ISSN: 2378-3672

Kumar et al. Int J Immunol Immunother 2024, 11:073
DOI: 10.23937/2378-3672/1410073
Volume 11 | Issue 1
Open Access

International Journal of
Immunology and Immunotherapy

RESEARCH ARTICLE

NMR-Based Blood Metabolomic Profiling and Immunological Parameters to Identify Early Biomarkers for Prostate Cancer Bone Metastases

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

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

[2]*Urology, All India Institute of Medical Sciences (AIIMS), New Delhi, India*

[3]*Radiodiagnosis, All India Institute of Medical Sciences (AIIMS), New Delhi, India*

[4]*Biostatistics, All India Institute of Medical Sciences (AIIMS), New Delhi, India*

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

Abstract

Metastasis to the bone is one clinically important feature of prostate cancer (PCa). Since PCa is treatable, existing diagnostic techniques are unable to predict metastatic disease. Identification of metabolic pathways involved in the growth of bone metastasis, therefore, has the potential to improve PCa prediction and treatment. Consequently, clinical treatment will benefit from sensitive and specific cancer biomarker/s for PCa bone metastases that may be used for diagnosis and prognosis. To investigate the blood plasma metabolomic profiles and immunological parameters to distinguish PCa patients with metastases from those without metastases using 1H-NMR spectroscopy for establishing potential biomarker/s. 40 Patients were recruited and blood samples were collected from metastases PCa patients and those without established metastases in the morning pre-prandial after overnight fasting. Proton spectra of blood plasma samples were carried out at a 700 MHz spectrometer using 1D CPMG with pre-saturation. To assign metabolite peaks, 2D TOCSY and COSY investigations were carried out. Both univariate (receiver operating characteristics (ROC) curve analysis) and multivariate analyses of partial least squares-discriminant analyses PLS-DA, variable importance to projection (VIP) score were carried out using MetaboAnalyst6.0. The metabolic profile analysis showed significantly higher concentrations of 3HOB, lactate, alanine, acetate, acetoacetate, glutamate, pyruvate, DMA, creatine, phosphocreatine, malonate, choline, GPC, glycine, creatinine, and glucosein PCa patients with metastases as compared to non-metastases. Furthermore, we demonstrated that bone metastasis patients with PCa had altered WBC subtypes neutrophil and lymphocyte thannon-metastasis PCa patients. NMR-based metabolomics and immunological parameters profiling provide novel insights into the pathophysiological mechanism of cancer progression to metastases stage of PCa to monitor treatment outcomes.

Keywords

Prostate cancer, NMR spectroscopy, Metabolomics, Bone metastases and Immunological parameters

Introduction

Prostate cancer bone metastatic (PCa) is a prominent and lethal disease that has to be detected early and treated well. PCa can eventually spread to the bone [1,2]. Even after decades of investigation, the effect of PCa on genetic modifications and initiating invasive progression and metastatic cascade remains unclear and less understood. In addition, there is not much information on metabolic profiling with correlates with clinical and pathology of metastases with PCa patients, on the underlying biochemical and tumorigenesis processes [1,2]. However, the current diagnostic techniques; testing blood samples for prostate-specific antigen (PSA) levels and using transrectal

Citation: Kumar P, Kumar V, Kumar R, Sharma S, Thulkar S, et al. (2024) NMR-Based Blood Metabolomic Profiling and Immunological Parameters to Identify Early Biomarkers for Prostate Cancer Bone Metastases. Int J Immunol Immunother 11:073. doi.org/10.23937/2378-3672/1410073

Accepted: October 10, 2024; **Published:** October 12, 2024

ultrasound (TRUS) guided to examine prostate biopsies are not very good at distinguishing between aggressive PCa cases and the even more prevalent and indolent forms of the disease, which are frequently curable, nor at differentiating cancer from other benign prostatic hyperplasia (BPH) [3-5]. As a result, certain investigators have attempted to identify new prognostic indicators and diagnostic strategies that can distinguish between metastasis and non-metastasistypes of PCa using a range of approaches [6-9]. A lot of research has gone into identifying the genetic and proteomic characteristics [7]. Limited study of PCa and metabolic alterations linked to bone metastases PCa have been studied using magnetic resonance spectroscopy [9-11]. Some important studies reported by Sreekumar, et al. and associates profiled the metabolome in tissue, urine, and plasma from PCa patients using liquid and gas chromatography-mass spectrometry, and they found changes linked to the advancement of the illness [9]. They found sarcosine, an N-methyl derivative of glycine, was specifically shown to be a potentially significant marker for PCa cell invasion, migration, and aggressiveness [9]. However, several studies reveal that the role of sarcosine as a biomarker is still controversial, and further validation studies are warranted.

The lack of an acceptable, sensitive, and specific cancer biomarker or biomarkers causes it to be challenging to diagnose PCa bone metastases. This has led to a focus on new biomarker/s from blood for patients with PCa and metastatic progression condition. To date, limited NMR studies have shown the potential of metabolic approach in blood plasma samples of PCa patients with bone metastases [9-11]. Earlier detection of PCa patients with metastases might improve their treatment and survival outcomes. Hence, further research in this domain is needed. Also, research is needed to understand PCa and metastatic progression of diseases. Further identification of metabolites between PCa patients with metastases and those without metastases may be very important for early diagnosis of the disease as well as the discovery of cancer-specific biomarker/s.

Thus, the aim of the present study was (a) To investigate the blood plasma metabolic profile to distinguish PCa patients with metastases from non-metastases using ^{1}H-NMR spectroscopy for establishing potential biomarker/s, (b) Furthermore, to discriminate blood parameters and WBC subtyping data of PCa patients with metastases and non-metastases for screening and monitoring of the aggressive PCa (c) To further attempt to identify a set of putative biomarker/s to their metabolic pathways for better understanding of the pathogenesis of PCa. It is the first of its kind to report the blood biomarker/s metabolomic profile and immunological data of PCa patients and metastases conditions *in vitro* settings using NMR Spectroscopy. It paves the way to enhance understanding of cancer pathogenesis and early diagnosis through biomarker/s identification and early detection systems.

