided in the examination of metabolic consequence correlating to disease, nutritional status, and environmental toxicity since excreted urine contains endogenous and exogenous metabolites, thereby providing a key biomarker fingerprint to diagnose, monitor, or predict for any pathophysiological condition [12]. More than 3100 metabolites have been characterized in human urine, and the list is expected to increase as more and more low-concentration metabolites are being characterized [9]. With the intended application of urine samples for biomedical research or clinical diagnosis, pre-analytical factors such as collection methods and pre-processing, transport and storage, freeze-thaw cycles, and sample normalization are taken into consideration when processing metabolomic analysis [13]. Standardization
of pre-analytical process is critical for minimizing inter-sample variability issues and maintaining the metabolic integrity of samples so that the metabolomic profile accurately reflects the in vivo biochemical status of the patient [13].

Generally, three kinds of collection modalities are employed for metabolomic studies involving urine samples: (a) first-morning void (b) spot urine samples, and (c) 24 h urine collection. The first-morning void urine samples are preferred since the overnight fast period helps to reduce the effect of any medication or the meal consumed from the previous day. Spot urine samples are the preferred sample type for dietary or pharmaceutical intervention studies and are usually collected during the daytime. However, pooled urine void samples during a 24 h duration reduce the influence of circadian cycle variation when compared to first void or spot urine samples [13].

Urine samples held at room temperature for short periods of time can lead to rapid degradation of metabolites. Considering the storage requirements of urine samples, it is optimal to freeze samples as soon as possible. Generally, while conducting clinical metabolomic studies, samples are refrigerated in autosamplers of MS and NMR instruments for time spans from hours to days. Ideally, it has been indicated by researchers that keeping samples at 4°C for 48 h or less does not significantly affect the urinary metabolome [9]. Although it would be prudent to minimize multiple freezing and thawing of urine samples, up to 9 freeze-thaw cycles are considered amenable for urine samples utilized for metabolomic studies. For long-term storage of up to 6 months or more, temperatures between −20 and −80°C are recommended. Another concern with urine collection and storage is bacterial contamination, which can be curtailed by collecting mid-stream urine and subsequently adding antibacterial agents such as sodium azide or sodium fluoride [9]. As an alternative, storage of samples at −80°C over the use of antibacterial additives can prevent the microbial metabolism of urinary metabolites, thereby rendering the urine sample suitable for downstream metabolomic applications. In addition, to reduce the metabolite transformation subsequent to sample collection, snap-freezing in liquid nitrogen aids in metabolic quenching (i.e., inactivation of enzymatic reactions), thereby obtaining an accurate picture of the metabolome at sampling time [9, 13].

The health status and fluid intake of sample submitters could also impact the solute concentration of urine. In such situations, pre-analytic normalization is essential to correct for variations in urinary constituents. Urine samples are normalized by measuring the osmolality or specific gravity, and subsequent dilution to the lowest concentration before running samples in separation and detection analytical platforms for metabolomics [9].

4. Analytical techniques used in metabolomics

Metabolomics provides a global analysis of several classes of metabolites with diverse physicochemical characteristics present in any biological sample. In general, metabolomic profiling employs two major analytical techniques for detection of metabolites, such as high-field NMR and MS. Advanced chromatographic separation techniques coupled with the aforesaid spectroscopic or spectrometric methods aid in efficiently separating complex matrices for effective detection. When comparing the detection methods, although NMR is a robust and nondestructive method, it has a lower sensitivity compared to MS. However, NMR is capable of structural elucidation of novel unknown compounds. On the other hand, MS is a very sensitive method.
that requires sample preparation steps coupled with suitable separation techniques to reduce ion suppression. For untargeted metabolomics, high-resolution MS instrumentation is required, whereas targeted metabolomics employ low-resolution MS platforms [5].

