ISSN: 2378-3419

Kumar et al. Int J Cancer Clin Res 2024, 11:192
DOI: 10.23937/2378-3419/1410192
Volume 11 | Issue 1
Open Access

International Journal of
Cancer and Clinical Research

RESEARCH ARTICLE

# Blood Plasma Metabolomic and Lipidomic Signatures of Prostate Cancer Using NMR Spectroscopy

***Pradeep Kumar[1*], Virendra Kumar[1], Rajeev Kumar[2], Sanjay Sharma[3], Sanjay Thulkar[3] and M A Khan[4]***

[1]*Department of NMR & MRI Facility, All India Institute of Medical Sciences, New Delhi, India*

[2]*Department of Urology, All India Institute of Medical Sciences, New Delhi, India*

[3]*Department of Radio-diagnosis, All India Institute of Medical Sciences, New Delhi, India*

[4]*Department of Biostatistics, All India Institute of Medical Sciences, New Delhi, India*

***Corresponding author:** *Dr. Pradeep Kumar, Department of NMR & MRI Facility, All India Institute of Medical Sciences (AIIMS), New Delhi-110029, India, Tel: +91-9873282071*

## Abstract

**Background:** Prostate cancer (PCa) with metastasis remains an incurable disease requiring early diagnosis and effective treatments. Bone metastasis is the predominant cause of mortality from PCa. Aggressive PCa metastasizes or spreads to bones, such as the hip, spine, and pelvis. Bone metastatic PCa is considered an advanced stage. This study was designed to determine the association of these metabolites/lipids with the progression of metastases cascade in PCa. Thus, the present study is based on 1H-NMR to investigate the blood plasma metabolomic and lipidomic profile to distinguish PCa patients with metastases from those without metastases for determining potential biomarker/s for progression to metastases cascade.

**Methodology:** Blood samples were collected from PCa patients (n = 35) and bone metastases (n = 30). Proton spectra of blood plasma samples were carried out at a 700 MHz spectrometer using 1D CPMG with pre-saturation. Partial least squares-differential analysis (PLS-DA) orthogonal PLS-DA (OPLS-DA), and VIP score and pathways analysis were using MetaboAnalyst 6.0.

**Results:** Significantly higher concentrations of lactate, alanine, glutamate, pyruvate, glycine, and creatinine while lower levels of phenylalanine were found in metastases PCa as compared to non-metastases patients using metabolic profiling. Lipidomic profiling analysis, 3-hydroxybutyrate (3-HOB), Acetate, Acetoacetate, dimethylamine (DMA), choline, and glycerophosphocholine (GPC) were found in higher concentrations in blood plasma samples of metastases PCa as compared to non-metastases PCa patients. Pathway enrichment analysis using the KEGG revealed the potential involvement of pyruvate metabolism & fatty acid biosynthesis in metastases PCa progression and cascade.

**Conclusion:** The present study highlights that amino acids, phospholipids, ketone bodies, and energy metabolites are potential biomarker/s for the progression to bone metastases cascade of PCa. NMR-based metabolomic and lipidomics profiling provides novel insights into the pathophysiological mechanism of cancer progression to the metastases of PCa to monitor treatment outcomes.

## Keywords

Prostate cancer, Metastases, Blood plasma, Metabolomic, Lipidomic, NMR spectroscopy

## Introduction

Prostate cancer (PCa) is one of the most commonly diagnosed malignancies in men aged over 50-years-old [1]. PCa patients may not present with specific symptoms during the initial course of the disease [2]. The most common screening methods are based on the measurement of serum prostate-specific antigen (PSA) and/or digital rectal examination (DRE), followed by transrectal ultrasound (TRUS) guided systematic biopsy [3,4]. Thus, the major problems are unnecessary repeat invasive biopsies and over-diagnosis. Hence, there is a need for potential and specific robust biomarker/s to differentiate PCa from non-aggressive tumors. Therefore, detection and evaluation of the metabolites and lipid species in patients with PCa are the hotspots in current research.

Metabolomics is a rapidly developing area to provide

**Citation:** Kumar P, Kumar V, Kumar R, Sharma S, Thulkar S, et al. (2024) Blood Plasma Metabolomic and Lipidomic Signatures of Prostate Cancer Using NMR Spectroscopy. Int J Cancer Clin Res 11:192. doi.org/10.23937/2378-3419/1410192

**Accepted:** November 23, 2024: **Published:** November 25, 2024

valuable information about diseases, of which a subset is lipidomics defined as a study of the content and function of whole lipids in the cell or tissue in biological systems [5,6]. To put it simply, lipidomics is the measurement of all the lipids inside a specific biological entity. Lipids play roles in membrane structure, energy storage, and signal transduction as well as in PCa [7,8]. Few studies indicated that lipids may serve as promising biomarker/s in the early diagnosis and individualized treatment of cancer [7-11]. Lipids are crucial in many cellular processes, including cell survival, proliferation, interaction, and apoptosis. Dysfunction of lipids metabolism was found to be associated with the pathogenesis of many diseases including PCa [11]. Both metabolomic and lipidomics are quantitative of metabolites and lipids determined in cells, tissue, body fluid, or organisms at a scheduled time, as an emerging field about the systemic analysis of lipids and their metabolites and interactions [12]. Studies on lipidomics suggested that lipids play many important functions in living organisms, especially in the transformation, progression, and metastasis of PCa [12].

High-throughput analytical chemical techniques such as chromatography coupled to mass spectrometry (MS) and nuclear magnetic resonance (NMR) spectroscopy have been used in metabolomics and lipidomics along with univariate and multivariate analyses to provide information on a large number of metabolites/lipids in particular those with altered level between healthy and cancer groups [12]. Metabolomics and lipidomics in PCa have been mainly performed by NMR and MS, according to the purpose of the study and the characteristics of measured metabolite and lipids.

However, limited comprehensive studies have been performed on the blood plasma of PCa patients using proton ($^1$H) NMR-based metabolomics and lipidomics for simultaneous measurement and quantification of metabolites and lipids [6-12]. Thus, the present study investigates the differences in metabolomic and lipidomics profiling of blood plasma in metastases with PCa patients and non-metastases PCa patients using $^1$H NMR spectroscopy.

The purpose of this study is to explore the diagnostic value of metabolites and lipids for PCa. Thus, the aim of the present study will be (a) To investigate the blood plasma metabolomics and lipidomics profiles to distinguish patients with metastases PCa and non-metastases PCa using $^1$H-NMR spectroscopy for establishing potential biomarker/s, (b) Further attempt to identify a set of putative biomarker/s to correlate with metabolic pathways for better understanding of the pathogenesis of metastasis PCa.

