Review Article

Potential of nuclear magnetic resonance metabolomics in the study of prostate cancer

R. Ravikanth Reddy[1], Naranamangalam R. Jagannathan[1,2,3]*

[1]Department of Radiology, Chettinad Hospital and Research Institute, Chettinad Academy of Research and Education, Kelambakkam, [2]Department of Radiology, Sri Ramachandra Institute of Higher Education and Research, [3]Department of Electrical Engineering, Indian Institute Technology Madras, Chennai, Tamil Nadu India

*E-mail: jagan1954@hotmail.com

ABSTRACT

Nuclear magnetic resonance (NMR) metabolomics is a powerful analytical technique and a tool which has unique characteristics and capabilities for the evaluation of a number of biochemicals/metabolites of cancer and other disease processes that are present in biofluids (urine and blood) and tissues. The potential of NMR metabolomics in prostate cancer (PCa) has been explored by researchers and its usefulness has been documented. A large number of metabolites such as citrate, choline, and sarcosine were detected by NMR metabolomics from biofluids and tissues related to PCa and their levels were compared with controls and benign prostatic hyperplasia. The changes in the levels of these metabolites aid in the diagnosis and help to understand the dysregulated metabolic pathways in PCa. We review recent studies on *in vitro* and *ex vivo* NMR spectroscopy-based PCa metabolomics and its possible role as a diagnostic tool.

INTRODUCTION

Prostate cancer (PCa) is the second most common malignancy in men older than 50 years of age.[1,2] In some individuals, the disease may have a slow growth rate and these patients may remain unaffected for several years, while in others, rapid progression leads to dissemination of the disease. Prostate-specific antigen (PSA) is widely used for the diagnosis of PCa[3] and helps in early detection of PCa.[4-6] Chronic prostatitis, benign prostatic hyperplasia (BPH), ejaculation, and vigorous exercise may also lead to an increase in the PSA levels and therefore it is not specific to PCa.[7] Conventional methods of cancer detection such as clinical (digital rectum examination), pathological (biopsy), and biochemical parameters (PSA) are limited both in sensitivity and specificity and provide inadequate information on the grading and staging of PCa. In addition, trans-rectal ultrasound, which is used to diagnose PCa, also has a poor specificity due to inaccurate sampling.[8] Thus, a combination of PSA and prostate biopsy is widely used for the diagnosis of PCa. However, when lesions are small and are randomly distributed throughout the prostate, they may be missed on the biopsy resulting in false-negative findings.[9]

Metabolomics is an approach to identify small molecules generally called "metabolites" which are sensitive to various biotic and abiotic stimuli. Numerous metabolites (biochemicals) are present in seminal fluid, prostatic fluid, blood, urine, intact tissues, tissue extracts, and cell extracts.[10] Metabolomics provides metabolic fingerprinting, which is associated with a specific phenotype that is produced as a result of various functions of genes, transcripts, and enzymes. Systematic qualitative and quantitative evaluation of the metabolite levels in biofluids and tissues would facilitate a better understanding of the different physiological states of the organism, diagnosis, and management of the disease.[10]

Access this article online	
Quick Response Code:	Website: www.indianjurol.com
	DOI: 10.4103/iju.iju_416_21

Received: 06.11.2021, **Revised:** 16.12.2021,

Accepted: 09.02.2022, **Published:** 01.04.2022

Financial support and sponsorship: Nil.

Conflicts of interest: There are no conflicts of interest.

Metabolites are present in a wide range of concentration in human biofluids and tissues, and thus reliable methods are required for their identification and accurate quantification. In metabolomics, two leading analytical techniques, nuclear magnetic resonance (NMR) spectroscopy, and mass spectrometry (MS) are most widely used to analyze the biofluids and tissues. MS is highly sensitive and detects a vast number of metabolite species in a single measurement.[11] However, MS is strongly dependent on the ionization efficiency of the metabolites, absolute quantification requires multiple internal standards, and sample preparation is relatively tedious and cumbersome. For these reasons, metabolomics using MS is often performed with a combination of chromatography techniques such as liquid chromatography or gas chromatography. On the other hand, NMR spectroscopy provides a broader picture on both the identification of the biomarkers and estimation of the concentration of metabolites in a noninvasive way with high specificity.[12]

The phenomenon of NMR is based on the property of nuclear spin.[13] It is defined as the study of molecules that are interacting with the radiofrequency waves of the electromagnetic radiation with the nuclei of molecules placed in a strong static magnetic field.[14] NMR spectroscopy provides the chemical structure of the matter, while, magnetic resonance imaging is an off-shoot of NMR that provides the images of the anatomy of various organs of the human body. When performed on the living systems, NMR spectroscopy is generally referred to as "*in vivo*" NMR or simply magnetic resonance spectroscopy (MRS). It provides biochemical (metabolites) information from a specified region of interest of the imaged organs. If NMR is performed on the biofluids, tissue extracts, etc., it is referred to as *"in vitro"* NMR and when intact tissues were used, it is known as *"ex vivo"* NMR.

^{1}H (Proton), ^{13}C (Carbon), ^{15}N (Nitrogen), and ^{31}P (Phosphorus) are the commonly used nuclei for the NMR spectroscopic study of body fluids and tissues.[11,12] Of these, ^{1}H is the routinely used nucleus and the lines or peaks, called as "resonances," observed in an NMR spectrum provide information on the presence of various protons of functional groups, such as of the protons present in a chemical moiety of metabolites or biochemical species present in the sample. To illustrate the capability of NMR for the identification of various types of protons present in the functional groups within a molecule, we recorded the ^{1}H NMR spectrum [Figure 1] of a mixture of alanine (Ala), glycine (Gly), citrate (Cit), and choline (Cho). The assignment of protons from each of these individual compounds is shown in the figure, which can easily be extended to larger biological molecules such as biofluids and tissues.

In the last two decades, NMR has emerged as a versatile and powerful tool that is widely used to analyse body fluids and tissues.[15,16] Although it has a limitation of lower sensitivity and a poor resolution as compared to the MS, it has excellent reproducibility with a possibility of quantitative estimate of the present metabolites. Further, its accuracy and a simple sample preparation protocol enable an unparalleled ability to analyze the intact biological samples (tissues) and the ability to trace dysregulated metabolomic pathways.[11] In addition, progress in instrument hardware and technological developments in software have enhanced the resolution and sensitivity of NMR spectroscopy in metabolomic studies.[11]

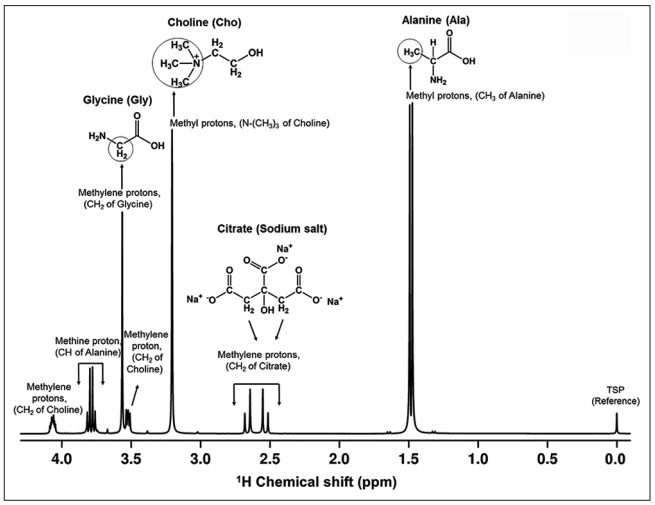

Figure 1: ^{1}H nuclear magnetic resonance spectrum of a mixture of alanine (Ala), glycine (Gly), citrate (Cit) and choline (Cho) recorded at 400 MHz. The assignment of protons from each of these compounds are shown in the figure

In PCa NMR metabolomics, a wide variety of samples such as tissues, tissue extracts, cell lines, and body fluids like seminal fluid, prostatic fluid, blood, and urine have been studied.[11,15-18] Advantages of NMR include an atomic-level information on the structure of different known/unknown metabolites of the sample under investigation and the identification and estimation of the concentration of a large number of metabolites.[18]

The potential of *in vivo* MRS,[8,19-23] *in vitro* MRS,[15,16,22-25] and high-resolution magic angle spinning (HRMAS) NMR spectroscopy of PCa has been reviewed.[25-30] We review the recent results and findings of *in vitro* and *ex vivo* NMR metabolomics of PCa. The focus is on the evaluation of the clinical potential of NMR metabolomics as an aid to the diagnosis of PCa and to study its metabolism.

