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RESEARCH ARTICLE

Targeted Metabolomic Profiling of High-Grade Gleason Score Distinguished from Low-Grade Gleason Score with Prostate Cancer in Blood Plasma using NMR Spectroscopy

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Abstract

Background: Separating low-grade from high-grade Gleason score (GS) prostate cancer (PCa) is an important clinical challenge for identifying patients eligible for active surveillance, thereby reducing the risk of overtreatment. PCa is one of the most prevalent cancers among men, characterized by varying degrees of aggressiveness. Despite recent progress in precisely determining some of the molecular mechanisms that contribute to cancer progression, more targeted and focused predictive techniques are still needed to differentiate between cancer stages. The present study demonstrates the proton nuclear magnetic resonance (1H NMR) based metabolic profiling of blood plasma for distinguishing between prostate cancer (PCa) patients having Gleason score GS < 7 from (GS) ≥ 7 coupled with machine learning or AI analysis.

Methodology: A total of 50 patients were included and Blood samples were in this study. 1H-NMR spectra of blood plasma samples were carried out at a 700 MHz spectrometer using 1D CPMG with pre-saturation. 2D COSY and TOCSY experiments were carried out for assignments of metabolite peaks. Blood plasma metabolite and clinical data analysis were carried out by machine learning (ML) or artificial intelligence (AI) using Metabo Analyst 6.0.

Results: A total of 25 metabolites were identified in the blood plasma of patients. A significantly higher concentration of choline, phosphocreatine, lactate, and taurine while a lower concentration of histidine and tyrosine was observed in the blood plasma of PCa patients with GS ≥ 7 as compared to GS < 7. Phosphocreatine, choline, and taurine showed the highest discrimination using PLS-DA, OPLS-DA, and random forest models. MSEA revealed significantly altered methylhistidine metabolism, beta-alanine metabolism, phospholipid biosynthesis, gluconeogenesis, betaine metabolism, and methionine metabolism associated with high GS PCa progression.

Conclusion: This present study established that a selected panel of metabolites may accurately detect PCa in blood plasma samples. Our results suggest that metabolic profiling of blood plasma may provide biomarker/s for distinguishing PCa patients with various histological grades.

Keywords

Prostate cancer, Gleason score, Metabolomics, Non-invasive biomarker, Machine learning

Introduction

Prostate cancer (PCa) is the most frequently diagnosed solid-organ malignancy in men over the age of 50-years [1,2]. The prostate-specific antigen (PSA) and the digital rectal examination (DRE) followed by transrectal ultrasound (TRUS) guided biopsy are used for diagnosis of PCa. PCa exhibits considerable heterogeneity in metabolic profiles, which the Gleason score (GS), a key prognostic indicator, can influence [3]. The Gleason score is calculated based on the patterns of cancer cell growth observed in biopsy samples. The score

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is a sum of the primary and secondary patterns, each graded on a scale from 1 to 5 [4,5]. Cancers are thought to progress from low grade to higher grade GS, leading to efforts to detect and eliminate low-grade disease to prevent morbidity and mortality from high-grade disease [6]. The grade of PCa is characterized by the GS) on a scale of 2-10, with 10 representing the most poorly differentiated tumors. Currently, there are no clinical tools that can accurately discriminate aggressive from indolent PCa. The Gleason scoring system is the most important prognostic tool in treatment planning, but it is dependent on subjective factors in the evaluation of aggressiveness and is limited by underestimation due to the under-sampling of biopsies [4]. New diagnostic and prognostic tools for evaluating PCa aggressiveness are therefore urgently needed. Metabolic alteration is an emerging hallmark of cancer and metabolic profiling of blood plasma using NMR can provide additional information about tumor behaviour, especially with the possibility of translating findings to potential biomarker/s [7,8]. PCa can be classified as low-grade GS or high-grade GS, it is depending on the degree by which prostate cancer cells histologically differ from normal cells, being high-grade PCa more aggressive and invasive than low-grade cancer [9]. We analyse how these metabolic differences can impact disease progression and treatment strategies, supported by current research findings. The overall aim of this study was to investigate the possibility of assessing PCa aggressiveness by NMR analysis of human blood plasma and urine and to identify specific metabolites as biomarker/s for tumor aggressiveness. Metabolic profiles and individual metabolite concentrations were used to discriminate between the histologically determined GS which was evaluated from blood samples. All these facts underline the urgent need for novel biomarker/s to improve clinical decision-making and management of PCa. However, there are limited studies that have reported the difference between high-grade GS and low-grade GS based on the difference in their metabolic profile [10-15].

Thus, the present study based on ^{1}H-NMR investigates the differences in metabolic profiling of blood plasma of PCa patients with GS ≥ 7 and GS < 7 for determining biomarker/s of PCa coupled with machine learning (ML) or artificial intelligence (AI) tools for better explaining and understanding metabolome of PCa.