## Materials and Methods

### Clinical and pathological details of PCa patients

All participants were recruited in this study based on clinical diagnosis of lower tract urinary symptoms (LUTS) and elevated PSA level, and/or DRE, biopsy results, Gleason score, and bone scan. Blood samples were collected from all the subjects and categorized as follows PCa patients with metastases and without metastases. 30 patients with PCa metastases and 35 patients with non-metastases PCa were recruited from the PCa Department of Urology at AIIMS, New Delhi, India. Informed consent was taken and Institute Ethics Committee approved the study. Demographic and clinical characteristics of patients with metastases and patients with non-metastases PCa are given in Table 1.

### Sample collection and processing

A total 65 of blood were collected from patients with metastases PCa (n = 30) and patients with non-metastases PCa (n = 35) in the morning pre-prandial after overnight fasting in sodium heparin vacutainer. The samples were centrifuged at 5,000 rpm at 4 °C for 10 min. The supernatant (blood plasma) was aliquot into 2 mL sterile vials and immediately stored at -80 °C until NMR analysis. Then 400 µL D2O was added to 200 µL of blood plasma sample with 0.5 mM sodium formate and 0.5 mM sodium trimethyl-silyl-[ 2, 2, 3, 3- H4]-propionate (TSP). The total 600 µL volume of the sample was transferred into a 5 mm NMR sample tube. TSP was added as an internal standard for chemical shift reference at 0.00 ppm. Since TSP interacts with large biomolecules (proteins and lipids) present in blood plasma, it may influence the quantitative measurement of metabolites. Hence formate was used as a concentration standard at 8.46 ppm for quantification of metabolites.

### 1D and 2D proton NMR spectroscopy

The $^1$H-NMR experiments were performed using a spectrometer operating at 700 MHz. A one-dimensional (1D) NMR spectrum with water suppression was acquired followed by CPMGT2 sequence. Two-dimensional (2D) correlated spectroscopy (COSY) and total correlation spectroscopy (TOCSY) were acquired using standard software provided by the manufacturer.

### NMR parameters for blood plasma sample

All proton (1H) NMR spectra were acquired 700 MHz spectrometer operating at 699.94 MHz at 25 °C. The one-dimensional (1D) spectrum was acquired using a Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence with a pre-saturation pulse for water peak suppression. The typical parameters used for 1H-NMR experiments were: number of scans = 64; data points = 32K; spectral width = 9124.1 Hz; a total spin echo delay of 15 ms and a repetition time (TR) of 70 s.

Two-dimensional (2D) experiments such as correlation spectroscopy (COSY) and total correlation spectroscopy (TOCSY) were also carried out for the unambiguous assignment of various metabolite peaks. The parameters used for TOCSY experiments were: data points 2K in F2 dimension; spectral width 9800 Hz; with

relaxation delay of 2.5 s. The number of t1 increments was 400 with 16 scans and a mixing time of 80 ms.

### NMR data processing

The $^{1}$H-NMR spectra were processed on a Dell 39N, PC, Red Hat Enterprise Linux workstation using the Varian software, Vnmrj 2.3A. The free induction decays (FID) were zero-filled to 64 K and an exponential weighting function corresponding to 0.3 Hz line broadening was applied before the Fourier transformation (FT) for blood plasma samples. The 2D NMR spectrum data was processed using automated processing with the data size of (2K × 2K) with Gaussian weighting function in both F2/F1 dimensions.

### Quantitation of blood plasma metabolites

The concentrations (µM) of metabolites were determined by using Chenomx NMR Suite 7.5 software (Chenomx Inc. Edmonton, Canada). The concentration of only those metabolites that showed well-resolved resonance in the proton 1D (CPMG) NMR spectra of blood plasma samples of PCa patients with metastasis and those PCa patients without metastasis. Chenomx software allows fitting spectral lines using the standard metabolite library for 700 MHz $^{1}$H-NMR spectra. Peak fitting concerning the internal formate signal facilitated the determination of concentrations for identified metabolites. In addition, the identification and quantification of blood plasma metabolites were also cross-checked from the Human Metabolome Database (HMDB) and literature.

### Statistical analysis

The statistical analysis was done using SPSS 20.0 (SPSS Inc. Chicago, IL, USA) software and MetaboAnalyst 6.0 web server.

### Univariate analysis

The concentration values of metabolites were reported as median and range. A p-value < 0.05 was taken as significant. Metabolomic and lipidomics data analyses were carried out using univariate analyses such as the Mann-Whitney U test. The Mann-Whitney U test was used for the comparison of metabolite concentration between the two groups.

### Multivariate analysis

The supervised orthogonal partial least-squares discriminate analysis (OPLS-DA) and variable importance to projection (VIP) score methods were applied to improve class discrimination between groups of samples and to further minimize the possible contribution of inter-group variability. The robustness and validation of the model of tenfold internal cross-validation (ICV) were applied, from which R2Y (goodness of fit parameter) and Q2 (predictive ability parameter, estimated by cross-validation) were calculated. Finally, these OPLS-DA models were validated by random permutation analysis (n = 100). Multivariate analyses based on the hierarchical clustering heatmap analysis and random forest analysis were performed using MetaboAnalyst 6.0 MetaboAnalyst 6.0 was used Metabolic Set Enrichment Analysis (MSEA) for potential metabolic analysis and Kyoto Encyclopaedia of Genes and Genomes (KEGG) database were carried out.

## Results

Figure 1 shows the representative $^{1}$H spectrum (1D CPMG) of a patient with metastases PCa (a) and from non-metastases patient (b). Table 1 shows the demographic and clinical characteristics of patients with metastases PCa and patients with non-metastases

