VALIDATION GENE EXPRESSION OF RNA-BINDING PROTEINS AND BIOMARKERS IN BENIGN PROSTATIC HYPERPLASIA AND PROSTATE CANCER

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ABSTRACT

Prostate cancer is the most common cancer in the world specifically in Iraq, compared to other cancers, that affects men of old ages of 50 years and more, but it rarely affects men that lesser than this age category. Benign prostate hyperplasia (B.P.H) is not cancer and does not develop into cancer. But it can be a serious medical problem for some men. Benign prostatic hyperplasia (BPH) is a histologic diagnosis that refers to the proliferation of smooth muscle and epithelial cells. The current study included extracting RNA from white blood cells and then converting it to cDNA. The gene expression of the Sam 68 was estimated in patients with Prostate Cancer and Benign Prostate Hyperplasia using Real time-PCR. The thesis also included the estimation of the studied chemical parameters by ELISA, which are: AKT1, β-catenin. The purity and concentration of RNA was estimated in two ways: using two techniques, the first is Nano Photometer, where the concentration was 55 ±13, and another technique called Quantus Fluorometer, and the concentration was 58 ±4.2. The current study proved that the sam68 gene was high gene expression in prostate cancer, where were highly significant P˂0.001, when compared to the gene expression in healthy people by using real time- pcr in presence the house keeping gene. Also, the gene expression was higher at the greater the age of the patient, as it was of high significance P˂0.001 whenever the age of the patient with prostate cancer was greater than 50 years, while the gene expression was of less significance when the patient is less than 50 years old, but in a lesser degree. There was no statistical significance in gene expression between benign prostate patients compared with healthy people, where a p value of more than 0.001. The studied chemical markers was estimated in the serum of patients with prostate tumors (60 with PCa and 60 BPH), using the ELISA technique. The current study showed that the enzyme AKT1 was highly activity in prostate cancer and benign prostate patients when compared with the it’s activity in healthy people and was of high significance, as it recorded a P <0.001. Statistical results of the Beta-catenin protein were highly significant in patients with prostate cancer and BPH and the value of P <0.001

I. INTRODUCTION

Prostate cancers (PCa) could be a heterogeneous age-related health problem that’s a essential purpose of most cancers associated deaths among men [1]; it’s miles the other most common cancer among guys world [2], and also the most common most cancers among adult males [3]. 1.1 million cases of PCa are cautioned in 2012, of that seventieth had been in growing countries [4]. The prevalence of PCa, that varies among ethnic organizations, could also be associated with genetic and environmental factors. However, globally each year some 270,000 men die from PCa [4]. PCa, the 0.33 most common most cancers in guys in Persia, could be a haggle less common than in Western nations [4,5]. Persia contains a low incidence of PCa (9.11 in step with 100,000), at the identical time as PCa patients World Health Organization were admit ted to a middle Euphrates River most cancers middle in Al-Najaf Al-Ashraf Province for the period of 2018 are eight.27 constant with 100,000, such versions of PCa incidence in marvelous countries could also be associated with such components as style and socioeconomic conditions [6].

Benign prostate Hyperplasia could be a a histologic analysis related to the growth of simple muscle and tissue in the prostate transition sector, the precise illness is unknown [7,8]. Benign prostate Hyperplasia (BPH) isn’t cancer and will not become cancer. while it's ready to be an intense clinic drawback for a few men[9]. Benign prostatic
hyperplasia is an increase in volume of the prostate and absence malignancy present, BPH is common as to be normal with go forward age[10]. Sam68 is upregulated and plays central roles in numerous human tumors[11], including PCa [12,13]. Sam68 mRNA yield high expression of this protein in PCa cells. Sam68 appears to guide the same capabilities, as its depletion impaired PCa cellular proliferation and caused neural stem cellular differentiation[14]. Additionally Up-regulation of Sam68 has been stated in a small number of PCa specimens at each protein and mRNA degree[12].

II. MATERIALS AND METHODS

A case-control study recruited 60 BPH patients, 60 PCa patients (aged 47-75 years), and 60 healthy subjects (aged 25-30 years) who were admitted to a urological center which including Al-Najaf Medical City in Al-Najaf Al-Ashraf Province. During the period from august/ 2020 to december/ 2020. The information has been reported using a questionnaire on every individual by face-to-face interviews, to obtain information on their status.

Blood Collection

Blood samples were collected using a disposable syringe from individuals. About 5 ml of blood were obtained from each individual by vein puncture, 2 ml was collected in EDTA tubes, the remaining 3 ml pushed into a disposable gel tube.

RNA Purification Protocol Procedure

Total RNA was extracted from WBC according to GENEral™ TriTAN Pure Kit from Geneaid company.

Determination the Concentration and Purity of RNA Sample

Two methods were used to determine the RNA yield including Nano Photometer and Quantus Fluorometer. The two methods were useful but have varying requirements such as equipment required, ease to use and calculation needed.

cDNA preparation

cDNA was prepared according to High Capacity cDNA Reverse Transcription Kit (thermo fisher).