5. Clinical research studies utilizing urine metabolomics as a diagnostic platform

Several research studies have focused on evaluating urinary biomarkers for clinically assessing the progression of a disease or different stages of disease evolution. These biomarkers can aid in the clinical assessment of patients without apparent disease, with suspected disease, or rather the progression or remission of overt disease [14]. The following sections describe some of the clinical conditions that have been studied by researchers for potentially identifying biomarkers in urine for diseases in general, such as cancers, metabolic syndromes, and infectious and renal diseases.

5.1 Cancer

Early advances in metabolomics technology have provided insight into the metabolism of cancer by focusing on how the Warburg effect in cancerous cells uses glycolysis effectively to produce amino acids, nucleotides, and lipids necessary for tumor proliferation. Furthermore, metabolomics research has also been used to discover novel diagnostic cancer biomarkers to better understand its complex heterogeneous nature, to discover pathways involved in cancer that could be used for identification of new targets, and for monitoring metabolic biomarkers for therapeutic aspects of cancer treatment. The metabolomics approach also provides ways to personalize cancer treatments by yielding essential information regarding the cancer patient’s response to medical interventions. Urine contains metabolic signatures of many biochemical pathways and thus gives way for a cohesive metabolomic approach. Techniques such as hydrophilic interaction chromatography (HILIC-LC-MS), reversed-phase ultra-performance liquid chromatography (RP-UPLC-MS), and gas chromatography time-of-flight mass spectrometry (GC-TOF-MS) are used for urine analysis [15].

Gas chromatography/mass spectrometry (GC/MS) has been recently used to explore disease biomarkers from urine or serum samples. Both primary and secondary metabolites can be analyzed as a metabolomic approach for biomarker analysis. The importance of early diagnosis is especially true in kidney cancer, especially prior to metastatic spread, and can improve survival rates from 10% to greater than 90%. The ultimate composition of molecules in the urine is the result not only of glomerular filtration but also of tubular secretion and reabsorption. Normal urine contains approximately 150 mg/24 h of protein, and compounds such as inulin (5 kDa) and lysozyme (14 kDa) are reported to appear freely in the urine. Although kidney cancer is the sixth leading cause of cancer death and represents only 3% of cancer incidence, it is a major cause of death in 11,000 patients per year in the United States. The disease is very resistant to chemotherapy, and one-third of the cases are metastatic at diagnosis. Thus when detected with symptoms, prognosis of renal cell carcinoma (RCC) is poor and once metastatic, it has only a 5% five-year survival rate. Therefore, a novel, convenient, and noninvasive approach is essential for identifying RCC at an earlier stage prior to metastasis [16]. Renal cell carcinoma entails abundant
primary and secondary metabolites as potential tumor markers. The human kidney injury molecule-1 (hKIM-1), when normalized to creatinine, appears in the urine of RCC patients and disappears or decreases in concentration after nephrectomy. Hence, detection of such metabolites in urine can be crucial to cancer diagnosis. It is highlighted that metabolomics is ideally suited for such approaches and analysis of these small molecule metabolites that appear in both serum and urine can be crucial [15].

Metabolomics has also been used to investigate the urinary metabolite differences between hepatocellular carcinoma (HCC) male patients and normal male subjects. The urinary endogenous metabolome was assayed using chemical derivatization followed by GC/MS. After GC/MS analysis, 103 metabolites were detected, of which 18 metabolites were shown to be significantly different between the HCC and control groups. Subsequently, a diagnostic model was constructed with a combination of 18 marker metabolites. This noninvasive technique of identifying HCC biomarkers from urine has potential application in clinical diagnostic oncology. Overall, these findings underscore that metabolomic analysis is a potent and promising strategy for identifying novel biomarkers of HCC [17].

5.2 Metabolic syndrome

The “metabolic syndrome” (MetS) can be understood as a clustering of components that reflect overnutrition, sedentary lifestyles, and resultant excess adiposity. MetS is a cluster of different conditions and not a single disease. The prevalence of the MetS is increasing to epidemic proportions in the United States and also in developing nations. MetS is associated with doubling of incidence of cardiovascular disease risk and an increased risk for incident type 2 diabetes mellitus [18].

