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A study of the relationship between the *Galectin -9 gene (LGALS9)* in three types of cancer patients in Basrah, Iraq

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ABSTRACT

Background. One essential member of the galectin family, galactose lectin-9 (Gal-9) serves several functions as a multi-subtype lectin. According to the latest research, tumour interaction with Gal-9 is a separate component that affects how quickly tumours grow.
This is the first investigation of the expression of the Galectin-9 gene with BC, CRC, and PC in Iraq, particularly in the Basrah Governorate.
Methods. A total (101) blood samples from patients with prostate, colorectal, and bladder cancer were obtained for this study. However, as a control group, 101 blood samples from people who were cancer-free were gathered. The first step are included extracting RNA form the blood samples, the extracted RNA converting into cDNA, then Gal-9 mRNA was measured by qPCR technique.
Results. *Gal-9* showed high expression level in bladder cancer as compared with the control group, wherever, expression levels were 14.63 and 4.97 respectively. In patients, there was a 4.97-fold increase in gene expression compared to a healthy control group. In colorectal cancer (CRC), the *Gal-9* gene was overexpressed, with expression levels measured at ± 2.84 for controls and ± 9.95 for patients. This indicates that the patients' gene expression differed by 3.5 fold from the healthy control group. Prostate cancer (PC) patients had an expression level of ± 9.08 for the *Gal-9* gene, whereas controls had an estimated expression level of ± 3.02. When compared to a healthy comparison group, the patients' gene expression was three times different.
conclusion: In the present study, *Gal-9* gene was expressed at a significantly higher level in the BC, CRC, and PC patients compared in the controls group.

Keywords: Gal-9 gene, Bladder cancer, Prostate cancer, colorectal cancer, qPCR, gene expression

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Introduction

Novel immune checkpoints have been identified throughout the last several decades, reviving cancer immunotherapy due to breakthroughs in immunological understanding. Immunotherapy—exemplified by immune checkpoint inhibitors—has emerged as the front-runner in the precision cancer treatment space, ushering in a new era for the majority of cancer patients' care [1]. Galectins are a class of animal lectins that modulate immunity and have been preserved throughout evolution. They are found in several immune cells, including macrophages, fibroblasts, and T cells. Merely eleven of them have been documented in human subjects engaged in various biological activities [2]. Gal-9 sometimes referred to as LGALS9, is expressed protein associated with immune modulation and tumour pathogenesis that influences the prognosis of many forms of cancer [3]. Galectin-9 is situated on chromosome 17. Numerous research has examined the role of (Gal-9) in the control of both innate and adaptive immunological responses, the induction of malignant cell death, and the modification of cellular polarity and adhesion [4]. Gal-9 was subsequently shown to activate regulatory T cells after it was first believed that it was the cause of the downregulation of T cell immunological responses by inducing apoptosis in CD4 + T helper 1 (Th1) and T helper 17 cells [2].

In 2023, bladder cancer (BC) accounted for 4% of cancer-related fatalities and 6% of anticipated new cancer cases in males. It was the fourth most common malignancy in this demographic [5]. According to [6], 70–75% of patients have non-muscle invasive bladder cancer at first presentation, 25–20% have muscle-invasive bladder cancer, and 5% have metastatic disease. Urothelial cell carcinoma accounts for around 90% of bladder cancer cases; squamous cell carcinoma, adenocarcinoma, and neuroendocrine carcinoma make up the majority of the remaining instances [7]. Both morphological and molecular heterogeneity are very variable in BC. However, there has been little progress in a number of promising biomarker therapeutics despite its well-characterized molecular signature and high incidence of potentially actionable genetic changes [8].

Globally, colorectal cancer is cause of cancer-related. Colorectal carcinogenesis involves a multitude of pathophysiological pathways, including aberrant cell proliferation, differentiation, resistance to apoptosis, invasion of tissues close to colorectal tumour cells, and distant metastasis [9]. Numerous genetic and environmental variables, such as a sedentary lifestyle, obesity, alcohol intake, smoking, or gut flora, combine intricately to launch these processes. There has been a notable rise in the prevalence of colorectal cancer in those under 50 years of age, despite the tremendous advancements made in the diagnosis and treatment management of patients with the disease [10]. The characteristics of early-onset colorectal cancer include a unique DNA methylation profile, a greater incidence of mucinous histology, a more distal location, and worse survival rates [11]. Identifying risk factors and conducting screening programs for people exposed to infection greatly improves the diagnosis of patients [12].