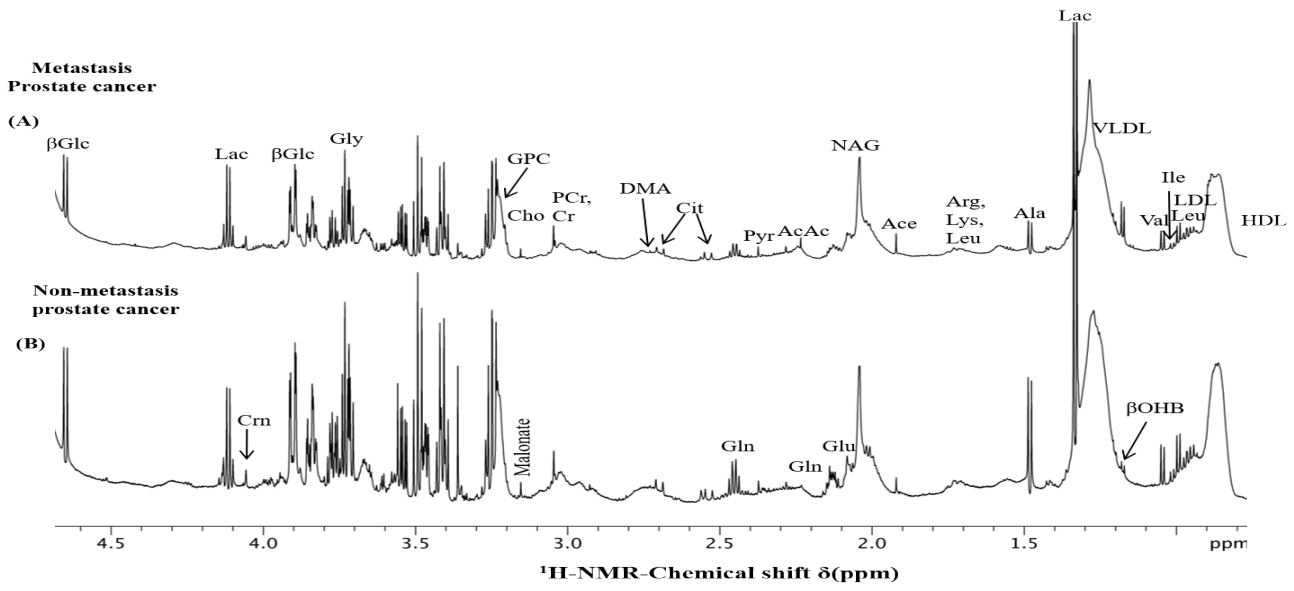


**Figure 1:** Representative one-dimensional (1D) aliphatic region and lipid region of $^{1}$H NMR (700 MHz) spectrum of blood plasma of a bone metastases PCa patient and non-metastases PCa patients for metabolomics and lipidomics profiling.

**Table 1:** Demographic and clinical characteristics of patients with metastases and patients with non-metastases PCa.

| Variables | Metastases with PCa patients N = 30 Median, (range) | Non-metastases PCa patients N = 35 Median, (range) |
|---|---|---|
| Age (years) | 69.5 (55-83) | 69 (47-75) |
| Weight (kg) | 54.5 (45-76) | 65 (46-83) |
| BMI (kg $m^{-2}$) | 21.8 (18.5-25.3) | 23.13(19.8-26.2) |
| PSA (ng/mL) | 205.38 (50.20-4166.6) | 21.42 (6.03-350.33) |
| Prostate size (ml) | 37.4 (12.7-64) | 46 (18- 89) |
| Gleason scores | | |
| 3 + 3 | NA | 7 |
| 3 + 4 | 4 | 10 |
| 4 + 3 | 5 | 8 |
| 4 + 4 | 3 | 3 |
| 3 + 5 | 2 | 2 |
| 4 + 5 | 12 | 5 |
| 5 + 5 | 4 | 0 |

BMI: Body Mass Index; PSA: Prostate-Specific Antigen; NA: Not Applicable

**Table 2:** Comparison of the concentration (M) of metabolites in the blood of PCa patients with metastases to PCa patients. Statistically significant ($p < 0.05$) metabolites involved in the discrimination between the two groups were calculated according to the Mann-Whitney U test.

| Metabolites | Concentration of blood plasma metabolites/lipids (μM) | | | | | | p-value |
|---|---|---|---|---|---|---|---|
| | Patients with metastases PCa n = 30 (Median, range) | | | Patients with PCa N = 30 (Median, range) | | | |
| | Median | Min | Max | Median | Min | Max | |
| **Metabolomic profiling** | | | | | | | |
| Leucine | 789.86 | 435.19 | 1698.70 | 943.67 | 536.98 | 1634.46 | 0.01 |
| Isoleucine | 281.68 | 145.35 | 450.05 | 324.11 | 116.91 | 706.91 | 0.02 |
| Valine | 349.90 | 188.97 | 579.11 | 386.75 | 120.42 | 743.73 | 0.02 |
| Lactate | 2684.39 | 1314.31 | 5691.74 | 4563.20 | 2260.23 | 10056.14 | < 0.001 |
| Alanine | 547.47 | 309.56 | 1251.30 | 871.45 | 342.45 | 1386.03 | < 0.001 |
| Glutamate | 457.16 | 284.29 | 865.03 | 639.48 | 409.21 | 1434.29 | < 0.001 |
| Pyruvate | 68.68 | 32.72 | 110.17 | 130.68 | 51.16 | 404.27 | < 0.001 |
| Glutamine | 869.93 | 197.22 | 1538.60 | 960.78 | 505.21 | 1666.93 | 0.14 |
| Creatine | 86.80 | 43.63 | 156.44 | 165.72 | 61.74 | 456.78 | < 0.001 |
| Phosphocreatine | 60.49 | 7.58 | 193.95 | 50.25 | 15.35 | 485.79 | 0.09 |
| Glycine | 283.04 | 43.13 | 810.72 | 509.14 | 179.26 | 1007.50 | < 0.001 |
| Creatinine | 147.91 | 43.22 | 312.72 | 273.21 | 113.47 | 759.86 | < 0.001 |
| Tyrosine | 186.51 | 87.13 | 559.54 | 253.90 | 117.08 | 501.05 | 0.04 |
| Histidine | 136.36 | 75.61 | 255.18 | 161.34 | 48.60 | 290.29 | 0.01 |
| Phenylalanine | 195.62 | 82.29 | 592.26 | 292.76 | 126.81 | 790.58 | 0.004 |
| Glucose | 4689.70 | 3240.37 | 8333.94 | 5367.08 | 2683.88 | 17999.70 | 0.11 |
| **Lipidomic profiling** | | | | | | | |
| Betaine | 203.85 | 87.07 | 1798.20 | 392.17 | 169.42 | 855.22 | 0.33 |
| 3-HOB | 511.15 | 218.48 | 800.67 | 762.91 | 213.54 | 2158.81 | < 0.001 |
| Acetate | 57.11 | 36.88 | 120.98 | 128.18 | 21.13 | 356.20 | < 0.001 |
| Acetoacetate | 102.44 | 44.48 | 320.35 | 234.08 | 84.91 | 423.69 | < 0.001 |
| DMA | 57.15 | 6.30 | 140.85 | 124.42 | 57.60 | 312.75 | < 0.001 |
| Malonate | 61.05 | 0.67 | 1033.14 | 113.96 | 33.91 | 461.96 | 0.05 |
| Choline | 85.92 | 51.58 | 160.59 | 162.92 | 64.92 | 491.98 | < 0.001 |
| GPC | 95.55 | 49.05 | 192.75 | 147.97 | 77.10 | 520.50 | < 0.001 |

3HOB: 3 hydroxybutyrate; DMA: Dimethylamine; GPC: Glycerophosphocholine

PCa. In all, 24 metabolites were assigned using 1D, and 2D NMR are given in Table 2. A p-value < 0.05 was considered significant. PLS-DA score plots and permutation tests are shown in Figure 2. Metabolites/ lipids showed discriminate performance using OPLS-DA score plots and validated models by permutation tests (n = 100) are shown in Figure 2. Figure 3 shows VIP scores for metabolites/ lipids with the highest contribution to the separation of the studied groups are presented. Figure 4A (metabolomic profiling) and Figure 4B (lipidomic profiling) show a heatmap analysis of significant metabolites/lipids differentially abundant in blood plasma samples of metastases patients with PCa as compared to non-metastases patients. MSEA analysis using the KEGG revealed the potential involvement of a list of prominent metabolic pathways shown in Figure 5 and Figure 6, respectively.

