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Palmitoyl Carnitine, an altered biomarker in ADT responsive and ADT non-responsive prostate cancer patients

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Abstract- Prostate cancer (PCa) ranks as the second most prevalent cancer among males worldwide and Androgen deprivation therapy (ADT) stands as a crucial treatment in managing PCa. However, patients commonly develop resistance to ADT within 2-3 years, posing a significant challenge to its effectiveness. This resistance greatly impacts the success of ADT administration. The intricate nature of PCa is evident through its extensive array of metabolic and functional alterations within affected tissue, showcasing a broad spectrum of underlying mechanisms and drivers. Metabolites associated with PCa, can be evaluated with the application of H Nuclear Magnetic Resonance (NMR) spectroscopy. NMR metabolomics helps to understand the alteration in metabolite concentration in various biological samples. NMR metabolomics can also give information of therapeutic response in PCa patients. An integral feature of PCa lies in its altered lipid metabolism, leading to the accumulation of acyl carnitines due to metabolic dysregulation. Notably, the elevation of palmitoyl carnitine, a long-chain acyl carnitine, has been observed in PCa patients. Analyses of serum samples from PCa patients who do not respond to ADT (ANR) exhibit elevated levels of palmitoyl carnitine compared to both PCa patients responsive to ADT (AR) and healthy control subjects. This study highlights the potential of palmitoyl carnitine as a marker for evaluating ADT response in PCa patients.

Key words: prostate cancer, metabolomics, metabolism, ADT, NMR spectroscopy

INTRODUCTION

Prostate cancer stands as one of the prevalent non-cutaneous solid cancers affecting males globally. Key clinical predictors like Prostate Specific Antigen (PSA) levels, digital rectal examination (DRE), trans-rectal ultrasound (TRUS), and Gleason score (GS) have been established, yet they possess limited sensitivity and specificity. A more accurate early diagnosis leading to improved treatment options is crucial for enhancing patient survival. Therefore, there's an urgent need to uncover novel and potent biomarkers for detecting disease progression.

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The metabolism of prostate cancer (PCa) cells differs significantly from that of normal cells. Prostate cancer metabolomics, a burgeoning research field, focuses on the relationship between metabolites and pathology. Irregularities in the normal metabolic state have been linked to the initiation and advancement of prostate cancer, as well as resistance to Androgen Deprivation Therapy (ADT). ADT is the most common treatment option for hormone sensitive prostate cancer either localized or metastasized prostate cancer. In due course of 2-3 years of ADT the disease becomes resistant to it and progresses to the prostate cancer associated death.[1] There is not much idea about the changes in the metabolic pathways and mechanism as a response to ADT. We predicted that the

understanding of any changes occurring in metabolites during this change of ADT responsive (hormone sensitive) to ADT non-responsive (hormone resistant or castration resistant) prostate cancer can help in the better perceptive of disease progression.[2]

Metabolomics is "the quantitative measurement of the dynamic multiparametric metabolic response of living systems to pathophysiological stimuli or genetic modification".[3] Numerous advanced yet minimally invasive metabolomic techniques play a pivotal role in cancer research. High-performance liquid chromatography (HPLC), nuclear magnetic resonance (NMR), and mass spectrometry (MS; encompassing liquid chromatography mass spectrometry LC-MS and gas chromatography mass spectrometry GC-MS) are actively utilized to probe the metabolic profiles of diverse biological samples.[4] NMR, specifically, stands out as a promising method for analyzing metabolites within biological specimens. Notably, it offers exceptional reproducibility and enables the quantitative assessment of metabolites through straightforward sample preparation procedures. Its application in prostate cancer (PCa) research is notable, being employed for the analysis of various biological samples. NMR is proficient in unveiling the atomic and chemical structure of metabolites present in a sample, facilitating their identification and quantification.