Materials and Methods

Patients

A total of 40 patients diagnosed with PCa were recruited in this study at the Department of Urology, AIIMS, New Delhi. To search for signs of possible metastases to the skeletal locations, a bone scan was performed. Men with age > 40 years with elevated PSA level (> 4 ng/mL) who underwent 12 core TRUS guided prostate biopsy and bone scan. Subjects with high risks of tumors and the presence of bone metastases with higher GS (7 or 8-10) were included in the metastasis group. In another group, no evidence of metastasizing in the bone scan. Patients with diabetes mellitus, hypertension, and other metabolic disorders were excluded. Blood samples were collected from bone metastases patients with PCa (n = 20 mean age: 66.0 ± 8.8 years), PSA: 268 (7.94-20500) ng/mL, and non-metastases patients (n = 20, mean age: 64.6 ± 8.3 years), PSA: 18.28 (5.02-54.40) ng/mL, in morning pre-prandial after overnight fasting. Blood reports of all patients were collected from the Department of Urology at AIIMS, New Delhi. Blood parameters such as hemoglobin (HB) (gm/dL),total leukocyte count (thou/mm3), neutrophil (%), lymphocyte (%), eosinophil (%), monocyte (%), basophil (%), absolute neutrophil count (thou/mm3), absolute lymphocyte count (thou/mm3), absolute eosinophil (thou/mm3), absolute monocyte count (thou/mm3), absolute basophil count (thou/mm3), RBC (mill/mm3), hematocrits (%), MCV (fL), MCH (pg), MCHC (g/dL), and platelet count (thou/mm3) value included in these study. Informed consent was taken and the institute's ethics committee approved the study.

Sample collection and processing

Blood samples (2 ml) were collected in a heparinized vacutainer and centrifuged at 5000 rpm for 10 minutes at 4 °C. The blood plasma samples were mixed with 0.5 mM TSP, 0.5 mM sodium formate, and deuterium oxide (D_2O) in preparation for proton NMR spectroscopy. 200 µl blood plasma, 30 µl formate (0.5 Mm), and 340 µl D_2O were used to make a 600 µl overall volume of sample.

NMR Spectroscopy

Proton NMR spectroscopy of the samples was carried out on a narrow bore spectrometer operating at 700 MHz (Agilent, U.S.A.) using a 1D CPMG sequence with pre-saturation. The parameters for the 1D experiment: Spectral width of 0.0 to 12 ppm; data points 32 K; the number of scans of 64; spin echo delay, τ of 16 ms, and a relaxation delay of the 70s. To assign resonances, two-dimensional (2D) total correlation spectroscopy (TOCSY) and correlation spectroscopy (COSY) studies were conducted.

Quantitative measurement of blood metabolites

By comparing the intensity of metabolite-isolated peaks generated when integrating the signal with that of formate (blood plasma), the quantity of metabolites was determined. The concentration of only those metabolites, which showed a well-resolved peak in the 1D spectrum of blood plasma was calculated using Chenomx software (Chenomx NMR Suite 9.0).

Statistical analysis

Statistical analysis was performed using SPSS (SPSS Inc., Chicago, IL, USA). To examine the variations between the two groups, the Mann-Whitney U test was used. The p-values < 0.05 were considered significant. The data were normalized and subjected to multivariate pattern recognition analysis using Metabo Analyst 6.0 software. Metabolites were considered significant at VIP > 1.0 for further analysis of metabolomic data analysis. Partial least square discriminant analysis (PLS-DA) and orthogonal partial least square discriminant analysis (OPLS-DA) loading plots were carried out for significant metabolite identification and heatmap analysis. Variable importance in projection (VIP) scores in PLS-DAs of metabolic profiles in the serum of patients, VIP scores for some important metabolites with the highest contribution to the separation of the studied groups are presented. The boxes on the right refer to the relative concentrations of the appropriate metabolites in the studied groups. In all statistical analyses value of $p < 0.05$ was considered to be statically significant.

Pathway analysis

The blood-identified metabolites altered metabolic pathways were analyzed using Metabo Analyst 6.0. It used metabolites from the blood plasma sample, which included metabolites from Kyoto Encyclopedia of Genes and Genomes (KEGG) and other databases. With a huge collection of pathways and metabolite libraries produced by the Human Metabolome Database (HMDB), this web-based program facilitates the discovery of changed metabolic pathways. For every comparison, a dataset with the chemical KEGG identities of the identical components was entered into the software.

Results

Figure 1 shows the representative 1D-CPMG ^{1}H spectrum of the blood plasma sample of a PCa patient with metastases (A) and from the patient without metastases (B). In all, metabolites were assigned using 1D and 2D NMR. The 2D line chart shows the median values of immunological blood parameters in PCa patients with bone metastases as compared to non-metastases PCa patients in Table 1 and Figure 2. The NMR-based metabolomics profile analysis. VIP score and AUC are shown in Figure 1. Significantly higher concentrations of 3-hydroxybutyrate (3-HOB), lactate, alanine, acetate, acetoacetate, glutamate, pyruvate, dimethylamine (DMA), creatine, phosphocreatine, malonate, choline, glycerophosphocholine (GPC), glycine, creatinine, and glucosewere found in bone metastases PCa as compared to non-metastases patients were shown in