## Univariate analysis result

The NMR-based metabolomic profile analysis showed significantly ($p < 0.05$) higher concentrations of metabolites such as lactate, alanine, glutamate, pyruvate, creatine, phosphocreatine, glycine, creatinine, and lower concentrations of tyrosine, histidine, phenylalanine, leucine, isoleucine, valine in blood plasma metastases PCa as compared to non-metastases patients are given in Table 2.

Furthermore, the NMR-based lipidomic profiling identifies higher concentrations of lipids like 3-hydroxybutyrate (3HOB), choline, dimethylamine (DMA), glycerophosphocholine (GPC), malonate acetate, and acetoacetate were observed in blood plasma samples and show in Table 2.

## Multivariate results

The results obtained from supervised OPLS-DA and VIP score of metabolites concentration in blood plasma showed separation between PCa patients with metastases and those without metastases. According to the VIP scores, the higher the value, the greater the discriminatory power of the metabolites. The metabolites such as pyruvate, creatine Lactate, creatinine glutamate, glycine, and alanine with VIP score of > 1.0 (see in Figure 3A) while lipids such as 3HOB, GPC, malonate, and DMA with VIP score of > 1.0 were the most discriminate metabolites in PCa patients with metastases and those without metastases (see Figure 3B). The present work investigates the differential

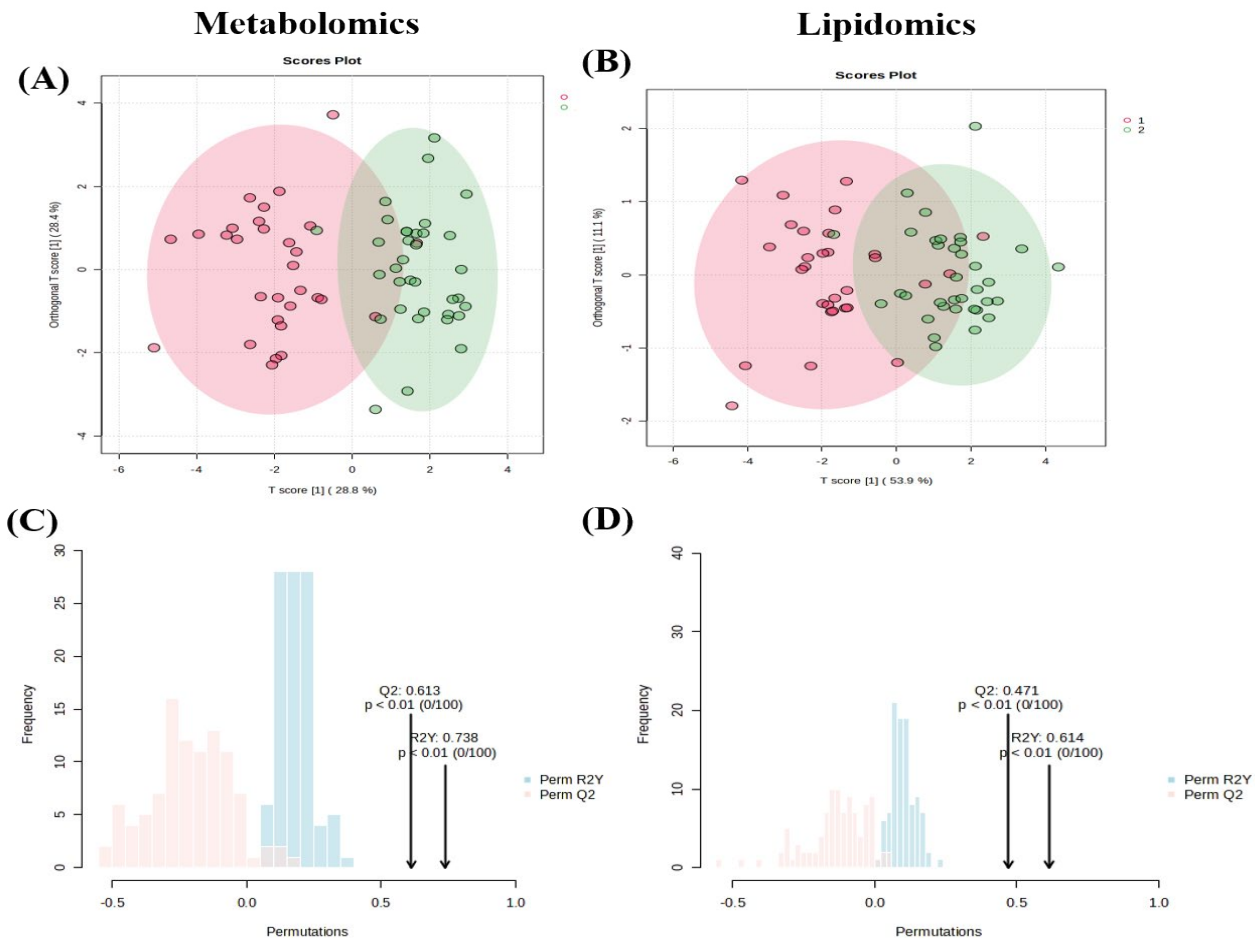


**Figure 2:** Results of multivariate analysis of metabolomic and lipidomic profiles. Score plots were obtained in OPLS-DAs of metabolite profiles of patients with metastases PCa (n = 30) and non-metastases PCa patients (n = 35) in blood plasma metabolomic (A) and lipidomics (B), The OPLS-DA model obtained for blood plasma profiling was cross-validated with permutation analysis (n = 100) and observed statistical results at $p < 0.01$ metabolomic (C) and lipidomics (D).

