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PSCA gene expression in bladder, colorectal and prostate cancer patients from Basrah governorate southern of Iraq

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Abstract. Cancer causes death in all countries of the world, with approximately (9.6) million deaths in 2018. Developing countries have high cancer rates. Furthermore, there are risk factors that lead to cancer, including economic and political instability and bad lifestyles. This is the first study of *PSCA* gene expression with BC, CRC and PC in Iraq, especially in Basrah governorate. This paper involved one hundred and one blood samples being collected from bladder, colorectal and prostate cancer. On the other hand, one hundred and one blood samples with no cancer were collected as a control group. Two ml of peripheral blood was drawn for RNA extraction, then total RNA was reverse-transcribed to cDNA, and PSCA mRNA was measured using qPCR. The results showed *PSCA* gene was over expressed in bladder cancer (BC) with an expression level at ± 8.63 for patients and controls estimated with ±4.16. The gene expression was a 2-fold change in patients compared to healthy control. While the *PSCA* gene was over-expressed in colorectal cancer (CRC) with an expression level ± 8.16 for patients and controls estimated at ±3.30. That means the gene expression was a 2.47-fold change in patients compared to healthy control. The *PSCA* gene was over expressed in prostate cancer (PC) with an expression level ± 9.47 for patients and controls estimated at ±4.22. The gene expression was a 2.24-fold change in patients compared to healthy control. In the present study, *PSCA* gene was expressed at a significantly higher level in the BC, CRC and PC patients compared in the controls group.

Keywords: *PSCA* gene, bladder, prostate cancer, colorectal cancer, qPCR

INTRODUCTION

Cancer causes death in all countries of the world, with approximately (9.6) million deaths in 2018. Developing countries have high cancer rates. Furthermore, there are risk factors that lead to cancer, including economic and political instability and bad lifestyles (Fitzmaurice *et al*., 2019). Even though Iraq has had national cancer registries and control programs in place since 1974, the worrisome rise in the number of cancer cases and deaths that have occurred since then is cause for concern (Board, 2018). Breast cancer, lung cancer, leukemia, bladder cancer, and colorectal cancer are the top five types of cancer common in Iraq, according to recent estimates provided by (IARC). Twenty-five thousand new cancer cases and more than fourteen thousand deaths accompanied by cancer occurred in Iraq in 2018 (Bray *et al*., 2018). Early detection techniques and appropriate therapy approaches, the latter of which is based on accurately forecasting a cancer's prognosis, are essential to the successful treatment of cancer. As a result, finding improved tumor biomarkers is essential to us for increasing the specificity and sensitivity of cancer diagnosis and prognosis (Wang *et al*., 2018). Bladder cancer (BC) ranks as the eleventh most prevalent kind of cancer to be diagnosed in people throughout the globe (Giannarini *et al*., 2018). More than 400,000 people all over the globe get a diagnosis of Bladder cancer each year, of whom 30% have

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muscle-invasive cancer (Funt and Rosenberg, 2017).

During first diagnosis, up to eighty percent of bladder cancers are not muscle invasive. (Van *et al*., 2009). The majority of urothelial malignancies, around 90–95%, are caused by BC. It is the eighth most prevalent cancer in women and the fourth malignancy in males. (Richters *et al*., 2020). Clinically, a bladder tumor will be suspected when there is macroscopic hematuria or a bladder mass, and the histology will confirm the diagnosis (Engbang *et al*., 2022). The variables that increase a person's likelihood of developing bladder cancer at an older age are the male gender, white, exposure to the chemical substance, radiation of the pelvic area, chemotherapy use, chronic irritation or infection of the bladder, family history, and smoking. (DeGeorge *et al*., 2017). Colorectal cancer (CRC) mostly affects people in their later years (Wang *et al*., 2015). Lowering rates of CRC in individuals with fifty years or more in many regions of the globe, whereas the incidence rate of early-onset CRC has grown 1% to 3% per year for those younger than 50 years (Lee *et al.,* 2018; Vuik *et al.,* 2019). On the other hand, as a consequence of globalization, industrialization, and urbanization, there is a current movement toward an increase in the prevalence of these illnesses in the more underdeveloped regions of the globe. Fortunately, Iraq has a low rate of colon and rectal cancer (6.12/100 000 people), although the rate of increase has been steady during the last (20) years (Ibrahem *et al.,* 2022).