METHODOLOGY USED FOR NUCLEAR MAGNETIC RESONANCE METABOLOMIC STUDIES

The available literature related to PCa NMR metabolomics published from 2013 to the first quarter of 2021was reviewed. An extensive literature search was carried out on PubMed, Web of Science, Cochrane and Google scholar data bases to identify NMR based metabolomics studies using

appropriate keywords such as *in vitro* NMR spectroscopy of PCa, *ex vivo* NMR spectroscopy of PCa, and HRMAS of PCa, and a combination thereof. A total of 26 studies fulfilled our search criteria. Of these 26, eight used urine samples, eight investigated blood samples, six were related to prostate tissues, two used both blood and urine, one was on the biofluids and tissue and another one was on seminal plasma. This review is limited to discussing the utility and potential role of *in vitro* and *ex vivo* NMR metabolomics related to the diagnosis and metabolism of PCa, since the use of *in vivo* multiparametric MR in PCa has been recently reviewed.[23] Studies related to PCa cell line model systems were excluded from the present review.

Biofluids and tissue collection and preparation

Urine is a common biological specimen studied widely for metabolomic profiling due to the abundance of potential disease biomarkers. It is readily available and involves a simple noninvasive collection procedure. The nature and the concentration of urinary metabolites are significantly affected by diet, physical activities and medications and complicate the analysis and interpretation of NMR.[31,32] For NMR experiments, preprandial urine is collected and a few mM of sodium azide is added to prevent bacterial infection.[32] After adjusting the pH with sodium phosphate buffer (pH 7.4), the urine sample is dissolved in a mixture that contains 90% H_2O and 10% deuterium oxide (D_2O), for NMR experiments.[31,32]

Another biofluid that is widely used is blood serum or plasma. It has certain advantages compared to urine such as (a) serum samples are less sensitive towards exogenous substrates, and (b) metabolomic profiling has good sensitivity to differentiate low grade (LG) from high grade (HG) PCa. The challenges include the deleterious effects of serum proteins and lipoproteins.[11,33] Pre-prandial blood is collected in a prechilled heparin vacutainer and is centrifuged at 2000 rpm for 15 min at 4°C and the resultant supernatant plasma is used for NMR.[32]

In addition to biofluids, metabolomics of prostate tissues can be studied; however, the tissue collection involves an invasive procedure. It offers a direct identification of the tumor tissue biomarkers that aid in the diagnosis of cancer and prediction of metabolism.[26,27] Tissues that are obtained from radical prostatectomy or from transurethral resection are snap frozen in liquid nitrogen to arrest degradation and enzymatic activity. NMR metabolomic analysis of the prostate tissue requires chemical extraction using (a) perchloric acid for the extraction of water-soluble low-molecular-weight metabolites (polar) and (b) the lipophilic metabolites are extracted using chloroform/methanol/water mixtures.[32,34]

Additionally, NMR metabolomics of seminal/prostatic fluids provides a richer information on the metabolites as compared to other biofluids and is less prone to confounding factors. Seminal fluid is obtained through ejaculation, while, the prostatic fluid is obtained after prostate massage. These fluids are stored below –20°C until the NMR analysis.[35-39]

Nuclear magnetic resonance experiments

NMR-based metabolomic experiments on the biofluids and tissue extracts require a high-field NMR spectrometer (operating at a ^1H frequency between 400 and 1000 MHz) equipped with a 5 mm probe or a 3 mm cryo probe to achieve higher sensitivity. The one-dimensional (1D) water-suppressed ^1H NMR spectra are acquired at 25°C.[11,32,40] For intact tissues, HRMAS NMR method is used. The intact tissue sample is packed in a special tube, called a rotor, made of zirconium oxide along with 10 L of D_2O. The rotor containing the tissue pieces is spun at a magic angle of 54.74° (with respect to main magnetic field of the NMR spectrometer) at 5 KHz or more at 20°C. HRMAS ^1H NMR spectra is acquired using a conventional solvent suppressed pulse sequence.[11,41] In an NMR spectrum, the line positions (chemical shifts) seen are with respect to a standard reference compound and for this purpose, in most of the cases 0.1% of trimethyl silylpropionic acid-d_4 (TSP) is added. For plasma, 0.5 mM of sodium formate serves as the chemical shift reference at 8.46 ppm. NMR spectra are processed by the software provided by the instrument vendors. Figure 2 shows the flow chart for performing *in vitro and ex vivo* NMR spectroscopy of biofluids and tissues.

DISCUSSION

In vitro nuclear magnetic resonance metabolomics of biofluids

NMR metabolomics of PCa is an emerging area of research and can be used for the identification of metabolite biomarkers in biofluids and tissues, for the diagnosis and for the monitoring of the treatment response. The method is useful to differentiate cancer from healthy subjects cohort (HC) and benign conditions. Additionally, the advantage of NMR metabolomics is the identification and quantification of a large number of unknown metabolites that aid in understanding the metabolic pathways, and the enzyme activity involved in cancer progression. However, small differences may be seen in the concentration of PCa biomarkers depending on the source of the sample studied (urine, blood, prostate tissue, semen, and prostate fluids), multiplatform analytical techniques used (NMR, mass spectrometry, and chromatography), and the use of different statistical analyses. PCa metabolomic profiling using all the three specimens namely, blood, urine, and tissue has also been reported.[42] These studies generated a large dataset and reported several metabolites, however, the concentration of the metabolites was not available in most of the studies and hence a meta-analysis was not attempted.