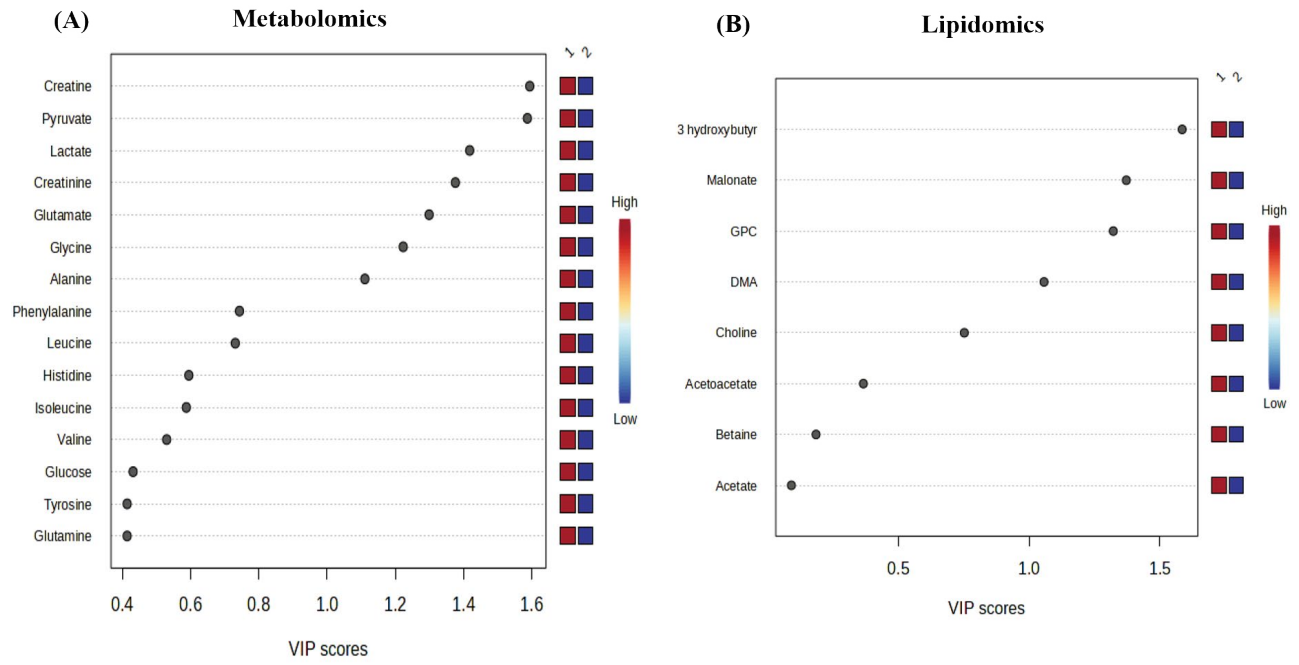


**Figure 3:** Variable importance in projection (VIP) scores in OPLS-DAs of blood plasma metabolomic (A) and lipidomics (B) profiles in two studied groups: patients with metastases PCa (n = 30) and non-metastases PCa patients (n = 35). VIP scores for metabolites/ lipids with the highest contribution to the separation of the studied groups are presented.

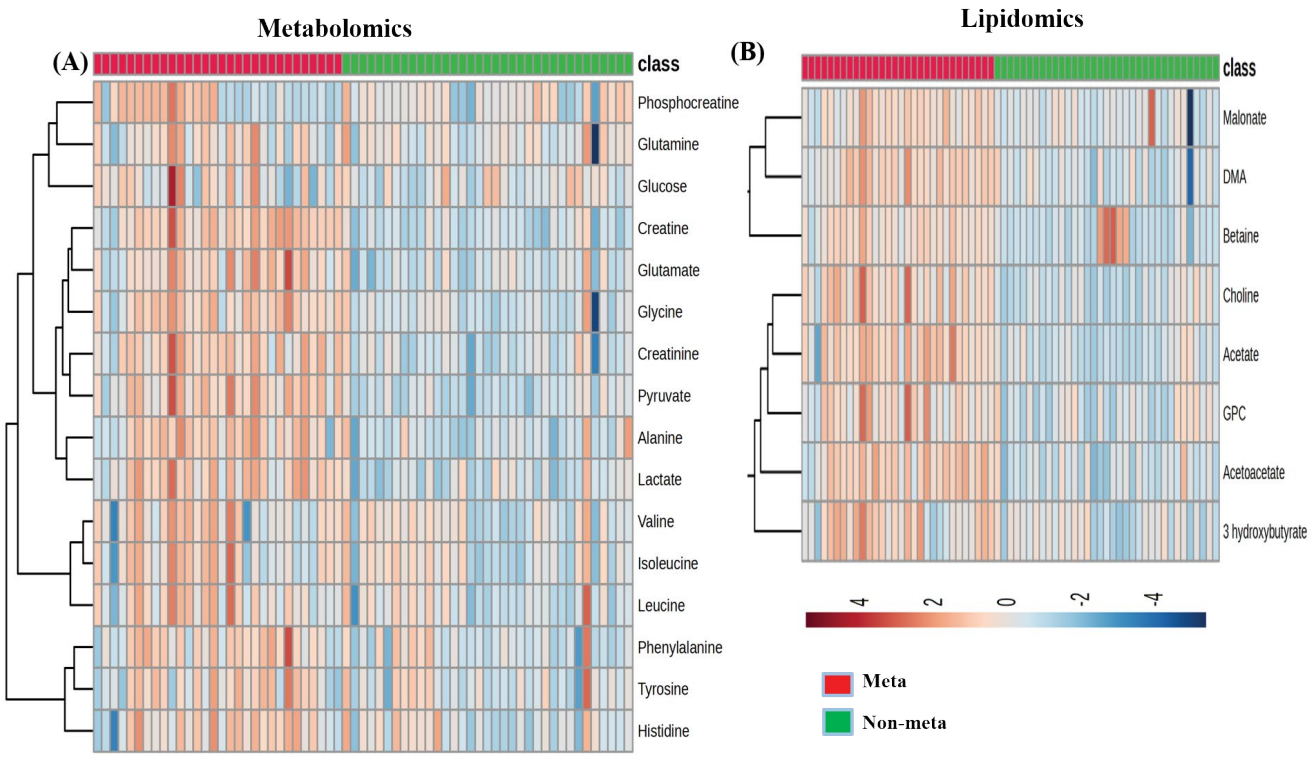


**Figure 4:** Heatmap analysis of significant metabolites/lipids differentially abundant in blood plasma samples of metastases patients with PCa as compared to non-metastases patients (A) Metabolomic (B) Lipidomic profiling.

abundance of important metabolites and lipids in blood plasma samples from PCa patients with metastases compared to non-metastases patients in a metabolomic and lipidomic study using a heatmap analysis shown in Figure 4.

The PCa patients with metastases and those without metastases patients were discriminated with R2Y = 0.738, Q2 = 0.613 for metabolomic profiling which suggested that the supervised OPLS-DA model was robust (see Figure 2C). The OPLS-DA model obtained for blood plasma metabolomic profiling was cross-validated

with permutation analysis (n = 100) and observed statistical results at $p < 0.01$ were shown in Figure 2C. Furthermore, patients with PCa metastases and those without metastases PCa patients were discriminated with R2Y = 0.614, Q2 = 0.471 for lipidomic profiling which suggested that the supervised OPLS-DA model was robust (see Figure 2C). The OPLS-DA model obtained for blood lipidomics profiling was cross-validated with permutation analysis (n = 100) and observed statistical results at $p < 0.01$ were shown in Figure 2D.

