

Novel biomarker for prostate cancer diagnosis by MRS

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1. ABSTRACT

Magnetic resonance spectroscopy (MRS) is a prospective tool for characterization of the chemical composition of tissues. *In vivo* MRS can be used for metabolite profiling in the prostate tissue to discriminate non-invasively carcinomas and healthy prostate. In this article different prostate metabolites have been discussed and how to exploit the MRS technique for the estimation of metabolites in prostate tissue quantitatively is elucidated. Choline, citrate, creatine, myo-inositol metabolites can be considered as biomarker for localization of malignancy in the prostate and their ratio can be used for the determination of cancer tissue in the prostate gland.

2. INTRODUCTION

The new 'omics' science like 'genomic', 'proteomics' 'metabolomics' is a promising field of science which can provide valuable information for the detection of cancer. Metabolite profiling is one component of metabolomics, which can be used for the investigation of cancer biology. Many cell regulatory processes are characterized through several low-molecular-weight metabolites (1). Metabolomics can detect changes in the distribution and concentration of a broad range of metabolites in biological systems. The metabolite concentration is important rather than the rate of chemical reaction. Determination of metabolic concentration, which

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is directly related to metabolic fluxes, is helpful for the understanding of the enzyme activities (2-4). Several modern analytical techniques viz., high-performance liquid chromatography (HPLC), fourier transform infrared spectroscopy (FTIR), chromatography–mass spectrometry (CG-MS), NMR, protein microarray analysis etc. are used to address the change of metabolites. However NMR in conjunction with chromatography is a practical tool to assign and quantify the metabolites in the course of the metabolic pathway (5).

Since 1970, NMR spectroscopy (NMRS) and GC–MS have been extensively used for metabolic profiling (6-8). NMRS is also utilized to examine the metabolic processes that occur in cancer cells (9-11). Prostate cancer, one of the most common cancers, is diagnosed by prostate-specific antigen (PSA) estimation and biopsy. But biopsy can cause enhancement in metastasis of cancer cells. Moreover, these tests are unable to predict this cancer at an early stage as well as the spreading profile of the cancer (12-13). Presently the physicians are being attracted to use NMRS to detect the prostate cancer. ^{31}P NMR spectroscopy has been used for the biochemical profiling of hormone during prostate cancer (14). Sreekumar *et al.* (15) has already discovered several biomarkers that can be utilized for the diagnosis of prostate cancer through NMR. It becomes one of the essential methodology for metabolic profiling of prostate cancer. With the technological advancement, metabolite detection with NMR spectroscopy happens to be cheaper and less time consuming (16). In this article, we depict the applicability of NMRS to explore the prostate metabolites. The advancement of the metabolomic profiling with NMR in prostate cancer is described.

3. NMR AS CLINICAL DIAGNOSTIC AND METABOLITE PROFILING TOOL

NMRS in the form of imaging and voxel based spectroscopy has been widely used to diagnosis and prognosis of different diseases (17,18). Through *in vivo* NMRS, peaks and resonates are detected and assigned to proper chemical functional group to identify the metabolites for quantification, and then a correlation is established between the peaks and biochemical reactions occurring in the disease cell to make clinical decisions. Using 3D localization schemes and software, *in vivo* and *ex vivo* NMR studies can measure metabolite concentrations in tissues. Though 3D localisation is not a quick procedure and is rather uncomfortable for patients especially using endo-rectal coil (19), there is an enormous potential of this non-invasive technique which can provide the detailed ideas about the metabolite concentrations and spatially distribution of metabolites with high resolution. However several factors like chemical shift, peak assignments, and resolution should be properly executed to avoid any ambiguity and false positive error in determining the diseases.

Most of the metabolite-profiling strategies target large populations of low-molecular-weight (LMW) metabolites within a biological system. NMRS is an important analytical technique utilized to profile the LMW

metabolites (20-22). Some biological samples like body fluids such as plasma and urine contain hundreds of small molecules which are LMW metabolites (5). NMRS has significant advantages for metabolite-profiling of biological samples. It needs a little preparation of samples or some time no need for sample preparation. This technique is non-destructive and competent to produce a broad profile of LMW metabolites of tissues or biofluids (23). However, NMR investigation in the form of metabolomics needs lot of post processing data analysis in the form of statistical to test some hypothesis (5).

4. CANCER METABOLITE PROFILING

The understanding of metabolite alterations is very important to trace the neoplast progression in tumors. Metabolite variation results from a combination of time dependent sequential activities at the intracellular site in the form of gene expression, cell signaling, protein synthesis and metabolic responses (21). Since each stage in this temporal metabolic occurrence is expressed through metabolic fluxes, the record of metabolite in different stages is very important to correlate the macroscopic consequences in the organism with the molecular responses in different times. Despite the complicity of the investigation of the metabolic fluxes, NMRS becomes the potential tool for assigning and quantifying the metabolite changes occurring in the abnormal tissues. Variation in metabolite concentration can be attributed to the biochemical incoherent status of organisms. Researchers are giving more importance to NMR-based metabolomics using serum samples for the diagnosis and prognosis of disease (24-28). Due to presence of hydrogen, carbon and phosphorus in the major biological compound, ^1H , ^{13}C and ^{31}P magnetic resonance spectroscopy is useful to explore the metabolic signatures in carcinomas cell in differentiating tumors and normal tissues (29-32). During assigning the metabolites in NMR spectra, it has been found that the choline / creatine ratio is higher in squamous cell carcinoma in comparison to normal tissue (33,34). Phospholipid metabolism in the cell membrane is characterized by choline and its derivatives as markers of cellular proliferation.