Accurate predictors of cardiometabolic diseases are of particular importance since the condition can be present long before the symptoms become clinically apparent. Nuclear magnetic resonance spectroscopy and gas- or liquid-chromatography coupled with MS are the major platforms applied to identify predictive biomarkers, monitoring therapeutic response as well as in basic mechanism studies of obesity, metabolic syndrome, type 2 diabetes, and cardiometabolic diseases for early diagnosis. Nicotinuric acid is correlated with cardiometabolic risk factors such as body mass index (BMI), blood pressure, HbA1c, blood lipids, and C-reactive protein, thus suggesting that it could be a potential biomarker of important features of MetS such as altered lipid metabolism and increased insulin resistance [18, 19].

Metabolic profiling of urine samples has also been used as a diagnostic tool in the predicting liver disease progression because traditional clinical chemistry tests for liver function only aid in diagnosis after substantial liver damage has occurred. With the current diagnostic methods incapable of predicting typical Jaundice syndrome (JS) in hepatic dysfunction, Wang et al. [20] conducted a study for the identification of potential biomarkers from JS disease by using a nontarget metabolomics method and testing their usefulness in human JS diagnosis. To identify the potential biomarkers, multivariate data analysis methods were utilized revealing 44 marker metabolites contributing to the complete separation of JS from healthy controls [20]. Targeted metabolite analysis revealed alterations in critical JS metabolic pathways, such as glutamate metabolism, synthesis and degradation of ketone bodies, alanine and aspartate metabolism strongly associated with JS development [20]. In another study, researchers compared the urine metabolome panel of three human subject group categories, namely individuals with nonalcoholic fatty liver disease (NAFLD), nonalcoholic steatohepatitis (NASH), as well as age and sex-matched healthy controls. Urine
metabolomic analysis performed using tandem LC-MS revealed differences in 31 metabolites between NASH and NAFLD groups, including variations in nucleic acids and amino acids. Among the overlapping metabolites, it was inferred that pathways of energy and amino acid metabolism, as well as the pentose phosphate pathway, were closely associated with progression of NAFLD and NASH [21].

UHPLC-Q-TOF-MS based metabolomics approach was applied to gain understanding of the global profiling of endogenous metabolites in urine from high-fat diet-induced obese rats [22]. Integrated with multivariate analysis, metabolic variations between the obese rats and healthy rats were differentiated. Twenty potential biomarkers were identified in response to high-fat diet-induced obesity. Seventeen of them are novel potential biomarkers that are independent of the known risk indicators for obesity, except hippurate, phenylacetylglutamine (PAG), and creatinine. Using the correlation between these biomarkers, a network diagraph was generated based on search results from the Kyoto Encyclopedia of Genes and Genomes (KEGG) database. Tryptophan metabolism, phenylalanine and tyrosine metabolism, and gut microbiota metabolism were found to be significantly disturbed in obese rats [22].

5.3 Urinary metabolites associated with inflammatory diseases

Immune-mediated inflammatory diseases are a group of diseases that share common molecular mechanisms and are characterized by aberrant and chronic activation of the immune system. Rheumatoid arthritis, psoriasis, psoriatic arthritis, systemic lupus erythematosus, Crohn’s disease, and ulcerative colitis are the most prevalent immune-mediated inflammatory diseases. Although these diseases target different tissues and organs, they share many genetic loci, and clinically similar inflammatory diseases are known to share specific hub metabolites such as citrate. Numerous high-throughput analysis technologies are available that can generate comprehensive profiles of multiple metabolites. However, most of these techniques require invasive sampling procedures. Understanding and identifying biological markers in urine that accurately correlate with the inflammatory disease can prove vital for easy and early disease diagnosis under routine clinical settings.