Prostate cancer (PC) is a widespread health issue that affects males all over the globe. (Mohammed *et al*., 2021). The prostate gland enlarges with age and is situated at the front of the rectum beneath the urine bladder. Its size may change. In younger men, it is approximately the size of a walnut; however, in older men, it is bigger. The role of the prostate is to produce part of the fluid that safeguards and sustains sperm cells, making the semen thinner (Al-Tmemi., 2014). Both the incidence and mortality of prostate cancer are dramatically different among nations and regions, with the greatest rates being seen in African-American males. In the United States of America, PC is the visceral malignant neoplasm that occurs most often in males and is the second-largest cause of death due to cancer (Attar, 2010). PC typically decreases noticeable clinical signs until the disease develops to an advanced stage. One reason that has caused the rise in cases is the development of the (PSA) antigen test used in the 1980s and considered early diagnosis rather than digital rectal examination (Catalona *et al*., 1994). Prostate stem cell antigen (PSCA) is a protein produced from the bladder epithelial, esophagus, kidney, and stomach (Zhang *et al*., 2017). PSCA is a glycoprotein linked to the cell surface by glycosylphosphatidylinositol (GPI), a member of the Thy-1/Ly-6 family. In 1998, PSCA was an elevated gene in prostate cancer cells on the 8q24 chromosome (Dorff *et al*., 2022). PSCA has a better disease-specific than other markers, making it the most promising candidate for molecular staging of PC. (Hara *et al*., 2002).

B-actin is characterized by unique gene promoter (housekeeping genes) elements that dictate that is produced in every cell. However, just because their expression is expressed in all the cells does not necessarily indicate that it is not controlled (Vedula *et al.,* 2023). It must not be susceptible to any sickness that humans have. In molecular biology, HKGs using assays depends on the assumption that their expression levels stay same in the cells. It is difficult to detect whether HKG translation has biological variability since there is no proven approach for normalizing the information on expression, while all methods need to be tested, evaluating whether HKG translation has variation in biology is especially challenging. (Mahmood *et al*., 2021). The study aims to clarify the prognostic role of *PSCA* gene expression in BC, CRC and PC.

MATERIALS AND METHODS

Collection of sample

One hundred and one blood samples were collected from BC, CRC and PC. Patients from Basrah Oncology and Hematology center from November 2021 to March 2022 included 32 samples with BC, 39 samples with CRC and 30 samples of PC. The range of their ages from between (29 - 94) years old.

And one hundred and one blood samples which had no cancer were collected as a control group, their ages between (27 - 90) years old. The

excluded criteria in this study were no viral infections, no metabolic disorders, no parasitic invasion, no diabetes and hypertension. 2 ml of peripheral blood were drawn by sterilized syringe from the two groups. After that, they are kept in sterilized EDTA tubes for RNA extraction, then the total RNA was transformed into cDNA then gene expression of *PSCA* and *B-actin* genes were estimated using RT-PCR were estimated using SYBR Green.

