Investigate the impact of ICAM-2 and Neprilysin biomarkers in prostate cancer patients infected with JC virus in AL-Najaf AL-Ashraf Province

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Abstract. A total of 74 clinical samples Formalin-Fixed Paraffin-Embedded (FFPE) were collected from patients diagnosed with prostate cancer (PCa) aged between 41 and 90 years and these samples were obtained from patients treated at notable medical institutions like Al-Sadr Medical City and leading clinical laboratories in Al-Najaf City, Iraq, during the period of January to December 2023. The current study indicated the potential role of the JCV virus in provoking prostatitis, which may lead to the emergence and development of prostate cancer in males compared to males who do not suffer from viral infection. The present study showed the presence of JCV virus DNA, as the percentage of positive samples reached (11, 14.864%) compared to negative samples (63, 85.135%). The current study showed a significant increase in the level of ICAM-2 biomarker in patients with JCV-positive prostate cancer, reaching (818.500±42.748 pg/ml) compared with patients with JCV-negative, reaching (502.925±58.037 pg/ml). Neprilysin (NEP) levels in the current study were significantly high in for JCV-positive patients and those with prostate cancer, reaching (4.275±0.376 pg/ml) compared with JCV-negative patients, reaching (3.131±0.232 pg/ml).

1 Introduction

Prostate cancer develops in the prostate gland, situated beneath the bladder in males and encircling the upper part of the tube that carries urine from the bladder (urethra) and Infection in the prostate gland, also referred to as prostate cancer-related infection, is when infections occur in individuals diagnosed with prostate cancer. [1]. In the past ten years, global cancer epidemiology has revealed that Prostate Cancer (PC) ranks as the second most common cancer in men and approximately 1,276,106 new cases and 358,989 deaths were reported and the incidence rate of prostate cancer varies worldwide, being lower in Africa and Asia compared to developed countries, moreover, mortality rates differ across regions and for instance, central america has the highest mortality rate at 10.7 per 100,000 people, while south-central asia records the lowest at 3.3 per 100,000 people and increasing awareness is crucial for recognizing the risk factors associated with PC and developing

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Effective prevention strategies and studies indicate that several factors, including age, race/ethnicity, family history, smoking habits, diet, and obesity, play a significant role in the development of prostate cancer [2]. Polyomaviruses are a family of small DNA viruses that have been known to infect a wide range of animals, including humans. The name "polyomavirus" is derived from "poly-" (meaning many) and "-oma" (meaning tumors), as these viruses were initially discovered for their ability to cause tumors in laboratory mice [3]. The JC virus is common in humans and usually become dormant after infection, allowing them to remain in the body without causing any symptoms and however, in specific situations these viruses can become active again and lead to illnesses, especially in people with weakened immune systems [4]. In recent years, there have been numerous discoveries of polyomaviruses, including the identification of Merkel cell polyomavirus (MCMV) in 2008 and MCMV is linked to Merkel cell carcinoma, a rare type of skin cancer and polyomaviruses remain a topic of active research, with scientists exploring their biology, transmission, association with diseases, and potential therapeutic approaches [5]. JCV polyomavirus, also known as JC virus (JCV), is a member of the Alpha polyomavirus genus within the family Polyomaviridae and is a small, non-enveloped DNA virus that infects humans and it was first identified in 1971 and is named after the initials of the patient in whom it was isolated [6]. JCV is a ubiquitous virus, with a high prevalence in the general population and infection with JCV usually occurs in early childhood, and the virus establishes a persistent, lifelong infection in the kidneys of healthy individuals and most JCV infections are asymptomatic and go unnoticed, as the virus remains in a latent state [7]. JCV infection is common among the general population, with seroprevalence studies revealing varying rates across different populations and geographical regions and the prevalence of JCV antibodies tends to rise with age, suggesting that the majority of individuals encounter the virus in childhood or early adulthood [8]. Intercellular Adhesion Molecule 2 (ICAM-2) is a cell surface protein that is part of the immunoglobulin superfamily and it is primarily involved in the regulation of leukocyte adhesion and migration, playing a critical role in immune responses [9]. ICAM-2 is found on endothelial cells and certain leukocytes, enabling interactions with integrins, particularly lymphocyte function-associated antigen 1 (LFA-1), present on T cells and other leukocytes. While the involvement of ICAM-2 in immune cell movement and inflammation is well understood, its potential link to polyomavirus infection and prostate cancer is an increasingly intriguing area of research [10]. Neprilysin, also recognized as the neutral endopeptidase (NEP), CD10, and common acute lymphoblastic leukemia antigen (CALLA), functions as a zinc-dependent metalloprotease that performs a vital role in managing peptide signaling by breaking down different bioactive peptides and this enzyme participates in multiple physiological functions such as regulating blood pressure, sensing pain, and adjusting immune responses and its presence can be noted in diverse tissues like the kidneys, intestines, lungs, and the prostate gland [11]. The importance of neprilysin in prostate cancer research has been increasingly recognized, with studies linking neprilysin to various aspects of cancer progression such as cell growth, programmed cell death, formation of new blood vessels, and metastasis. Researchers have explored neprilysin expression levels in prostate cancer, suggesting that reduced neprilysin levels may be associated with tumor advancement and unfavorable disease outcomes. This decrease in neprilysin levels could lead to an accumulation of substances it typically breaks down, potentially including peptides that promote tumor growth and spread [11,12].
2 Material and Methods