A study conducted by Alonso and coworkers identified multiple urinary metabolites (citrate, alanine, methyl succinate, trigonelline, N-acetyl Amino Acids, and an unknown metabolite) that can be associated with three or more of the immune-mediated inflammatory diseases [23]. Most of these urinary metabolites were found in lower concentrations in patients with inflammatory diseases compared to controls. Citrate, the strongest hub metabolite, for example, was present at lower concentrations in the urine of inflammatory bowel disease, rheumatoid arthritis, and, systemic lupus erythematosus patients [23–25]. Additionally, inflammatory diseases with similar phenotypes exhibited similar urinary metabolomes. Low levels of carnitine were identified in chronic arthritis diseases, namely rheumatoid arthritis and psoriatic arthritis. Similarly, reduced concentrations of hippurate were observed in patients with inflammatory bowel diseases such as Crohn’s disease and ulcerative colitis. In short, immune-mediated inflammatory diseases can be aggregated into three distinct clusters based on the urinary metabolite profiles: (1) psoriasis and psoriatic arthritis, (2) Crohn’s disease and ulcerative colitis, and (3) rheumatoid arthritis and systemic lupus erythematosus, all sharing between 3 and 6 metabolite associations [23]. However, since the treatment strategies of Crohn’s disease and ulcerative colitis are entirely different, a single test that distinguishes the two will be of utmost clinical value. In this context, researchers have identified that hippurate
and 4-cresol sulfate levels were lower in patients with Crohn's disease when compared to control and ulcerative colitis patients. Although similar studies showed that urinary metabolome is useful for the differential diagnosis between ulcerative colitis and Crohn's disease, complicated nature of the disease and the confounding factors such as surgical resections, drug and dietary therapy can interfere with the metabolic changes in observational studies. Therefore, the aforesaid confounders should be considered before such analysis [24, 26]. Urinary metabolome also has the potential for predicting both the disease activity and disease recurrence (especially at the site of surgery) in patients with Crohn's disease. For example, metabolites namely citrate, hippurate and 3-hydroxyisovalerate were found in much lower levels in patients with high disease activity for Crohn's disease than in low disease activity for Crohn's disease patients [23, 27]. Higher levels of three urinary metabolites (L-3,4-dihydroxy phenylalanine, levoglucosan, ethyl malonate), and lower concentrations of propylene glycol were associated with endoscopic recurrence in Crohn's disease in patients who have undergone ileocolonic resection [27]. Furthermore, urinary metabolites, octanoyl glucuronide, pyridoxic acid, and pantothentic acid were shown as dietary biomarkers for clinical remission in pediatric patients with inflammatory bowel disease, who had undergone either exclusive enteral nutrition or corticosteroid therapy [28].

In addition, there are distinct urinary metabolites such as phenyl acetyl glycine and tyrosine that were specific for ulcerative colitis and rheumatoid arthritis, respectively [23]. Urinary excretion of prostaglandins thromboxane synthase and prostacyclin metabolites were increased in patients with severe atherosclerosis [29]. Similarly, elevated levels of acotinic acid, isocitric acid, and citric acid were observed in the urine of osteoarthritic patients and these provide an indication of mitochondrial dysfunction leading to impaired cartilage and chondrocyte metabolism in osteoarthritis. Moreover, significant urinary metabolomic variations in histidine and histamine were observed between two different phenotypes of osteoarthritis (with and without knee effusion) [30]. The NMR-based approach also demonstrated the metabolic fingerprints of urine samples that distinguished chronic inflammatory rheumatoid diseases from healthy individuals. Several urinary metabolites (including leucine, valine, 3-hydroxyisobutyric acid, 3-hydroxyisovaleric acid, glycine, citric acid, creatinine, hippuric acid, and methyl Nicotinamide) were downregulated in patients with chronic inflammatory rheumatoid diseases. Some of the changes could be explained as a consequence of urinary tract infections, increased demand for muscle turnover events, or due to distal renal tubular acidosis [31]. As for pelvic inflammatory diseases, a clinical trial conducted by Zou and coworkers showed the presence of eighteen differential metabolites in the urine of rats inoculated with *Ureaplasma urealyticum* and pathogenic *Escherichia coli* to mimic multi-pathogenic infection of the upper genital tract leading to pelvic inflammation [32].

