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Current Approach of NMR-Based Metabolomics Practices as Diagnostic Potential

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Research Article

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ABSTRACT

Nuclear Magnetic Resonance (NMR) spectroscopy is a quantitative analytical tool commonly utilized for metabolomics analysis. The benefits afforded by advancing NMR techniques include robust, reproducible results that can provide rapid and accurate information about a variety of diseases. New technologies allow for quick, straightforward, and cost-effective screening for a range of disease markers. Some of the fields in which NMR may be useful include diagnosis of liver disease, kidney disease, neurological disorders, cardiovascular disease, and cancer. In particular, NMR is being more broadly used in metabolomics whereby metabolites are analyzed to more effectively diagnose and treat disease. Quantitative NMR (q-NMR) is a field of NMR spectroscopy dedicated to the measurement of analytes through signal intensity and its linear relationship with analyte concentration. Metabolomics-based NMR exploits this quantitative relationship to identify and measure biomarkers within complex biological samples such as serum, plasma, and urine. In this review of quantitative NMR-based metabolomics, the advancements and limitations of current techniques for metabolite quantification will be evaluated as well as the applications of q-NMR in biomedical metabolomics. This review discusses metabolomics biotechnology with a focus on recent applications of metabolomics as a powerful tool to elucidate metabolic disturbances and the related mechanisms of diseases.

Keywords: Biomarker; Metabolomics; Nuclear Magnetic Resonance spectroscopy (NMR); qNMR

Abbrevations: CKD: Chronic Kidney Disease; DMA: Dimethylamine; MBC: Metastatic Breast Cancer; VLDL: Very Low Density Lipoprotein; YDYHS: Yin-Deficiency and Yang-Hyperactivity Syndrome; YYDS: Yin-Yang Deficiency Syndrome; BC: Breast cancer; HCC: Hepatocellular Carcinoma; LC: Liver Cirrhosis; CD: Crohn's Disease; UC: Ulcerative Colitis; IBD: Inflammatory Bowel Disease; CC: Chronic Cholecystitis; AECOPD: Acute Exacerbations of COPD; PBC: Primary Biliary Cholangitis; XGC: Xantho Granulomatous Cholecystitis; GBC: Gallbladder Cancer; EPUFA: Essential Polyunsaturated Fatty Acids

INTRODUCTION

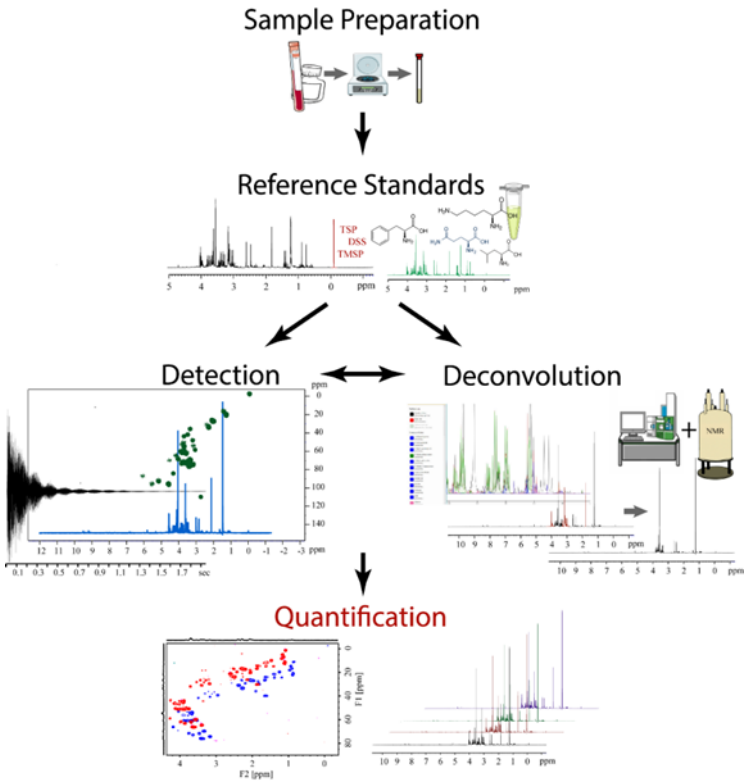
The analytical tool of metabolomics offers measurements of metabolites that are both quantitative and qualitative. For studying the systemic reactions to developmental and/or physiological change as well as exposure to external stimuli, such as nutrition and xenobiotics, metabolomics has shown to be an invaluable tool [1,2]. Recent research has demonstrated that the genome, proteome, and transcriptome can interact and be modulated by the metabolome [3]. As a result, the study of metabolomics is now a growing area of clinical research that aims to develop diagnostic tools and therapeutic possibilities while also unraveling the molecular causes of disease. In order to study small molecule metabolites of diverse metabolic route matrices and products, metabolomics analyzes numerous samples, such as blood, urine, and feces [4]. Nuclear Magnetic Resonance (NMR), Mass Spectrometry (MS), and chromatography are technologies used in metabolomics. Clinical research, disease therapy, medication characterization, animal and plant research, agricultural research, and nutrition all benefit greatly from mass spectrometry-based metabolomics [5].