2.1 Sample Collection

A total of 74 prostate tumor tissue blocks were from the archives of AL-Sadder Teaching Hospital and private histopathology laboratories in AL-Najaf city. The samples encompassed a timeline from January 2023 to December of the same year, providing a diverse representation of prostate cancer cases during this period.

2.2 DNA Extraction from FFPE Samples

For the efficient extraction of DNA from Formalin-fixed, paraffin-embedded (FFPE) tissue, the HiPure FFPE DNA Kit was employed. This kit utilizes silica gel column purification technology, obviating the need for labor-intensive methods such as phenol-chloroform extraction or alcohol precipitation. The entire extraction process was completed in a short span of 20 minutes, excluding digestion time.

2.3 Principle of ICAM-2 and Neprilysin kit

This ELISA kit uses the Sandwich-ELISA principle. the micro-ELISA plate provided in this kit has been pre-coated with an antibody specific to human ICAM-2 and Neprilysin samples (or standards) are added to the micro-ELISA plate wells and combined with the specific antibody. then a biotinylated detection antibody specific for human ICAM-2 and Neprilysin and Avidin-Horseradish Peroxidase (HRP) conjugate are added successively to each micro plate well and incubated. free components are washed away. the substrate solution is added to each well. only those wells that contain human ICAM-2 and Neprilysin, biotinylated detection antibody and Avidin-HRP conjugate will appear blue in color. the enzyme-substrate reaction is terminated by the addition of stop solution and the color turns yellow. the optical density (OD) is measured spectrophotometrically at a wavelength of 450 ± 2 nm. The OD value is proportional to the concentration of human ICAM-2 and Neprilysin. you can calculate the concentration of human ICAM-2 and Neprilysin in the samples by comparing the OD of the samples to the standard curve.

2.4 Statistical Analysis

All values are expressed as mean ± standard deviation of mean and the study results were statistically analyzed using the software statistical package for the social sciences (SPSS) version 24. The Chi-square test (χ²) was employed for categorical data analysis A P ≤ 0.05 was considered significant [13].

3 Results

The study including examination 74 FFPE samples from prostate cancer patients, JCV was investigated by using Real Time qPCR and 11 samples were positive for the presence of JCV viral DNA as shown in figure (1).
The data displayed in the table (1) and figure (2), showcases the relationship between ICAM-2 levels and prostate cancer patients concerning JC virus (JCV) infection. to sum up, the findings indicate a high significant difference (p≤0.001) in ICAM-2 levels among prostate cancer patients based on their JC virus infection status and notably patients who tested positive for the JC virus exhibited markedly elevated ICAM-2 levels (818.500±42.758) pg/ml compared to those who tested negative (502.925±58.037) pg/ml.

**Table 1.** Relation of ICAM-2 levels with prostate cancer patients based on viral infection (JCV).

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>NO. of examination of prostate cancer patients blocks (74)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>JCV +ve (Mean ±S. D)</td>
</tr>
<tr>
<td>ICAM-2 (pg/ml)</td>
<td>818.500±42.758</td>
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\[X^2 = 357.282 \quad P \text{ value: 0.001 Highly Significant (S)}\]

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**Fig. 1.** Amplification plots for detection JCV in FFPE PCa by Real-time qPCR.

**Fig. 2.** Relationship between ICAM-2 and PCa FFPE and JCV infected.
The data displayed in this table (2) and figure (3) illustrates the relationship between neprilysin levels and prostate cancer patients, categorized by the presence or lack of JC virus (JCV) infection. The findings from this data indicate a notable statistical difference in neprilysin levels between prostate cancer patient’s infection with JC virus (4.275 ± 0.376) pg/ml and without JC (3.131 ± 0.232) pg/ml virus infection and which showing statistical elevated levels in patients with JCV infection.