Primers Preparation and Storage [15]

Table(1) Sequences of nucleotides in Sam68 and GAPDH

<table>
<thead>
<tr>
<th>Supplementary Table S1</th>
<th>Primer sequences used for qPCR (5’ to 3’)</th>
</tr>
</thead>
<tbody>
<tr>
<td>qPCR</td>
<td>Gene</td>
</tr>
<tr>
<td>Sam68</td>
<td>CTCCTGCTAGGCCAGTGAA</td>
</tr>
<tr>
<td>GAPDH</td>
<td>GACTCATGACCAAGTCATGC</td>
</tr>
</tbody>
</table>

Real time PCR Amplification.

The SYBR green method was used to quantify PCR product, and condition cycle was set as following table.

Table(1) condition of real time- PCR cycle

<table>
<thead>
<tr>
<th>qPCR reaction</th>
<th>x1 cycle</th>
<th>x40 cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reaction step</td>
<td>Enzyme activation</td>
<td>Denaturation</td>
</tr>
<tr>
<td>Cycle length</td>
<td>10min</td>
<td>15sec</td>
</tr>
<tr>
<td>Temperature</td>
<td>95°C</td>
<td>95°C</td>
</tr>
</tbody>
</table>
Determination of Human RAC-alpha Serine (Threonine-protein Kinase) and Beta Catenin ELISA Kit

This kit is an Enzyme-Linked Immunosorbent Assay (ELISA) according to Bioassay Technology Laboratory.

**Total RNA concentration and purity**

**NanoDrop Spectrophotometer**

Total RNA was extracted from blood specimens prepared from patients with malignant prostate tumors as described previously in section 2.3.1. The concentration of extracted RNA was measured by Eppendorf biophotometer. Results (Mean±SD) exhibited a level of 55.5 ± 13.61 ng/µl. The purity of the extracted RNA was estimated by measuring the ratio of A260/A280. It was found to be 1.93 ± 0.04 & A260/A230 was 1.8±0.03

**Quantus Fluorometer**

RNA was extracted from blood specimens prepared from patients with prostate tumors as described previously in section 2.3.2. The concentration of extracted RNA was measured by Quantus™ Fluorometer. Results (Mean±SD) showed that the total RNA concentration was a level of 58 ± 4.21 ng/µl

**Sam68 gene expression in PCa and BPH**

RNAs of Sam 68 gene were extracted from 120 blood patients, converted to cDNA and successfully amplified with appropriate efficiencies. To our knowledge, this is the first study dealt with such extraction and amplification carried out in Iraq. Moreover, the comparison with other studies carried out abroad highlights a remarkable notice which is the high number of samples investigated relative to those enrolled abroad. The relative quantification of Sam 68 gene expression in malignant prostate tumors was achieved through the calibration against the expression of the same gene in normal blood (calibrators). A normalized gene (GAPDH) was used as a control for the experimental variability in this quantification. Thus, the expression folds of Sam 68 gene were calculated with respect to the internal control gene (the housekeeping gene), i.e.,GAPDH. gene expressions were found to be significantly (p<0.001) raised in Prostate cancer by 2 fold, depending on One Way ANOVA Statistically. The mean expression of prostate cancer (PCa) patients 2.98 ± 1.0 and with benign prostate hyperplasia (BPH) 1.0 ± 0.25 was significantly increase than in the control group 8.67 ± 0.33

In the current study Sam 68 gene was found to be expressed in blood PCa patients 2 folds relative to those of blood healthy people(control). Such results suggested the up regulation of the expression of both genes during carcinogenesis. In the present study, Prostate cancer patients have showed elevated Sam68 gene expression levels in respect to the normal prostate blood. The elevation is an expected observation and implicates a possible association of Sam68 up-regulation with enhanced proliferation and invasiveness of cancer cells.

On another hand of the study, and as far as the gene is related to BPH, it has been observed that the studied gene did not show an increase in the gene expression of the Sam68 gene in the blood of BPH patients, gene expressions...
were found to be not significantly (p value > 0.001). This does not mean that there is no increase in the gene expression of the sam68 in prostate tissue. But the difference in the fact that the gene showed an increase in gene expression in prostate cancer patients indicates that prostate cancer cells were destroyed and released in the blood, and what proves the validity of the study is that there was no increase in the gene expression in the blood of prostate cancer patients in patients who had their prostate removed. Or perhaps the reason is that the sam 68 gene expression is normal and without an elevated in BPH patients, and this reason may be the most accurate because no study has yet been recorded that shows the gene expression of the gene is high in mm in BPH patients as shown in figure (1)

Others studies shown Post-transcriptional regulation of gene expression is often aberrant in cancer cells and changes in both alternative splicing and translational regulation of specific mRNAs have been reported [16, 17]. The results demonstrate that Sam68 is frequently upregulated in human PCas and that downregulation of its expression or activity affects prostate cancer cell proliferation and survival. Sam68 has been shown to be frequently upregulated in some tumors, like breast cancer [18] prostate [12,13]. Signaling pathways attributed to growth factors receptor activation, such as ERK1/2 pathway, seem to modulate alternative splicing through Sam68 phosphorylation, promoting some tumoral effects. Sam68 also directly interacts with the androgen receptor and binds to androgen responsive elements (AREs) within the promoter region of the prostate-specific antigen (PSA) gene, where Sam68 seems to have some effect on AR-regulated transcriptional activity independently of its ARN binding capacity [13]

Sam68 may also be acting as a co-activator of ER-dependent transcription in mammary development and tumorigenesis [19].