### 5.4 Infectious diseases

Urine metabolomics has been increasingly used for the study of biomarker discovery in infectious diseases, as it offers significant methodological advantages. The application of NMR spectroscopy metabolomics has the potential for infectious disease diagnosis since it can differentiate between various viral and bacterial infections. A specific metabolomic response comes from the host in the form of immune cells and apoptosis signaling when a pathogen causes infection [33, 34].

Urinary tract infection (UTI) is one of the most common bacterial infections in humans. Main causative organisms of UTI include *Escherichia coli*, *Klebsiella*
pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus, and Enterococcus faecalis. Ultra-Fast Liquid Chromatography Mass Spectrometry (UFLC-MS) was used to differentiate UTI by E. coli in a case study involving 17 individuals. Metabolic discriminators identified were related to TCA cycle, terpenoid backbone biosynthesis, amino sugars and nucleotide sugars metabolism, arachidonic acid, and steroid hormone biosynthesis [35]. In another study, nontargeted exploratory UPLC-MS-based approach was used for the investigation of UTI-related changes in urine associated with E. coli infection in 117 subjects. A C-terminal glycopeptide of the human fibrinogen alpha-chain was identified as the discriminator metabolite [36]. NMR-based screening showed that K. pneumoniae causing UTI can metabolize glycerol to 1,3-propanediol (1,3-PD), acetate, ethanol, and succinate. The quantity of 1,3-PD was found to be proportional to the bacterial count. However, other bacteria causing UTI cannot metabolize glycerol under similar conditions [37, 38]. Urinary NMR spectroscopy of samples infected with E. coli, K. pneumoniae, P. aeruginosa, and P. mirabilis showed peaks of nonspecific metabolites such as succinate, acetate, lactate, and ethanol compared to healthy groups in a case-control study done in 617 people. Lactate metabolism, nicotinic acid, and methionine metabolism were altered in the affected individuals [39].

Pneumonia is caused by a wide range of microorganisms, including bacteria, fungi, viruses, and parasites. Conventional methods of diagnosis are time-consuming and include isolation of organisms from blood, sputum, pleural fluid, and bronchoalveolar lavage. Urinary metabolomics can be used as a tool to differentiate Streptococcus pneumoniae infection, responsible for community-acquired pneumonia from other infections. A study was conducted on 641 individuals, including healthy volunteers, patients with metabolic stress, fasting individuals, patients with pneumococcal pneumonia, other lung infections, and asthma or chronic obstructive pulmonary disease (COPD). NMR spectra comparison of 61 metabolites in urine from age and gender-matched S. pneumoniae infected and noninfected groups were performed. Among these metabolites, 6 were significantly decreased while 27 were significantly increased. Six metabolites that decreased are associated with TCA cycle intermediates (citrate, and succinate), nicotinamide metabolism (1-methylnicotinamide), food intake (levoglucosan and trigonelline), and protein catabolism (1-methylhistidine). Increased concentration was observed in amino acids (alanine, asparagine, isoleucine, leucine, lysine, serine, threonine, tryptophan, tyrosine, and valine), fatty acid oxidation (3-hydroxybutyrate, acetone, carnitine, and acetylcarnitine), inflammation (hypoxanthine and fucose), metabolites involved in glycolysis (glucose and lactate), osmolytes (myo-inositol and taurine), acetate, quinolinate, adipate, dimethylamine, and creatine. TCA cycle intermediates 2-oxoglutarate and fumarate also appeared to increase upon pneumococcal infection. Metabolites that were not affected by pneumococcal infection included creatinine, 3-methylhistidine, aconitate (trans and cis), metabolites related to gut microflora (3-indoxylsulfate, 4-hydroxyphenylacetate, hippurate, formate and TMAO (trimethylamine-N-oxide), dietary metabolites (mannitol, propylene glycol, sucrose, and tartrate) and certain amino acids (glycine, glutamine, histidine, and pyroglutamate) [38, 40]. In another study, metabolic profiling of an independent sample set of 145 urine samples from healthy individuals or patients with various conditions showed around 86% and 94% in sensitivity and specificity, respectively, for the diagnosis of pneumococcal pneumonia [40].