Metabolomics, as compared to other omics, provides more direct and comprehensive information about the biological system from an individual or a group [6]. This information can be utilized for illness diagnosis and therapy, drug toxicological mechanism study, and precision medicine, among other things. Quantitative NMR (qNMR) is a specific branch of the field of NMR that focuses on extremely precise and repeatable measurements of molecular concentrations. For the study of substances in complicated mixtures, qNMR is frequently used. To measure active pharmaceuticals from a variety of complex matrices (e.g., body fluids, drug formulation, natural product extracts, etc.), For the study of substances in complicated mixtures, qNMR is frequently used. To measure active pharmaceuticals from a variety of complex matrices (e.g., body fluids, drug formulation, natural product extracts, etc.), qNMR has been widely employed in the pharmaceutical business for decades. As a result, qNMR has seen increased application in metabolomics since it is a suitable approach for determining metabolite concentrations [7]. Figure 1 depicts a schematic overview of the use of qNMR in metabolomics. The five steps of the qNMR methodology are as follows:

- A streamlined sample preparation.
- The choice of reference standards.
- The detection of analytes.
- Metabolite deconvolution.
- Metabolite quantification.

Absolute and relative quantification are the two fundamental approaches used in metabolite quantification by qNMR [8]. Measuring molar concentrations in relation to control samples is a component of relative quantification. Among other sorts of comparisons, this quantification technique is frequently applied in biomedical metabolomics as a tool to identify disease state models from healthy control groups. Through multivariate and univariate statistical analysis of binned NMR spectrum data, a relative quantification is frequently accomplished in NMR-based metabolomics [9]. The groups are then separated and the metabolites that differ between the healthy and illness groups are identified using the statistical models that were produced. Metabolite changes are not evaluated separately for relative quantification, particularly multivariate model analysis, but rather are connected with other metabolites. In contrast, absolute quantification is the precise determination of a compound's content in isolation from all other compounds present in a biological sample. Absolute quantification is commonly facilitated by the use of internal standards and metabolite deconvolution.

Figure 1. Overview of NMR-based metabolite quantification in biomedical metabolomics.



Step 1: Sample preparation through deproteinization and/or centrifugation of biofluids.

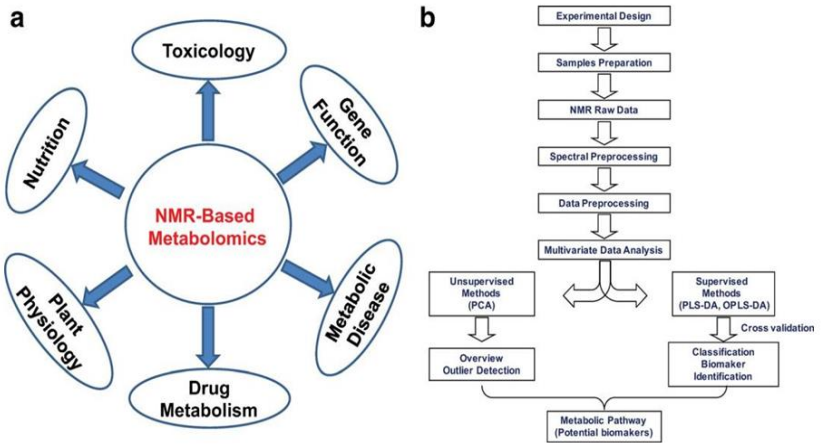
Step 2: Selection of reference standard(s) for determination of unknown metabolite concentration.

Step 3: Detection of analyte signal through NMR spectroscopy.

