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

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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 PCa³ 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

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.
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. 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.

The phenomenon of NMR is based on the property of nuclear spin. 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. 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 “ex vivo” NMR.

\[ ^1H \text{ (Proton), } ^{13}C \text{ (Carbon), } ^{15}N \text{ (Nitrogen), and } ^{31}P \text{ (Phosphorus) are the commonly used nuclei for the NMR spectroscopic study of body fluids and tissues.} \]

Of these, \(^1H\) 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 \(^1H\) 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. 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.

In addition, progress in instrument hardware and technological developments in software have enhanced the resolution and sensitivity of NMR spectroscopy in metabolomic studies.

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. 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.

The potential of in vivo MRS, in vitro MRS, and high-resolution magic angle spinning (HRMAS) NMR spectroscopy of PCa has been reviewed. 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 2021 was 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. 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. For NMR experiments, preprandial urine is collected and a few mM of sodium azide is added to prevent bacterial infection. After adjusting the pH with sodium phosphate buffer (pH 7.4), the urine sample is dissolved in a mixture that contains 90% H$_2$O and 10% deuterium oxide (D$_2$O), for NMR experiments.

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. 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.

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. 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.

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.

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. 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$_2$O. 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 are acquired using a conventional solvent suppressed pulse sequence. 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. 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
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profiling with the use of multivariate analysis.\(^\text{[43]}\) \(^1\)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.\(^\text{[44]}\) Decreased guanidinoacetate, phenyl acetyl glycine, and Gly levels along with increased Lac and Ala levels distinguished PCa from HC.\(^\text{[44]}\) 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.\(^\text{[50]}\) 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.\(^\text{[60]}\)

For the discrimination of PCa from BPH, a large dataset using urine NMR metabolomic profile has been reported.\(^\text{[56]}\) The authors reported that the multivariate analysis failed to distinguish PCa from BPH, while the univariate analysis could discriminate PCa from BPH.\(^\text{[56]}\) 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%.\(^\text{[57]}\) 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.\(^\text{[57]}\) Also, the metabolic alterations due to mitochondrial dysfunction, cell proliferation, energy demand, oxidative stress and protein turnover have been documented.\(^\text{[61]}\)

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.\(^\text{[58]}\) 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-methylnicotinamide in urine.\(^\text{[58]}\)
NMR metabolomics of blood plasma was able to distinguish patients with high GS (≥7) PCa from those with low GS (<7) PCa \cite{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 \cite{63} and blood plasma \cite{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. \cite{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. \cite{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. \cite{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. \cite{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. \cite{55} Metabolic alterations were also seen in blood plasma of PCa patients with and without metastases. \cite{65} Alterations in the amino acids, phospholipids, fatty acids oxidation and glutaminolysis were reported to be associated with PCa progression and tumorgenesis. \cite{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. \cite{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. \cite{46} The data showed changes in fatty acid (acylcarnitines), Cho (glycerophospholipids), and amino acid metabolism (arginine) in PCa patients as compared to BPH. \cite{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. \cite{35-39} NMR of seminal plasma was shown to be helpful for the diagnosis and monitoring of low and intermediate-grade PCa. \cite{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 PCa. 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 1H NMR | 21 BPH 29 PCa | Acetylcarnitines, 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[23] | Serum 1H 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. |
| Kumar *et al.*, 2016[47] | Serum 1H NMR | 65 | 70 BPH 75 PCa | Gly↑, Cit↑, sarcosine↑Ala↑, Cr↑, xanthine↑ and hypoxanthine | Differentiation between LG and HG PCa based on the Ala, pyruvate and Gly biomarkers. |
| Madhu *et al.*, 2016[48] | Tissue HRMAS NMR | 10 benign (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 | | Cit↑, Spm↑, Cho | Noticeable metabolic alteration occurred in TMPRSS-ERGhigh samples of gene TMPRSS-ERG. Increased cho-containing metabolites in TMPRSS-ERGhigh. |
| Pérez-Rambla *et al.*, 2017[50] | Urine 1H NMR | Not studied | 64 PCa 51 BPH | Glutamate↑Pseudouridine↑, Gly↑, dimethylglycine↑Fumarate↓4-imidazole acetate↓ | Decreased Cit and poly in TMPRSS-ERGhigh. 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 1H 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 1H 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. |
| Braadland *et al.*, 2017[53] | Tissue HRMAS NMR | Not studied | 110 radical prostatectomy | Smp, Cit, Cr, t-Cho | Notable reduction seen in urinary taurine level during the development of PCa. Several amino acids contributed for the classification of the stages of PCa. |
| Vandergrift *et al.*, 2018[54] | Tissue HRMAS NMR | 27 338 | m1↑, GPCho↑, PCho↑, valine | Identification of patient with less aggressive PCa to avoid overtreatment. Identification tumor grade, classification of stage, and prediction of recurrence. |

Contd...
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, creatine, 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.\[59\]
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Ex vivo nuclear magnetic resonance metabolomics 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.\(^{45}\) Bradland \textit{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$\text{high}$ and ERG$\text{low}$ 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 \textit{in vitro} and \textit{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 improved 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 1H, 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 metabolic 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 metabolic 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.

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