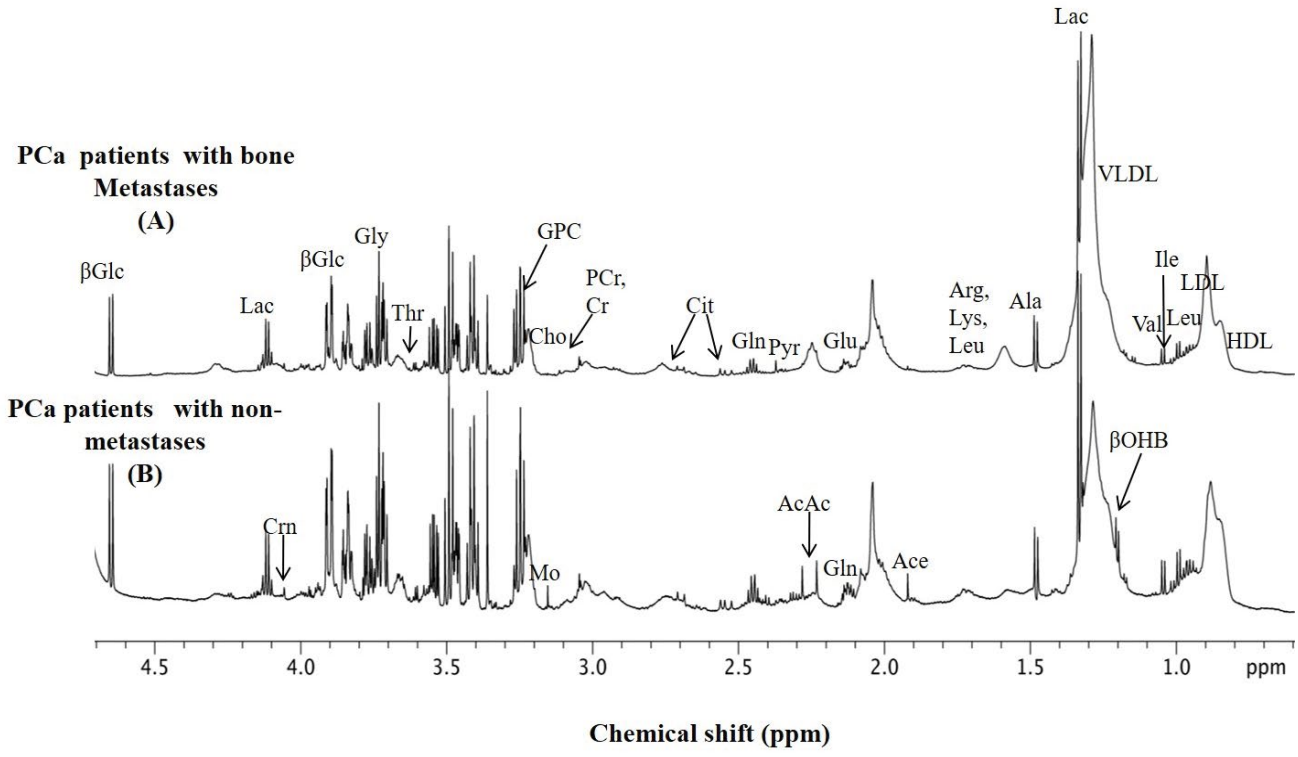

Figure 1: Representative one-dimensional (1D) aliphatic region of 1H NMR (700 MHz) spectrum of blood plasma of a bone metastases PCa patient and non-metastases.

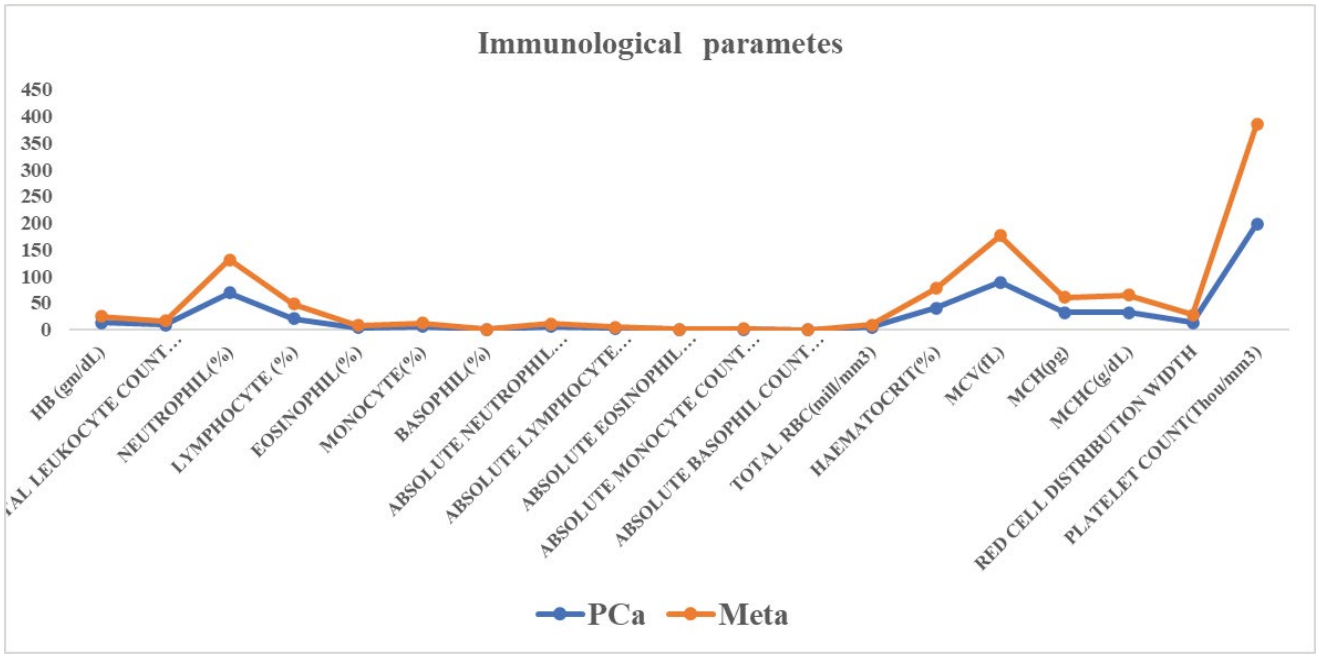

Figure 2: The median values of immunological blood parameters in PCa patients with bone metastases as compared to non-metastases PCa patients.

Table 1: The median values (Minimum and Maximum Values) of blood immunological parameters in PCa patients with Bone metastases as compared to non-metastases with PCa patients.