According to the *p*-values from the pathway enrichment analysis, the pathways containing at least two components of the prominent metabolites are shown in Figure 5, and lipidomics-derived pathways are shown in Figure 6. Based on KEGG database analysis, pyruvate metabolism, and glycine & serine metabolism

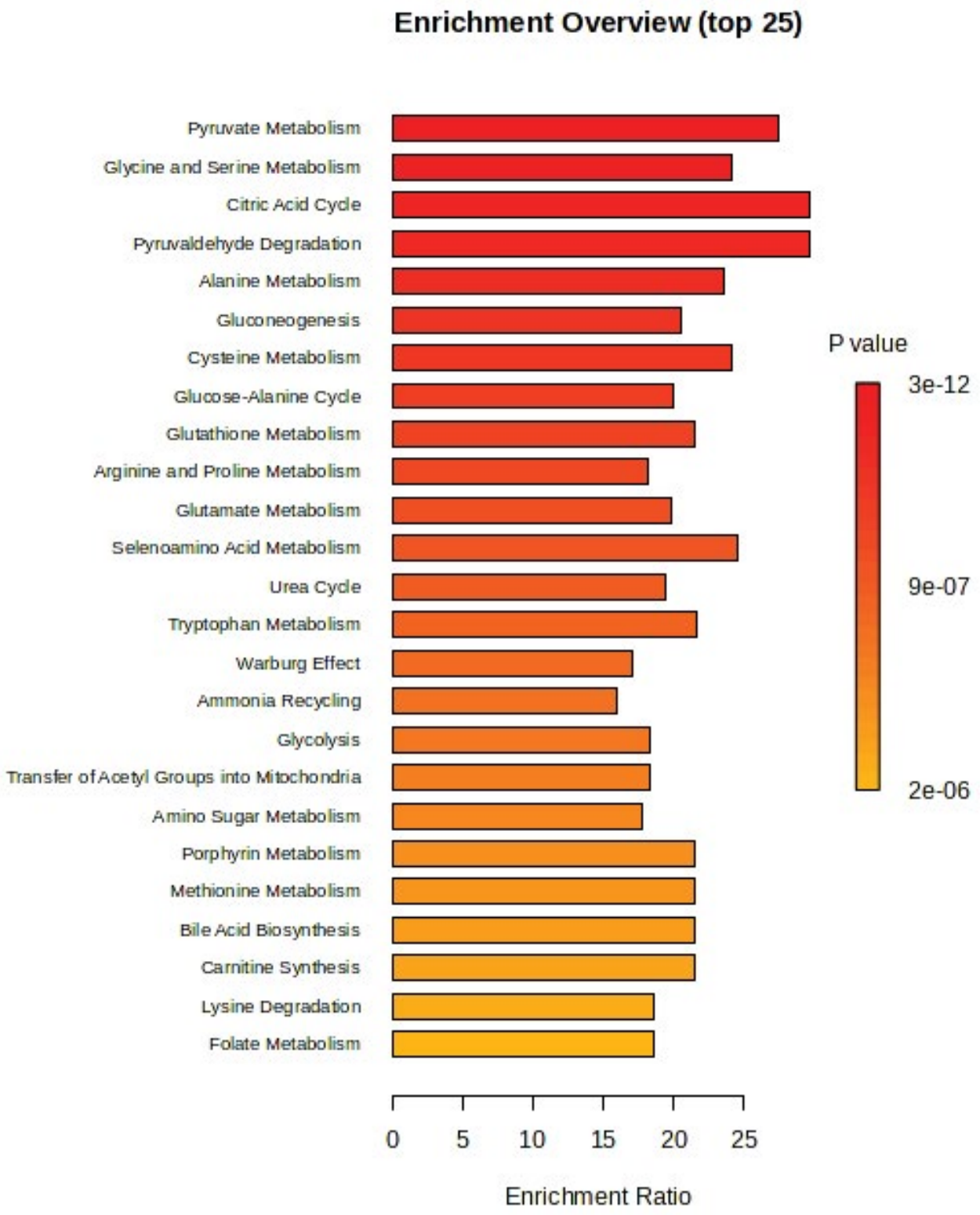


**Figure 5:** Metabolic Set Enrichment Analysis (MSEA) showing altered significant metabolites revealed in bone metastases PCa patients.

were the associated pathways with p-values < 0.05 shown in Figure 5. Figure 6 shows the lipids-associated pathways based on KEGG database analysis, fatty acids biosynthesis, and phospholipids biosynthesis.

## Discussion

In this study, we integrated data from metabolomics and transcriptomics and found significant alterations in several metabolic pathways in prostate cancer at both the metabolic and transcriptional levels. It is important to thoroughly define lipid metabolism in PCa to gain a new understanding of the genesis and evolution of prostate tumors as well as to identify prospective biomarkers that may be more accurately used for enhanced detection. Significant changes in metabolites/lipids were found in bone metastases PCa as compared to non-metastases patients. Pathway enrichment analysis using the KEGG revealed the potential involvement of pyruvate metabolism and fatty acid biosynthesis in PCa. The present study highlights those amino acids, phospholipids, ketone bodies, and energy metabolites are potential biomarker/s for the progression to bone metastases cascade of PCa.