NMRS has been employed to determine metabolite changes in tumor cells in mouse (35). In the study, it was observed that there is a lacking in the transcription factor, hypoxia-inducible factor-1 (HIF-1). One of the main activities of HIF-1 is to up-regulate the genes of certain glycolytic enzymes and the glucose transporters (GLUT-1 and GLUT-3). It was also observed that ATP production was extensively damaged in HIF-1-deficient tumor cells having lower content of glycine, betaine and various choline metabolites. However, in the magnetic resonance imaging (MRI) and post-mortem histological analysis, it has been noted that there was no difference in vascularity between the wild-type and HIF-1 β -deficient tumours (34). ^1H NMR spectra in conjunction with multivariate statistics for biochemical data interpretation have been used by Odunsi *et al.* (36) for early detection of epithelial ovarian cancer (EOC). Based on ^1H -NMR spectra and principal component analysis (PCA)

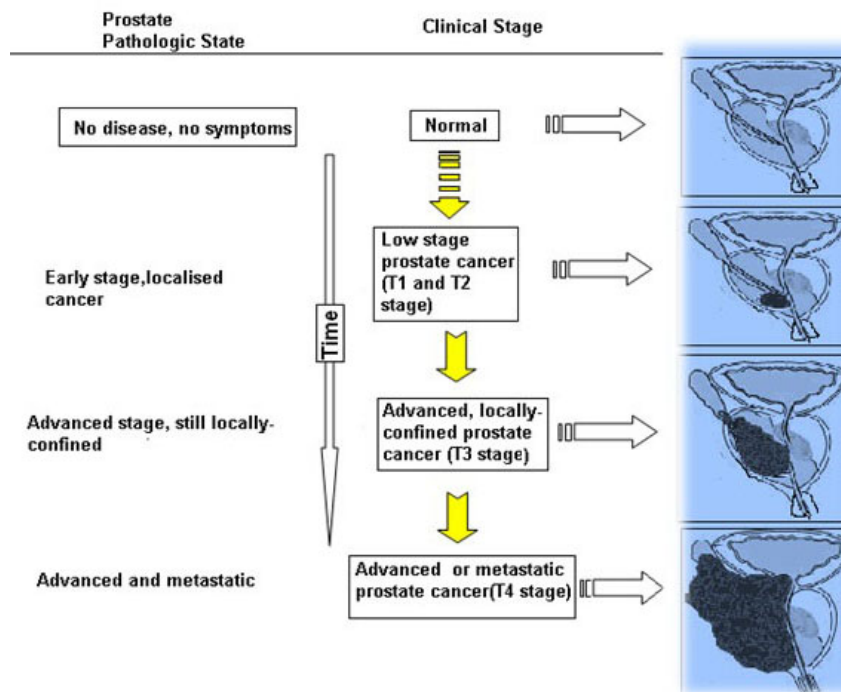


Figure 1. A cartoon picture for Different stages in prostate malignancy. (Stage T1: Tumor is non palpable. It cannot be diagnosed through imaging and can diagnose PSA test. Using PSA test, cancer can be found only in T1c sage. However, the cancer can be diagnosed using histological examination of tissue after removal through prostate surgery. Stage T2: Tumor still confined to the prostate gland which does not exceed the capsule. In T2a stage, palpable lump confined one side of the gland .In T2b/ T2c stage, it can spread more than one half of one lobe of the prostate. In this stage, it has grown enough to become palpable at digital transectal examination or visible at ultrasound or other imaging methods. Stage T3: In this stage, palpable lump no longer confined to the prostate capsule (T3a). The tumor occupies the seminal vesicles (T3b).Stage T4: During this stage, the lump of the tumor has occupied prostate adjacent structures such as rectum or bladder, the external sphincter and the muscles of the pelvic wall. At this stage specific symptoms can appear).

serum specimens were successfully separated from patients with EOC and control serum. Several reports are available to identify metabolic signatures of oral cancer using ^1H and ^{31}P magnetic resonance spectroscopy (29, 37, 38). It has been observed that the choline / creatine ratio is significantly higher in oral squamous cell carcinoma (OSCC) than in normal tissue (32, 39). Using NMR, a study observed the high concentrations of choline, lactic acid glutamic acid, and lipids during OSCC in squamous cell (29). The diagnosis of colon cancer was performed using NMR spectroscopy of blood supplemented by analysis using neural networks (40). Metabolic profiling of human brain tumors using quantitative in vivo ^1H magnetic resonance spectroscopy can discriminate meningiomas, grade II astrocytomas, anaplastic astrocytomas and glioblastomas using the different metabolites ratios of lactate, alanine, saturated lipid, myo-inositol and choline (41). In the investigation of breast carcinoma, single voxel spectroscopy is obtained for the detection of metabolites which includes choline, phosphocholine (PC), glycerophosphocholine (GPC), myo-inositol and taurine (42). Metabolite characterization in the course of pre and post therapy of the breast cancer is performed very successfully with NMR (43). NMR spectroscopy is emerging as very effective technique for discovery and characterization of metabolites in the malignant tissue.

5. FACTORS RELATED TO PROSTATE CANCERS

In the prostate gland, reasons behind malignancy in most of the cases are unknown. Figure 1 displays different stages of the metastasis in the cancerous prostate gland. The variation in prostate metabolites in the process of metabolism in malignant tumor is most probably linked with the activity of enzyme (s) and related defective gene. The ‘up’ and ‘down’ regulation of enzyme activities are subjected to alterations by several factors. For prostate cancer, it has been recorded that factors like ethnicity, diet, and genetic mutations are the important causes for the cancer initiation (44). African-Americans having prostate cancer possess two fold higher mortality rates in comparison to whites (45). Gene mutations whether inherited or acquired, are another factor of prostate cancer development. It has been revealed that mutations in breast cancer type 1 susceptibility gene (BRCA1) and breast cancer type 2 susceptibility gene (BRCA2) may contribute to the development of prostate cancer (46). Mutations in germline BRCA1, checkpoint homolog kinase 2 (CHK2) confer an increased prostate cancer risk (47). In a review article, Dong describes a dozen of genes related to the prostate cancer (48). It includes the germline mutation genes; somatic mutation genes for sporadic prostate cancer (AR, ATBF1, EPHB2 (ERK), KLF6, mitochondria DNA,

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p53, PTEN, and RAS); germline genetic variants genes (BRCA1, BRCA2, CYP17, CYP11B1, CYP3A4 etc.); and genes related to changes of genomic copy number that affecting gene function (ANXA7, KLF5, etc). Some of these genes may not be directly associated with cancer formation but can promote the risk of cancer indirectly.