Nuclear magnetic resonance metabolomics of urine

Discrimination of PCa from HC is a clinically relevant problem that can be addressed by ^1H NMR metabolic

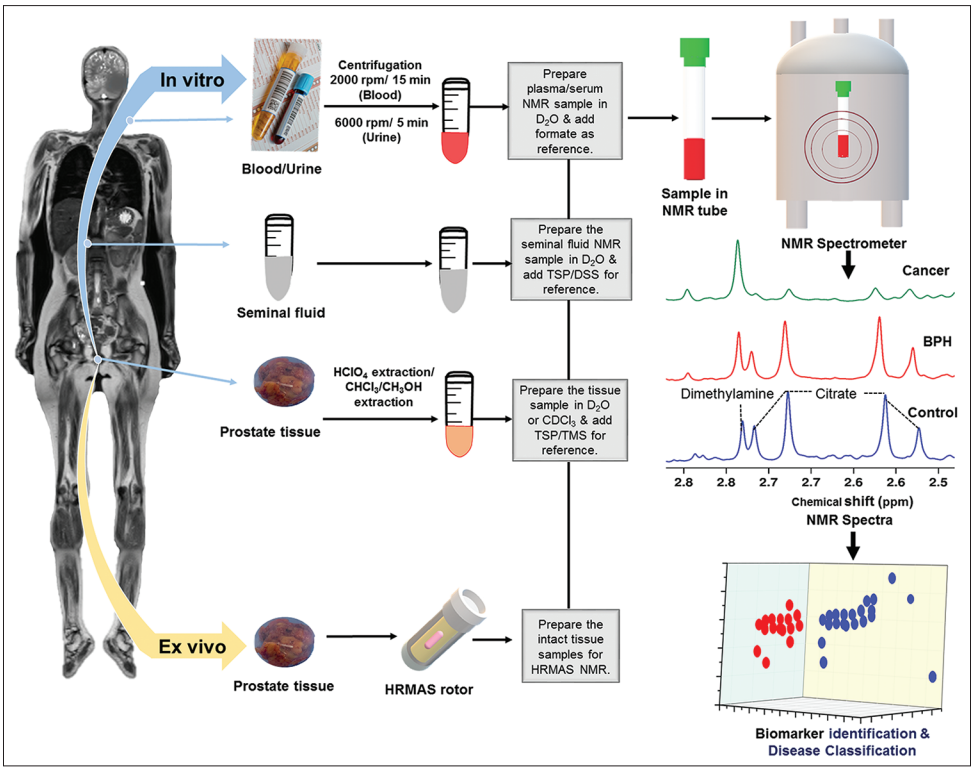

Figure 2: Schematic representation of preparation of bio-fluids and tissues for *in vitro* and *ex vivo* nuclear magnetic resonance spectroscopy

profiling with the use of multivariate analysis.[43] ^{1}H NMR metabolomic profiling identified several known/unknown metabolites related to PCa, BPH and HC that may be used as possible biomarkers for the diagnosis [Figure 3]. Major findings are the discovery of the Cit, creatine (Cr), Gly, and sarcosine for the identification and discrimination of PCa from BPH and HC. Yang *et al.* reported twenty metabolites such as guanidinoacetate, phenyl acetyl glycine, Gly, L-lactate (Lac), and L-alanine (Ala) as the potential biomarkers of PCa.[44] Decreased guanidinoacetate, phenyl acetyl glycine, and Gly levels along with increased Lac and Ala levels distinguished PCa from HC.[44] The published NMR data on biofluids and prostate tissues of PCa, BPH, and HC are summarised in Table 1.

Increased levels of branched-chain amino acids (BCCA), glutamate, and pseudouridine and a reduction in the levels of Gly, dimethylglycine, fumarate, and 4-imidazole-acetate discriminated PCa patients from BPH.[50] In contrast, a lower concentration of BCCA, isoleucine, leucine, valine and other metabolites such as hippurate, dimethylglycine, glycerophosphocholine, glutamine, Gly, taurine and creatinine was reported in PCa patients as compared to the BPH patients.[60]

For the discrimination of PCa from BPH, a large dataset using urine NMR metabolomic profile has been reported.[56] The authors reported that the multivariate analysis failed to distinguish PCa from BPH, while the univariate analysis could discriminate PCa from BPH.[56] Further, this study identified alterations in the metabolic pathways related to glycolysis and urea cycle. However, Lima *et al.* showed that multivariate analysis can distinguish PCa from cancer-free controls with a sensitivity in the range of 67%–89% and a specificity between 74% and 89% and an accuracy of 73% - 86%.[57] Thus, the various studies evaluating urine showed some variations in the metabolite data implying the need for further research on NMR metabolomics at various centers with a larger cohort of patients.

Combination of gas chromatography-MS with ^{1}H NMR can detect larger number of metabolites in urine. Altered metabolites in PCa were reported to be associated with dysregulations of 14 metabolic pathways that provided insight on the development and progression of cancer.[57] Also, the metabolic alterations due to mitochondrial dysfunction, cell proliferation, energy demand, oxidative stress and protein turnover have been documented.[61]

Monitoring the progression of PCa in patients with different grades of tumor (low Gleason score [GS] [GS <7] vs. high GS [GS ≥7]) is clinically relevant and can be achieved by a simultaneous analysis of serum and urine ^{1}H NMR metabolomics.[58] Such complementary approaches (NMR metabolomics profile + transcriptomic profile) unveil significant metabolic alterations in the patients with high GS, particularly the increased levels of serum glucose (Glc), Gly, and elevated levels of 1-methlynicotinamide in urine.[58]

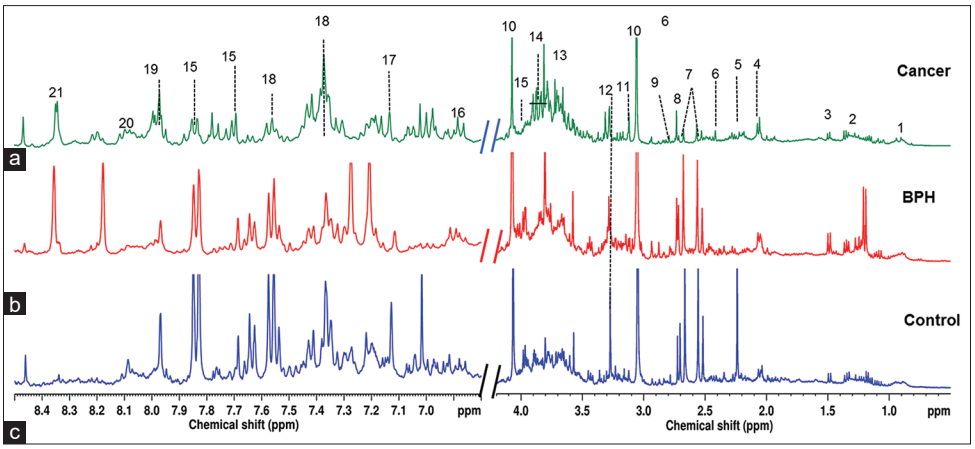

Figure 3: ^{1}H nuclear magnetic resonance spectra of urine acquired at 400 MHz from: (a) cancer, (b) benign prostatic hyperplasia (BPH), and (c) control. Assignment of metabolites: 1 = Branched-chain amino acids, 2 = Lactate, 3 = 2-hydroxyisobutyrate, 4 = N-acetyl groups, 5 = 2-hydroxy glutarate, 6 = Pyruvate, 7 = Citrate, 8 = Dimethylamine, 9 = Sarcosine, 10 = Creatinine, 11 = Cis-aconitic acid, 12 = Trimethylamine-N-oxide, 13 = Glycine, 14 = Serine, 15 = Hippurate, 16 = 4-hydroxybenzoate, 17 = 3-methylhistidine, 18 = phenylalanine, 19 = Histidinem, 20 = Trigonelline, 21 = Formate

NMR metabolomics of blood plasma was able to distinguish patients with high GS (≥7) PCa from those with low GS (<7) PCa[62] with Cho, Ala and dimethylamine serving as the biomarkers.

Another important clinical question is to distinguish the patients with PCa from the biopsy-negative population. Using urine[63] and blood plasma[64] NMR-derived metabolomic profiles, it is possible get an insight on the metabolic alterations that occur in biopsy-negative versus biopsy-positive cases.