Method

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, and Gleason score. Blood samples were collected from all the subjects and categorized based on biopsy and GS in the Department of Urology at AIIMS New Delhi, India. A total of 50 patients were recruited and blood samples were collected from patients in the morning pre-prandial after overnight fasting in a sodium heparin vacutainer before TRUS-guided prostate biopsy. Blood samples were collected from PCa patients with GS ≥ 7 (GS 7 to 10) (n = 35 mean age: 67.2 ± 7.6 years), PSA: 228.28 (6.20-1796.10) ng/mL, and GS < 7 (GS 3 + 3 = 6) (n = 15, mean age: 68.7 ± 7.0 years), PSA: 16.97 (4.11-61) ng/mL. Informed consent was taken and Institute Ethics Committee approved the study.

Sample collection and processing

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 format 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. Hence format was used as a concentration standard at 8.46 ppm for quantification of metabolites.

1D and 2D proton NMR Spectroscopy

All proton (1H) NMR spectra were acquired from a 700 MHz spectrometer operating at 699.94 MHz at 25 °C. The one-dimensional (1D) spectrum was acquired using the Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence with a pre-saturation pulse for water peak suppression. The typical parameters for ^{1}H-NMR experiments were: number of scans = 64; data points = 32K; spectral width = 9124.1 Hz; a total spin echo delay of 16 ms and a repetition time (TR) of 70 s.

The parameters used for TOCSY experiments were: data points 2K in F2 dimension; spectral width 9800 Hz; with a 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 metabolites

The metabolite concentrations (mM) were determined using Chenomx NMR Suite 7.5 software (Chenomx Inc. Edmonton, Canada). The concentration (Targeted) of only those metabolites that showed well-resolved resonance in the proton 1D (CPMG) NMR spectra of blood plasma samples of all patients.

Statistical Analysis

Statistical analyses were carried out using machine learning tools or AI software SPSS 20.0 (SPSS Inc. Chicago, IL, USA) and MetaboAnalyst6.0 web server (www.metaboanalyst.ac). For, the comparison between the two groups study was analyses using the Mann-Whitney U test. In all statistical analyses value of $p < 0.05$ was considered to be statically significant. Further analysis, receiver operating characteristics (ROC) curve analysis) multivariate partial least squares discriminatory analysis (PLS-DA), orthogonal PLS-DA variable importance in projection (VIP) score, and discriminant function analysis were carried out. 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). Variables with VIP score > 1.0 were selected as significant metabolites that can differentiate patients with PCa from low-grade GS of blood plasma samples. The variable selection algorithms in PLS-DA, OPLSDA, and random forest analysis were for diagnostic performance. Metabolites panel consisting of metabolites common in four selection algorithms so-called overlapping set (OL). Hierarchical clustering heatmap of blood plasma metabolome data. Each column shows the metabolic pattern of individual PCa patients having PCa patients with GS ≥ 7 and GS < 7 group. Pathway analysis using Metabolic Set Enrichment Analysis (MSEA) was carried out.

Result

Figure 1 shows the representative aliphatic region of 1D ^{1}H NMR spectrum of the blood plasma sample of a PCa patient having ≥ 7 (4 + 4) (**A**) and GS < 7 (3 + 3) (B). In all, 25 metabolites were assigned using 1D and 2D NMR. For the concentration, [mM median (range)] of 6 metabolites that showed significant ($p < 0.05$) differences, AUC values, and VIP score (> 1) between PCa patients with GS ≥ 7 and GS < 7, are presented in Table 1. Figure 2 shows the PLS-DA and OPLDSA score plots, VIP plot, and predicted model showing discrimination of PCa patients with GS ≥ 7 from GS < 7. Table 2 shows the identified metabolites in blood plasma samples of PCa patients using NMR spectroscopy and their chemical shift (ppm) positions of resonances ^{1}H of metabolites. Furthermore, the code (identifiers) of the metabolites of data sets was searched in different metabolites Databases (KEGGID and HMDB) are shown in Table 2.

The blood plasma sample of PCa patients with GS ≥ 7 showed a significantly higher concentration of choline, phosphocreatine, lactate, and taurine than PCa patients with GS < 7 while lower concentrations of tyrosine and histidine are given in Table 1.

The results obtained from PLS-DA OPLS-DA of metabolites concentration in blood plasma showed a separation between patients with GS ≥ 7 and SG < 7. PCa patients with GS ≥ 7 from patients with GS < 7 were discriminated with R2Y = 0.704, Q2 = 0.412, and with an accuracy of 0.78 were shown in Figure 2. The significant metabolites in blood plasma such as choline, phosphocreatine, lactate, taurine, tyrosine, and histidine showed high VIP scores (> 1.0) in GS ≥ 7 compared to GS < 7 patients are shown in Table 1 and Figure 2C. The model obtained for blood plasma profiling was validated