Sometimes diet habit appears to be correlated to cancer formation. There may be a relation between the carbohydrate pattern and prostate cancer (49). Consumption of dairy products, the main source of calcium, has significant correlation with prostate cancer (50). The diet like milk, dairy, calcium, saturated fat, etc. are reported to increase the risk of prostate cancer. Conversely, diets like tomatoes / lycopene, carotenoids, cruciferous vegetables, fish / marine omega-3 fatty acids, vitamin E, soy etc. are described as prostate cancer protective agents (51).

6. PROSTATE CANCER AND ITS METABOLOMICS

The physiological and biochemical activity occurring in the intra and inter cellular spaces manifested by the abnormal metabolism has direct connectivity with cancer. It has been reported that different metabolic biomarkers associated with glycolysis, mitochondrial citric acid cycle, choline and fatty acid metabolism, can give the information on the process of cancer development (52). Recently, Sreekumar and his coworkers (15) have characterized prostate cancer metabolomics through the investigation on 262 clinical samples (including tissue, urine and plasma) to compare the metabolomes of different sub-types of prostate cancer. They identified 1,126 metabolites from such samples, and 87 of them can be used as biomarker to differentiate prostate cancer from benign prostate tissue. Sarcosine, a derivative of the smallest amino acid glycine is described as one of the significant biomarker. Several other researchers suggest that sarcosine can be a potential biomarker not only for prostate-cancer (53), but also for other cancers (54). One can predict that there should be a correlation among the genes of the biomarkers, their promoter related to oncogene and their pathways. Some software has been developed to identify biomarkers (proteins) to address the cellular signaling pathways. This method has been utilized in one current study, where six cancer cell lines are used to study the secretome (55). Secretome, secreted from human mammary epithelial cells, can be used as biomarker of cancer. It can also be used as biomarker in metastasis of prostate, based on the hypothesis that several secretome proteins are interconnected via intracellular pathways during cancer (55). Isotope-coded affinity tag (ICAT) technique was introduced by Gygi and co-worker (56). Using the ICAT approach, Martin and his coworkers (57) analyzed androgen-regulated secreted proteins from neoplastic prostate tissue. This group discovered 52 androgenic hormone regulated proteins like PSA, neuropilin-1, amyloid-like protein 2 etc. These protein biomarkers can be used for metabolomics study of prostate cancer.

17 β -hydroxysteroid dehydrogenases (17 β -HSDs) have physiological role in steroid and lipid metabolism. Several human diseases like cancer (breast or

prostate cancer), endometriosis, metabolic syndrome and mental diseases were associated with dysfunctions of 17 β -HSDs (58). Another two candidate biomarkers were discovered for prostate cancers - Mac-2 binding protein and macrophage inhibitory cytokine 1. Mac-2 binding protein was screened through LC-MS/MS and verified by western blot/ELISA (59). Macrophage inhibitory cytokine 1 was screened through oligonucleotide microarray, genome-based computational prediction as well as LC-MS/MS and verified by RT-PCR/ELISA/IHC (60). In human body, sarcosine is produced from *S*-adenosylmethionine to glycine catalyzed by the enzyme glycine-*N*-methyltransferase (GNMT). The GNMT is significant enzyme which is available at prominent levels in several organs like mammalian liver, pancreas, prostate and might be important biomarker for diagnosis of prostate cancer (61). Using 2-DE coupled to MALDI-TOF MS, it has been identified that urinary calgranulin B/MRP-14 can be used as a novel marker for prostate cancer detection (62,63).

Despite all the above mentioned bio-marker, some very basic metabolites related to the abnormal metabolism in tissue also can be taken as good sensor for the prediction of the prostate cancer. Such group includes for example, choline, creatine, citrate, inositol etc. (64). These four metabolites can be considered as potential clinical biomarker for the detection of cancer. Their metabolic pathways are very important for better understanding how they get modified (Figure 2). In the subsequent sections their detail chemical and metabolism characterization is discussed.

7. PROSTATE METABOLITES AND NMR SPECTROSCOPY

There is specific application of NMR to characterize the metabolites for differentiating benign prostate, clinically localized prostate cancer, and metastatic disease. It is used in the form of MR spectroscopic imaging (MRSI) (Figure 3) to investigate the changes of metabolites like creatine, choline compounds (choline, glycerophosphocholine, phosphorylcholine), inositol, lactate, alanine, citrate etc. that act as potential biomarkers in the assessment of prostate cancer (65-67).

Generally transrectal ultrasound (TRUS)-guided needle biopsy done in the prostate in conjunction with prostate specific antigen (PSA) test is used for screening benign prostate hyperplasia (BPH) and prostate cancer (PC) patient from healthy subject. But histological evaluation in the process of biopsy is an invasive procedure and hypothesizing is sometimes subject to some uncertainty. It has been reported that TRUS-guided biopsy executed on the patients with elevated PSA level has shown a low sensitivity (~0.6) with high false-negative rate (0.15–0.3.0) (68). Even though the serum PSA is the most widely used biomarker for prostate cancer screening, PSA level is not specific for prostate cancer but also undergoes uncertainty as a function of age or other prostatic conditions such as benign prostatic hyperplasia (BPH) or prostatitis (69). There is 15% risk factor of prostate malignancy for men with a PSA level below 4.0. ng/ml,