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The cancer of the prostate is a complex cancer, and that cancer progression is affected by genetics and environmental factors [13]. Prostate cancer is sixth kind of cause's deaths globally. Prostate-specific antigen testing, MRI scans, and prostate tissue biopsies are the major methods used for diagnosis; however, PSA testing for screening purposes is still debatable [14]. There are now new diagnostic tools accessible, such as germline testing, different PET scans, and risk stratification bioassay tests. When prostate cancer is the only affected organ, it is regarded as localized and perhaps treatable. Bisphosphonates, rank ligand inhibitors, hormone therapy, focused radiation, chemotherapy, immunotherapy, and radiopharmaceuticals may be employed if the illness has progressed outside the prostate. While the exact causation of prostate cancer is unknown, heredity is undoubtedly a factor. Prostate cancer risk is known to be influenced by genetic background, ethnicity, and family history [15, 16].

Patients who have genetic or hereditary cancer of the prostate often have earlier onset of the disease, faster progression, a greater likelihood of localized advanced disease, and a higher risk of post-operative recurrence. Of all the main cancers in males, hereditary prostate cancer is the most heritable [17]. With an average age of 66 years old, the incidence and mortality of prostate cancer correspond with advancing age globally. When prostate cancer is detected early on, it may not even need any treatment at all. The most common complaints are nocturia, frequency, and difficulty urinating. However, as the axis skeleton is the most prevalent location for metastasis, urinary incontinence and back discomfort may be symptoms indicating an advanced stage of the cancer [16].

Because of its high sensitivity, precision, and real-time analytic capabilities, RT-qPCR has used for mRNA quantification in gene expression study [18,19]. Reverse transcription, amplification efficiency, and the extraction, processing, and storage of RNA are among the technical concerns that must be taken into account. In order to ensure the reliability of the qPCR data, it is therefore necessary to properly normalize the data towards an appropriate reference gene that control on the experimental errors by the process [20]. This is explained in the guidelines for the Minimum Information for Publication of Quantitative Real-Time PCR Experiments. A number of genes are often used as reference genes, including glyceraldehyde-3-phosphate dehydrogenase (Gapdh) and beta-actin (B-actin). Since there is no universal references gene and it is impossible to know which gene would work under all experimental circumstances, finding appropriate reference genes to validate is a crucial first step in RT-qPCR gene expression research [21]. Clarifying the predictive significance of Gal-9 expression in BC, CRC, and PC is the goal of this research.

The study aims to clarify the prognostic role of *Gal-9* gene expression in BC, CRC and PC.

Material and method

Collection of sample

A total of 101 blood samples were drawn from PC, CRC, and BC. Patients from November 2021 to March 2022 at the Basrah Oncology and Hematology Centre comprised 32 BC samples, 39 CRC samples, and 30 PC samples. The age range of the individuals ranges from 29 to 94 years old.

Additionally, 101 blood samples from healthy individuals between the ages of 27 and 90 were taken as a control group. No viral infections, metabolic diseases, parasite invasion, diabetes, or hypertension were the exclusion criteria in this investigation. Using a sterile syringe, 2 ml of the blood samples were extracted from each of the two groups. Following that, they are stored in sterile EDTA tubes for RNA extraction. The total RNA was then converted into cDNA, and SYBR Green was used to evaluate the expression of the *Gal-9* and *B-actin* genes by RT-PCR.