RNA isolation and RT reaction

Total cellular RNA was extracted from the blood samples by GENEzol ™ Tri RNA, a pure kit rendered to the manufacturer protocol. The quality and concentration of total RNA for all the samples were determined by a Nanodrop spectrophotometer and the A 260/280 ratio was calculated after making the blank with RNase free water. Total RNA was reverse transcribed by Accupower® Rocket script™ RT PreMix kit as the following: lyophilized random hexamer was dissolved completely and mixed by vortex/centrifuge or by pipetting then diluted in a 1:9 ratio (10:90 µl), while Oligo dT primer was liquid diluted in a 1:1 ratio, the samples and reagents were thawed before used. The total RNA (400ng) from each one (sample) was transformed to cDNA using Rocket script™ RT PreMix kit. The primer (Oligo dT or random hexamer), total RNA and DEPC-water were added to the cDNA master mix tubes. In the final step, the PCR tubes were placed in the thermal cycler and run using the conditions: Primer annealing: at 37°C for 10 min, cDNA synthesis at: 60°C for 1 hr and Heat inactivation: at 95°C for 95°C. All cDNA samples were stored at -20°C until more analysis. (Abd Al-Wahid & Abd Al-Abbas, 2023). qPCR was performed using the qthermo cycler and SYBR® Green I dye, to examine the mRNA expression levels of the target gene (*PSCA*) also the housekeeping gene (*B-actin*). The primer sequences were:

***PSCA* Forward:**
5'- AAAGCCCAGGTGAGCAACGAG-3'
***PSCA* Reverse:**
5'-CTGTGAGTCATCCACGCAGTTC-3'
***B-actin* Forward:**
5'-ATGGGTCAGAAGGATTCCTATGT-3'
***B-actin* Reverse:**
5'-AGCCACACGCAGCTCATT-3'

The *PSCA* primers produced a 147-bp product (Zheng *et al*., 2015), whereas the *B-actin* primers produced a 153-bp product (Yang *et al*., 2014). Each PCR reaction (final volume, 20 µl) contained 10 µl Go Taq qPCR Master Mix, 1 µl complementary DNA, 1 µl forward primers, 1 µl reverse primers and 7 µl Nuclease –Free water.

The thermal cycling conditions for the qPCR were: Denaturation at 95°C for 4 min; and 45 cycles of denaturation at 95°C for 30 sec, annealing at 52°C for 30 sec and extension at 72°C for 40 sec.

Statistical analysis (the qPCR data analysis):

The obtained qPCR data were analyzed using the ΔΔCT method as the CT values for each gene (PD-L1, Gal-9 and PSCA) as well as for the HK gene (*B-actin*) were determined (Livak and Schmittgen, 2001). The following steps were performed to obtain the expression of each gene:

$\Delta CT_{patient} = CT_{patient} - CT_{HK\ gene}$
$\Delta CT_{control} = CT_{control} - CT_{HK\ gene}$
$\Delta\Delta CT = \Delta CT_{patient} - \Delta CT_{control}$
Gene expression (Exp.) = 2^ - ΔΔCT
$\text{Fold change (FC)} = Exp_{patients} / Exp_{controls}$

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

RESULTS

PSCA Gene expression experiment

The results of *PSCA* gene expression levels of patients and healthy controls. The genes of interest were estimated using SYBR Green. The melting curve showed one peak for all samples as shown in Figure 1, demonstrates the specific binding of the SYBR Green for *PSCA* in patients and healthy controls. Also, the amplification curve was shown in Figure 2.

The expression of PSCA gene in BC

The *PSCA* gene was over-expressed in BC with an expression level ± 8.63 for patients and controls estimated at ±4.16. The gene expression

was a 2-fold change in patients compared to healthy control. The standard deviation for control group was ± 8.63 and was ±15.323 for patients, at p-value = 0.156 as shown in Figure 3.

The expression of PSCA gene in CRC

The *PSCA* gene was over-expressed in CRC with an expression level ± 8.16 for patients and controls estimated at ±3.30. The gene expression was a 2.47-fold change in patients compared to healthy control. The standard deviation for the control group was ± 6.838 and was ± 7.623 for patients, p-value = 0.003 as shown in Figure 4.

The expression of PSCA gene in PC

The *PSCA* gene was over-expressed in PC with an expression level ± 9.47 for patients and controls estimated at ±4.22. The gene expression was a 2.24-fold change in patients compared to healthy control. The standard deviation for the control group was ± 8.616 and was ± 8.527 for patients. p-value = 0.021 as shown in Figure 5.