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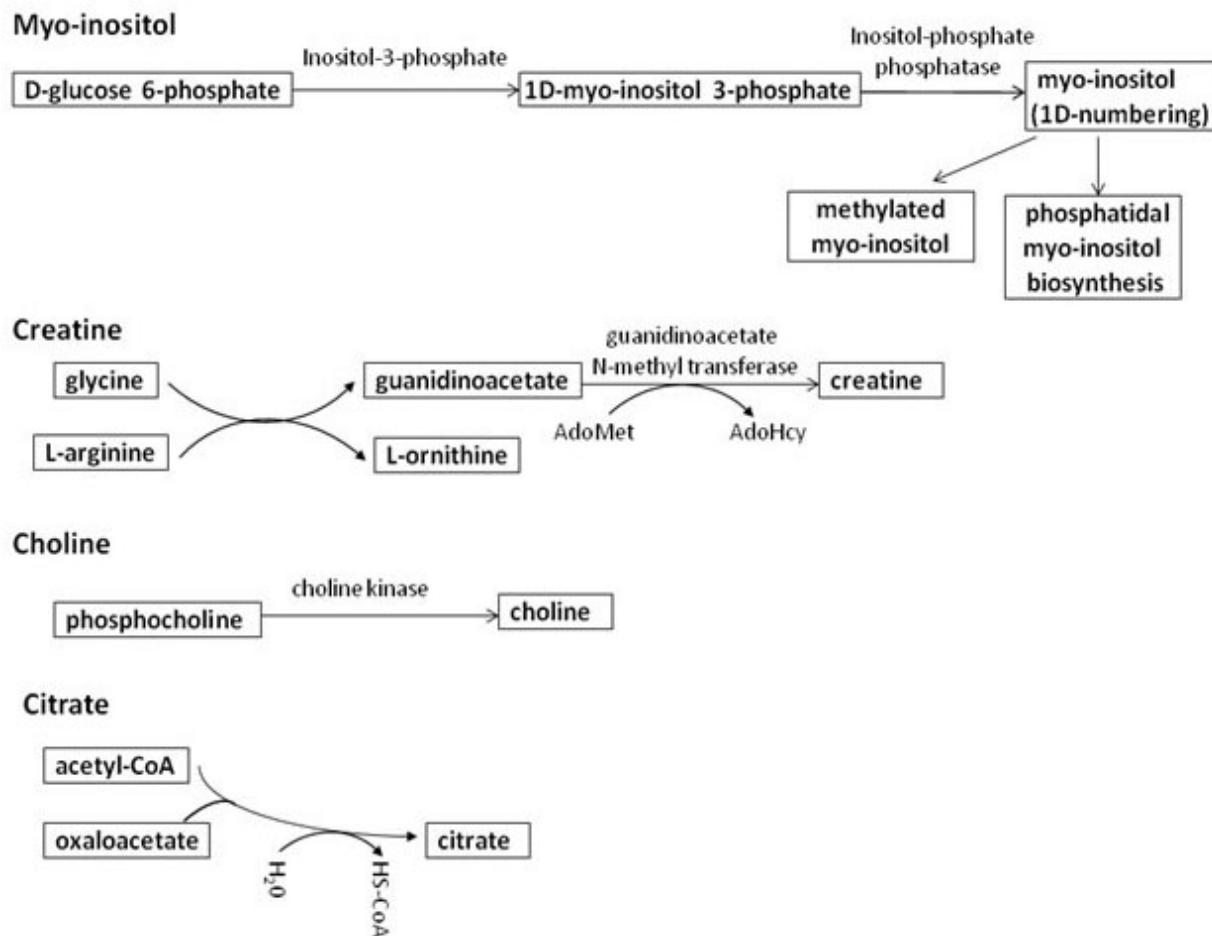


Figure 2. Metabolic pathway and their metabolites of myo-inositol, creatine, choline, citrate. These pathways are using as potential biomarkers for diagnosis and prognostic evaluation of prostate cancer.

25% chance of having prostate cancer for men with a PSA level in between 4.0. and 10 ng/ml and serum PSA levels exceeding 10 ng/ml shows 50% chance of having prostate cancer. Sometimes it is difficult to diagnose accurately localized prostate cancer in patients with PSA level in the range of 4–20 ng/ml with repetitive TRUS-guided biopsy. This fact needs to explore a very sensitive and specific marker for the local malignancy detection non-invasively. In such a case NMR modalities are becoming very popular for non-invasive identification and localization of prostate cancer (70-74). Mainly two types of NMR modalities are used, magnetic resonance imaging (MRI) with different contrast such as T₂ contrast, diffusion contrast etc. (75) and MRS (76). The combination of MRI and MRS is known as magnetic resonance spectroscopic imaging (MRSI). For example while MRI traces anatomy with good resolution, ¹H MRSI is used to investigate the spatial distribution of metabolites in tissue. Only single use of imaging even with very good resolution is not sufficient to detect prostate cancer localization and to address the disease staging (77). MRI sensitivity varies from 62-80% in the detection of tissue architecture anomalies in the prostate with a specificity of 56-72% (78, 79) with some uncertainty in

false-positive findings (80). In such a case MRS in conjunction with MRI could be an appropriate protocol to characterize the malignant tissue at the cellular level (81-83). The proton spectrum possessed from ¹H MRSI demonstrates the signature of metabolites such as choline (Cho), creatine (Cr) and citrate (Cit) in different part of the prostate with high spatial resolution (84).

Researchers are involved to characterize metabolites through the access of MR spectra in the *in-vivo* gland as potential marker for prostate pathology, especially to discriminate between the signature of adenocarcinomas and benign prostate hyperplasia (BPH) (65, 85-87). Citrate, choline, creatine are the significant metabolites assigned in the spectra (88, 89). Citrate as a natural abundance in the healthy prostate is the most prominent metabolite appearing as resonant peak in the spectra. The wealth of information contained in the tissue content of citrate in prostate can be helpful for the better understanding of biochemistry alteration taking place in different zone of the gland (65). Other resonant peak arising from the methyl proton signal of (phospho) cholines in ¹H MR spectrum is also an important metabolite signature which can be utilized for the