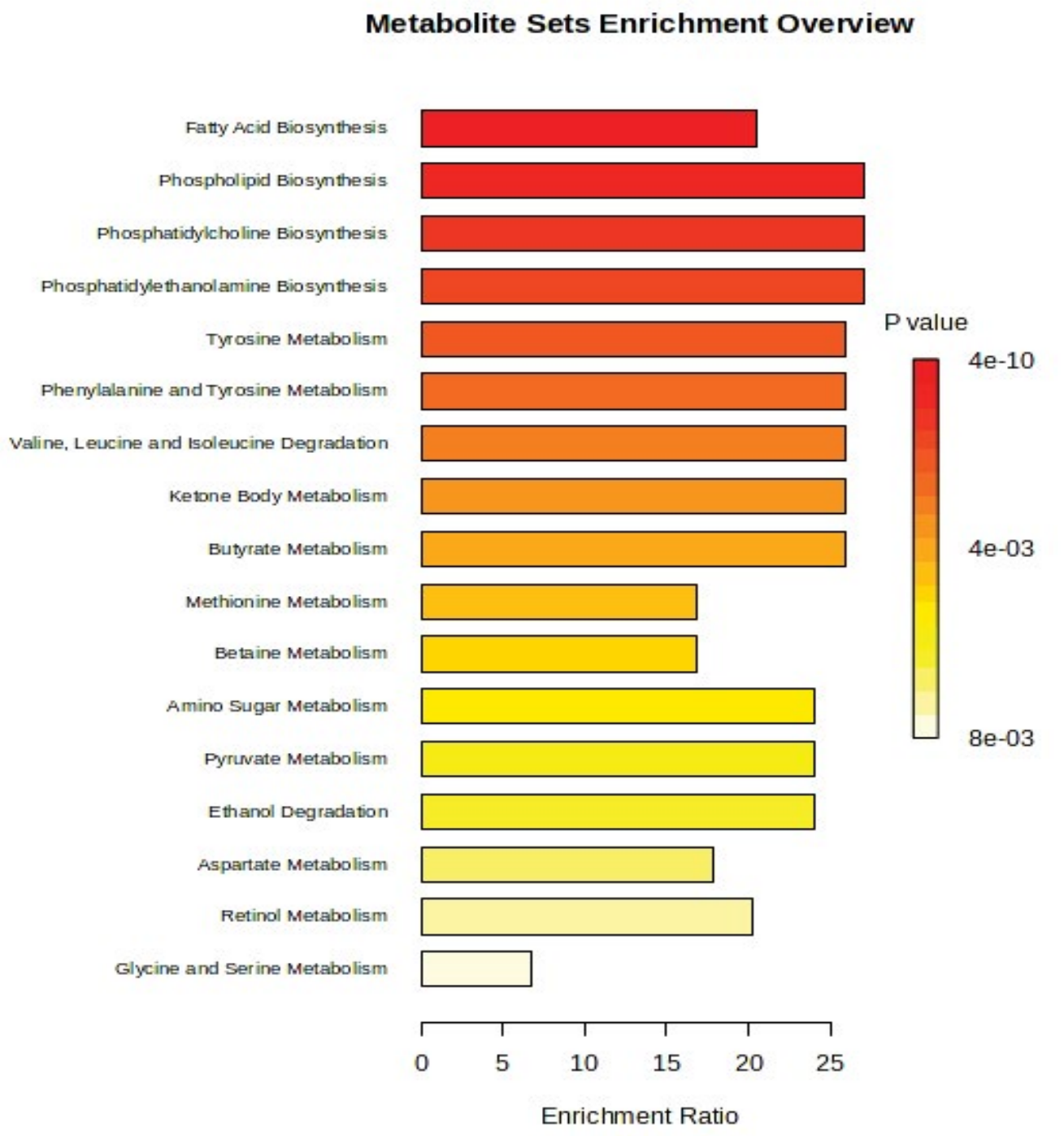


**Figure 6:** Metabolic Set Enrichment Analysis (MSEA) showing altered significant lipids and their derived fatty acids revealed in bone metastases PCa patients.

### Metabolomic profiling

Significantly higher concentration of metabolites such as lactate, alanine, glutamate, pyruvate, creatine, phosphocreatine, glycine, and creatinine while lower levels of tyrosine, histidine phenylalanine, leucine, isoleucine, and valine were found in blood plasma sample of bone metastases PCa as compared to non-metastases patients.

The data showed a significantly lower concentration of amino acids such as valine, isoleucine, and leucine in the blood plasma of patients with metastases PCa in comparison to non-metastases conditions. They are the only amino acids or BCAA that are generally metabolized in skeletal muscle. Thus, in the case of the shortage of energy demand in muscle, BCAA Can be converted into the acetyl-CoA, organic molecules that enter the TCA cycle [13]. The metabolic flexibility afforded by multiple inputs into the TCA cycle allows cancer cells to adequately respond to other fuels available in the changing microenvironment during the evolution of the malignant transformation of cancer cells [13]. Additionally, patients with PCa had a significantly lower concentration of aromatic amino acids such as tyrosine and phenylalanine. Phenylalanine is an essential amino acid obtained from the diet and is required for the production of the nonessential amino acid tyrosine [13]. In cancer cells, tyrosine breaks down into pyruvate and fumarate and enters the TCA cycle as an energy requirement for tumorigenesis. The reduction of these aromatic amino acids may thus be associated with high protein turnover needed for rapid cell proliferation [13]. The significantly higher concentration of alanine in bone metastases PCa compared to non-metastases PCa is possibly accompanied by overexpression of the metabolism of relevant amino acids and altered TCA cycle in hypoxia [14,15]. The augmented level of alanine is consistent with the earlier observation that the speed of amino acid metabolism befits augmenting in PCa and alanine was found to be at a considerably higher level in PCa cell lines, serum, and biopsy tissues [14,15]. Our findings of a higher level of pyruvate in bone metastases PCa compared with PCa also confirm the outcomes of an augmented level of transamination from pyruvate to alanine, which concurs with earlier [14,15]. Further higher concentration of glycine was found in patients with bone metastases PCa as compared to PCa, which was similar to that seen in colorectal, head, and neck cancers studies by NMR [15-18]. The reliance on glycine for the proliferation and development of cancer cells was studied by gene expression analysis, which revealed enhanced expression of glycine biosynthesis enzymes in tumor cells. Further, glycine is an important source of one carbon unit for purine synthesis to promote rapid cancer cell proliferation and tumorigenesis [14]. Significantly higher levels of energy metabolites such as creatinine, phosphocreatine, and creatine were seen in blood plasma samples of patients with bone metastases PCa as compared to PCa [15,19]. Our results revealed elevated levels of energy demand for the aggressiveness of the tumor. Our data showed elevated blood plasma lactate in bone metastases PCa patients in comparison to non-metastases PCa. It was reported that a higher concentration of lactate seen in PCa patients was positively correlated with an increased risk of cancer [20,21].

### Lipidomics profiling

Eight lipids were assigned and quantified in the proton NMR spectrum acquired from blood plasma samples of PCa patients with metastases and non-metastases. Subsequently, univariate and multivariate such as OPLS-DA plot and VIP Score were applied to find lipids to distinguish metastases PCa from the non-metastases PCa group. Significantly higher concentration of 3-HOB, choline, GPC, malonate acetate, acetoacetate, and DMA in the blood plasma of metastases PCa from the non-metastases PCa group.

Our data revealed that a significantly higher concentration of membrane components such as choline and GPC were seen in patients with bone metastases PCa as compared to non-metastases, signifying an alteration in phospholipid metabolism related to tumor proliferation and progression [22,23].

A higher level of ketone body including acetoacetate, acetate, and 3-HOB was seen in bone metastases PCa patients [24]. These are produced by the liver from fatty acid oxidation and converted into acetyl CoA, which then enters the TCA cycle. Higher levels of acetate may reflect increased utilization of lipid demands to meet the energy requirements for cell growth and proliferation in metastatic cancer cells [24]. Dimethylamine is an intermediate product of Cho metabolism. Results showed a significantly higher concentration of DMA in patients with PCa metastases indicating a change in choline metabolism. The higher levels of DMA in PCa may be associated with membrane phospholipids metabolism needed for cancer cell proliferation [25]. A higher concentration of malonate was seen in the blood plasma of the patients with PCa metastases. Malonate is a crucial component of lipid synthesis and membrane biogenesis for rapidly proliferating cells in PCa [26].

Integrating metabolomic and lipidomic data can enhance our understanding of prostate cancer biology, enabling the identification of novel biomarkers for early detection and monitoring strategies. Overall, NMR-based metabolomic and lipidomic analyses hold promise for advancing precision medicine in PCa management.

## Conclusion

NMR-based metabolomic and lipidomics profiling provides novel insights into the pathophysiological mechanism of cancer progression to the metastases of PCa to monitor treatment outcomes. The present

study highlights that amino acids, phospholipids, ketone bodies, and energy metabolites are potential biomarker/s for the progression to bone metastases cascade of PCa.