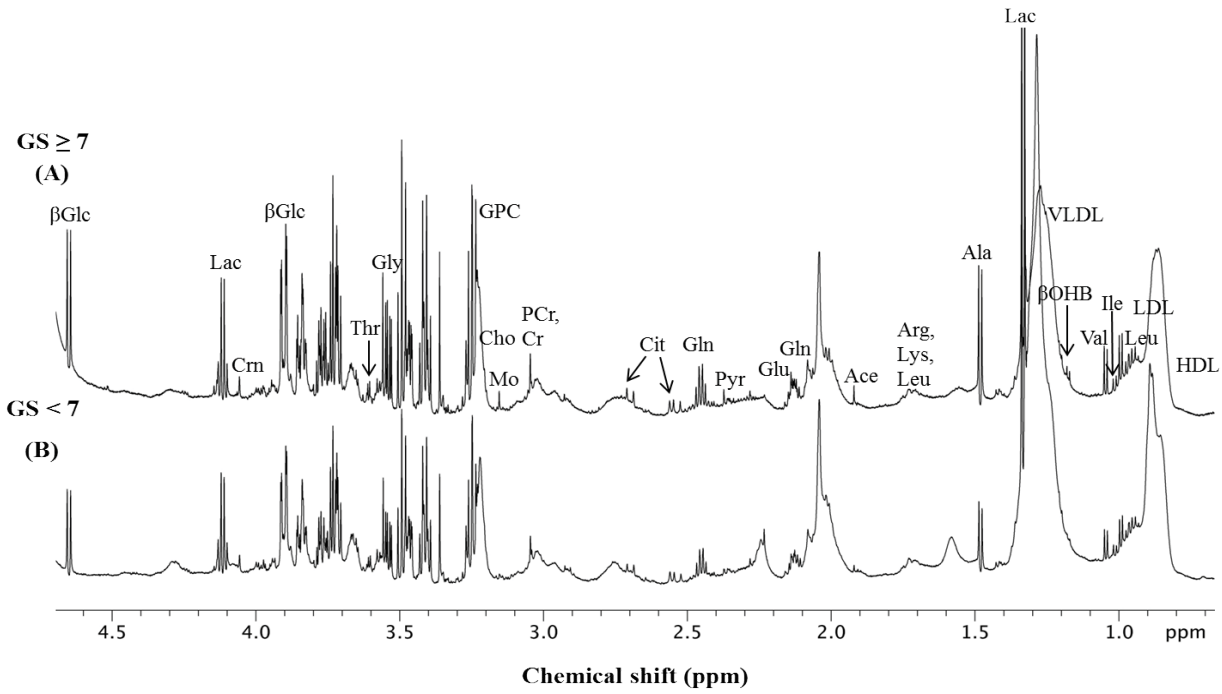

Figure 1: Representative one-dimensional (1D) aliphatic region of 1H NMR (700 MHz) spectrum of blood plasma of a PCa patient with GS ≥ 7(A) and GS < 7(B).