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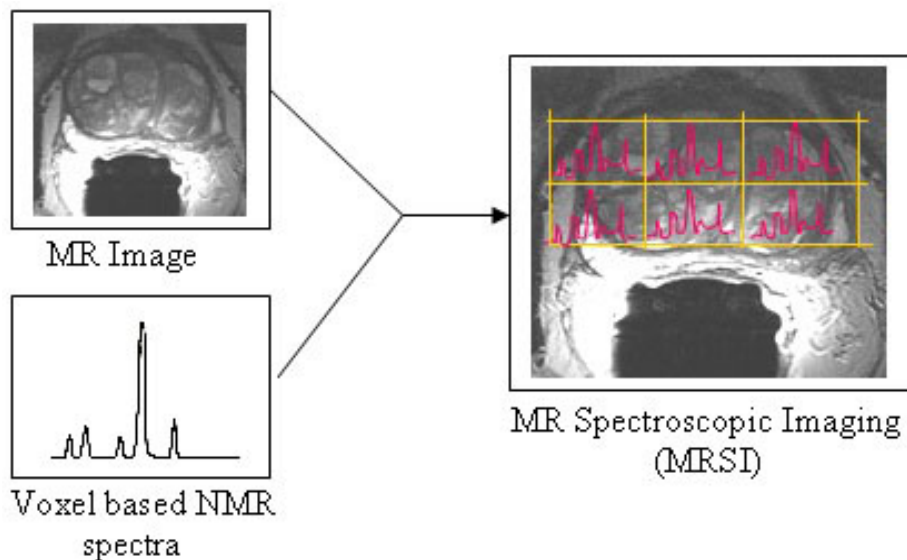


Figure 3. A cartoon picture of the MRSI Technique.

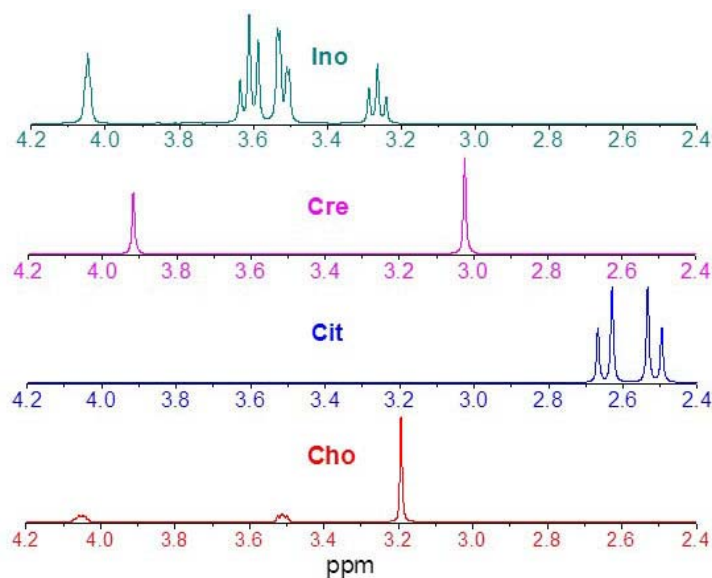


Figure 4. ^1H NMR spectra (simulated) of Cho, Cit, Cre and Ino. The peak positions can be used for referencing and assigning for in-vivo ^1H spectra.

pathological examination of the prostate tissue (87). Such kind of metabolite detection in voxel based MRS has been used to investigate the metabolite distribution over region in the prostate gland (90, 91). Generally the metabolite ratios are determined to predict the pathological change in the prostate gland. Using MRSI the value of $\text{Cit}/(\text{Cho}+\text{Cr})$ ratio has been found to be lower in adenocarcinomas tissue than in benign prostate hyperplasia (91). The lowering of $\text{Cit}/(\text{Cho}+\text{Cr})$ ratio is attributed to the increase in choline-containing compounds and / or decrease in citrate. A great deal of works has been reported on the application of MRSI for analysis of post therapeutic metabolic response (92-95) and detection and localization of prostate cancer (96-103).

In the process of localization and characterization of prostate cancer through ^1H MRSI, the metabolic pattern is used for discrimination between benign and malignant tissue.

8. BIOMARKERS IN PROSTATE CANCER

8.1. Choline

Generally choline at prostate tissue is present at a comparatively lower concentration than the other; still it has a large peak as a singlet at 3.2.2 ppm (Figure 4) that arises from nine magnetically equivalent protons in three methyl groups (Table 1). There are also other two resonates

Table 1. ¹H containing prostate metabolites in prostate

Compound	Structure	Active chemical group	Chemical shift (ppm)	Splitting pattern	J (Hz)
Choli		CH ₂ (C-1) CH ₂ (C-2) CH ₃	4.0.5 3.5.4 3.2.2	t t s	--
		CH ₂	2.5.8	AB	15.5.
		CH ₂ CH ₃	3.9.3 3.0.3	s s	--
		5-CH 1-CH, 3-CH 4-CH, 6-CH 2-CH	3.2.8 3.5.3 3.6.1 4.0.5	t dd dd t	

at 3.5.4 ppm and 4.0.5 ppm as doublets of triplets with intensity much lower than that of the 3.2.2 ppm resonante. These two resonantes are not observed *in vivo* due to overlapping with other resonantes. The metabolite peaks from choline (RO.CH₂.CH₂.N⁺(CH₃)₃) containing compounds observed in *in vivo* NMR spectra is characterized by R group (Table 1) which attributes to free choline, phosphorylcholine and glycerophosphorylcholine; and it is often referred to as ‘total choline’ or simply Cholin (Cho). It is very difficult to differentiate the different choline compounds in *in vivo* ¹H MRS due to the small chemical shift difference relative to the linewidth. In such a case, ³¹P MRS would be the good protocol for the determination of the relative contribution of phosphorylcholine and glycerophosphorylcholine. Cho is a metabolic marker of phospholipids synthesis and degradation (104), thereby addressing the membrane synthesis and degradation. Choline level increases in malignant tumor due to cellular turnover in neoplastic tissues. Recently from the literature, it has been found that total choline concentration in benign prostate tissue is ~ 0.4. mmol/kg and that in cancer prostate tissue is ~ 1 mmol/kg (105). In this way Cho becomes a good marker for detection of prostate cancer.