RNA isolation and RT reaction

Using a pure kit that followed the manufacturer's instructions, GENEzol TM Tri RNA was used to extract total cellular RNA from the blood samples. Using a Nanodrop spectrophotometer, the total RNA level were assessed, and the A 260 /280 ratio was computed after the creation of the blank using RNase-free water. Using the Accupower® Rocket scriptTM RT PreMix kit, total RNA was convert as follows: Before being used, the samples and reagents were thawed. Lyophilized random hexamer was thoroughly dissolved and combined by pipetting or centrifuging, then diluted in a 10:90 µl. Oligo dT primer was liquid-diluted in a 1:1 ratio. Each sample's 400ng of total RNA was converted to cDNA using the Rocket scriptTM RT PreMix kit. The cDNA master mix tubes were filled with whole RNA, DEPC-water, and the primer (Oligo dT or random hexamer). PCR tubes put in the heat cycler and operated under the following settings: Heat inactivation: at 95˚ C for 95˚ C, primer the annealing stage are done for ten min at 37˚ C, and cDNA synthesis: at 60˚ C for 1 hr. All of the cDNA samples were kept at -20°C until further examination. [22]. SYBR® Green I dye and the qthermo cycler were used in qPCR to measure the target gene's (PD-L1) and housekeeping gene's (B-actin) mRNA expression levels. The primer sequences for Gal-9 [23] were F 5'-CTTTCATCACCACCATTCTG -3' and reverse R=5'-AGCCACACGCAGCTCATT-3'. For B-actine [23], the sequences were F5'-ATGGGTCAGAAGGATTCCTATGT-3' and reverse R=5'-AGCCACACGCAGCTCATT-3'. These results produced a 153 bp product. 10 µl of Go Taq qPCR Master Mix, 1 µl complementary DNA, 1 µl forward primers, 1 µl reverse primers, and 7 µl nuclease-free water were added to each PCR reaction (final volume: 20 µl). The denaturation stage needs four minutes at 95˚C (45)cycles, the annealing stage needs for half minutes at 52˚C, and the extension stage needs for forty seconds at 72˚C were done thermocycler.

Statistical analysis (the qPCR data analysis):

The obtained qPCR data were analyzed using the ΔΔCT method as the CT values for each gene *Gal-9*, as well as for the HK gene (B-actin) were determined [24]. The following steps were performed to obtain the expression of each gene:

ΔCT patient= CT patient - CT HK gene.

ΔCT control = CT control - CT HK gene.

ΔΔCT = ΔCT patient - ΔCT control.

Gene expression (Exp.) = 2^ - ΔΔCT

Fold change (FC) = Exp. patients / Exp. Controls.

One–way ANOVA was performed to evaluate the difference among cancer patients and healthy controls using SPSS version 17.0, at confidence interval ≤ 0.05 were considered as statistically significant.

The result

Gal-9 Gene expression experiment

The results of *Gal-9* gene expression patients and healthy controls. The gene of interest was estimated using SYBR Green. The melting curve showed one peak for all samples as shown in Figure (1) which demonstrate the specific binding of SYBR Green for *Gal-9* in patients and healthy controls also, the amplification curve was shown in Figure (2).