Step 4: Metabolite deconvolution by which data is filtered for significant biomarkers of interest. Deconvolution can be achieved through computational methods after data collection and/or experimental design prior to data collection.

Step 5: Metabolites are quantified by comparison of known metabolite concentration to unknown analytes (Figure 2).

Figure 2. Schematic diagram for NMR-based metabolomic application.



LITERATURE REVIEW

Recent advances and applications

There have been more metabolomics investigations using MS. Drug, toxin, and disease-related metabolite effects have been investigated using MS-based metabolomics techniques. Numerous disorders, including breast cancer, kidney cancer, cardiovascular disease, bladder cancer, esophageal cancer and gastric cancer, kidney disease, natural metabolic errors, toxicological effects, and nutrition, have been studied using MS-based metabolomics methods [10-16].

Disease diagnosis

Compared to genomes and proteomics, metabolomics has a closer relationship to physiology. The pathophysiological processes of the body are altered by the disease, which eventually results in corresponding changes in metabolites. The search for illness biomarkers involves examining certain metabolites and contrasting them with typical human metabolites. A novel way of illness diagnostics will be provided by metabolomics.

Chronic Kidney Disease (CKD): Chronic Kidney Disease (CKD) is a term used to describe a clinical condition that is impacted by both genetic and environmental factors and is brought on by a variety of pathogenies, including glomerulonephritis, chronic interstitial nephritis, hypertensive nephrosclerosis, and diabetic nephropathy. Membranous glomerulonephritis, focal segmental glomerulosclerosis, and IgA nephropathy are examples of high-risk chronic kidney disease that may progress to end-stage renal diseases. Taherkhani A et al., discovered novel diagnosis approaches based on biomarkers, recent effective antigens, and new therapeutic procedures related to these conditions. A sizable number of metabolites and proteins that have been previously identified and suggested as prospective biomarkers of different CKDs employing '-omics-' technologies, proteomics, and metabolomics were also reviewed. Protein/peptide biomarkers of the main causes of CKD, such as diabetic nephropathy, IgA nephropathy, lupus nephritis, focal segmental glomerulosclerosis, and membranous nephropathy, are the subject of research by Chebotareva NV et al. The most data on urine peptide and protein contents in various nephropathies was obtained by Mass Spectrometry (MS) methods. Urinary proteomic-peptide profiles may replace renal biopsy as a reliable early non-invasive diagnostic tool for particular morphological forms of kidney disease thanks to new analysis techniques. The development of novel strategies for targeted therapy may also benefit from MS research of the main pathogenetic pathways underlying the course of renal illness.

Corneal diseases: In the early stages, corneal diseases may show modest symptoms, which can delay a diagnosis and prompt treatment. A permanent loss of vision may result from this. An additional tool for the early identification and treatment of corneal illnesses is the fast emerging science of metabolomics, which enables the analysis of metabolites in a system. Alvin W and colleagues found new biomarkers can be found in the examination of tear, cornea, and aqueous humor thanks to the development of nuclear magnetic resonance and mass spectrometry. New perspectives on disease pathways are found, and they offer crucial details for future targeted therapeutics. To provide further insight into the effectiveness of current treatments, molecular analysis is conducted. In this paper, we present a thorough analysis of the metabolomic research done on the cornea and in diverse pathologies, including dry eye disease, Sjogren's syndrome, keratoconus, post-refractive surgery, contact lens wearers, and diabetic corneas. Using Nuclear Magnetic Resonance (NMR), Lee et al. Rat urine, plasma, and tears Plasma levels of 2-hydroxybutyrate, citrate, and succinate, which are important players in inflammatory pathways, were raised, as were pro-inflammatory cytokines such IL-6, IL-1, and TNF. Potential biomarkers for DED may be found in the identified metabolites. Matrix-Assisted Laser Desorption Ionization Mass Spectrometry Imaging (MALDI-MSI) was employed by Chen et al. Rat conjunctiva, aqueous humor, and cornea. The integrity of the tear film and the metabolism of phenylalanine and glycerophospholipid were both affected. A rise in betaine may indicate an early cytoprotective defense against dry eye. The

varied metabolic responses of complex eye components in DED can be accurately analyzed by MALDI-MSI, which may be used to personalize treatment. NMR Tears were discovered by Galbis Estrada et al. [17]. Essential Polyunsaturated Fatty Acids (EPUFA) supplementation resulted in a rise in choline, which stabilizes the tear film and has anti-inflammatory effects on the ocular surface. Antioxidants and EPUFAs taken orally can modify changes in the metabolic profile of DED's tears. NMR and high-performance liquid chromatography were developed by Kryczka et al. [18]. Dead people's corneal buttons and keratoconic corneas. There are no significant metabolic differences between keratoconic and older, normal corneas. Young keratoconic corneas share biochemical characteristics with older normal corneas, which could mean that the cornea in keratoconus is aging more quickly.