Neonatal sepsis is an infection that occurs in the bloodstream of newborn infants less than 28 days old, and it can be divided into early-onset and late-onset. A case study was conducted to analyze the difference in the urinary metabolome of infected
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and healthy neonates [41]. Urine metabolic profiles were assessed using nontargeted NMR spectroscopy and targeted liquid chromatography-tandem mass spectrometry analysis in 16 septic neonates and 16 nonseptic ones. The metabolic profile of neonates with sepsis was found to be different compared to those without sepsis. Metabolites from energy-producing biosynthetic pathways and basic structural components of the organism showed clear separation. Elevations in urinary taurine and hypotaurine were noted in septic neonates. Depletion in glutamine levels was also seen in critically ill adults. Hypo- or hyperglycemia was also common in adults. Increased amounts of pyruvate and lactate in the urine can be due to sepsis-associated hypoperfusion and/or hypoxia [42]. In septic preterm infants, early metabolic responses include lactic acidosis and increases glucose requirements. A product of urine degradation called inosine was also observed in higher quantities in this condition because of cellular destruction. Trimethylamine N-oxide (TMAO) levels and certain vitamins such as riboflavin and nicotinamide were found to be reduced in neonatal septic patients [41].

Clostridium difficile is a spore-forming bacterial pathogen, which is the leading cause of healthcare-associated infective diarrhea [43, 44]. In the United States, more than 500,000 cases of C. difficile infection (CDI) are reported annually accruing $6.3 billion in healthcare costs [44]. Intestinal dysbiosis is the reason for recurrent CDI. Urinary metabolites might be used as a prognostic method for patients with recurrent CDI as no diagnostic tests are available to predict the risk of CDI recurrences in patients [45]. With regards to C. difficile infections, Kao et al. performed NMR studies on urine of 31 infected subjects (age- and sex-matched to 31 healthy controls) and detected 53 metabolites. Choline appeared to be the most relevant for the diagnosis of C. difficile infection. This finding has been possibly attributed to the absence of choline-metabolizing microorganisms in this infection [33]. Isa et al. proposed four urinary metabolites as biomarkers for active tuberculosis patients [46]. Using an untargeted HPLC-MS and MS/MS, 102 urine samples were collected from infected individuals. The majority of the metabolites were neopterin, kynurenine, spermine, N-acetylated sugars, and sialic acids, which are host-derived metabolites involved in immune cell activation [46].