## Author Contributions

Pradeep Kumar, Virendra Kumar, Rajeev Kumar, Sanjay Sharma, Sanjay Thulkar, M A Khan. Pradeep Kumar was patient data collection, NMR data acquired, analyzed, and wrote the manuscript draft. Pradeep Kumar, Virendra Kumar, Rajeev Kumar, Sanjay Sharma, Sanjay Thulkar, and M A Khan contributed to the discussion, manuscript draft reviewed the final manuscript.

## Acknowledgments

The NMR facilities (700 MHz NMR spectrometers) provided by the Department of NMR, AIIMS, New Delhi are greatly acknowledged and appreciated.

## Conflicts of Interest Statement

The authors declare there are no conflicts of interest.

## References

1. Siegel RL, Giaquinto AN, Jemal A (2024) Cancer statistics, 2024. CA Cancer J Clin 74: 12-49.
2. Prensner JR, Rubin MA, Wei JT, Chinnaiyan AM (2012) Beyond PSA: The next generation of prostate cancer biomarkers. Sci Transl Med 4: 127rv3.
3. Bokhorst LP, Bangma CH, van Leenders GJ, Lous JJ, Moss SM, et al. (2014) Prostate-specific Antigen-Based Prostate Cancer Screening: Reduction of prostate cancer mortality after correction for nonattendance and contamination in the rotterdam section of the european randomized study of screening for prostate cancer. Eur Urol 65: 329-336.
4. Descotes JL (2019) Diagnosis of prostate cancer. Asian J Urol 6: 129-136.
5. Belhaj MR, Lawler NG, Hoffman NJ (2021) Metabolomics and lipidomics: Expanding the molecular landscape of exercise biology. Metabolites 11: 151.
6. Lima AR, Carvalho M, Aveiro SS, Melo T, Domingues MR, et al. (2022) Comprehensive metabolomics and lipidomics profiling of prostate cancer tissue reveals metabolic dysregulations associated with disease development. J Proteome Res 21: 727-739.
7. Pardo-Rodriguez D, Santamaría-Torres M, Salinas A, Jiménez-Charris E, Mosquera M, et al. (2023) Unveiling disrupted lipid metabolism in benign prostate hyperplasia, prostate cancer, and metastatic patients: Insights from a colombian nested case-control study. Cancers (Basel) 15: 5465.
8. Burch TC, Isaac G, Booher CL, Rhim JS, Rainville P, et al. (2015) Comparative metabolomic and lipidomic analysis of phenotype stratified prostate cells. PLoS One 10: e0134206.
9. Buszewska-Forajta M, Pomastowski P, Monedeiro F, Walczak-Skierska J, Markuszewski M, et al. (2021) Lipidomics as a diagnostic tool for prostate cancer. Cancers 13: 2000.
10. Ingram LM, Finnerty MC, Mansoura M, Chou CW, Cummings BS (2021) Identification of lipidomic profiles associated with drug-resistant prostate cancer cells. Lipids Health Dis 20: 15.
11. Li J, Ren S, Piao HL, Wang F, Yin P, et al. (2016) Integration of lipidomics and transcriptomics unravels aberrant lipid metabolism and defines cholesteryl oleate as potential biomarker of prostate cancer. Scientific Reports 6: 20984.
12. Lin X, Lécuyer L, Liu X, Triba MN, Deschasaux-Tanguy M, et al. (2021) Plasma metabolomics for discovery of early metabolic markers of prostate cancer based on ultra-high-performance liquid chromatography-high resolution mass spectrometry. Cancers 13: 3140.
13. Dereziński P, Klupczynska A, Sawicki W, Pałka JA, Kokot ZJ (2017) Amino acid profiles of serum and urine in search for prostate cancer biomarkers: A pilot study. Int J Med Sci 14: 1-12.
14. Kumar D, Gupta A, Mandhani A, Sankhwar SN (2015) Metabolomics derived prostate cancer biomarkers: Fact or fiction? J Proteome Res 14: 1455-1464.
15. Kumar D, Gupta A, Mandhani A, Sankhwar SN (2016) NMR spectroscopy of filtered serum of prostate cancer: A new frontier in metabolomics. Prostate 76: 1106-1119.
16. Chan ECY, Koh PK, Mal M, Cheah PY, Eu KW, et al. (2008) Metabolic profiling of human colorectal cancer using high-resolution magic angle spinning nuclear magnetic resonance (HR-MAS NMR) spectroscopy and gas chromatography mass spectrometry (GC/MS). J Proteome Res 8: 352-361.
17. Somashekar BS, Kamarajan P, Danciu T, Kapila YL, Chinnaiyan AM, et al. (2011) Magic angle spinning NMR-based 192 metabolic profiling of head and neck squamous cell carcinoma tissues. J Proteome Res 10: 5232-5241.
18. Zheng H, Dong B, Ning J, Shao X, Zhao L, et al. (2020) NMR-based metabolomics analysis identifies discriminatory metabolic disturbances in tissue and biofluid samples for progressive prostate cancer. Clin Chim Acta 501: 241-251.
19. Wyss M and Kaddurah-Daouk R (2000) Creatine and creatinine metabolism. Physiol Rev 80: 1107-1213.
20. Albers MJ, Bok R, Chen AP, Cunningham CH, Zierhut ML, et al. (2008) Hyperpolarized 13C lactate, pyruvate, and alanine: Noninvasive biomarkers for prostate cancer detection and grading. Cancer Res 68: 8607-8615.
21. Yang B, Zhang C, Cheng S, Li G, Griebel J, et al. (2021) Novel metabolic signatures of prostate cancer revealed by $^{1}$H-NMR metabolomics of urine. Diagnostics (Basel) 11: 149.
22. Giskeødegård GF, Hansen AF, Bertilsson H, Gonzalez SV, Kristiansen KA, et al. (2015) Metabolic markers in blood can separate prostate cancer from benign prostatic hyperplasia. Br J Cancer 113: 1712-1719.
23. Glunde K, Bhujwalla ZM, Ronen SM (2011) Choline metabolism in malignant transformation. Nat Rev Cancer 11: 835-848.
24. Zhang X, Xia B, Zheng H, Ning J, Zhu Y, et al. (2022) Identification of characteristic metabolic panels for different stages of prostate cancer by $^{1}$H NMR-based metabolomics analysis. J Transl Med 20: 275.
25. Hasim A, Ma H, Mamtimin B, Abudula A, Niyaz M, et al. (2012) Revealing the metabonomic variation of EC using 1 H-NMR spectroscopy and its association with the clinicopathological characteristics. Mol Biol Rep 39: 8955-8964.
26. Bag S, Banerjee DR, Basak A, Das AK, Pal M, et al. (2015) NMR ($^{1}$H and $^{13}$C) based signatures of abnormal choline metabolism in oral squamous cell carcinoma with no prominent Warburg effect. Biochem Biophys Res Commun 459: 574-578.