Cancer diseases: Cancer is one of the most serious diseases that cause an enormous number of deaths all over the world. Tumor From that of normal tissues, metabolism can distinguish itself very well. One of the best strategies to discover biomarkers for cancer detection, diagnosis, and to offer fresh insights into internal physiological states where small changes in metabolite concentrations may occur is to investigate the metabolism of the tumor. Since the Nuclear Magnetic Resonance (NMR) technology may provide a wealth of biochemical information at the molecular level for tumor research, it is currently widely used to evaluate cell extracts, tissues, biological fluids, etc. [19].

Colorectal cancer: Colorectal Cancer (CRC) is one of the most prevalent tumor types. Understanding the metabolic profile of colorectal cancers is crucial for treatment approaches and molecular diagnostics. Amiot et al, investigated the fecal metabolic profile of patients with advanced colorectal cancer and controls using ^{1}H-NMR and multivariate modelling [20]. Beatriz et al, employed High Resolution Magic Angle Spinning (HR-MAS) NMR to examine metabolites in intact tumor tissues and samples of the surrounding mucosa obtained from colorectal cancer patients.

By using PCA and OPLS-DA, the results showed significant biochemical differences between the two types of tissues. Furthermore, using Tumor Node Metastasis (TNM) classification, metabolic profiles were able to distinguish between tumors of various T- and N-stages (T stage has a higher weight than N stage and the former affects survival more significantly). NMR was utilized by Bertini et al, to profile the blood metabolome in people with metastatic Colorectal Cancer (m CRC) and see if there might be a disease sign that might accurately predict Overall Survival (OS). Supervised prediction models permitted a separation of 96.7% of patients from the healthy controls in the validation set. Wang et al.'s analysis of the ^{1}H-NMR profiling data using PCA, PLS-DA, and OPLS-DA to pinpoint the distinct metabolites of rectal cancer. The separation between the various phases of rectal cancer tissues and healthy controls was very good, according to the results. These altered metabolites showed abnormalities in the metabolism of choline, amino acids, ketone bodies, and energy, which may be associated to the development of human rectal cancer. In order to define the metabolic fingerprint of tumoral and normal tissue samples acquired from a cohort of patients suffering from primary colorectal cancer, Piotto et al, used HR-MAS NMR. By utilizing PLS-DA to analyze the data, it was shown that whereas normal tissues had higher levels of myo-inositol and b-glucose, tumor tissue samples are richer in taurine, glutamate, aspartate, and lactate. Then, a blind test was conducted on tumor and healthy tissue using the statistical model.

Breast cancer: Breast Cancer (BC) has the greatest incidence, death, and recurrence rates of any malignant disease affecting women worldwide. Identification of markers for BC diagnosis and management, including prognosis, early diagnosis, and customized care, is essential. Li et al, used multivariate modeling to assess 31 breast tissue samples (13 cancer, 9 benign, and 9 normal) acquired by core needle biopsy. Cancer and non-cancer samples can be distinguished well with OPLS-DA on the NMR spectra, despite the fact that benign tumors and normal tissues cannot be distinguished from one another. Beathe et al.'s analysis of the metabolic profiles of tissues obtained from 85 breast cancer patients included meticulous monitoring of the concentration levels of GPC, PC, and choline. Choline and glycine concentrations were significantly greater in tumor