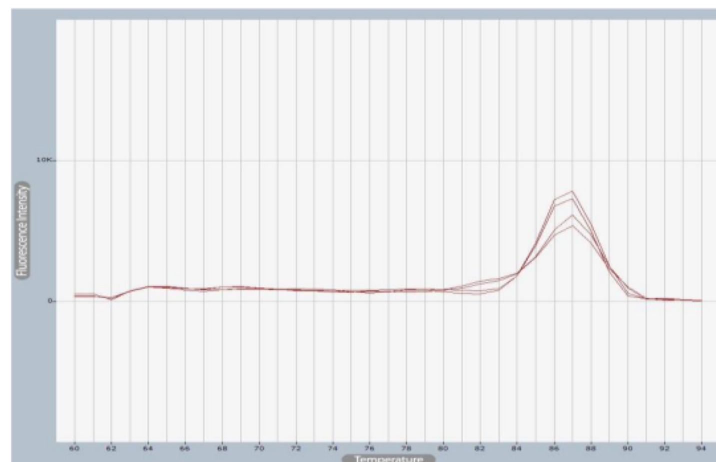

Figure 1: The melting curve of *Gal-9* gene in patients and healthy controls

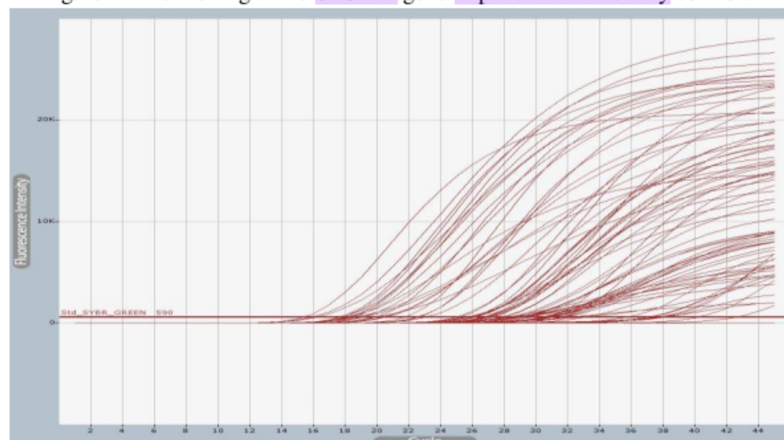

Figure 2: The amplification curve of *Gal-9* gene in patients and healthy controls

The expression of *Gal-9* gene in BC

The *Gal-9* gene was over expressed in BC with expression level ± 14.63 for patients and controls estimated with ±2.94. The gene expression was 4.97 fold change in patients compare to healthy control, at p-value =0.049. As shown as in figure (3).

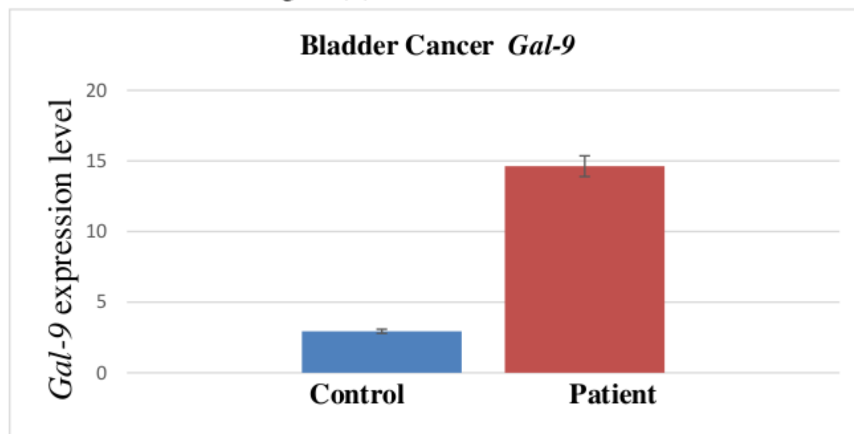

Figure 3: The gene expression was 4.97 fold change in BC patients compare to healthy controls.

3.3. The expression of *Gal-9* gene in CRC

The *Gal-9* gene was over expressed in CRC with expression level ± 9.95 for patients and controls estimated with ±2.84. The gene expression was 3.5 fold change in patients compare to healthy control, at p-value = 0.007. As shown as in figure (4).

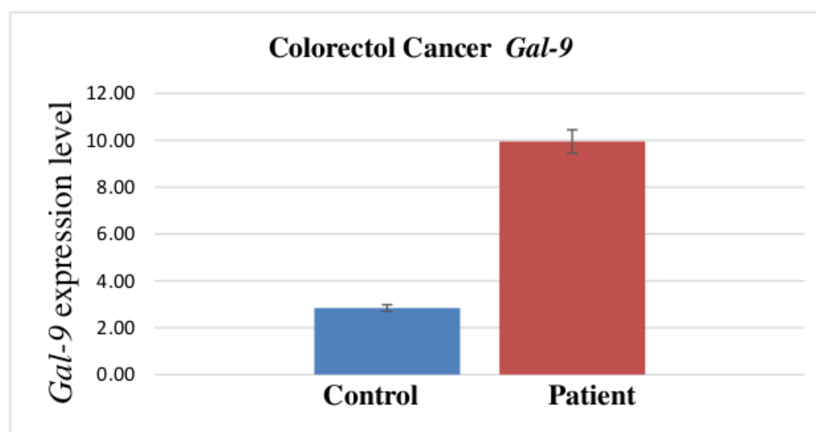

Figure 4: The gene expression was 3.5 fold change in CRC patients compare to healthy controls.