	Clinical and immunological variables						
	Bone metastases with PCa patients (n = 20)			Non-metastases with PCa patients (n = 20)			
Variables	**Median**	**Min**	**Max**	**Median**	**Min**	**Max**	**p-value**
Haemoglobin HB (gm/dL)	**12.10**	**8.10**	**14.80**	**13.30**	**10.10**	**16.00**	**0.02**
Total leukocyte count (thou/mm3)	8.09	3.90	11.83	8.43	6.90	12.50	0.14
Neutrophil (%)	**63.80**	**46.00**	**80.00**	**68.00**	**58.20**	**82.00**	**0.02**
Lymphocyte (%)	**25.45**	**13.00**	**50.00**	**20.10**	**10.00**	**35.00**	**0.01**
Eosinophil (%)	3.00	1.00	16.70	2.35	1.00	12.40	0.93
Monocyte (%)	7.00	0.00	12.20	5.80	2.00	11.00	0.75
Basophil (%)	0.30	0.00	1.20	0.40	0.00	1.50	0.52
Absolute neutrophil count (thou/mm^3)	4.88	2.24	7.24	5.48	4.18	74.72	0.23
Absolute lymphocyte count (thou/mm^3)	1.94	0.95	3.05	1.91	1.23	14.47	0.18
Absolute eosinophil (thou/mm^3)	0.25	0.06	1.97	0.20	0.07	1.34	0.91
Absolute monocyte counts(thou/mm^3)	0.57	0.22	1.20	0.53	0.24	5.08	0.39
Absolute basophil count (thou/mm^3)	0.03	0.01	0.10	0.05	0.01	0.39	0.23
Total RBC (mill/mm^3)	4.26	3.21	6.04	4.68	3.00	5.24	0.52
Haematocrit (%)	37.20	26.60	55.80	40.85	30.30	46.60	0.08
MCV (fL)	87.90	76.50	97.30	92.20	43.80	103.90	0.52
MCH (pg)	28.65	24.70	32.30	29.65	26.00	93.50	0.18
MCHC (g/dL)	31.79	28.10	40.40	32.55	29.40	34.60	0.80
Red cell distribution width	**14.70**	**10.90**	**19.90**	**13.80**	**9.40**	**15.30**	**0.02**
Platelet count (thou/mm^3)	193.00	1.91	333.00	189.00	104.00	295.00	0.65

Table 2: Comparison of the concentration (M) of metabolites in the blood of PCa patients with metastases to those without metastases patients. Statistically significant ($p < 0.05$) metabolites involved in the discrimination between the two groups were calculated according to the Mann-WhitneyU test.

Metabolites	Blood metabolites concentration (μM)						p-value
	Bone metastases with PCa patients (n = 20)			Non-metastases with PCa patients (n = 20)			
	Median	Min	Max	Median	Min	Max	
3HOB	868.79	213.54	2158.81	511.16	218.49	800.68	< 0.001
Lactate	4559.15	2260.24	10056.14	2429.58	1314.31	4428.71	< 0.001
Alanine	896.46	503.32	1386.04	533.06	309.56	1007.99	< 0.001
Acetate	129.31	21.14	254.56	53.97	36.88	105.69	< 0.001
Acetoacetate	230.49	84.92	423.69	106.04	44.48	223.75	< 0.001
Glutamate	624.63	409.22	1100.03	441.29	284.29	746.86	0.0002
Pyruvate	125.38	51.17	404.27	63.84	32.73	101.88	< 0.001
DMA	122.18	57.60	312.75	57.83	23.85	140.85	< 0.001
Creatine	155.28	61.74	456.79	85.88	52.46	126.27	< 0.001
Phosphocreatine	170.39	15.36	485.79	58.80	10.40	193.96	0.003
Malonate	122.11	33.92	461.96	61.39	33.24	107.18	0.002
Choline	171.47	64.93	491.99	73.18	51.58	111.11	< 0.001
GPC	154.13	77.10	520.50	90.00	49.05	192.75	0.001
Glycine	507.46	179.26	899.68	291.13	195.44	572.83	< 0.001
Creatinine	279.97	113.47	759.86	147.58	67.54	273.55	< 0.001
Glucose	5866.18	3008.06	17999.70	4539.10	3240.38	8333.95	0.04
3HOB: 3 hydroxybutyrate; DMA: Dimethylamine; GPC: Glycerophosphocholine							

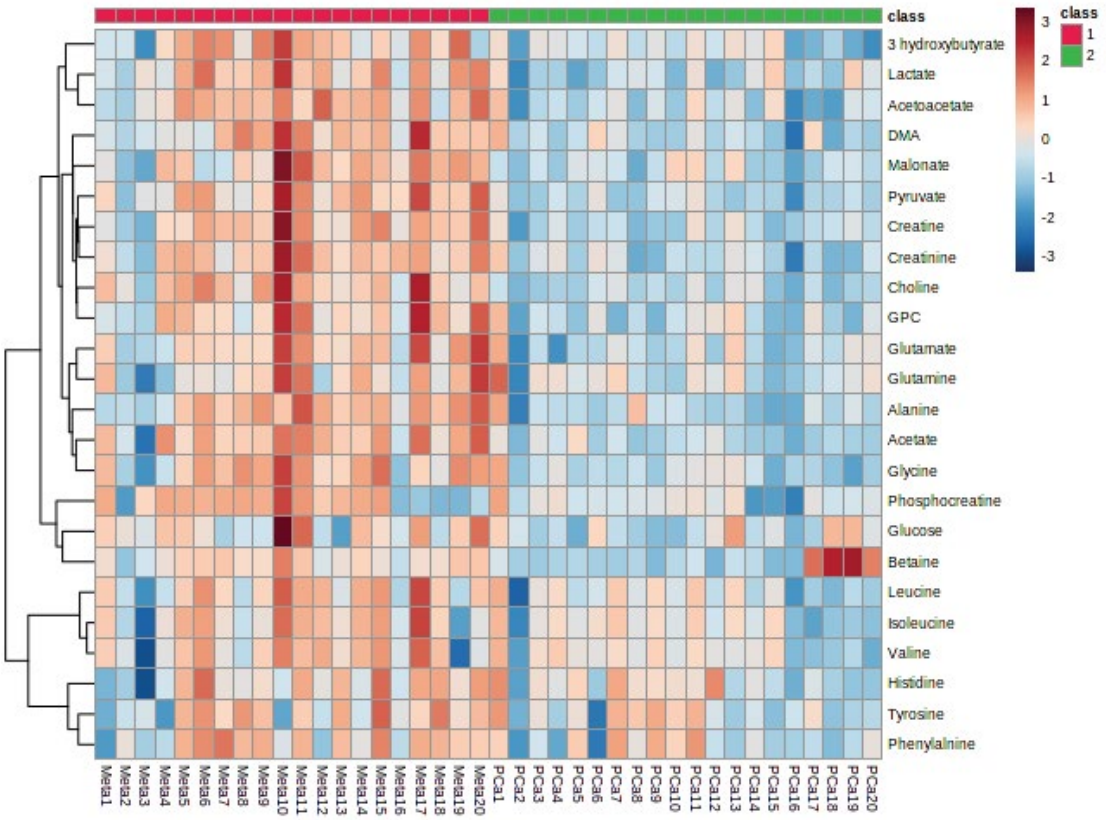

Figure 3: Heatmap analysis of significant metabolites differentially abundant in blood plasma samples of metastases patients with PCa as compared to metastases Patients. Each column shows the metabolic pattern of individual PCa patients in and without metastasis groups. The amount of each metabolite in individual blood plasma samples is expressed as a relative value obtained by the autoscaling method and is represented by the color scheme in which red and blue indicate high and low concentrations of metabolites, respectively.