samples bigger than 2 cm compared to smaller tumor samples, suggesting that HR-MAS NMR may be a useful technique for BC diagnosis that can also determine the stage of the disease. The next step was to measure metabolites in intact BC samples using electronic reference to access *in vivo* concentrations (ERETIC) using HR-MAS NMR. B-glucose, lactate, glycine, myo-inositol, taurine, GPC, PC, choline, and creatine were the nine significant discriminatory metabolites. In contrast to early localized disease (EBC), Elodie et al, sought to discover metabolic serum abnormalities connected to advanced Metastatic Breast Cancer (MBC). With 89.8% sensitivity and 79.3% specificity, the results clearly distinguished between EBC and MBC samples, with higher levels of serum concentrations of acetoacetate, 3-hydroxybutyrate, glycerol, pyruvate, mannose, N-acetylglycoproteins, and glutamate and lower concentrations of histidine, alanine, and betaine metabolites being found in MBC tissues. Giskeodegard et al, performed biopsies from BC patients that were removed after surgery and examined by HR-MAS NMR. PLS-DA, Probabilistic Neural Networks (PNNs), and Bayesian Belief Networks (BBNs) were used to analyze the data. Additionally, it was discovered that the estrogen and progesterone receptor status can be accurately predicted to enhance forecasts of breast cancer hormonal therapy. Based on a set of 257 retrospective serial samples, Vincent et al, used a mix of NMR and MS approaches to develop and test a model for early BC recurrence identification. Over 55% of patients' recurrences could be identified using the model as early as 13 months before the recurrence was discovered.

Liver cancer: Hepatocellular Carcinoma (HCC) is the most common cancer in the world in terms of incidence and mortality due to patient neglect. By using HR-MAS NMR, Yang et al, evaluated the amounts of metabolites in the sera of HCC tumor and unaffected surrounding liver tissues. Lactate, amino acids such as glutamate, glutamine, Glycine, leucine, and alanine, Choline, triglycerides, glucose, phosphoryl ethanolamine and glycogen were the distinguishing potential biomarkers. Using single blood samples, Gao et al, tracked the changes in endogenous metabolites of Liver Cirrhosis (LC) and HCC. The findings showed that HCC patients had greater levels of acetate, pyruvate, Gln, a-ketoglutarate, glycerol, tyrosine, 1-methylhistidine, and phenylalanine and lower levels of LDL, isoleucine, Valine, acetoacetate, creatine, Choline and unsaturated lipids.154 cirrhotic individuals with and without HCC had their metabolic profiles from Nahon et al, evaluated. According to the findings, cirrhotic and big HCC tumors may clearly be distinguished from one another using serum NMR spectra in combination with the OPLS analysis model. By using ^{1}H-NMR in conjunction with pattern-recognition and visualization techniques, Debora et al, studied the metabolic profiles from primary HCC, cirrhotic tissues (CIR), hepatic metastases from colorectal carcinomas, and non-cirrhotic normal tissues. The findings showed distinct metabolic variations in the examined grades, and tissue signals of lactate and glucose were principally used to distinguish between the various histological samples, which can be very helpful in the diagnosis of liver tumors.

Lung cancer: One of the most serious medical conditions and the leading cause of cancer death is lung cancer. In this area, there has been a lot of research. Claudia et al.'s application of PCA and HCA followed by ^{1}H-NMR produced good discriminating between tumor and unaffected tissues, demonstrating how the two tissue types have inherently different metabolic profiles. Liu et al, developed a new algorithm called PSO-SVWL-PLSDA to restore the effectiveness that was diminished by the metabolic profiles' great similarity when paired with ^{1}H-NMR metabolomics. The metabolites lactate, glucose, threonine, valine, taurine, trimethylamine, glutamine, glycoprotein, proline, and lipid that showed the strongest data clustering for the detection of lung cancer. Using ^{1}H-NMR and multivariate data analysis techniques, Chen et al, performed the metabolomic features of 51 lung samples from 17 individuals with lung cancer. The data unmistakably revealed metabolomic traits of lung cancer tissues at different sites. Durate et al, combined analysis of the NMR data comprising PCA, PLS-DA, and OPLSDA to look for the metabolic indicators of lung cancer in urine. The data obtained demonstrated great sensitivity and complete specificity. Adenocarcinomas could also be distinguished from carcinoid tumors and epidermoid carcinomas using PLS-DA of a subset of tumor samples, which revealed variations in metabolite levels across these histological categories.