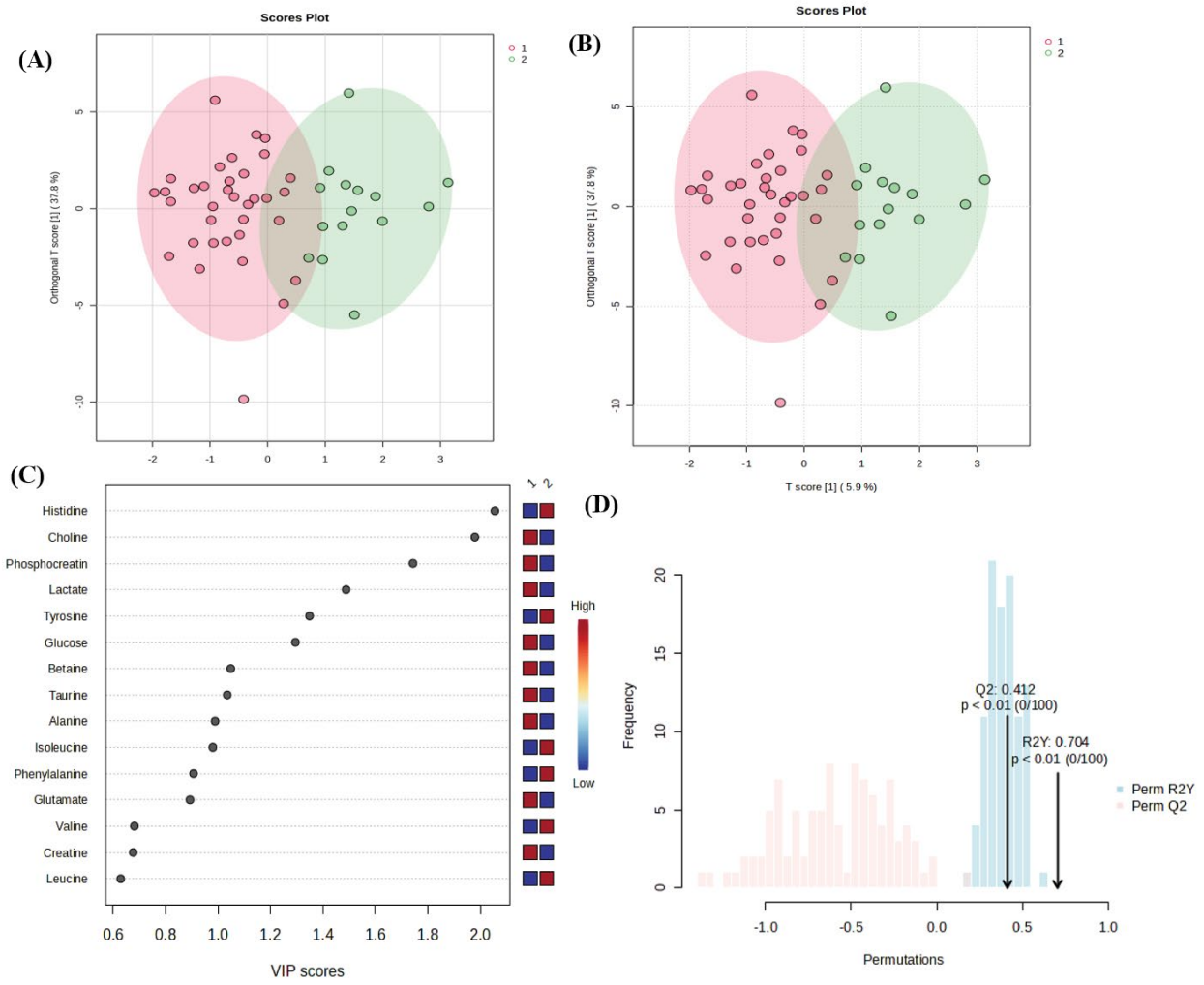

Figure 2: A and B) PLS-DA scores OPLS-DA plot showed clear discriminate patients with GS ≥ 7 (red) and GS < 7 (green); C) Variable in projection (VIP) scores of metabolite profile in blood plasma in two study groups; D) VIP scores for 15 metabolites with the highest contribution to the separation of the present study groups and Validation of OPL-DA by permutation test performed with 100 random permutations and models showing Q2 = 0.412 and R2Y = 0.704 values from permuted analysis (n = 100).

Table 1: The concentration of metabolites (mM) in blood plasma samples of PCa patients with GS ≥ 7 and GS < 7. AUC values were obtained in univariate ROC curve analyses and VIP scores.

S.No.	Metabolites	Metabolite concentration(mM)		p-value	AUC	VIP Score
		PCa with GS ≥ 7 N = 35 (Mean ± SD)	PCa with GS < 7 N = 15 (Mean ± SD)			
1	Leucine	0.78 (0.53-1.88)	0.90 (0.43-1.18)	0.36	0.68	0.69
2	Valine	0.28 (0.11-0.66)	0.34 (0.14-0.43)	0.31	0.67	0.76
3	Isoleucine	0.34 (0.12-0.79)	0.37 (0.19-0.52)	0.22	0.71	0.92
4	3HOB	0.55 (0.21-1.31)	0.59 (0.25-0.80)	0.62	0.55	0.02
5	Lactate	**3.26 (1.50-7.53)**	**2.29 (1.31-4.43)**	**0.04**	**0.67**	**1.37**
6	Alanine	0.60 (0.37-1.24)	0.54 (0.30-1.00)	0.19	0.66	1.05
7	Acetate	0.06 (0.02-0.17)	0.06 (0.04-0.10)	0.56	0.55	0.26
8	Acetoacetate	0.10 (0.04-0.34)	0.10 (0.04-0.22)	0.52	0.52	0.35
9	Glutamate	0.46 (0.35-0.97)	0.43 (0.28-0.74)	0.24	0.61	1.00
10	Pyruvate	0.07 (0.04-0.23)	0.06 (0.05-0.09)	0.10	0.62	0.83
11	Glutamine	0.86 (0.50-1.50)	0.87 (0.53-1.48)	0.87	0.51	0.16

12	DMA	0.07 (0.03-0.13)	0.06 (0.04-0.14)	0.49	0.57	0.38
13	Creatine	0.08 (0.05-0.16)	0.08 (0.05-0.12)	0.53	0.57	0.62
14	Phosphocreatine	**0.11 (0.01-0.64)**	**0.05 (0.01-0.19)**	**0.001**	**0.80**	**1.74**
15	Malonate	0.06 (0.03-0.24)	0.06 (0.03-0.10)	0.49	0.50	0.16
16	Choline	**0.11 (0.06-0.17)**	**0.07 (0.05-0.11)**	**0.001**	**0.75**	**1.88**
17	GPC	0.09 (0.04-0.22)	0.09 (0.04-0.19)	0.62	0.55	0.42
18	Glycine	0.33 (0.17-0.78)	0.31 (0.21-0.57)	0.75	0.50	0.07
19	Creatinine	0.15 (0.08-0.73)	0.15 (0.09-0.27)	0.14	0.52	0.50
20	Tyrosine	**0.18 (0.06-0.30)**	**0.21 (0.09-0.32)**	**0.04**	**0.66**	**1.36**
21	Histidine	**0.11 (0.04-0.19)**	**0.16 (0.07-0.25)**	**0.005**	**0.76**	**2.09**
22	Phenylalanine	0.20 (0.07-0.33)	0.23 (0.10-0.38)	0.18	0.61	0.91
23	Glucose	5.45 (2.75-9.38)	4.51 (3.23-8-.32)	0.11	0.65	1.16
24	Taurine	**1.27 (0.78-2.83)**	**1.11 (0.71-1.68)**	**0.05**	**0.63**	**1.10**
25	Betaine	0.21 (0.14-0.45)	0.20 (0.14-0.28)	0.15	0.60	0.94