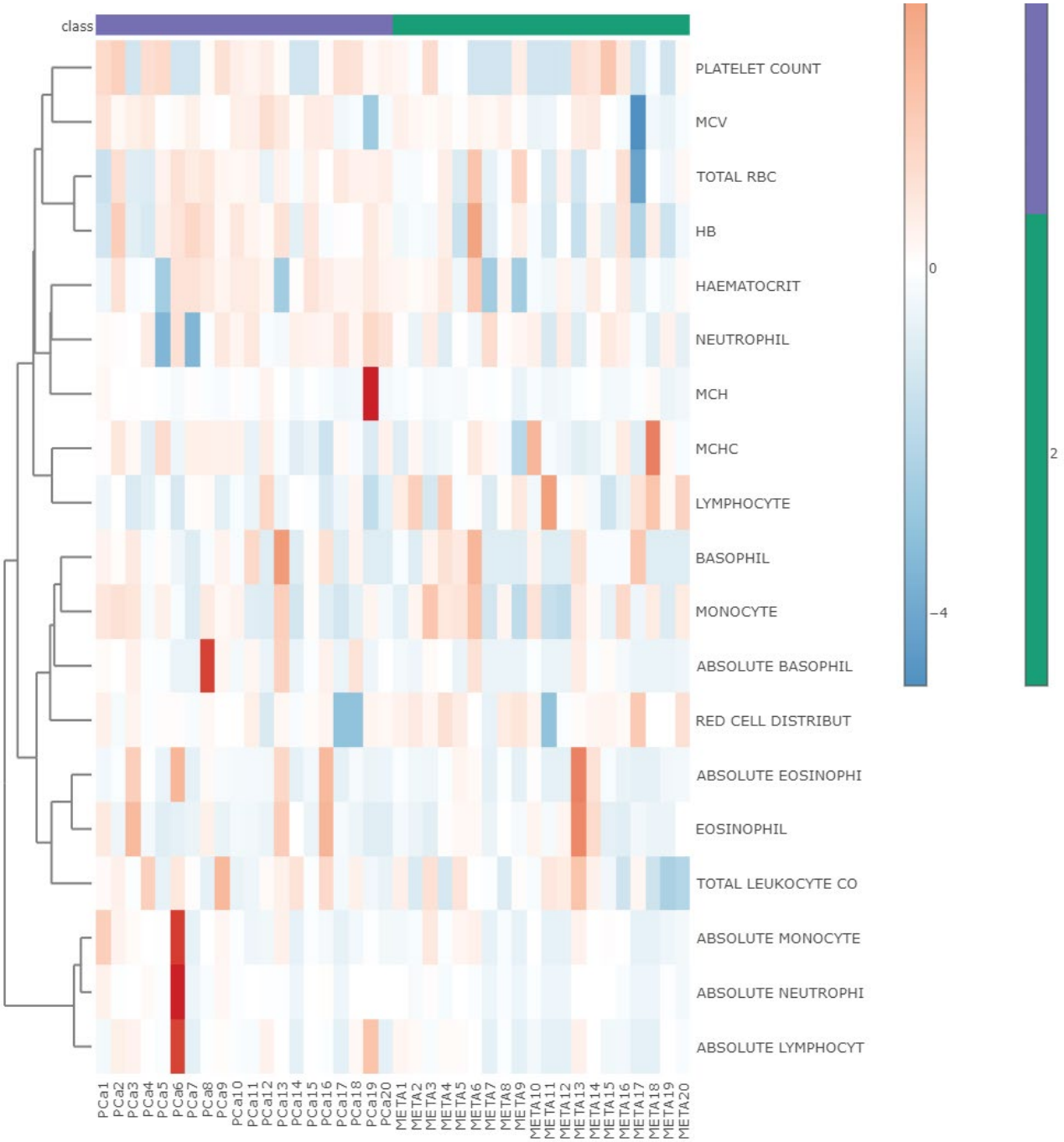

Figure 4: Heatmap analysis of significant metabolites differentially abundant in blood parameters and WBC subtype of metastases patients with PCa as compared to metastases Patients. Each column shows the blood parameters and WBC subtype pattern of individual PCa patients in the metastases group and without metastases group. The amount of each WBC subtype in individual blood plasma samples is expressed as a relative value obtained by the auto-scaling method and is represented by the color scheme in which red, oranges, and blue indicate high, intermediate, and low levels of blood parameters and WBC subtype respectively.

Table 2. Furthermore, an analysis of the statistically significant difference between white blood cells and other immunological parameters of PCa patients is given in Table 2. Heatmap corrections such as blood metabolites and immunological parameters are shown in Figure 3 and Figure 4.