Pancreatic cancer: A tumor that is malignant is pancreatic cancer. Early identification of pancreatic cancer is essential for its treatment because, once detected, surgical cure is rarely an effective choice for the majority of patients. NMR was used to examine the serum taken from patients with pancreatic cancer by OuYang et al. Results demonstrated clear separation between the cancerous and benign groups. When compared to the control group, pancreatic cancer patients had greater levels of isoleucine, leucine, creatinine and lower levels of 3-hydroxybutyrate, 3-hydroxyisovalerate, lactate, triglyceride, and TMAO. These metabolite changes may serve as metabolic indicators for pancreatic cancer early detection. Oliver et al, used ^{1}H-NMR followed by statistical analyses to examine the serum from patients with pancreatic cancer and healthy volunteers. By eliminating individuals with overt diabetes mellitus, the diagnostic model accurately discriminated benign from malignant pancreatic tumors. Lin et al, profiled the plasma metabolites derived from 19 pancreatic cancer patients using ^{1}H-NMR spectra. While lactate, 3-hydroxybutyrate, HDL, LDL, alanine, glutamate, glycine, histidine, isoleucine, lysine, and valine were found to be reduced in the Patient control group, levels of N-acetyl glycoprotein, DMA, VLDL, and acetone increased.

Heart diseases: It has been possible to study the various circulatory system illnesses using NMR metabolomics. In the entire world, hypertension is a relatively frequent disease. Li et al, analyzed plasma samples by ^{1}H-NMR and GC-MS spectroscopy and identified several potential biomarkers of YDYHS and YYDS including betaine, mevalonic acid, corticosterone, leucine, propionic acid, methionine, D-glucose, glycine, tyrosine and malic acid (the first five metabolites were identified by NMR analysis) which indicated that abnormal glucose metabolism might be the main common pathway from YDYHS to YYDS. 32 coronary blood samples from healthy individuals and myocardial ischemia patients, including those with Stenotic Disease (SD) and microvascular disease ("Micro"), were examined using ^{1}H-NMR-based metabolomics by Deidda et al. When compared to the control group, Micro patients showed higher levels of 2-hydroxybutirate, alanine, leucine, isoleucine, and N-acetyl groups as well as lower levels of creatine/phosphocreatine, creatinine, and glucose. In contrast, SD patients showed higher levels of 3-hydroxybutirate and acetate and lower levels of 2-hydroxybutirate. Additionally, when compared to micro patients, SD patients exhibited lower levels of 2-hydroxybutirate, alanine, leucine, and N-acetyl groups and greater levels of 3-hydroxybutirate and acetate. As a result, the metabolites listed above may serve as possible biomarkers to identify and classify various clinical and developmental stages of coronary artery disorders. Valine, alanine, glutamine, inosine, and adenine were specifically mentioned in the study by Ameta et al, and were five biomarkers of statistical significance between UA and HC.

Inflammatory disease: Inflammatory Bowel Disease (IBD) has two forms: Ulcerative Colitis (UC) and Crohn's Disease (CD). By using NMR-based metabolic profiles, Dong et al, analyzed the plasma samples from acute UC mice model and they found eight metabolites changed significantly: Reduced levels of acetate and glucose, and increased levels of valine, lipid, glycerol, ω-3 fatty acid, lysine and phenylalanine. The study analyzed serum samples from healthy individuals and IBD patients, including UC and CD, using ^{1}H-NMR-based metabolomics. In contrast to a control group, active IBD patients had higher amounts of leucine, isoleucine, 3-hydroxybutyric acid, N-acetylated substances, acetoacetate, glycine, phenylalanine, and lactate and lower levels of creatine, dimethyl sulfone, histidine, and choline and its derivatives. NMR-based metabolomics analysis of blood samples from Primary Biliary Cholangitis (PBC) patients indicated that 33 metabolites changed significantly compared to HC and four metabolites among them (3-hydroxyisovalerate, 4-hydroxyproline, citraconate and pyruvate) were identified as potential biomarkers for primary biliary cholangitis diagnosis. Analysis of serum samples from healthy individuals and IBD patients, including UC and CD, using ^{1}H-NMR-based metabolomics. In contrast to a control group, active IBD patients had higher amounts of leucine, isoleucine, 3-hydroxybutyric acid, N-acetylated substances, acetoacetate, glycine, phenylalanine, and lactate and lower levels of creatine, dimethyl sulfone, histidine, and choline and its derivatives. Additionally, active IBD patients showed lower levels of low-density lipoproteins and very low-density lipoproteins and higher levels of N-acetylated substances and phenylalanine compared to IBD patients in remission. The best biomarkers for identifying and

diagnosing the various stages of IBD disease are these metabolites.