COVID 19 is a pneumonia caused by coronavirus (SARS-CoV 2) responsible for producing a global pandemic and still continues to be a worldwide emergency [47]. During SARS-CoV-2 infection, research studies have been conducted to correlate urinary constituent abnormalities for COVID-19 disease severity and outcome. A case study was done in four cohorts namely, healthy control (n = 27), non-COVID-19 control (n = 17), patients with nonsevere COVID-19 (n = 48), and patients with severe COVID-19 (n = 23) [48]. Peptide yields from urine samples in patients with severe and nonsevere cases were found to be greater compared to serum samples in the healthy control group. A total of 16,148 peptides and 1494 proteins were obtained from sera using tandem mass-tag (TMT)-based proteomics, while 19,732 peptides and 3854 proteins were identified from urine. Similarly, 80% of detectable serum proteins and 62% of serum metabolites were found in urine of the infected group. Cytoplasmic proteins (26%) and membrane proteins (21%) were the most abundant protein groups in the urinary proteome, whereas the proportion of secreted proteins was only 16%. Further, more intracellular compartment proteins released from tissues were seen in the urinary proteome of the infected group compared to the serum proteome. Also, 197 cytokines and their receptors were observed in urine of infected group, whereas 124 cytokines were seen in serum. In the same study, the reduction in endosomal sorting complexes required for transport (ESCRT) complex proteins and downregulation
of CXCL14 in urine was reported to be associated with an increase in SARS-CoV-2 replication [48]. In another study consisting of 142 infected volunteers and 104 healthy volunteers, urine samples were subjected to mass spectrometry. Significant alterations in nitrogen metabolism, D-glutamine and D-glutamate metabolism, aminoacyl-tRNA biosynthesis, arginine biosynthesis, glutathione metabolism, pantethenate and CoA biosynthesis, glyoxylate and dicarboxylate metabolism were noticed among infected group and control group. Nineteen amino acids such as alanine, leucine, glutamine, tryptophan, and 15 acylcarnitines were obtained from urine analysis. Glycine level was decreased in the infected group. Alteration in valine was also observed. This study suggested acylcarnitines as important markers for COVID-19 infection [49].

Acquired Immunodeficiency Syndrome (AIDS) is responsible for causing a severe immunosuppressive state on the immune system of humans. AIDS has emerged as a global health hazard and no effective methods are available for the characterization of affected patients. Urinary metabolomics can be a promising method to differentiate affected and nonaffected AIDS individuals, and also for monitoring the progress of HIV therapeutics. Studies using biofluids, such as urine, whole blood, and serum, have also been employed to identify metabolite markers correlating to HIV-induced oxidative stress (OS). Munshi et al. suggested that urinary neopterin could be used as a metabolic biomarker of AIDS infection. Urinary glutamic acid and formic acid levels were higher in HIV/AIDS patients compared to healthy controls. When comparing HIV-infected patients treated with or without antiretroviral therapy (ART), ART naïve patients had lower levels of urinary methionine, 2-methylglutaric acid, l-alanine, and glycolic acid, however, patients receiving ART had even reduced levels of the aforesaid metabolites [50]. Hence, urinary amino acids and their metabolites can help to serve as markers for assessing the progress of ART in AIDS patients.

Malaria is a mosquito-borne parasitic illness caused by Plasmodium falciparum, Plasmodium vivax, and Plasmodium berghei. Morbidity and mortality caused by these parasites are greater in tropical and sub-tropical countries. Prospect of infection biomarkers in biofluids is therefore important in a population to control the impact of the infection. Urinary metabolites could be used as a tool to differentiate the infected groups from the noninfected ones. In a case-control study of 21 P. falciparum-infected individuals and 25 controls, urine samples subjected to high-performance liquid chromatography-high resolution mass spectrometry (HPLC/HRMS) revealed altered levels of 1,3-diacetylpropane, N-acetylputrescine, and N-acetylspermidine between patients and control cohorts, thereby suggesting these molecules as potential biomarkers for malarial infections [51]. NMR spectroscopy of urinary samples from patients infected with P. vivax was also studied. Urinary ornithine and pipecolic acid were higher in malarial patients and could be used as a potential biomarker to differentiate between malarial and nonmalarial cases [52]. In another study using a mouse model of P. berghei infection, 4-amino-1-[3-hydroxy-5-(hydroxymethyl)-2,3-dihydrofuran-2-yl]pyrimidin-2(1H)-one and 2-amino-4-[[5-(4-amino-2-oxopyrimidin-1(2H)-yl)-4-hydroxy-4,5-dihydrofuran-2-yl]methyl]sulfanyl] butanoic acid were the two urinary metabolites detected in infected mice groups compared to healthy mice [53]. Therefore, the aforementioned catabolites in urine may aid to assess the progression of the disease among affected individuals.