The blood metabolic profile of PCa patients with bone metastases as determined by NMR is distinct from PCa patients with non-metastases. The supervised OPLS-DA score plot shows clear discrimination between bone metastases PCa patients and PCa patients with non-metastases. Variable in projection (VIP) scores of metabolite profile in urine in two study groups. VIP scores for 15 metabolites with the highest contribution to the separation of the present study groups. The boxes on the right refer to relative concentrations of appropriate metabolites in study groups. Box & whisker plots (choline and pyruvate) presentation are shown in Figure 5. The heatmap showed that the patients with PCa metastases and those patients with PCa non-metastases

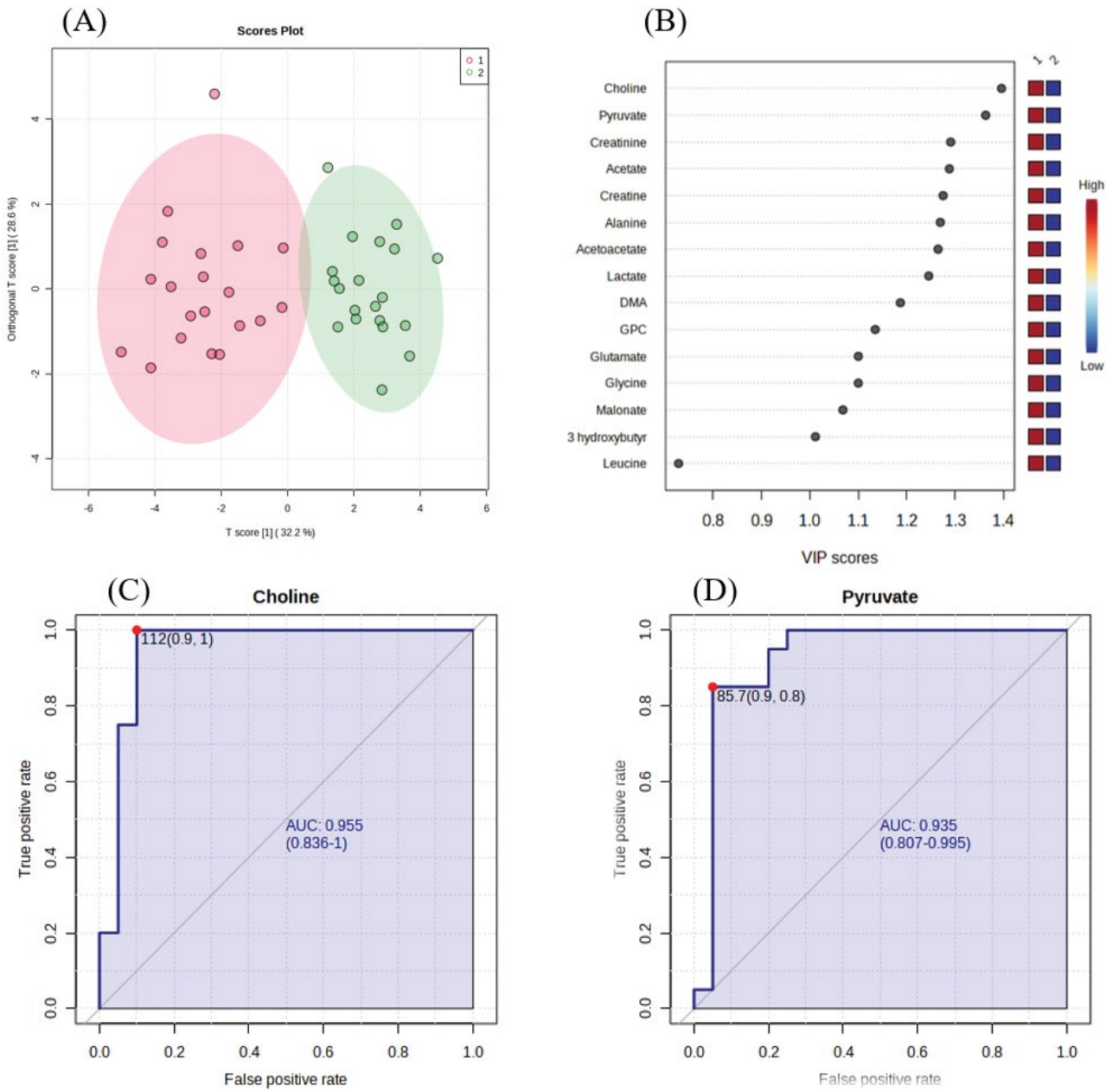

Figure 5: Results of PLS-DAs of metabolic profiles. Score plots obtained in OPLS-DAs of metabolites profiles in blood plasma (A), VIP) scores (B) in two studied groups: metastases PCa (n = 20) and non-metastases patients (n = 20), Univariate ROC curve analysis of choline (C) and Pyruvate (D).

were almost clearly separated from each other. It could be observed that the metabolic state of patients with PCa metastases results in significantly increased levels of 3 HOB, lactate, alanine, acetate, acetoacetate, glutamate, pyruvate, DMA, creatine, phosphocreatine, malonate, choline, GPC, glycine, creatinine, and glucose. The result of heatmap analysis further demonstrated that these metabolites could discriminate between patients with PCa metastases from those without metastases, so these endogenous metabolites could be used as potential biomarker/s. Furthermore, Figure 3 and Figure 4 show the heatmap visualization based on blood metabolites in patients with PCa metastases and those patients without PCa metastases. Pathway enrichment analysis using the KEGG and SMPDB revealed the potential involvement of metabolism in PCa shown in Figure 6. Pathway enrichment analysis using the revealed potential involvement of KEGG, pyruvate metabolism, phospholipids and phosphatidylcholine and phosphatidylethanolamine biosynthesis and citric acid cycle, alanine metabolism, fatty acid biosynthesis, methionine metabolism were dominated in bone metastases PCa are shown in Figure 6.

Discussion

Utilizing Nuclear Magnetic Resonance (NMR) spectroscopy for metabolomics, the research explores how metabolic profiles in the blood can serve as biomarkers for early-stage bone metastases. The integration of immunological parameters further