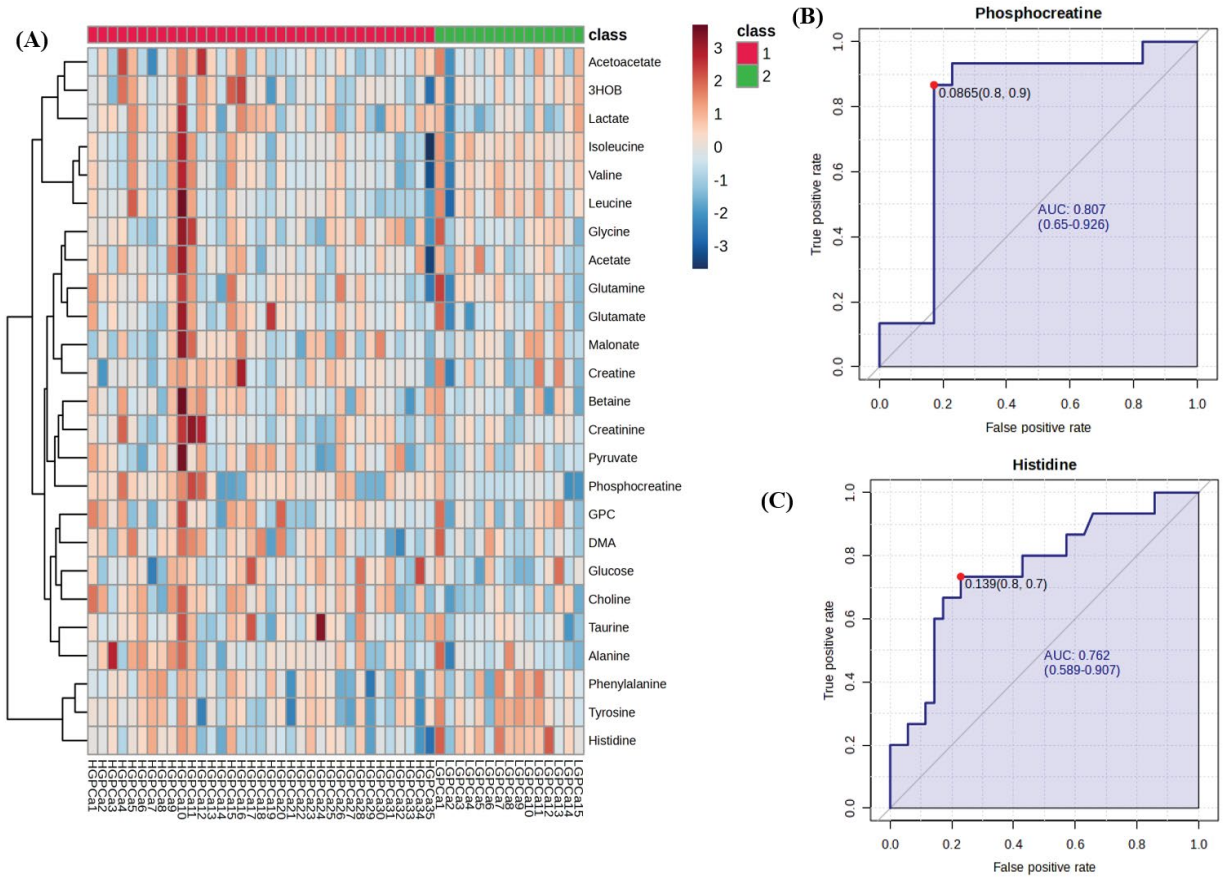

Figure 3: Hierarchical clustering heat map of blood plasma metabolome data. Each column shows the metabolic pattern of individual PCa patients with GS ≥ 7 and GS < 7 (A). The univariate ROC curves analysis for AUC for phosphocreatine (B) and histidine (C).

with permutation analysis (n = 100) and with observed statistic results at $p < 0.01$. Further, these metabolites were used for univariate ROC curve analysis. The AUC for ROC curve analysis calculated at optimal cutoffs for the significant metabolites are summarized in Table 1.

Figure 3A shows a hierarchical clustering heat map of blood plasma metabolome data. Each column shows the metabolic pattern of individual PCa patients with GS ≥ 7 and GS < 7. The univariate ROC curves analysis for AUC for phosphocreatine and histidine are displayed in Figure 3B and Figure 3C, respectively.