5.5 Renal dysfunction

Acute kidney injury is defined as a sudden phase of kidney failure that occurs in a few hours. This condition is characterized by an increase in serum creatinine and a
notable decrease in urine output [54]. Chronic kidney disease (CKD) is a condition, where glomerular filtration rate is progressively affected along with kidney damage [55]. In a study conducted in patients with acute kidney injury, urine samples from patients subjected to LC-MS and 1H NMR scanning revealed an increase in urea cycle, proline metabolism, nitric oxide pathway, and its metabolite, asymmetric dimethyl-arginine (ADMA), serotonin metabolism and homovanillic acid. On the other hand, a decrease in Kreb’s cycle and citrate, benzoate metabolism and hippurate, pyruvate metabolism, and lactate were observed [56–58]. In another study conducted by Matin-Lorenzo et al., urine samples were collected from 24 control subjects and 38 patients with acute kidney injury. LC-MS/MS analysis revealed urinary 2-hydroxybutyric acid, pantothenic acid, and hippuric acid were significantly downregulated and urinary N-acetylneuraminic acid, phosphoethanolamine and serine were upregulated in diseased patients [59]. It was also reported that a low risk of chronic kidney disease is associated with urinary glycine and histidine, increased urinary lysine, and NG-monomethyl-L-arginine (NMMA) [60]. However, carnitine metabolism, beta-oxidation and acylcarnitines, phenylacetylglutamine, urea cycle, proline metabolism and their metabolites, proline, and citrulline were increased in chronic kidney disease. Conversely, urea cycle, proline metabolism, nitric oxide pathway and ADMA, Krebs cycle and citrate, bile acid metabolism, and taurocholate were reported downregulated in chronic kidney disease conditions [61, 62].

Diabetes is a chronic (DM) metabolic disease that result in unusually higher preprandial plasma glucose levels as a result of defects in insulin secretion, insulin activity, or both. Chronic diabetes can lead to several pathophysiological conditions that range from cardiovascular abnormalities to renal failure. The hemodynamic dysregulation caused by diabetes mediates renal injury by inducing abnormal morphological and functional changes in the renal nephrons [63]. Gas chromatography-mass spectrometry was used to quantify 94 urine metabolites in screening cohorts of patients with diabetes mellitus (DM) and chronic kidney disease CKD (DM + CKD), in patients with DM without CKD (DM–CKD), and in healthy controls. Thirteen metabolites were significantly reduced in the DM + CKD cohorts compared to healthy groups. Twelve of them are related to mitochondrial metabolism, suggesting a suppression of mitochondrial activity in diabetic kidney disease [64].

Autosomal dominant polycystic disease is a hereditary disorder which is characterized by cyst formation in ductal organs, mainly the kidney and the liver, and also by gastrointestinal, musculoskeletal, and cardiovascular abnormalities [65]. Research conducted in a mouse model for autosomal dominant polycystic kidney disease was used to assess whether metabolomic shifts prior to renal cystogenesis can aid in early diagnosis for the condition. Utilizing GC-MS time of flight spectroscopy, urine samples collected from mice prior to exhibiting any serological evidence of kidney dysfunction revealed that purine and galactose metabolic pathways were affected, with elevation of biomarkers such as allantoic and adenosine [66].

6. Conclusion

Urine metabolomics is a powerful diagnostic technique that can be utilized to diagnose several diseases, including cancers, hereditary diseases, immune-mediated and metabolic disorders, and renal dysfunction. Urine metabolite constituents essentially serve as biomarker signatures to identify pathways related to specific diseases as well as to detect abnormal concentrations of the metabolites that may be associated with
the disease. Furthermore, the analysis of urinary metabolome can be used to evaluate disease activity, response to treatments and to monitor the progression or remission of the disease. Additionally, the standardization and curation of urine metabolomic databases for health and pathological phenotypes can potentially be developed and employed in routine clinical settings for disease diagnosis.

Conflict of interest

The authors declare no conflict of interest.
References


Application of Urine Metabolomics as a Marker in Health and Disease
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