Relevance of age of patients with sam 68 gene expression

blood prostate cancer (60) were classified into 2 groups. The first group consisted of 12 samples of patients of ages <50 years, while the second group consisted of 48 samples of patients who have ages >50 years. To explore the relevance of sam68 gene expression with the ages of prostate cancer, Way ANOVA Statistically was used to evaluate the data. A significant (p<0.001) elevation of sam68 gene expression was found in patients of ages >50 years when they were compared with healthy people

III. BIOCHEMICAL PARAMETERS

AKT serine/threonine kinase 1

The mean concentration of prostate cancer (PCA) patients 3.94± 1.03 and with benign prostate hyperplasia (BPH) 4.10± 1.19 was significantly increase than in the control group 3.16± 0.6 as shown in Figure (2). The p-value is < 0.001.

![Figure (2) : concentration of AKT (pg/ml) in PCA, BPH patient and the total number of control group](image-url)
Akt acts as a key factor in cell survival and proliferation and is overexpressed or activated by mutation in a variety of human cancers, including lung, breast, ovarian, gastric and pancreatic carcinomas. Multiple studies demonstrated the significance of Akt as a mediator of cellular proliferation and as an effective target for drug development. However, the drugs that have emerged directly targeting Akt and other major components of the Akt signaling pathway have limited pharmaceutical and clinical properties. Immunotherapy specific to Akt might hold promise for the targeted therapeutic approach in human cancer [20].

Correlation of Sam68 gene expression with AKT concentration in PCa

In this study the mean correlation in prostate cancer (PCa) between gene expression of sam 68 with AKT concentration was significantly increase, where the p-value is < 0.001, as shown in figure (3).

Sam68 promoted cell proliferation and stifled caspase-mediated cell death by enhancing phospho-AKT expression [23]. It's antecedently been reportable that Sam68 regulates proliferation of carcinoma via the AKT pathway [24]. Systematically, down regulation of SAM68 in cervical cancer cells stifled cellular motility and invasion by the inhibition of the AKT/GSK-3 β/Snail pathway [25].

β-Catenin:

In this study the mean concentration of β-catenin in prostate cancer (PCA) patients 3.94±1.03 and with benign prostate hyperplasia (BPH) 4.10±1.19 was significantly increase than in the control group 3.16±0.6 as shown in Figure (6). The p-value is < 0.001. As shown in figure (4).
β-catenin is a kind of multifunctional protein encoding by CTNNB1. In epithelial, β-catenin regulates epithelial cell growth and intracellular adhesion. In the Wnt signaling pathway, it is a crucial effector controlled by Wnt proteins and modulates transcription of genes[26]. have reported that changes in Wnt signalling can lead to carcinogenesis and the progression of malignancies, including prostate cancer.1-3 In addition, some investigators have suggested that Wnt/β-catenin signalling and the androgen receptor (AR) play critical roles in prostate cancer progression[27,28,29]

Correlation of Sam68 gene expression with β-catenin concentration in PCa

In this study the mean correlation in prostate cancer (PCa) between gene expression of sam 68 with β-cat concentration was significantly increase, where the p-value is < 0.001. As shown in figure (5)

Figure(5) Correlation between Gene expression and β-catenin concentration

Sam68 suppresses cell proliferation via inhibiting Wnt/β-catenin signaling[30]. Sam68 may promote the nuclear accumulation of β-catenin, facilitate Wnt/β-catenin signal activation and upregulate TCF/LEF transcription activity in carcinoma cells [31]. moreover, our mechanistic studies disclosed that knockdown of Sam68 suppresses cell proliferation via inhibiting Wnt/β-catenin signal, that is conspicuously activated in NSCLC and plays a vital role in respiratory organ tumorgenesis and metastasis. However, the elaborate mechanisms of Sam68 in promoting cell proliferation through activating Wnt/β-catenin signal stay unclear[32]

IV. CONCLUSION

The current study concluded several concepts. The gene expression of the sam68 gene is evident in prostate cancer, the sam68 gene can be used as a marker for prostate cancer detection. Gene expression of the sam68 gene was not evident in BPH. Elevated serum AKT-1 concentration in Pca and BPH. Increase of serum β-catenin concentration in Pca and BPH

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