Nuclear magnetic resonance metabolomics of blood samples

In addition to urine, either blood serum or plasma samples can be used to identify the potential metabolite biomarkers for the diagnosis of PCa and for differentiating PCa from BPH and HC. One study utilised both the serum and the plasma samples to identify the potential biomarkers of PCa.

Serum NMR metabolomics distinguished HC from LG and HG PCa patients based on the changes seen in the metabolites such as Ala, Gly, pyruvate, and sarcosine.[33] These biomarkers differentiated 90.2% of the PCa cases with a sensitivity of 84.4% and a specificity of 92.9% as compared to HC.[33] The NMR coupled with discriminant function analysis indicated that five biomarkers Ala, sarcosine, creatinine, Gly, and Cit have the potential to discriminate PCa from BPH.[47]

NMR metabolomics requires robust data processing methods for the analysis of urine and blood plasma/serum. Also, since the metabolomic data are generally obtained from different samples or using different analytical techniques, data fusion may be used to improve the classification accuracy. Urine and serum NMR metabolomics combined with partial least squares-discriminant analysis (PLS-DA) showed that several amino acids contributed to the classification of different stages of PCa.[52]

Evaluation of tumor recurrence is clinically important and NMR metabolomics can be used as a tool to monitor the disease process. NMR combined with liquid chromatography – MS method showed alterations in amino acid metabolism, purine and pyrimidine synthesis, tricarboxylic acid cycle, tryptophan catabolism, and changes in Glc, and Lac levels.[55] Metabolic alterations were also seen in blood plasma of PCa patients with and without metastases.[65] Alterations in the amino acids, phospholipids, fatty acids oxidation and glutaminolysis were reported to be associated with PCa progression and tumorgenesis.[65] Plasma samples from PCa patients showed higher levels of valine, glutamine, Cr, tyrosine, phenylalanine, histidine, 3-methylhistidine and lower levels of urea indicating the possibility of higher risk of developing cancer in these patients during 13 years of follow-up.[66]

Discrimination of PCa from BPH using both the blood serum and plasma samples is possible with the help of NMR, MS, and gas chromatography.[46] The data showed changes in fatty acid (acylcarnitines), Cho (glycerophospholipids), and amino acid metabolism (arginine) in PCa patients as compared to BPH.[46]

In addition to urine and blood, NMR metabolomics of seminal/prostatic fluids can also be carried out and studies have reported decreased levels of Cit, spermine (Spm), and myo-inositol (mI) in PCa as compared with HC.[35-39] NMR of seminal plasma was shown to be helpful for the diagnosis and monitoring of low and intermediate-grade PCa.[51] HG PCa samples showed dominant lipids/lipoproteins with less contributions from other metabolites while the metabolites such as Cho, Lac, and Cr had minimal predictive value.

Table 1: *In vitro* and *ex vivo* nuclear magnetic resonance reports on human urine, blood, and prostate tissue related to HC, benign prostatic hyperplasia and prostate cancer

Authors, year and (reference)	Biological specimen studied; NMR method used	Control	Disease	Metabolites observed	Salient findings
Giskeødegård *et al*., 2013[45]	Tissue HRMAS NMR	47	LG=30 HG=81	Spm↓, Cit↓, Cr↓Taurine↓, Cho↑, Gly↑	HG PCa tissue are distinguished from LG Decreased concentrations of Spm, Cit in LG PCa tissue Increased level of clinically accepted measure of CCP/C ratio in PCa Accurately distinguished normal tissue from cancer with 86.9% specificity and 85.2% sensitivity
Giskeødegård *et al*., 2015[46]	Serum Plasma ^{1}H NMR	21 BPH	29 PCa	Acylcarnitines, cho, arginine	Discrimination of BPH from PCa using different analytical techniques Changes in fatty acid, Cho metabolism and amino acid metabolism (arginine) in PCa reported to be good markers for distinction of PCa from BPH
Kumar *et al*.,2015[33]	Serum ^{1}H NMR	32	LG=40 HG=30	Ala↑, Pyruvate↑, Sarcosine↑and Gly¯	Differentiation of HC from PCa (LG, HG) with higher sensitivity (84.4%) and specificity (92.9%) by using metabolites like Ala, pyruvate, Gly and sarcosine Differentiation between LG and HG PCa based on the Ala, pyruvate and Gly biomarkers
Kumar *et al*.,2016[47]	Serum ^{1}H NMR	65	70 BPH 75 PCa	Gly↓, Cit↓, sarcosine↑Ala↑, Cr↑, xanthine↑and hypoxanthine	DFA-based categorization accurately determines abnormal prostate (BPH + PC) based on Gly, sarcosine, Ala, Cr, xanthine, and hypoxanthine Differentiation of PCa from BPH with an accuracy of 88.3% by NMR and 75.2% by clinical laboratory method Gly, sarcosine, Ala, Cr, xanthine, and hypoxanthine determine abnormal prostate (BPH + PC)
Madhu *et al*.,2016[48]	Tissue HRMAS NMR	10 benign	7 PCa (untreated) 6 PCa (treated)	Ala↑, Lac↑, t-Cho↑Cit↓, polyamine↓	Higher level of Lac, Ala, and t-Cho exist in HG PCa samples compared to BPH Significant reduction of Lac and t-Cho concentrations after treatment with degarelix
Hansen *et al*.,2016[49]	Tissue HRMAS NMR	95	34	Cit↓, Spm↓, Cho	PLS-DA based analysis differentiated prostate samples of gene TMPRSS-ERG$_{high}$ from TMPRSS-ERG$_{low}$ Noticeable metabolic alteration occurred in TMPRSS-ERG$_{high}$ Increased cho-containing metabolites in TMPRSS-ERG$_{high}$ Decreased Cit and poly in TMPRSS-ERG$_{high}$
Pérez-Rambla *et al*., 2017[50]	Urine ^{1}H NMR	Not studied	64 PCa 51 BPH	Glutamate↑Pseudouridine↑, Gly↓, dimethylglycine↓Fumarate↓4-imidazole acetate↓	Increased concentration of BCAA, glutamate, and pseudouridine in PCa compared to BPH Decrease in the concentrations of Gly and dimethylglycine in PCa and useful to discriminate PCa from BPH
Roberts *et al*.,2017[51]	Seminal plasma ^{1}H NMR	Not studied	151	Lipids/lipoproteins	Seminal plasma of HG PCa samples exhibit dominant lipids/lipoproteins while other metabolites (Cho, Lac, and Cr) have minimal predictive value Useful for the diagnosis and monitoring of low and intermediate-grade PCa
Zheng *et al*.,2017[52]	Blood/urine ^{1}H NMR	Not studied	19 BPH 16 EPCa 12 APCa 25 MPCa 8 CRPC	Cit↓, Glc↓Cho↓, taurine↓, phosphocholine↑	Optimized data preprocessing model for the better classification of different stages of PCa Several amino acids contributed for the classification of the stages of PCa Notable reduction seen in urinary taurine level during the development of PCa
Braadland *et al*.,2017[53]	Tissue HRMAS NMR	Not studied	110 radical prostatectomy	Spm, Cit, Cr, t-Cho	Cit and Spm concentrations are likely to be linked with increased risk of recurrence tChoCr/Spm ratio and ChoCr/Cit ratio are linked with increased risk of recurrence
Vandergrift *et al*., 2018[54]	Tissue HRMAS NMR	27	338	mI↑, GPCho↑, PCho↑, valine	Patient with highly aggressive cancer showed an elevated level of mI Identification of patient with less aggressive PCa to avoid overtreatment Identification tumor grade, classification of stage, and prediction of recurrence

Contd...