Table 2. Relation of Neprilysin Levels with Prostate Cancer Patients based on Viral Infection (JCV).

<table>
<thead>
<tr>
<th>Biomarker</th>
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<tbody>
<tr>
<td></td>
<td>JCV +ve (Mean ±S. D)</td>
</tr>
<tr>
<td>Neprilysin (pg/ml)</td>
<td>4.275 ± 0.376</td>
</tr>
</tbody>
</table>

$X^2 = 97.544 \quad P. value: 0.001 \quad \text{Highly Significant (S)}$

Fig. 3. Relationship between neprilysin and PCa FFPE and JCV infected.

4 Discussions

The discovery of JCV DNA in samples of prostate cancer initiates a conversation about the possible link between JCV infection and the development of prostate cancer [1,14,15] and indicates there were a potential connection between JCV and different types of cancer, such as prostate cancer. The JCV virus is recognized for its capacity to induce cancer in various situations, mainly by disrupting the regulation of the cell cycle and preventing programmed cell death [16,17]. The outcomes of present study are improved by evidence from another studies that indicated that JCV may disrupt cellular signaling pathways in prostate cells and this disruption can affect cell proliferation and survival, potentially initiating tumorigenesis [18,19]. Exploring the connection between JCV status and treatment response might influence the decisions made in therapy [20,21] and certain researchers [22,23] have indicated that detecting the JCV virus in tumor samples has led medical professionals to implement more rigorous treatment plans for affected individuals, than in infected without virus as they opt for less aggressive therapies to mitigate potential treatment-related toxicity and those studies further reinforces the findings from our investigation into identifying the virus. The present study revealed increase levels of ICAM-2 in patients with JCV positivity and this observation implies a possible link between JCV infection and elevated ICAM-2 expression in individuals with prostate cancer [24,48] and the increased levels of ICAM-2
noted in JCV positive prostate cancer patients could have various implications, levels of ICAM-2 have the potential to be used as a diagnostic indicator to identify JCV infection in patients with prostate cancer [25,26]. This discovery could offer insights into the impact of viral infections on prostate cancer and could be relevant for diagnostic or therapeutic purposes and noticeable disparity in ICAM-2 levels, findings from the chi-square test reveal a substantial contrast in ICAM-2 levels among prostate cancer patients who were positive for JCV compared to those who were negative and this implies that the presence of JCV infection had relation with fluctuations in ICAM-2 levels in individuals with prostate cancer [27,28]. Increased levels of ICAM-2 could suggest the existence of JCV and assist in categorizing patients according to their viral status [29,30]. The connection between JCV infection and increased ICAM-2 levels could also have predictive consequences and individuals with elevated ICAM-2 levels may experience distinct disease progressions or responses to treatments in contrast to those with lower levels [31,32] and exploring the influence of JCV infection on ICAM-2 levels could provide valuable insights for devising specific treatment approaches. Investigating the modulation of ICAM-2 expression or focusing on pathways related to JCV could open up potential treatment avenues [33,34]. Finally, the results of this study illuminate the connection between ICAM-2 levels and JCV infection among individuals with prostate cancer and this information may hold significant for the detection, prediction, and therapy of JCV-related prostate cancer, indicating the need for additional research and medical confirmation. The increased neprilysin levels observed in individuals with JCV infection could indicate a possible link between JCV infection and modified neprilysin expression in prostate cancer [35,36]. Neprilysin, an enzyme responsible for breaking down peptides which including those linked to tumor growth and spread and may therefore play a role in the advancement or severity of prostate cancer in JCV-infected individuals [37,38] and the possibility exists that JCV infection could trigger the increase in neprilysin levels, either through direct interactions with viral proteins or immune responses activated by the infection [39]. Elevated levels of neprilysin in prostate cancer patients infected with JCV could potentially function as a biomarker indicating disease aggressiveness or prognosis [40,41]. Monitoring neprilysin levels in conjunction with other clinical parameters may assist in risk assessment and tailored treatment strategies [42,43] and regularly monitoring neprilysin levels, particularly in individuals with JCV infection, can offer crucial prognostic insights and help tailor treatment plans to suit each patient's needs [44,45] and these discoveries could impact the assessment of risk, prediction of outcomes, and decisions regarding treatment for individuals with prostate cancer [46,47].

The data in present study indicates a notable correlation between neprilysin levels and JCV infection in patients with prostate cancer and it is imperative to conduct additional research to confirm these results and delve deeply into their clinical significance.

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