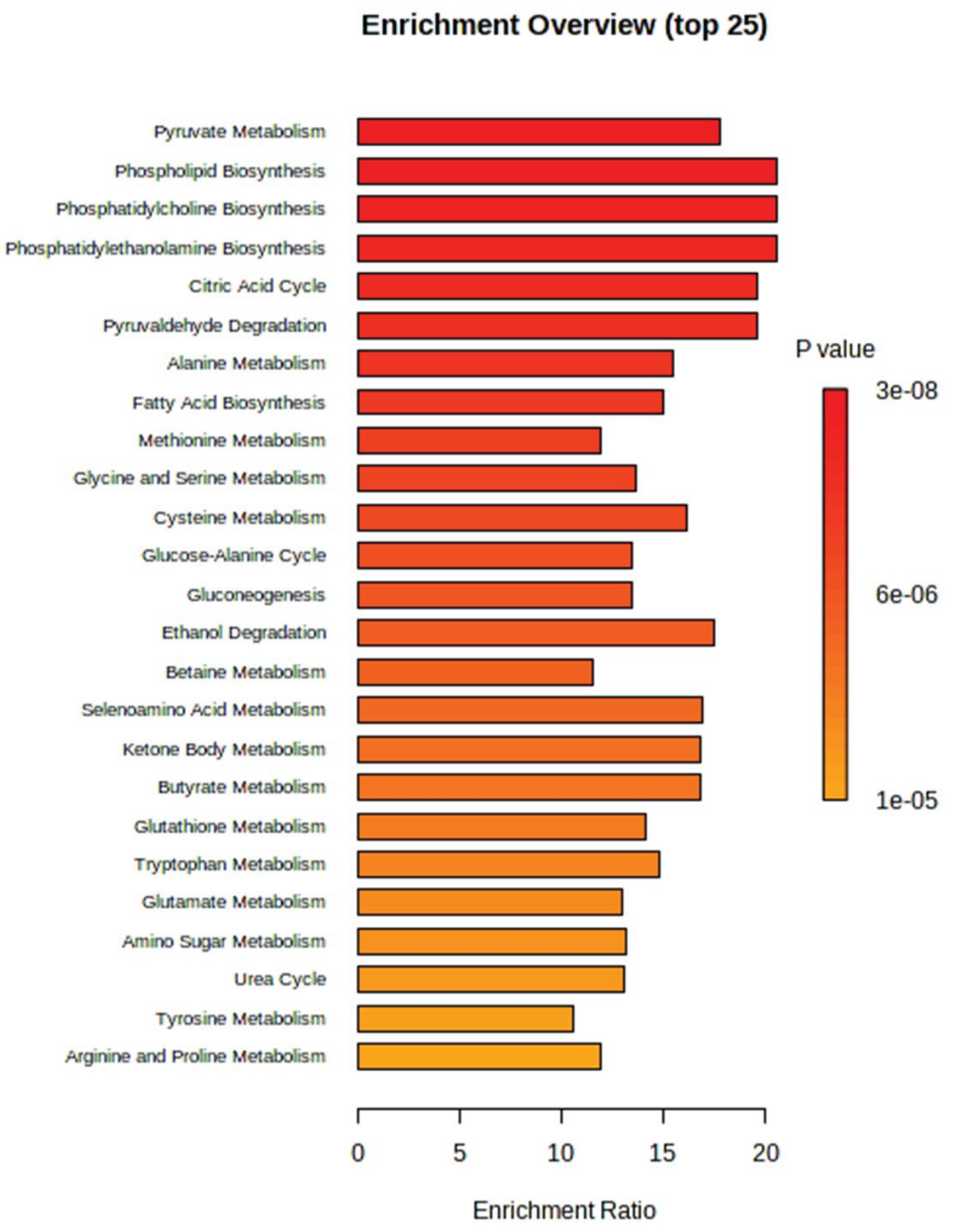

Figure 6: Metabolic Set Enrichment Analysis (MSEA) showing altered significant blood plasma metabolites associated with potential metabolic pathways of bone metastases PCa. Dominated pyruvate metabolism, phospholipids biosynthesis, and TCA cycle were observed.

enhances understanding of the disease's progression by revealing the role of immune system alterations. In recent years, with the advent of NMR spectroscopy, medical and oncology research has been focusing on the identification of metabolites as biomarker/s for early detection and treatment of cancer [12-14]. In The same way, researchers have been starting to look at metabolite identification in blood samples that can

be used as biomarkers for diagnosis and treatment of PCa. Blood contents are convenient biomarkers for the diagnosis of PCa because inexpensive and fast to harvest. Several studies have looked at differences in metabolite levels revealed blood between healthy or BPH and PCa subjects [15,16]. This study focused on the separation between PCa patients with bone metastases and non-metastases groups using univariate and multivariate analysis (OPLS-DA, VIP score, and ROC models). These findings match our results for the blood metabolites, which showed significant levels in the show in Table 2 comparing PCa patients with bone metastases and non-metastases. Therefore, we suggest that a higher level of lymphocytes may be useful in clinical practice in bone metastases patients with PCa who had a lower value of neutrophils compared to non-metastases. Therefore, we suggest that an altered WBC subtype may be useful in clinical practice in bone metastasis patients with PCa. The present study revealed significantly higher concentrations of alanine, pyruvate, and glutamate in the blood plasma of PCa patients with metastases as compared to non-metastases. A higher level of ketone body including acetoacetate, acetate, and 3-HOB was seen in bone metastases PCa patients [10]. 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. A significantly increased concentration of membrane metabolite choline, GPC, betaine, and DMA in bone PCa patients with metastases indicates a higher proliferation of metastatic PCa cells. Furthermore, alteration of glucose metabolism (glucose and lactate) energy metabolism (creatine, phosphocreatine, creatinine), and nucleic acid metabolism (glycine) were observed in bone metastases with PCa patients. These metabolic reprogramming of the amino acids, glycolysis, and Krebs cycle and interconnected with fatty acids, ketone body, and nucleic acid metabolism. The pathway enrichment analysis using the revealed potential involvement of KEGG “pyruvate metabolism, phospholipids and phosphatidylcholine and phosphatidylethanolamine biosynthesis and citric acid cycle, alanine metabolism, fatty acid biosynthesis, methionine metabolism” in bone metastases PCa.

The significantly higher concentration of alanine in bone metastases PCa compared to non-metastases PCa is possibly accompanied by over expression of the metabolism of relevant amino acids and altered TCA cycle in hypoxia [16]. 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 [16]. 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 [16]. 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 [16-18]. 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 [16]. 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 [15,19]. 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 [16,20]. Our results revealed elevated levels of energy demand for the aggressiveness of the tumor. Our data showed elevated blood plasma glucose and lactate in bone metastases PCa patients in comparison to non-metastases PCa. It was reported that a higher concentration of glucose and lactate seen in PCa patients was positively correlated with the increased risk of cancer [21-23].