8.2. Citrate

In a close spectra observation, citrate (Figure 4) shows four resonant peaks with a range of 2.4.9 ppm and 2.6.7 ppm. It has two doublets centered on 2.5.8 ppm with a

scalar coupling 15.5. Hz. The proton signal of citrate arises from its two magnetically equivalent CH₂ moieties (Table 1) that form the molecular symmetry creating strongly coupled AB spin system. Citrate is an important metabolite as intermediate of the TCA or Krebs cycle, where it is formed when acetyl-CoA donates a carbonyl group to oxaloacetate. It has been reported that due to inhibition actions of zinc on the oxidation of citrate in the Krebs cycle in healthy prostate, the epithelial cells synthesize and secrete citrate in large amounts (~60 mM) (106). The citrate storage or net production is regulated by synthesis rate versus utilization rate. A huge amount of zinc in the range of ~3–5 μmol/g is accumulated in the epithelial cells of prostate peripheral zone while zinc accumulation in other tissues being ~0.2–0.4. μmol/g does enhance the levels of mitochondrial zinc, which in turn inhibits m-aconitase activity and citrate oxidation (107). Due to inactivity of m-aconitase, the first step of citrate oxidation is truncated in the Krebs cycle and hence it will increase the citrate level in normal prostate tissues. In carcinomas epithelial cells, zinc level is drastically reduced causing a great reduction in citrate levels. Thus monitoring citrate levels in the prostate is very significant to obtain direct biochemical information for the diagnosis of malignant adenocarcinoma and benign prostatic hyperplasia (65, 107).

8.3. Creatine

In ¹H NMR spectra, the creatine resonances appear as two singlet at 3.0.2 ppm and 3.9.2 ppm respectively (Figure 4) originated from the methyl and

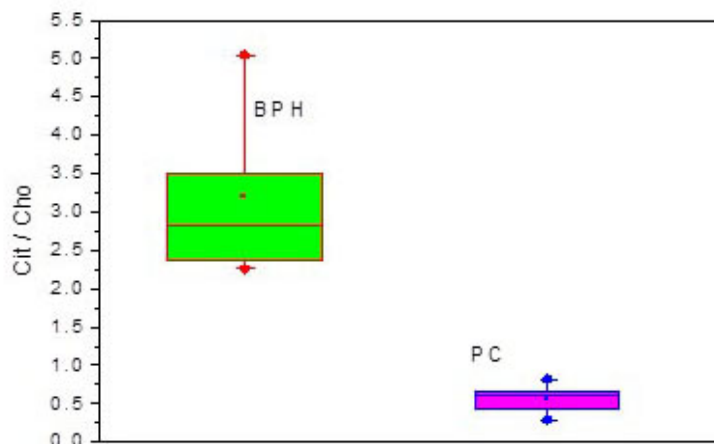


Figure 5. Cit / Cho ratio in benign prostatic hyperplasia (BPH) and prostatic cancer (PC). The ratio being lower in PC indicates the elevated Cho level. (Ref: 64).

methylene protons (Table 1) of creatine and phosphorylated creatine, i.e., phosphocreatine. The distinct difference in the position of two peaks is not well observed in *in vivo* ^1H MRSI due to much smaller chemical shift difference between corresponding protons in creatine and phosphocreatine in comparison to *in vivo* linewidth. *In vivo* ^1H spectra of typical prostate tissue show creatine resonance as “total creatine” (Cre) centered at around 3.0 ppm (Figure 4). The creatine/phosphocreatine (Cre) plays an important role in the cellular energy metabolism. Its activities can be classified in two forms. (1) Cre acts as energy buffer in cells maintaining the constancy of ATP levels through the creatine kinase reaction to meet up the fluctuation in high energy demands. Creatine is carried from liver to tissues and transported into cells through a large concentration gradient by creatine transporter protein (CrT) inhabited at the plasma membrane (108,109) and finally creatine kinase regulates the phosphorylation of creatine. (2) It acts as energy shuttle to facilitate the energy flux diffusion from mitochondria to intracellular energy consumer sites. In this dual activity total creatine (i.e., sum of creatine and phosphocreatine) remains almost constant in normal tissue and consequently creatine resonate in ^1H NMR spectra can be considered as suitable for *in vivo* concentration reference. In prostate tissue Cre is also a prominent peak besides two other major peaks (i.e., Cho and Cit).

8.4. Myo-inositol

Myo-inositol resonates at four distinct chemical shift positions 3.2.8, 3.5.3, 3.6.1 and 4.0.5 ppm of which protons 1 and 3 and protons 4 and 6 overlap (Figure 4, Table-1). The most pronounced resonance of Ino is from protons 1 and 3 at 3.5.3 ppm. Myo-inositol, which is a carbocyclic polyol acts as the structural basis in intracellular signaling pathways (110). Myo-inositol-1L-phosphate synthase (MIPS) (EC 5.5.1.4.) converts D-glucose 6-phosphate to 1-L-myo-inositol-1-phosphate (MIP) which is then dephosphorylated by myo-inositol monophosphatase (IMP) (EC 3.1.3.2.5.) to produce free myo-inositol. Even though myo-inositol is sometimes referred to as a vitamin and since, humans can make myo-

inositol endogenously, it is not a vitamin. Myo-inositol or inositol hexaphosphate (IP6) is the most abundant of nine positional isomers. It has been reported that IP6 has anticancer activity in the form of induced apoptotic cell death, cell cycle arrest and differentiation of cancer cell lines in prostate (111). Inositol 1,4,5-trisphosphate (IP3) plays the role of messengers of the Ca^{2+} by the process of mobilization of intracellular Ca^{2+} , in association with endoplasmic reticulum (ER) organelle acting as Ca^{2+} signal source (112). The activity of inositol 1,3,4,5-tetraphosphate (IP4) and inositol 1,3,4,5,6-pentaphosphate (IP₅) is related to the induction of intracellular Ca^{2+} sequestration. (113).