Altered lipid metabolism is a key feature in cancer metabolism. Deregulated lipid metabolism can indicate pathological pathways and therapy response as well. Palmitoyl carnitine is an example of long chain fatty acid that accumulates in prostate cancer as a result of deregulated metabolism. We conducted a ^{1}H NMR serum metabolomic analysis to examine the variations in palmitoyl carnitine levels among three groups: ADT responsive (AR) and non-responsive (ANR) prostate cancer patients, as well as healthy controls.

MATERIAL & METHODS

Blood sample collection from healthy controls, AR and ANR PCa patients

Ethical Approval for this study was taken by the Institutional Research Ethics Committee, Department of Zoology, University of Rajasthan and Bhagwan Mahavir Cancer Hospital and Research Centre, Jaipur. We included 20 healthy controls, 35 AR and 35 ANR PCa subjects in our study. Informed consents were taken from all patients and healthy controls. Control cohort did not have any chronic disease and any comorbidity. AR and ANR groups did not have any other therapy. All the pathological and clinical parameters of the disease were provided by the Bhagwan Mahavir Cancer Hospital and Research Centre.

To search metabolite marker serum samples were analysed using ^{1}H NMR. From fasting volunteers approx 10 ml blood was collected in the morning time. Blood was then left untouched for 30 minutes at room temperature. In the next step the serum was separated by centrifugation at 1,600g for 15 min at 4°C. After centrifugation 200 µL serum was stored in 1.5 mL aliquots. In this way each sample was stored in multiple aliquots (about 3-4) and immediately shifted to -80 °C freezer and stored there until the NMR measurements were performed.

^{1}H NMR based serum metabolite analysis

Serum samples stored at 80°C were taken and thawed. After thawing of serum samples they were vortexed for 10 seconds. Then 250µL serum from each serum sample was mixed with 200µL 0.9% normal saline followed by addition of 50µL D_2O (deuterium oxide). The mixture was vortexed for 10 seconds and left on ice for 3 seconds. In the next step mixture was centrifuged for 12,000g for 5 min at 4°C. 500µl clear supernatant was then taken and transferred to 5mm NMR tube for further ^{1}H NMR data acquisition.[5] Analysis was performed at the JEOL NMR spectrometer (operating at a ^{1}H frequency of 400 MHz) equipped with a 5 mm probe for greater sensitivity. The one-dimensional (1D) water-suppressed ^{1}H NMR spectra were acquired at 25°.

Further NMR data Processing, metabolite identification and quantification was done by using software NMR suite and Human Metabolome Database (HMDB).[6] Metabolite concentrations were measured according to their peak area with reference to total area of every NMR spectrum.

RESULT

The data obtained from 1H NMR underwent further analysis using Python programming tool to conduct statistical assessments. An ANOVA test was utilized to compare the means across three distinct groups, with the predetermined threshold for 'statistical significance' set at $p < 0.05$. The sample distribution among the healthy control, AR, and ANR groups consisted of 20, 35, and 35 serum samples, respectively.

The analysis revealed a substantial variation in the mean intensity of palmitoyl carnitine among all three groups, with a remarkably low p-value of 2.86E-13 (in scientific notation).Further examination of the mean differences between specific groups unveiled significant disparities: Between AR and ANR, the observed variation was at a p-value of 3.80E-09. Comparing the healthy control group with AR resulted in a p-value of 1.45E-06. Meanwhile, the comparison between the healthy control group and ANR yielded a p-value of 3.38E-09.

These findings underscore the considerable differences in palmitoyl carnitine intensity across the groups studied, reinforcing the significance of this metabolite in distinguishing between these distinct physiological states.

Box plots visually depict the relative intensity of significantly altered palmitoyl carnitine among the healthy control, AR, and ANR groups (Figure-1). Notably, the relative intensity of palmitoyl carnitine was observed to be highest in ANR subjects compared to both AR and healthy control subjects.The results derived from ANOVA and the subsequent box plots strongly indicate palmitoyl carnitine as a significant metabolite, showcasing varying levels among ANR, AR, and healthy control subjects. Specifically, our observations highlight markedly higher levels of palmitoyl carnitine in ANR subjects compared to both AR and healthy control subjects.