The results indicated that 5 metabolites with a high AUC above 0.65 were choline, phosphocreatine, lactate, tyrosine, and histidine respectively whereas AUC for taurine such as were AUC less than 0.65. Histidine showed the highest AUC value (0.80) followed by phosphocreatine and choline (0.76 and 0.75 respectively) (Table 1). Thus, the distinctive signature

Table 2: List of identified metabolites in blood plasma samples of PCa patients using NMR spectroscopy and their chemical shift (ppm) positions of resonances ^{1}H of metabolites. Code (identifiers) of the metabolites of data sets was searched in different metabolites Databases (KEGGID and HMDB).

S.No.	Blood plasma Metabolites	Chemical shift δ (^{1}H) ppm	Multiplicity	HMDB	KEGG
1	Leucine	0.96	t	HMDB0000687	C00123
2	Valine	1.00	d	HMDB0000883	C00183
3	Isoleucine	0.94	d	HMDB0000172	C00407
4	3HOB	1.20	d	HMDB0000442	C03197
5	Lactate	1.33	d	HMDB0000190	C00186
6	Alanine	1.48	d	HMDB0000161	C00041
7	Acetate	1.91	s	HMDB0000042	C00033
8	Acetoacetate	2.23	s	HMDB0000060	C00164
9	Glutamate	2.36	m	HMDB0000148	C00025
10	Pyruvate	2.37	s	HMDB0000243	C00022
11	Glutamine	2.45	m	HMDB0000641	C00064
12	DMA	2.73	s	HMDB0000087	C00543
13	Creatine	3.03	s	HMDB0000064	C00300
14	Phosphocreatine	3.05	s	HMDB0001511	C02305
15	Malonate	3.16	s	HMDB0000691	C00383
16	Choline	3.21	s	HMDB0000097	C00114
17	GPC	3.23	s	HMDB0000086	C00670
18	Glycine	3.56	s	HMDB0000123	C00037
19	Creatinine	4.06	s	HMDB0000562	C00791
20	Tyrosine	6.88	d	HMDB0000158	C00082
21	Histidine	7.08	s	HMDB0000177	C00135
22	Phenylalanine	7.33	m	HMDB0000159	C00079
23	Glucose	5.23, 4.64	d	HMDB0000122	C00031
24	Taurine	3.25	s	HMDB0000251	C00245
25	Betaine	3.91	s	HMDB0000043	C00719

3HOB: 3Hyroxybutyrate; DMA: Dimethyl Amine; GPC: Glycerophosphocholine; s: Singlet; d: Doublet, t: Triplet m: Multiplet

Table 3: Feature selected and identified metabolites in blood plasma samples of high and low-grade Gleason PCa using different multivariate models.

Feature selection methods	Random forest	PLS-DA	OPLS-DA	OL
Metabolites panels	Phosphocreatine	Histidine	Histidine	Phosphocreatine
	Choline	Choline	Choline	Choline
	Histidine	Phosphocreatine	Phosphocreatine	Histidine
	Lactate	Lactate	Lactate	Lactate
	Valine	Tyrosine	Tyrosine	Tyrosine
	Creatine	Alanine	Glucose	
	Tyrosine	Glutamate	Betaine	
	Taurine	Betaine	Taurine	
	Alanine	Taurine	Alanine	
	Glucose	Phenylalanine	Isoleucine	

PLS-DA: Partial Least Squares Regression Discriminant Analysis; OPLS-DA: Orthogonal Partial Least Squares Regression Discriminant Analysis; OL: Overlapping Set Panel; Random Forest Features Ranked by Their Contributions to Classification Accuracy (Mean Decrease Accuracy)

with the 5 metabolites achieved the highest AUC value and significantly increased the diagnosis performance of PCa patients having high-grade GS and low-grade GS. The choline, phosphocreatine, lactate, tyrosine, and histidine showed discriminate performance using PLS-DA, OPLS-DA, and random forest models are shown in Table 3.

Metabolic pathway analysis is essential for the understanding of cellular processes of specific diseases, providing insight into the development of treatment methods. The Pathway analysis integrates blood plasma metabolite set enrichment analysis to extract biologically meaningful information from the data given in Figure 4. According to the metabolic pathway analysis based exploratory analysis by using the relative concentrations of differential significant potential blood plasma metabolites, we found abnormal pathways and potential biomarkers involved with the patients with GS ≥ 7 and GS < 7. Table 4 shows that pathway analysis results showed the unique significantly altered targeted biochemical pathways seen were: Methylhistidine metabolism, beta-alanine metabolism, phospholipid biosynthesis, gluconeogenesis, betaine metabolism,