Table 1: Contd...

Authors, year and (reference)	Biological specimen studied; NMR method used	Control	Disease	Metabolites observed	Salient findings
Clendinen *et al*.,2019[55]	Serum ^{1}H NMR	Not studied	40 remission 40 recurrence	Purine, pyrimidine Glc, Lac.	Significant alterations in amino acid metabolism, purine and pyrimidine synthesis, tricarboxylic acid cycle, tryptophan catabolism, changes in Glc, and Lac seen in PCa Metabolic alterations are observed to be associated with PCa recurrence
Bruzzone *et al*.,2020[56]	Urine ^{1}H NMR	Not studied	453 PCa 202 BPH	Acetate↓, Lac↓, Glc↓Gly↓, Oxaloacetate↓	Cancer cells maximized the usage of nitrogen and carbon sources for cell growth and proliferation while minimizing metabolic waste Metabolic alterations were found in glycolysis and urea cycle Useful for discriminating PCa from BPH
Lima *et al*.,2020[57]	Urine ^{1}H NMR	42	41 PCa	2-hydroxy isobutyrate, 2-hydroxyvalerate Oxalate Propylene glycol d-threitol, l-threose Acetone, mannitol Hydroxyacetone 2-furoylglycine, ribitol trigonelline	Identified 14 biochemical pathways in the PCa development and progression Dysregulation of metabolic pathways were found to be linked with amino acids and energetic metabolism during PCa progression
Gómez-Cebrián *et al*., 2020[58]	Serum/urine ^{1}H NMR	Not studied	73 PCa	Gly↑, Glc↑, MNA	Analysis of urine/serum metabolomics profile from PCa patients at different stages were carried out 36 metabolic pathways were observed as. Dysregulated in low (GS <7) and high GS PCa (GS ≥7) groups Glc, Gly, and MNA metabolites were seen to be associated with energy metabolism and nucleotide synthesis
Zheng *et al*.,2020[59]	Tissue ^{1}H NMR	Not studied	39 BPH 39 HSPC 25 CRPC	Cit↓, Cr↓, glutamine↓Gly↓, Cho↑, formate↑, uridine	Identification of diagnostic biomarkers for the discrimination between BPH versus hormone-sensitive prostate cancer (HSPC), and BPH versus CRPC Altered metabolic pathways seen during PCa progression Useful for classification and diagnosis of PCa in clinical practice

NMR=Nuclear magnetic resonance, HRNMR=High-resolution NMR, HRMAS=High-resolution magic angle spinning, ↑=Increased, ↓=Decreased, HSPC=Chormone-sensitive prostate cancer, PCa=Prostate cancer, CRPC=Castration-resistant PCa, EPCa=Early PCa, APCa=Advanced PCa, MPCa=Metastatic PCa, DFA=Discriminant functional analysis, PLS-DA=Partial least squares discriminant analysis, ERG=Erythroblast transformation specific related gene, GS=Gleason score, BPH=Benign prostatic hyperplasia, LG=Low grade, HG=High grade, CCP/C=Cho+Cr+Poly/Cit, GS=Gleason score, ^{1}H NMR=Proton nuclear magnetic resonance, HC=Healthy control, PC=Prostate cancer, TMPRSS=Transmembrane protease, serine, BCAA=Branch chain amino acids, MNA=1-Methylnicotinamide

In vitro nuclear magnetic resonance metabolomics of prostate tissue

Apart from biofluids, NMR metabolomic studies can be extended to prostate tissues and tissue extracts. Alterations seen in the metabolites in PCa, BPH and controls [Figure 4] are readily observed in tissue samples and reflect the changes in the metabolic phenotypes during the progression of the cancer. Further, the changes seen in the tissues represent a true picture of the cancer or the diseased condition that is closer to the real situation when compared with the data obtained from biofluids. The use of NMR to probe the metabolite alterations at different stages of PCa, progression (early, advanced, and metastatic PCa), castration-resistant PCa, and BPH has been reported.[42] Cancer samples showed decreased Cit, creatinine, acetate, leucine, valine, Gly, lysine, histidine, glutamine, and Cho, with increased uridine and formate metabolites. This indicates possible disturbances in the energy, amino acid, Cho and fatty acid and uridine metabolisms.[42]

Androgen signalling plays an important role in the development and the progression of PCa and NMR-based metabolomics of prostate tissues after the treatment with androgen deprivation therapy has been reported.[59] Metabolites such as Ala, Cit, glutamate, Gly, Lac, mI, and taurine were found to be useful for differentiating BPH from hormone-sensitive PCa and castration-resistant PCa. These results revealed the metabolic pathways altered during the progression of PCa and are useful for the classification and diagnosis of PCa in clinical practice.[59]

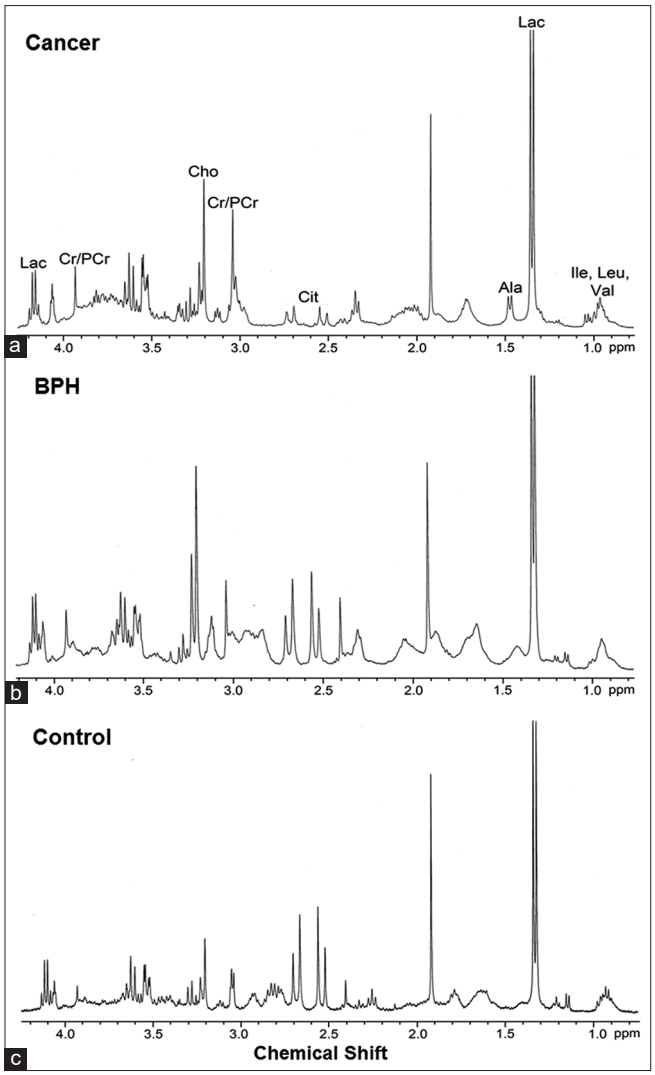

Figure 4: Representative examples of the *in vitro* proton (^{1}H) nuclear magnetic resonance (NMR) spectra of the prostate cancer tissues recorded using a 400 MHz NMR spectrometer.(a) Cancer tissue.(b) Benign prostatic hyperplasia (BPH).(c) Normal prostate tissue. Ala = Alanine, Cit = Citrate, Cho = Choline, Cr = Creatine, Ile = Isoleucine, Lac = Lactate, Leu = Leucine, PCr = Phosphocreatine, Val = Valine (Reproduced with permission from John Wiley and Sons. Kumar *et al*. 2014[27])

Ex vivo nuclear magnetic resonance etabolomics of intact prostate tissue

The advantage of NMR metabolomics is that it facilitates the evaluation of intact tissues using the HRMAS method for differentiating the benign from the malignant prostatic tissues. It is possible to obtain a well-resolved spectra, in a noninvasive way, of the individual metabolites that are present in intact tissues.[67-70] Another advantage is that the same tissue specimen can subsequently be used for the histopathological analysis.[67-70] Both quantitative estimation and qualitative description of individual metabolites for the grading and staging of PCa is possible.[26,27] Studies indicated that Cit, Ala, Cho, and Spm, can be used as the biomarkers of the prostatic tissues. Of these, Cit and Cho were the most frequently used potential biomarkers for the diagnosis of PCa. The *ex vivo* NMR studies of intact tissues performed using HRMAS are also presented in Table 1.