9. METABOLITE RATIO IN PROSTATIC PATHOLOGY

In the earlier section different important metabolites are described. Sometimes the variation in spectral peak integral or the absolute magnitude of the metabolites in the tissue can not reveal the exact pathological change occurring in tissue spatially as well as temporarily. In that case the variation in the ratio among various metabolites would be efficient to provide the information regarding the change in the tissue. Many have been reported on the change in metabolite ratio in the normal, benign and malignant prostate tissue (114-116). In a single voxel MRSI the voxel wise spectral examination gives the metabolite peak which in turn can be used to estimate the metabolite ratios. This type of analysis is derived from the gaining of spectral grid which further on superposition with the MR image is used to construct metabolite ratio mapping in the prostate (Figure 3).

The metabolite ratios such as Cit/Cho, Cre/Cho, Ino/Cho, Cit/(Cho+Cre), (Figures 5-8) are very significant to discriminate between BPH and PC. Keeping Cre level reference and depending on the physiological as well as biochemical changes occurring in the prostate tissue, Cho, Cit, Ino are estimated. The average value of Cit/Cho ratio

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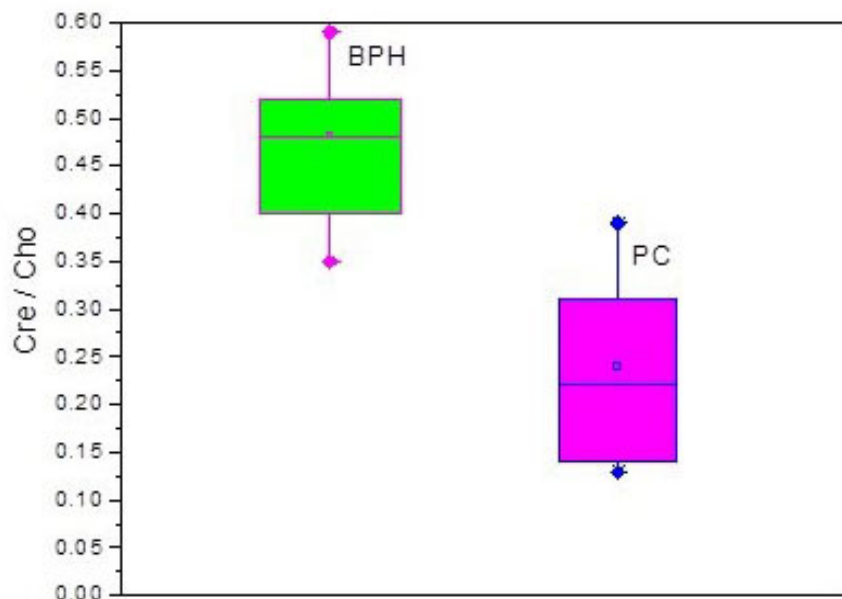


Figure 6. Cre / Cho ratio in benign prostatic hyperplasia (BPH) and prostatic cancer (PC). Same trend as in Figure 4 with consistency, Cre / Cho ratio being lower in PC also indicates the elevated Cho level. (Ref: 64).

(Figure 5) has been found as 3.1.5 and 0.5. respectively, in case of a typical BPH and PC group. The value of metabolite ratio with BPH and PC is consistent with the fact that in case of PC, the metabolism is related to lowering of citrate level and increase in choline level. The same hypothesis can be applied to explain the variation in Cre/Cho ratio. A typical Cre/Cho ratio has been found as 0.4.5 in BPH and 0.2.7 in PC (Figure 6). On the other hand, in the examination of the variation of Ino/Cho ratio (Figure 7), the value comes out lower in case of BPH than in case of PC. This finding apparently is not consistent with peak height (for Cho and Ino) appearing in ^1H spectra of prostate of BPH and PC, because Ino peak is not so high in case of PC whereas Cho peak is much higher. But the total Ino resonates through a wide region of spectra (3.2.8 to 4.0.5 ppm) and in that case the peak integral value will be larger. So it is worthy to assume that in case of PC the increase in total Ino is higher than that in Cho.

It should be also mentioned that Cho peak (3.2.2 ppm) and Cre peak (3.0.4 ppm) often do overlap with each other, and make it difficult to estimate the individual integral peak value. In such cases it is useful to estimate $\text{Cit}/(\text{Cho}+\text{Cre})$ ratio. From experimental results, it has been found that $\text{Cit}/(\text{Cho}+\text{Cre})$ ratio is less in case of prostate adenocarcinoma cell than in BPH cell (Figure 8) or $(\text{Cho}+\text{Cre})/\text{Cit}$ is higher in case of prostate adenocarcinoma cell than in BPH cell. This ratio is significant in the discrimination BPH from PC. The mean $\text{Cit}/(\text{Cho}+\text{Cr})$ was obtained as 2.1.5 with standard deviation 0.5.94 in BPH and the same in PC was obtained as mean 0.4.677 with standard deviation 0.1.41. So examination for prostate cancer detection can include a cutoff value for $\text{Cit}/(\text{Cho}+\text{Cr})$ having some standard deviations above the

mean value for the discrimination of the healthy prostate from the cancer tissue.

The metabolite ratio can be set on a threshold value for the discrimination of healthy and cancerous prostate. However the variation taking place in the determination of metabolite mostly depends on the experimental procedure, patient age, environment etc. So the threshold value is very difficult to be considered universally accepted. Still it is possible to set the threshold value based on a localized intuitive observation. Researchers found different ratio values (Table 2), but the trend in the findings is very consistent.