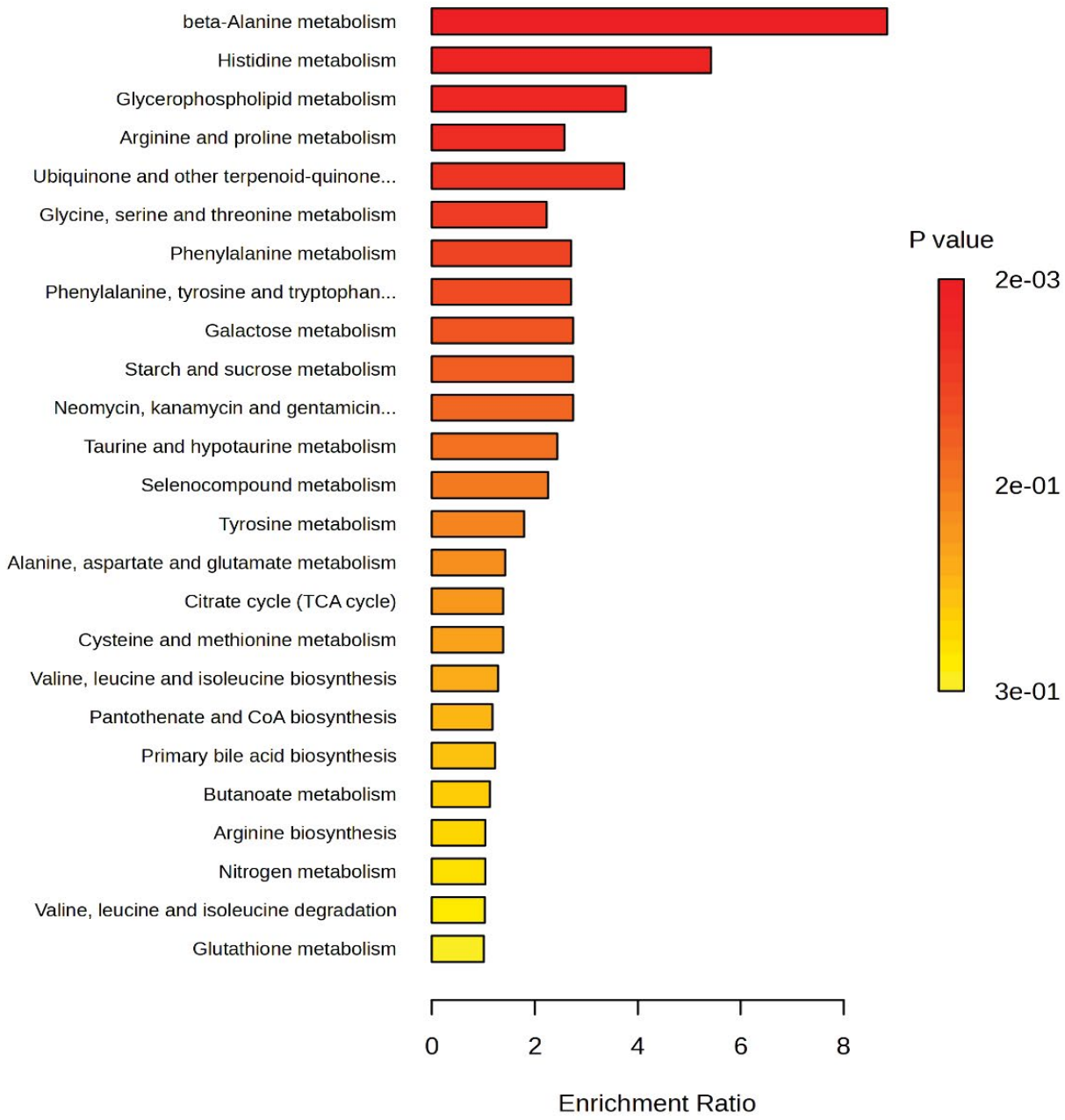

Figure 4: Metabolic Set Enrichment Analysis (MSEA) showing the altered significant metabolites revealed in the blood plasma of PCa patients. Metabolic Pathway Analysis (MetPA) shows the most altered potential significant metabolic pathways involved in PCa progression.

Table 4: Results from the blood plasma metabolites associated with dominated altered metabolic pathway analysis of PCa patients using Metabolic Pathway Analysis (MetPA).

Altered Metabolic pathways	Total Compounds	Hits	Raw p	Holm p	FDR
Methylhistidine Metabolism	4	1	2.11E-03	1.18E-01	5.76E-02
Beta-Alanine Metabolism	34	2	4.44E-03	2.44E-01	5.76E-02
Histidine Metabolism	42	2	4.44E-03	2.44E-01	5.76E-02
Phospholipid Biosynthesis	29	1	6.17E-03	3.27E-01	5.76E-02
Phosphatidylcholine Biosynthesis	14	1	6.17E-03	3.27E-01	5.76E-02
Phosphatidylethanolamine Biosynthesis	12	1	6.17E-03	3.27E-01	5.76E-02
Betaine Metabolism	21	1	1.77E-02	8.86E-01	1.42E-01
Methionine Metabolism	42	3	4.61E-02	1.00E+00	2.65E-01
Gluconeogenesis	33	3	4.92E-02	1.00E+00	2.65E-01

Raw p is the original p value calculated from the enrichment analysis; Holm p is the p value adjusted by Holm-Bonferroni method; FDR p is the p-value adjusted using False Discovery Rate

and methionine metabolism associated with PCa aggressiveness.