Clinical distinction between LG and HG PCa is necessary for treatment planning, and in this direction, HRMAS can be of immense use. Based on the metabolomic alterations, it is possible to distinguish the normal tissues from cancer (both LG and HG) with more than 85% specificity and 87% sensitivity.[45] Increased level of total Cho + Cr + Poly/Cit (CCP/C) ratio was detected in cancer tissues, while Spm and Cit were decreased in patients with LG PCa.[45] Braadland *et al*. showed that the concentration of Spm and t-Cho + Cr/Spm (tChoCr/Spm) ratio was an independent prognostic markers of recurrence.[53] In addition, HRMAS was used for differentiating healthy from cancerous prostate tissues with erythroblast transformation specific related gene (ERG) translocation.[49] Metabolic profiles revealed that Cit/Cr ratio can different between ERG_{high} and ERG_{low} PCa patients, which can act as a potential tool for the risk-stratification of PCa.[49]

HRMAS can also be used to monitor treatment induced metabolic changes in both the cancerous and benign tissue samples from patients with and without treatment with Degarelix.[48] The results showed that after treatment with Degarelix, a lower level of Lac and t-Cho was observed in PCa samples.[48] The combination of HRMAS and quantitative histology has also been used for tumor identification, staging and prediction of recurrence.[54] The authors reported that: (a) the patient with highly aggressive cancer showed an elevated level of mI and (b) metabolic profiles can predict the stage of PCa (less aggressive) to avoid overtreatment.[54]

SUMMARY

NMR metabolomics is a powerful analytical tool that supplements the existing diagnostic imaging and biochemical investigations. It provides the initial benchmark for the evaluation of body fluids and tissues and has the capability to simultaneously discover and identify a large number of potential metabolite biomarkers which can be used for clinical diagnosis, predicting the aggressiveness of the tumor and to monitor metastasis from PCa. The method can also be used to reliably discriminate HC and BPH from PCa. Furthermore, NMR metabolomic studies provide comprehensive information about the altered metabolic pathways such as the alterations in the amino acid metabolism and changes in the energy metabolism.

The state-of-the-art HRMAS NMR metabolomics facilitates distinction of intact cancerous prostate tissues from healthy and benign tissues that directly corroborates with the histopathological analysis. HRMAS can in fact be used as a complimentary diagnostic tool for the discrimination of PCa from benign tissues. The combination of *in vitro* and *ex vivo* NMR metabolomic analysis of blood plasma/serum,

urine, seminal/prostatic fluid and tissues associated with PCa revealed decreased levels of Cit, Spm and increased levels of Lac, Cho, and other amino acids. Recent developments in ultra-high field NMR has allowed us to achieve imporved MR spectral resolution of metabolites, which has implications in metabolomics research as it aids in identifying greater number of metabolites from biofluids and tissues. The NMR spectrometers of today, which use GHz operating frequency for ^{1}H, would enable the identification of many unknown compounds and complex mixtures of biological molecules, their metabolomics network, and trace the metabolic pathways related to cancer.

However, NMR metabolomics of biofluids and tissues have certain limitations preventing its routine use in a clinical setting as an independent diagnostic tool. Although several recent studies have suggested the use of certain metabolic biomarkers for the diagnosis and monitoring of PCa, none of them are currently ready to be used in the clinic. In addition, NMR metabolomics of biofluids and tissues are affected by various confounding factors such as diet, medication, and smoking. Further, most studies till date have dealt only with smaller cohorts, which are inadequate for a tool to be included as a diagnostic method. To implement the MR metabolomic findings in clinical use, a larger patient cohort dataset with proper validation is required. In addition, standardization of the methods with quality control is necessary along with large randomized clinical trials. The future metabolomic studies should include several metabolite biomarkers as well as many metabolic pathways that play a crucial role in cancer along with robust statistical, machine learning, and artificial intelligence methods for accurate diagnosis and better understanding the metabolism of cancer.

Acknowledgments

NRJ thank his students and collaborators for their help and support. NRJ also acknowledge the Science and Engineering Research Board, Department of Science and Technology, Government of India for the award of J. C. Bose Fellowship. RRR thanks Science and Engineering Research Board for the award of Research Associateship through J C Bose grant given to NRJ.