Our findings also suggest that the metabolomics profile of blood plasma from PCa patients may be reflected inpyruvate metabolism, phospholipids biosynthesis, citric acid cycle, and alanine metabolism, more serious in PCa patients with bone metastases. These metabolites represent promising biomarker/s that were changed along with the development of PCa and might be related to the occurrence and aggressive of this advanced cancer. These PCa-related distinctive metabolites could benefit from the development of novel types of advanced disease biomarker/s and assist in understanding the potential pathogenesis. Further study in a large cohort is needed to investigate whether the observed differences in creatine, choline, GPC DMA, pyruvate, glycine, andalanine are PCa-specific and the related mechanism underlying the aggressiveness of PCa bone metastases.

However, the reason for the increased lymphocytes and decreased neutrophils is still unknown. The study suggests that the changes in blood cell counts might be linked to the release of certain signaling molecules called cytokines and chemokines [24,25]. These molecules can affect WBC proliferation and might explain why lymphocyte levels increase and neutrophil levels decrease. In summary, the study identifies a distinct blood cell profile in PCa patients with bone metastases, potentially due to immune system changes. This profile

might be useful for clinical assessments, but additional research is necessary to confirm and elaborate on these findings.

Conclusion

NMR-based metabolomic profiling of blood plasma, the present study showed that bone metastases PCa could be distinguished from non-cancerous individuals by metabolites (3HOB, lactate, alanine, acetate, acetoacetate, glutamate, pyruvate, DMA, creatine, phosphocreatine, malonate, choline, GPC, glycine, creatinine, and glucose) immunological parameters (neutrophil and lymphocytes). The findings may add to our understanding of the progression and aggressiveness of bone metastases PCa and indicate that these metabolites and white blood cells are potential candidate biomarkers for bone metastases PCa. Moreover, the present study supported the interpretation that blood plasma metabolomics-derived biomarker/s for bone metastases PCa may be a new option for non or partially invasive bone metastases PCa diagnostics.

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.

Conflict of Interest

The authors declare that they have no conflict of interest.

Funding Information

This manuscript is not funded.

Acknowledgment

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

References

1. Goode EA, Wang N, Munkley J (2023) Prostate cancer bone metastases biology and clinical management (Review). Oncol Lett 25: 163.
2. Siegel RL, Giaquinto AN, Jemal A (2024) Cancer statistics, 2024. CA Cancer J Clin 74: 12-49.
3. Kdadra M, Höckner S, Leung H, Kremer W, Schiffer E (2019) Metabolomics biomarkers of prostate cancer: A Systematic Review. Diagnostics (Basel) 9: 21.
4. Prensner JR, Rubin MA, Wei JT, Chinnaiyan AM (2012) Beyond PSA: The next generation of prostate cancer biomarkers. Sci Transl Med 4: 127rv3.
5. Benedetti E, Chetnik K, Flynn T, Barbieri CE, Scherr DS, et al. (2023) Plasma metabolomics profiling of 580 patients from an Early Detection Research Network prostate cancer cohort. Sci Data 10: 830.
6. Zhang Y, Liang X, Zhang L, Wang D (2021) Metabolic characterization and metabolism-score of tumor to predict the prognosis in prostate cancer. Sci Rep 11: 22486.
7. Evin D, Evinová A, Baranovičová E, Šarlinová M, Jurečeková J, et al. (2024) Integrative metabolomic analysis of serum and selected serum exosomal microRNA in metastatic castration-resistant prostate cancer. Int J Mol Sci 25: 2630.
8. Huang J, Mondul AM, Weinstein SJ, Derkach A, Moore SC, et al. (2019) Prospective serum metabolomic profiling of lethal prostate cancer. Int J Cancer 145: 3231-3243.
9. Thysell E, Surowiec I, Hörnberg E, Crnalic S, Widmark A, et al. (2010) Metabolomic characterization of human prostate cancer bone metastases reveals increased levels of cholesterol. PLoS One 5: e14175.
10. 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.
11. Mondul AM, Moore SC, Weinstein SJ, Karoly ED, Sampson JN, et al. (2015) Metabolomic analysis of prostate cancer risk in a prospective cohort: The alpha-tocopherol, beta-carotene cancer prevention (ATBC) study. Int J Cancer 137: 2124-2132.
12. Hurwitz LM, Agalliu I, Albanes D, Barry KH, Berndt SI, et al. (2021) Recommended Definitions of Aggressive Prostate Cancer for Etiologic Epidemiologic Research. J Natl Cancer Inst 113: 727-734.
13. Kelly RS, Vander Heiden MG, Giovannucci E, Mucci LA (2016) Metabolomic biomarkers of prostate cancer: prediction, diagnosis, progression, prognosis, and recurrence. Cancer Epidemiol Biomarkers Prev 25: 887-906.
14. Nicholson G, Rantalainen M, Maher AD, Li JV, Malmodin D, et al. (2011) Human metabolic profiles are stably controlled by genetic and environmental variation. Mol Syst Biol 7: 525.
15. 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.
16. 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.
17. Chan ECY, Koh PK, Mal M, Cheah PY, Eu KW, et al. (2009) 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.
18. 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.
19. Glunde K, Bhujwalla ZM, Ronen SM (2011) Choline metabolism in malignant transformation. Nat Rev Cancer 11: 835-848.
20. Wyss M, Kaddurah-Daouk R (2000) Creatine and creatinine metabolism. Physiol Rev 80: 1107-1213.
21. Wulaningsih W, Holmberg L, Garmo H, Zethelius B, Wigertz

A, et al. (2013) Serum glucose and fructosamine in relation to risk of cancer. PLoS One 8: e54944.

22. Wright JL, Plymate SR, Porter MP, Gore JL, Lin DW, et al. (2013) Hyperglycemia and prostate cancer recurrence in men treated for localized prostate cancer. Prostate cancer Prostatic Dis 16: 204-208.

23. 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.

24. Cihan YB, Arslan A, Ergul MA (2013) Subtypes of white blood cells in patients with prostate cancer or benign prostatic hyperplasia and healthy individuals. Asian Pac J Cancer Prev 14: 4779-4783.

25. Bou-Dargham MJ, Sha L, Sang QA, Zhang J (2020) Immune landscape of human prostate cancer: Immune evasion mechanisms and biomarkers for personalized immunotherapy. BMC Cancer 20: 572.