Discussion

In this study, targeted approaches that map dysregulated metabolic pathways may be extremely important for understanding the processes behind cancer and aggressiveness. The Gleason score, derived from the histopathological evaluation of prostate biopsy samples, is crucial for determining the cancer's aggressiveness and guiding treatment decisions. The score ranges from 6 to 10, with higher scores indicating more aggressive and less differentiated cancer. The present study demonstrates the proton nuclear magnetic resonance (^{1}H NMR) based metabolic profiling of blood plasma for distinguishing between prostate cancer (PCa) patients having Gleason score GS < 7 from (GS) ≥ 7.

This study explores the metabolic differences in blood plasma between PCa patients with Gleason scores less than 7 and those with scores of 7 or higher GS. Our data revealed significantly lower concentrations of histidine and tyrosine in the blood plasma of PCa patients with GS ≥ 7 compared to GS < 7. While higher concertation of phosphocreatine, choline, lactate, and taurine were found in patients with ≥ 7 as compared to patients with GS < 7.

Higher concentrations of Lac are seen in the blood plasma of patients with ≥ 7 as compared to patients with GS < 7 which is associated with an increase in glycolytic flux as well as the Cori cycle or Lac cycle, and high Lac concentrations have been associated with more aggressive diseases and metastasis [16,17]. Since Lac is the endpoint product of glycolysis, its accumulation implied an increased anaerobic glycolysis. Additionally, the Warburg effect describes that cancer cells have increased anaerobic glycolysis and therefore may lead to the accumulation of Lac in the blood plasma of PCa progression [16,17].

The energy metabolite phosphocreatine was also observed to be high in the blood plasma of high-grade GS. Creatine and its phosphorylated form are well recognized as key intermediates in energy metabolism and elevated creatine is associated with high energy demand for rapid proliferation of cancer cells [18,19]. Creatine is mainly synthesized in the liver and kidney and stored in muscle as phosphocreatine. Phosphocreatine has a direct function in cellular energy transport. Creatinine is a nonenzymatic product of creatine and phosphocreatine, and the creatine-phosphocreatine system plays a crucial role in cellular energy transportation, especially in cells with altered energy metabolism [18,19].

The present study revealed a significantly higher concentration of choline in the blood plasma of PCa patients with GS ≥ 7 as compared to GS < 7. Choline is associated with the breakdown of membrane phospholipids during the malignant transformation of prostatic epithelial cells [20,21].

Our results showed significantly higher concentrations of taurine in the blood plasma of PCa patients with GS ≥ 7 compared to GS < 7. Taurine can regulate the signal pathways for cell proliferation and protein synthesis through AKT/FOXO1, JAK2/STAT3, PI3K/AKT, and mTOR/AMPK. Taurine is primarily engaged in the taurine and hypo-taurine metabolism pathway. Elevated taurine concentrations have been seen in the reactive stroma, which may indicate an inflammatory reaction [22,23]. On the other hand, we also observed a positive link between taurine levels and age, which would require further investigation in studies that are expressly intended for that purpose. Our data also showed a significantly lower concentration of tyrosine in the blood plasma of the patients with GS ≥ 7 compared to GS < 7 which might be attributed to the high protein turnover seen in PCa aggressiveness [24].

The proteinogenic amino acid histidine was also lower in patients with PCa compared to low GS. The

histidine is related to changes in anti-inflammatory responses and anti-oxidative stress and also may be related to protein energy wasting [25]. It is considered to have antioxidant properties such as scavenging singlet oxygen and free radicals. The histidine also provides intracellular buffering to stimulate anaerobic energy formation. The lower concentration may reflect the increased use of the carbon skeleton of this amino acid for the biosynthesis of cellular molecules needed for the rapid proliferation of malignant cells [24,26].

NMR based on metabolic profiling of blood plasma samples this study shows that low and high-grade GS PCa can be distinguished by the concentrations of phosphocreatine, choline, histidine, taurine, lactate, and tyrosine. In the future, by analyzing larger patient cohorts, concentration cut-off values can be determined based on the metabolic profiles and can become tools for assessing PCa aggressiveness. ^{1}H-NMR is feasible as a diagnostic supplementary tool for evaluating TRUS-guided prostate biopsies, providing metabolic profiles that can predict tumor aggressiveness. Thus, our results demonstrate the value of NMR in distinguishing and monitoring the follow-up of patients included in active surveillance programs.

Conclusion

This present study established that a selected panel of metabolites may accurately detect PCa in blood plasma samples. The significant difference in metabolite concentration of phosphocreatine, histidine, choline, lactate tyrosine, and taurine may be used as a measure to discriminate between PCa patients having GS ≥ 7 and < 7. Our findings indicate that metabolite alterations are associated with the tumor grade and progression of the disease. We believe that metabolite biomarker/s as presented herein could have future clinical utility for the diagnosis and monitoring of PCa if they can discriminate against low-grade-cancer patients. Based on these results further work is acceptable to validate these findings in a much larger cohort.

Author Contributions

Pradeep Kumar, Virendra Kumar, Rajeev Kumar, Sanjay Sharma, Sanjay Thulkar, MA 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 MA Khan contributed to the discussion, manuscript draft reviewed the final manuscript.

Conflict of Interest

The authors declare that they have no conflict of interest.

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