REFERENCES

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2018;68:394-424.
2. Euceda LR, Andersen MK, Tessem MB, Moestue SA, Grinde MT, Bathen TF. NMR-based prostate cancer metabolomics. In: Prostate Cancer. New York: Springer; 2018. p. 237-57.
3. Heidenreich A, Bastian PJ, Bellmunt J, Bolla M, Joniau S, van der Kwast T, *et al.* EAU guidelines on prostate cancer. Part 1: Screening, diagnosis, and local treatment with curative intent-update 2013. Eur Urol 2014;65:124-37.
4. Matlaga BR, Eskew LA, McCullough DL. Prostate biopsy: Indications and technique. J Urol 2003;169:12-9.
5. Philip J, Dutta Roy S, Ballal M, Foster CS, Javlé P. Is a digital rectal examination necessary in the diagnosis and clinical staging of early prostate cancer? BJU Int 2005;95:969-71.
6. Velonas VM, Woo HH, dos Remedios CG, Assinder SJ. Current status of biomarkers for prostate cancer. Int J Mol Sci 2013;14:11034-60.
7. Haythorn MR, Ablin RJ. Prostate-specific antigen testing across the spectrum of prostate cancer. Biomark Med 2011;5:515-26.
8. Engelbrecht MR, Barentsz JO, Jager GJ, van der Graaf M, Heerschap A, Sedelaar JP, *et al.* Prostate cancer staging using imaging. BJU Int 2000;86 Suppl 1:123-34.
9. Bjurlin MA, Wysock JS, Taneja SS. Optimization of prostate biopsy: Review of technique and complications. Urol Clin North Am 2014;41:299-313.
10. Trock BJ. Application of metabolomics to prostate cancer. Urol Oncol 2011;29:572-81.
11. Gowda GA, Raftery D. Overview of NMR spectroscopy-based metabolomics: Opportunities and challenges. In: Gowda GA, Raftery D, editors. NMR-Based Metabolomics: Methods and Protocols. Vol. 2037. New York, NY, USA: Humana Press; 2019. p. 3-14.
12. Dumas ME, Maibaum EC, Teague C, Ueshima H, Zhou B, Lindon JC, *et al.* Assessment of analytical reproducibility of 1H NMR spectroscopy based metabonomics for large-scale epidemiological research: The INTERMAP Study. Anal Chem 2006;78:2199-208.
13. Harris R. Nuclear Magnetic Resonance Spectroscopy. Longman, London, 1986.
14. Keeler J. Understanding NMR spectroscopy. Chichester: John Wiley & Sons; 2011.
15. Sharma U, Jagannathan NR. Metabolism of prostate cancer by Magnetic Resonance Spectroscopy (MRS). Biophys Rev 2020;12:1163-73.
16. Lima AR, Bastos Mde L, Carvalho M, Guedes de Pinho P. Biomarker discovery in human prostate cancer: An update in metabolomics studies. Transl Oncol 2016;9:357-70.
17. Fan TW, Lane AN. Applications of NMR spectroscopy to systems biochemistry. Prog Nucl Magn Reson Spectrosc 2016;92-93:18-53.
18. Nagana Gowda GA, Raftery D. Can NMR solve some significant challenges in metabolomics? J Magn Reson 2015;260:144-60.
19. George AK, Turkbey B, Valayil SG, Muthigi A, Mertan F, Kongnyuy M, *et al.* A urologist's perspective on prostate cancer imaging: Past, present, and future. Abdom Radiol (NY) 2016;41:805-16.
20. Kurhanewicz J, Dahiya R, Macdonald JM, Chang LH, James TL, Narayan P. Citrate alterations in primary and metastatic human prostatic adenocarcinomas: 1H magnetic resonance spectroscopy and biochemical study. Magn Reson Med 1993;29:149-57.
21. Kurhanewicz J, Vigneron DB, Nelson SJ, Hricak H, MacDonald JM, Konety B, *et al.* Citrate as an *in vivo* marker to discriminate prostate cancer from benign prostatic hyperplasia and normal prostate peripheral zone: Detection via localized proton spectroscopy. Urology 1995;45:459-66.
22. Gholizadeh N, Pundavela J, Nagarajan R, Dona A, Quadrelli S, Biswas T, *et al.* Nuclear magnetic resonance spectroscopy of human body fluids and *in vivo* magnetic resonance spectroscopy: Potential role in the diagnosis and management of prostate cancer. Urol Oncol 2020;38:150-73.
23. Kumar V, Bora GS, Kumar R, Jagannathan NR. Multiparametric (mp) MRI of prostate cancer. Prog Nucl Magn Reson Spectrosc 2018;105:23-40.
24. Kumar D, Gupta A, Nath K. NMR-based metabolomics of prostate cancer: A protagonist in clinical diagnostics. Expert Rev Mol Diagn 2016;16:651-61.
25. Kdadra M, Höckner S, Leung H, Kremer W, Schiffer E. Metabolomics biomarkers of prostate cancer: A systematic review. Diagnostics (Basel) 2019;9:21.
26. Decelle EA, Cheng LL. High-resolution magic angle spinning 1H MRS in prostate cancer. NMR Biomed 2014;27:90-9.