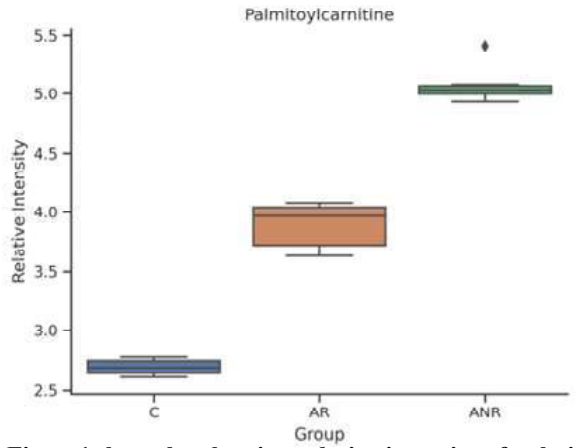

Figure1: box plot showing relative intensity of palmitoyl carnitine among healthy control, AR and ANR subjects.

DISCUSSION

The use of Androgen Deprivation Therapy (ADT) stands as a pivotal approach in the management of Prostate Cancer (PCa). However, a substantial obstacle to its effectiveness lies in the development of resistance over time. In PCa, various metabolites undergo alterations, offering promising insights into monitoring the outcomes of therapeutic interventions.

Within PCa, there are modifications in lipid metabolic pathways. Prostate cancer cells exhibit heightened levels of lipid oxidation, leading to increased production of acetyl CoA through β-oxidation. Acetyl CoA plays a pivotal role in generating acetyl carnitine and ketone bodies.7 Consequently, this process reduces the efficiency of the Tricarboxylic Acid (TCA) cycle. Moreover, a discrepancy arises between the TCA cycle and β-oxidation, resulting in the accumulation of long-chain acyl carnitines such as palmitoyl carnitine.[8]

Palmitoyl carnitine, derived from palmitic acid, has been associated not only with PCa but also with conditions like obesity, type 2 diabetes[9], and kidney malignancies. The accumulation of acylcarnitines has shown correlation with the advanced stages of kidney and liver cancers.[10] Higher levels of acyl carnitines have been identified in hepatocellular malignancies.[11] Research by Wu *et al.* (2014) demonstrated elevated levels of acylcarnitines in urine samples of patients with non-small cell lung carcinoma.[12]

In studies by Giskeodegard *et al.* (2015)[13] and Schmidt *et al.* (2017)[14], elevated levels of acylcarnitines were reported in the plasma of PCa subjects. Therefore, a reduction in their levels following ADT could signify a positive therapeutic response in Androgen Receptor (AR) subjects and potential resistance in Androgen Non-Receptor (ANR) subjects.

Chi *et al.* (2020)[15] observed variations in palmitoyl carnitine levels after ADT administration, reporting a decrease as a favorable response to ADT in PCa patients. Surprisingly, the association between ADT outcomes and serum palmitoyl carnitine levels has not been previously examined.

In our study, we measured serum palmitoyl carnitine levels using 1H Nuclear Magnetic Resonance (NMR) spectroscopy in control, AR, and ANR subjects. Significantly higher levels of serum palmitoyl carnitine were observed in ANR subjects compared to AR and control groups across a sample size of 20 controls, 35 AR, and 35 ANR subjects. The inclusion of a substantial number of samples suggests the potential of utilizing this

biomarker for monitoring the response to ADT in patients with prostate cancer.

CONCLUSION

In our study we observed higher levels of palmitoyl carnitine in ANR in comparison to AR and control subjects. It indicates that palmitoyl carnitine can be a good biomarker to monitor the response to ADT in prostate cancer treatment. However, a large number of samples are required to validate the study results. Further accumulated palmitoyl carnitine can interfere with the other metabolic pathways and can minimize the response to ADT while treatment. Further investigation with other metabolic dysfunction should also be done.

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