While Xantho Granulomatous Cholecystitis (XGC), which is based on Chronic Cholecystitis (CC), is a catastrophic inflammatory condition accompanied by the development of yellow granulomas, Chronic Cholecystitis (CC) is a benign disease characterized by repeated acute or subacute cholecystitis. Even though XGC is benign, it might be challenging to distinguish from Gallbladder Cancer (GBC). So, utilizing ^{1}H-NMR-based metabonomics analysis, one study discovered glaring discrepancies in serum metabolic characterizations between healthy persons, CC, XGC, and GBC patients. Importantly, they emphasized that lower glucose levels, some amino acids, Low Density Lipoprotein/Very Low Density Lipoprotein (LDL/VLDL) levels, and higher levels of lactate and pyruvate were found, and that these metabolites from various metabolic pathways could be identified as the biomarkers of the GBC to ^{1}H-NMR metabolomics analysis was used in a study to detect plasma samples from the non-surviving patients with Decompensated Cirrhosis (DC), stable cirrhosis patients and HC, the results showed elevated levels of lactate, tyrosine, phenylalanine and methionine and decreased levels of choline, phosphatidyl cholines, lipid (VLDL, LDL) in non-surviving DC patients. They also developed a new method to accurately forecast mortality in DC and distinguish between non-surviving and surviving patients, and their study even had high reference values for liver transplantation.

Chronic Obstructive Pulmonary Disease (COPD): Metabolomics application in respiratory illnesses, such as adult respiratory disease syndrome, Chronic Obstructive Pulmonary Disease (COPD), pneumonia, and acute lung injury were found. We also go through how metabolomics might be used to track worker and environmental exposure to aerosolized pollutants. We describe microbiome-derived compounds that originate from the gut and lung in asthma and COPD that have mechanistic implications for these diseases in light of the most recent developments in our understanding of the microbiome.

Airflow restriction caused by the prevalent chronic disease COPD can lead to respiratory failure and pulmonary heart disease. One study using ^{1}H-NMR spectroscopy examined serum from COPD patients and discovered elevated amounts of glycerol phosphocholine and lower levels of phenylalanine, tyrosine, alanine, valine, leucine, and HDL. Additionally, all of these markers they discovered were helpful for the early detection and management of COPD.

In an effort to discriminate between severe Acute Exacerbations of COPD (AECOPD) and other concomitant disorders including heart failure and pneumonia, which frequently occur with AECOPD and have symptoms that are identical, Fortis et al. used the NMR-metabolomics technique. AECOPD patients had lower levels of the following biomarkers compared to stable COPD patients: Glycine, glutamine, proline, citrate, histidine, formate, creatine, phosphate, alanine, and mannitol. Additionally, the first six metabolites showed statistically significant changes, which may be used as markers to distinguish AECOPD from COPD patients. They could not discover specific metabolic changes of AECOPD to distinguish it from heart failure and pneumonia, hence the different respiratory failure groups could not be distinguished by this method.

Diseases of the nervous system: The nervous system is in charge of controlling and coordinating all bodily functions. The Central Nervous System (CNS) and the Peripheral Nervous System (PNS) make up this system. Brain and nerve tissue degeneration, disorientation, personality changes, and other new symptoms that may appear with the disease's course and aging are all caused by neurodegenerative illnesses. They can also result in permanent harm and frequently trigger protracted neural inflammation.

The literature has reported on a number of metabolic indicators linked to several biochemical processes, including inflammation, oxidative stress, and homeostasis, among others. These indicators aren't enough of a differentiator, though. Changes in the tryptophan pathways and phosphocreatine breakdown were discovered by van der Velpen et al. The strong connection between this pathology and amino acids and tryptophan catabolites is suggested by their distinct association. Ibaez et al.'s classification of the population into AD Patients, a healthy control group, and patients with Mild Cognitive Impairment (MCI)—a term used to describe patients who are in between stages of normal aging and AD provides another

perspective. The initial proof and molecular readouts for sex-related metabolic differences in AD were presented by Arnold et al. eventually, indicating the potential for improved stratification could lead to personal medicine.

Glutathione is an antioxidant that protects against the oxidative damage that PD metabolism experiences. In samples of postmortem CSF from PD sufferers, LeWitt et al. noticed a decline in glutathione levels and an increase in 3-hydroxykynurenine levels. While 3-hydroxykynurenine is an excitotoxic metabolite, glutathione is an antioxidant molecule that protects the brain by lowering ROS from oxidative stress. The studies of amino acids (serine, threonine, histidine) as biomarkers of PD and other disorders can generate controversies because there are differences in sampling, storage, data processing, which do not allow to conclude how variations of amino acids concentration occur and how metabolic pathways are affected in PD.