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The expression of *Gal-9* gene in PC

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The *Gal-9* gene was over expressed in PC with expression level ± 9.08 for patients and controls estimated with ±3.02. The gene expression was 3 fold change in patients compare to healthy control, at p-value = 0.001. As shown as in figure (5).

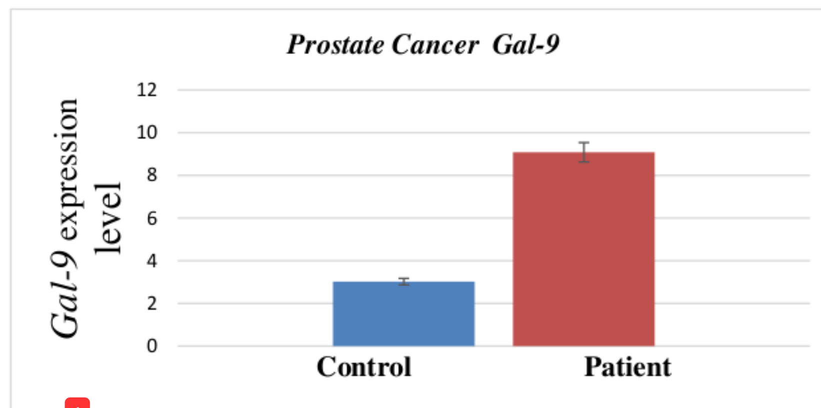

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Figure 5: The gene expression was 3 fold change in PC patients compare to healthy controls.

Discussion

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Galectin-9 (Gal-9) is a type of tandem-repeat galectin, it have role in the tumor development, Gal-9 has several functions in the angiogenesis, immune evasion, apoptosis, and control on the tumor cell cycle. Gal-9 is therapeutic target in cancer treatment [25,26]. Gal-9 is also found in the cytoplasm and on the surface of cells, and tumor cells appear to release it [27]. Recombinant Gal-9 therapy may stop the progression of cancer's metastatic stage [27].

According to this study, the expression of the gal-9 gene was 4.97 times higher in patients with bladder cancer than in healthy controls. Similarly, patients with colorectal cancer had a 3.5 fold increase in gene expression compared to healthy group, and patients with prostate cancer had a threefold change in gene expression compared to healthy controls (figures (3), (4), and (5)).

Many studies reveal a wide range of expression variations between tumor cell lines and normal tissue. Breast, lung, melanoma, renal, adrenal and PC cell lines all showed low or absent Gal-9 expression [28- 31,32], cleared the increased Gal-9 expression has reported in the colon cancer and leukemia cell lines.

Gal-9 gene regulates a number of illnesses, including T-cell-mediated conditions like asthma and autoimmune disorders [33]. It also has an impact on the management of bacterial and viral infections and may slow the growth of tumours. More studies aimed at figuring out Gal-9 binding

partners, signaling pathways, and the creation of novel recombinant Gal-9 forms that are well suited for immunotherapy are expected to pave the way for future Gal-9-based therapeutic therapies in a range of disorders.

Elevated levels of Gal-9 have been reported in ovarian carcinoma [34-36], chronic lymphocytic leukemia [37] and pancreatic ductal adenocarcinoma [38].Gal-9 levels and the cancer's clinical stage had a favorable correlation. Galectins have a significant role in controlling tumor angiogenesis, tumor immune escape, and tumor cell transformation [39].

5. Conclusion

Finally, our results show that there is a positive correlation between BC, CRC, and PC and the expression of Gal-9. The research indicates that Gal-9 may prove to be a beneficial target for the diagnosis and treatment of the malignancies, as well as for their detection.

6. Conflict of Interest

The authors have declared that no conflict of interest exists.

7. Ethical Standards

This study was conducted following the approval of the scientific and ethics committees of Basrah University and the Iraqi Ministry of Health (numbered 73 dated 24/1/2022). All participants were willingly agreed to be part of this study, written consent form was filled by all subjects participated in the study.

8. Abbreviations

qPCR, quantitative polymerase chain reaction ; BC, Bladder cancer; CRC, Colorectal cancer; PC, Prostate cancer; *Gal-9*, Programmed Cell Death Protein.

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