10. CONCLUDING REMARKS

Applicability of magnetic resonance spectroscopy for the detection of prostate cancer is described. Generally clinicians prescribe for PSA test, DRE, TRUS, and sextant biopsy for the understanding of localization and progression of cancer in the prostate gland. But these techniques are of limited accuracy for discriminating prostate cancer patient from healthy human, whose PSA value lies between 4 and 20 ng/ml. In such cases some prostate metabolites, mainly choline, citrate, creatine and inositol can be taken as good biomarker for the detection of early prostate cancer. A highly positive correlation exists between the variation in these metabolite fluxes and malignancy formation in the prostate even at the early stage. Identification and quantification of the metabolites of prostate tissue is fundamentally important for the characterization of the malignancy. To accomplish this purpose, it is necessary to make use of a range of analytical methods. Mass spectrometry is a powerful and rather

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Table 2. Metabolite ratio in different region of prostate

Metabolite ratio	Metabolite ratio			Reference
	Normal PZ	BPH	Cancer	
Cit/(Cho+Cr)	1.2.8 ± 0.1.4b	1.2.1 ± 0.2.9	0.6.7 ± 0.1.7	Kurhanewicz <i>et al.</i> (65)
Cit/(Cho+Cr)		1.4.6 ± 0.1.1	0.4.5 ± 0.0.6	Kim <i>et al.</i> (96)
Cit/(Cho+Cr)	2.1.6 ± 0.5.6	1.4.3 ± 0.5.8	0.3.1 ± 0.2.5	Kumar <i>et al.</i> (97)
(Cho+Cr)/Cit	0.5.4 ± 0.1.1		2.1 ± 1.3.	Kurhanewicz <i>et al.</i> (91)
(Cho+Cr)/Cit	0.5 ± 0.1.	0.6 ± 0.2.	1.9 ± 0.8.	Kurhanewicz <i>et al.</i> (98)
(Cho+Cr)/Cit		0.5.6 ± 0.2.	2.1 ± 1.3.	Parivar <i>et al.</i> (99)
(Cho+Cr)/Cit	0.2.2 ± 0.1.3		1.6.2 ± 2.0.8	Males <i>et al.</i> (100)
(Cho+Cr)/Cit	0.5.8 ± 0.3.8		4.6.8 ± 3.9.5	Kaji <i>et al.</i> (19)
(Cho+Cr)/Cit	0.6.3 ± 0.2.		1.7.4 ± 1.3.5	Zakian <i>et al.</i> (101)
(Cho+Cr)/Cit	0.3.9 ± 0.2.6		2.6.5 ± 2.3.7	Wetter <i>et al.</i> (102)
(Cho+Cr)/Cit		0.5.9 ± 0.0.3	1.0.8 ± 0.2.	Shukla-Dave <i>et al.</i> (103)
Cho/Cit	0.0.65 ± 0.0.5		0.9.2 ± 1.2.8	Reinsberg <i>et al.</i> (71)

PZ: peripheral zone; BPH: benign prostate hyperplasia

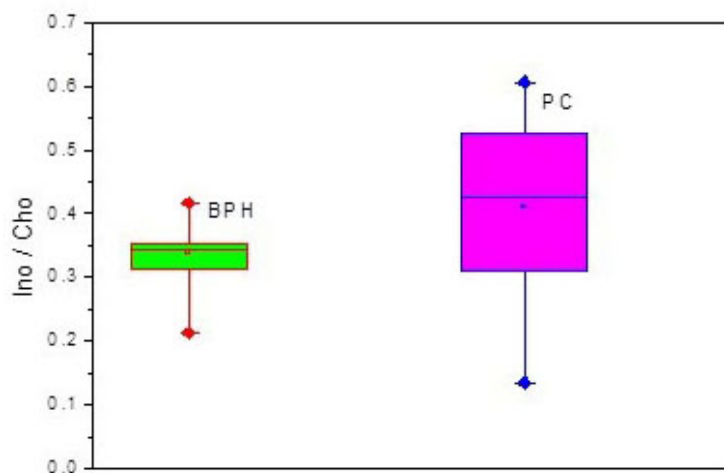


Figure 7. Ino / Cho ratio in benign prostatic hyperplasia (BPH) and prostatic cancer (PC). The ratio being higher in PC is attributed to the increase in Ino peak integral in *in-vivo* spectra. (Ref: 64).

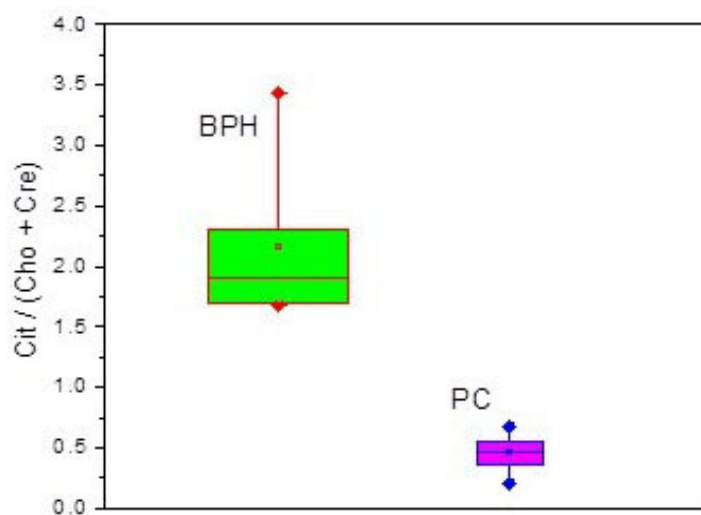


Figure 8. Cit / (Cho+Cre) ratio in benign prostatic hyperplasia (BPH) and prostatic cancer (PC). This investigation is important for the determination of the threshold limit on the Cit / (Cho+Cre) ratio for discrimination of carcinomas and healthy prostate (Ref: 64, 76).

straightforward tool for such types of analysis and is widely used by chemists and biologists, but complementary information from other techniques, particularly MRS, is invaluable for the identification of prostate metabolites. The diagnosis and localization of cancer in the prostate can be efficiently done by the estimation of metabolite ratio using NMR spectroscopy noninvasively. In the process of the determination of metabolite ratio, there exists some threshold value depending on the instrumentation, environment, and ethnicity for the differentiation of the prostate malignancy from healthy gland.

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