Other diseases: The metabolic signature of serum samples from severe burn patients was characterized by NMR analysis. Zhang et al, reported a set of biomarkers which offered a new approach to improve the diagnosis and reduce the mortality of severe burn patients, including 12 metabolites: butyric acid, dihydrobiopterin, aldosterone, 7-dehy-drocholesterol, biotin, odotyrosine, ketoisovaleric acid, deoxycorticosterone, 2-methoxyestrone, 2-hydroxy- butyric acid, 1,3-diaminopropane and 3-methyl histidine. Among these biomarkers, ketoisovaleric acid, 3-methyl histidine, and p-hydroxybutyric acid all showed significantly elevated levels in the early phases of burn injury. Additionally, 3-methyl histidine level is connected to skeleton catabolism following severe burns, ketoisovaleric acid is a novel biomarker for mitochondrial malfunction, and hydroxybutyric acid shows an increase in ketogenic metabolism.

The early and intermediate stages of Age-Related Macular Degeneration (AMD), which is a prevalent eye condition that causes vision loss in adults in their 50's and older, typically have no noticeable symptoms. In one study, 396 AMD patients (Coimbra group and Boston group) had blood samples analyzed using the NMR-based metabolomics technique. The researchers discovered variations in several amino acids, particular lipid moieties, dimethyl sulfone, and organic acids at various phases of AMD. Comparisons between the several groups revealed that diet and way of life had an impact on the variations of AMD in the metabolites. The metabolic changes in early AMD stage were distinct in Coimbra patients (unsaturated fatty acids, acetate, creatine, dimethyl sulfone, C18 cholesterol, and HDL-choline resonances) and Boston subjects (albumin, histidine, glutamine, and also unsaturated fatty acids). Additionally, all of these possible biomarkers may be used to differentiate between different AMD stages.

DISCUSSION

Numerous metabolomics studies focus on identifying the changes of small molecule metabolites in samples in order to find human disease biomarkers, but the identification of these changes can be influenced by a variety of factors such as individual differences, interactions with other small molecules or the environment in the human body, methods of sample collection, preparation, and storage, experimentation procedures, data analysis, data description, database storage, and experiment procedures. In recent years, metabolomics has been widely employed to look for disease related biomarkers. However, many parts of metabolomics investigations still have limitations and difficulties. As previously discussed in reviews, each analytical technique (such as NMR or MS) has its own flaws and restrictions with regard to sensitivity, repeatability, and equipment costs. In addition, no matter the analytical technique employed, metabolomics research encounters the following typical issues.

- Because the makeup of metabolites in samples is so complex it is currently impossible to accurately assess the whole metabolome in a single analysis.
- The analysis of metabolomics produces massive amounts of data, while it is difficult to comprehensively identify all the changed metabolites with the available metabolite databases. In addition, the functions and mechanisms of the

identified metabolite changes are also difficult to interpret.

- Since the metabolome is extremely susceptible to a variety of internal and external influences, even minute variations may have an impact on the samples metabolic profiles and make it difficult to interpret the findings.
- The number of patient samples is insufficient compared to the vast volumes of laboratory data, and this disparity makes it challenging to translate these laboratory discoveries into clinical applications.
- As was said in the preceding paragraph, standardization is another significant issue. The standardization of metabolomics investigations will help make the results from different studies more comparable and aid in the identification of biomarkers. As a result, the continuous development of metabolomics research techniques will help address the foregoing constraints.

CONCLUSIONS

In this study, we discuss the use of human blood samples for NMR-based metabolomics for searching and detecting illness biomarkers throughout the previous five years. NMR metabolomics makes a substantial contribution to the validation and quantification of biomarkers for many human diseases, such as neoplasms, digestive, neurological, respiratory, mental, infectious, parasitic diseases, and others, by assisting in the diagnosis and therapy. Blood is undoubtedly one of the most traditional and frequent samples, but recent studies have given significant attention to other body fluid samples such urine, saliva, sweat, and tears since it is important to create and improve non-invasive methods. In actuality, there are still numerous issues in sample preparation, experimental and statistical methods, as well as the validation and quantification that need to be resolved.

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