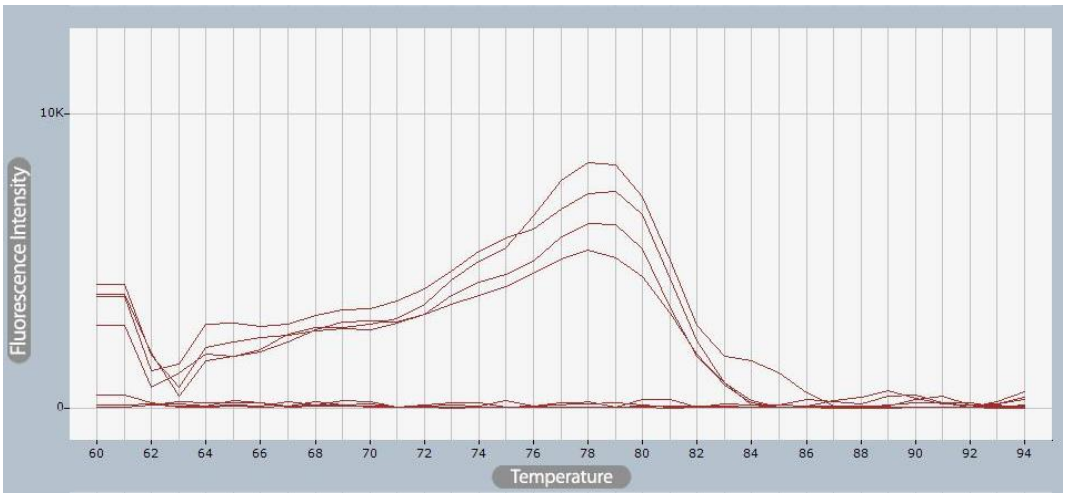

Figure 1. The melting curve of *PSCA* gene in patients and healthy controls

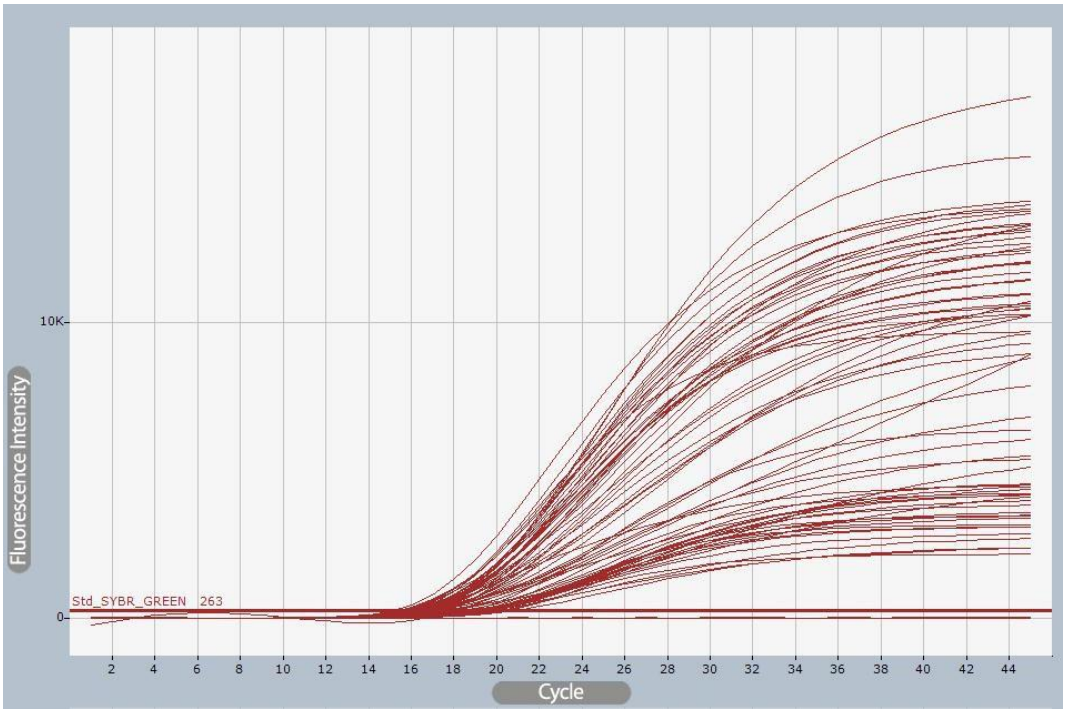

Figure 2. The amplification curve of *PSCA* gene in patients and healthy controls

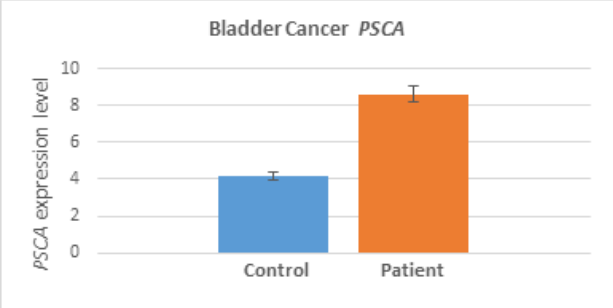

Figure 3. The gene expression was 2-fold change in BC patients compared to healthy controls, p-value = 0.156

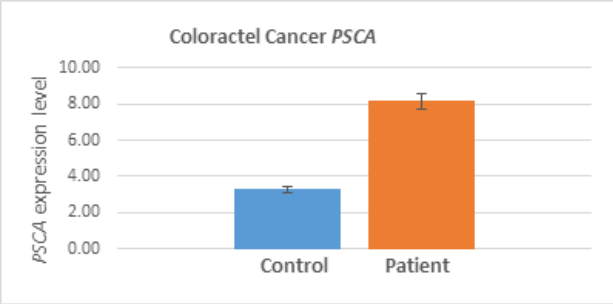

Figure 4. The gene expression was 2.47-fold change in CRC patients compared to healthy controls, p-value = 0.003

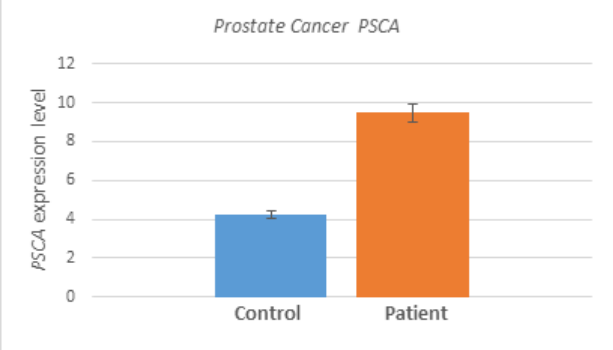

Figure 5. The gene expression was 2.24-fold change in PC patients compared to healthy controls, p-value = 0.021

DISCUSSION

This is the first study of *PSCA* gene expression with BC, CRC and PC in Iraq especially in Basrah governorate. The prostate stem cell antigen, also known as *PSCA*, is a protein attached to the cell's surface. Glycosylphosphatidylinositol (GPI) is the molecule that connects the two components. *PSCA* may be detected in the epithelial cells of the human bladder and the prostate (Classon and Coverdale, 1994). Reiter *et al.*, (2000) showed the molecular mechanisms of PC initiation and progression remain poorly understood. Alterations of chromosome arm 8q are frequently linked to recurrent and metastatic disease; nevertheless, the specific genes that are overrepresented within this region are not known. This study showed the gene expression was 2-fold change in BC patients and 2.47- fold change in CRC patients compared to healthy controls. Argani *et al.,* (2001) found that the *PSCA* gene was identified in the urothelial, kidney cell, pancreas, and lung cancer. Additionally, this gene was shown to be expressed in pancreatic cancer. Saeki *et al.,* 2010; Ono *et al.,* 2018, found partial normal tissue expression in the skin, stomach, esophagus, kidney, brain, and bladder. Saffran *et al.* (2001) and Zhao *et al.* (2020) conducted research showing that inhibiting *PSCA* activity in cancer cells reduced the cells' metastatic potential. This was the case even though the biological role of *PSCA* is not completely known. On the other hand, additional research suggests that PSCA inhibits the spread of metastasis (Wang *et al.*, 2015).