27. Kumar V, Dwivedi DK, Jagannathan NR. High-resolution NMR spectroscopy of human body fluids and tissues in relation to prostate cancer. NMR Biomed 2014;27:80-9.
28. Fuss TL, Cheng LL. Evaluation of cancer metabolomics using *ex vivo* High Resolution Magic Angle Spinning (HRMAS) Magnetic Resonance Spectroscopy (MRS). Metabolites 2016;6:11.
29. Dinges SS, Vandergrift LA, Wu S, Berker Y, Habbel P, Taupitz M, *et al*. Metabolomic prostate cancer fields in HRMAS MRS-profiled histologically benign tissue vary with cancer status and distance from cancer. NMR Biomed 2019;32:e4038.
30. Andersen MK, Giskeødegård GF, Tessem MB. Metabolic alterations in tissues and biofluids of patients with prostate cancer. Curr Opin Endocrine Metabol Res 2020;10:23-8.
31. Emwas AH, Luchinat C, Turano P, Tenori L, Roy R, Salek RM, *et al.* Standardizing the experimental conditions for using urine in NMR-based metabolomic studies with a particular focus on diagnostic studies: A review. Metabolomics 2015;11:872-94.
32. Beckonert O, Keun HC, Ebbels TM, Bundy J, Holmes E, Lindon JC, *et al.* Metabolic profiling, metabolomic and metabonomic procedures for NMR spectroscopy of urine, plasma, serum and tissue extracts. Nat Protoc 2007;2:2692-703.
33. Kumar D, Gupta A, Mandhani A, Sankhwar SN. Metabolomics-derived prostate cancer biomarkers: Fact or fiction? J Proteome Res 2015;14:1455-64.
34. Tyagi RK, Azrad A, Degani H, Salomon Y. Simultaneous extraction of cellular lipids and water-soluble metabolites: Evaluation by NMR spectroscopy. Magn Reson Med 1996;35:194-200.
35. Lynch MJ, Masters J, Pryor JP, Lindon JC, Spraul M, Foxall PJ, *et al.* Ultra high field NMR spectroscopic studies on human seminal fluid, seminal vesicle and prostatic secretions. J Pharm Biomed Anal 1994;12:5-19.
36. Lynch MJ, Nicholson JK. Proton MRS of human prostatic fluid: Correlations between citrate, spermine, and myo-inositol levels and changes with disease. Prostate 1997;30:248-55.
37. Averna TA, Kline EE, Smith AY, Sillerud LO. A decrease in 1H nuclear magnetic resonance spectroscopically determined citrate in human seminal fluid accompanies the development of prostate adenocarcinoma. J Urol 2005;173:433-8.
38. Kline EE, Treat EG, Averna TA, Davis MS, Smith AY, Sillerud LO. Citrate concentrations in human seminal fluid and expressed prostatic fluid determined via 1H nuclear magnetic resonance spectroscopy outperform prostate specific antigen in prostate cancer detection. J Urol 2006;176:2274-9.
39. Serkova NJ, Gamito EJ, Jones RH, O'Donnell C, Brown JL, Green S, *et al.* The metabolites citrate, myo-inositol, and spermine are potential age-independent markers of prostate cancer in human expressed prostatic secretions. Prostate 2008;68:620-8.
40. Piotto M, Saudek V, Sklenár V. Gradient-tailored excitation for single-quantum NMR spectroscopy of aqueous solutions. J Biomol NMR 1992;2:661-5.
41. Cheng LL, Lean CL, Bogdanova A, Wright SC Jr., Ackerman JL, Brady TJ, *et al.* Enhanced resolution of proton NMR spectra of malignant lymph nodes using magic-angle spinning. Magn Reson Med 1996;36:653-8.
42. Zheng H, Dong B, Ning J, Shao X, Zhao L, Jiang Q, *et al.* NMR-based metabolomics analysis identifies discriminatory metabolic disturbances in tissue and biofluid samples for progressive prostate cancer. Clin Chim Acta 2020;501:241-51.
43. Zaragozá P, Ruiz-Cerdá JL, Quintás G, Gil S, Costero AM, León Z, *et al.* Towards the potential use of (1)H NMR spectroscopy in urine samples for prostate cancer detection. Analyst 2014;139:3875-8.
44. Yang B, Zhang C, Cheng S, Li G, Griebel J, Neuhaus J. Novel metabolic signatures of prostate cancer revealed by ^{1}H-NMR metabolomics of urine. Diagnostics (Basel) 2021;11:149.
45. Giskeødegård GF, Bertilsson H, Selnæs KM, Wright AJ, Bathen TF, Viset T, *et al.* Spermine and citrate as metabolic biomarkers for assessing prostate cancer aggressiveness. PLoS One 2013;8:e62375.
46. Giskeødegård GF, Hansen AF, Bertilsson H, Gonzalez SV, Kristiansen KA, Bruheim P, *et al.* Metabolic markers in blood can separate prostate cancer from benign prostatic hyperplasia. Br J Cancer 2015;113:1712-9.
47. Kumar D, Gupta A, Mandhani A, Sankhwar SN. NMR spectroscopy of filtered serum of prostate cancer: A new frontier in metabolomics. Prostate 2016;76:1106-19.
48. Madhu B, Shaw GL, Warren AY, Neal DE, Griffiths JR. Response of Degarelix treatment in human prostate cancer monitored by HR-MAS ^{1}H NMR spectroscopy. Metabolomics 2016;12:120.
49. Hansen AF, Sandsmark E, Rye MB, Wright AJ, Bertilsson H, Richardsen E, *et al*. Presence of TMPRSS2-ERG is associated with alterations of the metabolic profile in human prostate cancer. Oncotarget 2016;7:42071-85.
50. Pérez-Rambla C, Puchades-Carrasco L, García-Flores M, Rubio-Briones J, López-Guerrero JA, Pineda-Lucena A. Non-invasive urinary metabolomic profiling discriminates prostate cancer from benign prostatic hyperplasia. Metabolomics 2017;13:52.
51. Roberts MJ, Richards RS, Chow CW, Buck M, Yaxley J, Lavin MF, *et al.* Seminal plasma enables selection and monitoring of active surveillance candidates using nuclear magnetic resonance-based metabolomics: A preliminary investigation. Prostate Int 2017;5:149-57.
52. Zheng H, Cai A, Zhou Q, Xu P, Zhao L, Li C, *et al.* Optimal preprocessing of serum and urine metabolomic data fusion for staging prostate cancer through design of experiment. Anal Chim Acta 2017;991:68-75.
53. Braadland PR, Giskeødegård G, Sandsmark E, Bertilsson H, Euceda LR, Hansen AF, *et al*. Ex vivo metabolic fingerprinting identifies biomarkers predictive of prostate cancer recurrence following radical prostatectomy. Br J Cancer 2017;117:1656-64.
54. Vandergrift LA, Decelle EA, Kurth J, Wu S, Fuss TL, DeFeo EM, *et al.* Metabolomic prediction of human prostate cancer aggressiveness: Magnetic resonance spectroscopy of histologically benign tissue. Sci Rep 2018;8:4997.
55. Clendinen CS, Gaul DA, Monge ME, Arnold RS, Edison AS, Petros JA, *et al.* Preoperative metabolic signatures of prostate cancer recurrence following radical prostatectomy. J Proteome Res 2019;18:1316-27.
56. Bruzzone C, Loizaga-Iriarte A, Sanchez-Mosquera P, Gil-Redondo R, Astobiza I, Diercks T, *et al.* 1H NMR-based urine metabolomics reveals sign of enhanced carbon and nitrogen recycling in prostate cancer. J Proteome Res 2020;192:419-28.
57. Lima AR, Pinto J, Barros-Silva D, Jerónimo C, Henrique R, Bastos ML, *et al.* New findings on urinary prostate cancer metabolome through combined GC-MS and ^{1}H NMR analytical platforms. Metabolomics 2020;16:70.
58. Gómez-Cebrián N, García-Flores M, Rubio-Briones J, López-Guerrero JA, Pineda-Lucena A, Puchades-Carrasco L. Targeted metabolomics analyses reveal specific metabolic alterations in high-grade prostate cancer patients. J Proteome Res 2020;19:4082-92.
59. Zheng H, Zhu Y, Shao X, Cai A, Dong B, Xue W, *et al.* Distinct metabolic signatures of hormone-sensitive and castration-resistant prostate cancer revealed by a ^{1}H NMR-based metabolomics of biopsy tissue. J Proteome Res 2020;19:3741-9.
60. Kumar P, Kumar R, Kumar V, Kumaran SS, Sharma S, Thulkar S, *et al.* Metabolomics Study of Urine Discriminates Prostate Cancer (PCa) Patients from Benign Prostatic Hyperplasia (BPH) by 1H NMR Spectroscopy. In: Proceedings of the Twenty-Seventh Annual Meeting of the International Society for Magnetic Resonance in Medicine (ISMRM), Paris, France; June 15-18, 2018. p. 2284.
61. Kumar P, Kumar R, Kumar V, Kumaran SS, Thulkar S, Gupta SD, *et al*. Study of Altered Urinary Metabolism in Prostate Cancer Patients using NMR Metabolomics. In: Proceedings of the Twenty-Seventh Annual Meeting of the International Society for Magnetic Resonance in Medicine (ISMRM), Paris, France; June 15-18, 2018. p. 4008.
62. Kumar P, Kumar R, Kumar V, Kumaran SS, Sharma S, Thulkar S,

et al. High-grade and low-grade prostate cancer discrimination via blood plasma NMR based metabolomics. In: Proceedings of the Twenty-Seventh Annual Meeting of the International Society for Magnetic Resonance in Medicine (ISMRM), Paris, France; June 15-18, 2018. p. 2285.
63. MacKinnon N, Ge W, Han P, Siddiqui J, Wei JT, Raghunathan T, *et al.* NMR-based metabolomic profiling of urine: Evaluation for application in prostate cancer detection. Nat Prod Commun 2019;14:1934578X19849978.
64. Jagannathan NR, Kumar P, Kumar R, Kumar V, Kumaran SS, Thulkar S, *et al.* Metabolic Profiling of Blood Plasma for Distinguishing Prostate Cancer in Patients with Positive Biopsy and Negative Biopsy using NMR Spectroscopy. In: Proceedings of the Twenty-Seventh Annual Meeting of the International Society for Magnetic Resonance in Medicine (ISMRM), Paris, France; June 15-18, 2018. p. 4007.
65. Jagannathan NR, Kumar P, Kumar R, Kumar V, Kumaran SS, Sharma S, *et al*. Blood Plasma Metabolic Profiling Discriminates Prostate Cancer Patients with Metastases from those Without Metastases. In: Proceedings of the Twenty-Seventh Annual Meeting of the International Society for Magnetic Resonance in Medicine (ISMRM), Paris, France; June 15-18, 2018. p. 2277.
66. Lécuyer L, Victor Bala A, Demidem A, Rossary A, Bouchemal N, Triba MN, *et al.* NMR metabolomic profiles associated with long-term risk of prostate cancer. Metabolomics 2021;17:32.
67. Griffin JL, Shockcor JP. Metabolic profiles of cancer cells. Nat Rev Cancer 2004;4:551-61.
68. DeFeo EM, Wu CL, McDougal WS, Cheng LL. A decade in prostate cancer: From NMR to metabolomics. Nat Rev Urol 2011;8:301-11.
69. Andrew E, Bradbury A, Eades R. Removal of dipolar broadening of nuclear magnetic resonance spectra of solids by specimen rotation. Nature 1959;183:1802-3.
70. Burns MA, He W, Wu CL, Cheng LL. Quantitative pathology in tissue MR spectroscopy based human prostate metabolomics. Technol Cancer Res Treat 2004;3:591-8.

How to cite this article: Reddy RR, Jagannathan NR. Potential of nuclear magnetic resonance metabolomics in the study of prostate cancer. Indian J Urol 2022;38:99-109.