Tang *et al.,* (2012) has demonstrated that the *PSCA* gene contains androgen-responsive domains, and there is also the potential that androgen might mediate PSCA expression. However, it is yet unknown whether PSCA is controlled by AR signaling. Fu *et al.* (2012) found that BC had better PSCA mRNA expression than the nearby normal bladder tissue, which resulted in increased levels of the gene's expression. In tumor samples, PSCA mRNA expression was 7.3 times greater than in normal bladder tissues. This study showed *PSCA* gene expression was a 2.24-fold change in PC patients compared to healthy controls. Reiter *et al.,* (1998) cleared PSCA has been described as expressed in a normal prostate and overexpressed in PC. Therefore, the *PSCA* gene is a result as a molecular marker for progression of a PC tumor (Gu *et al.*, 2000). This overexpression is in part due to an amplification and overrepresentation of a chromosomal region on 8q, but does also reflect an additional, amplicon-independent overexpression (Reiter *et al.,* 2000). Also, PSCA mRNA was detected in more than 80% of primary prostate tumors by in situ analysis. Moreover, mRNA expression in tumers frequently appeared to be stronger than in adjacent normal glands, raising the possibility that

PSCA may be overexpressed in some prostate tumors and might have some cancer specificity (Reiter *et al.,* 2000).According to Cher *et al.* (1994), a significant portion of advanced prostate tumors have genetic gain/amplification in the area where *PSCA* is located on chromosome 8q24.2. These results supported *PSCA* as a potential target for PC diagnosis and therapy (Gu *et al.,* 2000; Dorff *et al.,* 2022). Wente *et al.,* (2005); Gu *et al.,* (2000); Han *et al.,* (2004) showed *PSCA* expression in tumors relates with disease progression and prognosis in PC. For example, high levels of PSCA mRNA in peripheral blood have been linked to high-grade and extraprostatic illness (Fawzy *et al.,* 2015).

As a result, PSCA has been investigated for its potential as a target for PET imaging in the context of cancer diagnosis, staging, and follow-up (Lepin *et al*., 2010; Dorff *et al*., 2022). *PSCA* expression has been shown to have significant associations with Gleason score, tumor stage, and extraprostatic extension in studies conducted by Gu *et al*., (2000), Hara *et al*., (2002), and Zhao *et al*., (2012), respectively. Joung *et al.* (2007) found that individuals with an extraprostatic illness and a high Gleason rating were more likely to get a positive result for *PSCA* in their peripheral blood. It is yet unknown what triggers the overexpression of *PSCA* in PC. Amplification of the *PSCA* gene is one of the many mechanisms that might explain this phenomenon. According to Zhigang, & Wenlv (2004), the *PSCA* level corresponds with increased tumor stage to androgen-independence of PC. They also discovered that PSCA protein and mRNA have high expression in the marked rate of advanced stage, androgen-independent, poorly differentiated of the human PC, and metastatic cases.

CONCLUSION

In conclusion, our findings indicate a positive relationship exists between the expression of the *PSCA* gene and BC, CRC, and PC. According to the findings, *PSCA* has the potential to be a useful target for the detection and treatment of human BC, CRC, and PC and a valuable target for diagnosis and therapy of these tumors.

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CONFLICT OF INTEREST

The authors have declared that no conflict of interest exists.

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