Research in Molecular Medicine

Evaluation of IL-2 and IL-7 Expression in Patients With Prostate Cancer

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Citation Namazi F, Hadi N, Parnian F, Moghimi M. Evaluation of IL-2 and IL-7 Expression in Patients With Prostate Cancer. Research in Molecular Medicine. 2019; 7(2):33-38. https://doi.org/10.32598/rmm.7.2.83

https://doi.org/10.32598/rmm.7.2.83

Article Type:
Research Paper

Article info:
Received: 17 Jan 2019
Revised: 21 Feb 2019
Accepted: 5 Mar 2019

Keywords:
Interleukin, IL-2, IL-7, Prostate cancer

ABSTRACT

Background: Prostate cancer is a biologically heterogeneous disease which has become the fifth leading cause of cancer-related deaths in men worldwide. Interleukins (ILs) display required inflammation moderator. IL-2 and IL-7 play significant roles in regulating cancer-immune system interactions. So, the purpose of this investigation was to evaluate the expression level of IL-2 and IL-7 in prostate cancer patients and healthy subjects.

Materials and Methods: In this case-control study, expression of IL-2 and IL-7 was examined in peripheral blood of 40 prostate cancer patients and 40 healthy subjects by reverse transcription quantitative real-time polymerase chain reaction.

Results: Our findings showed that IL-7 has highly elevated expression in patients with prostate cancer compared to healthy subjects (P=0.0001). In contrast, no significant difference was observed in the expression of the IL-2 between the two study groups (P=0.12).

Conclusion: Based on the results, IL-7 may be used as a prospective biomarker or as a molecular target in designing new prostate cancer control strategies. However, the findings of this investigation revealed no association between IL-2 expression and prostate cancer. Nevertheless, more studies should be included to appraise the exact relevance of this gene to prostate cancer.

Introduction

Cancer is one of the most prevalent fatal illnesses in the world and almost fourteen million recently diagnosed cases is the leading cause of deaths globally [1]. Prostate cancer as a well-known cancer in the world. It is the third prevalent reason of cancer-associated fatality between male although the preface of Prostate-Specific Antigen (PSA) could help to earlier diagnosis of this cancer [2, 3]. In general, prostate cancer is the second most common cancer in male in the world, and the sixth most prevalent cancer in Iran [4].

Exposure to the environmental/lifestyle risk factors may raise the risk of developing prostate cancer. However, epidemiological investigations have consistently eminent highlighted the familial clustering of the disease

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[5]. Different factors lead to the occurrence of prostate cancer, such as age, family history, genetic aptitude, and race [6]. In recent years, gaining knowledge about cancer biomarkers could help to better management of patients; biomarkers of cancer include nucleic acids, proteins, and lipids, cytogenetic and cytokinesis parameters [7].

Although localized forms of prostate cancer could be treated via surgery or radiotherapy. Some patients having withstand such interpositions are at risk of illness relapse [8]. Based on the diversity of treatments with no clear best option, decisions for prostate cancer are highly priority-sensitive to ensure that decisions are stable with each patient's priority [9]. The occurrence of prostate cancer in Iranian men is higher compared to the other countries in Asia [10].

Interleukins (ILs) belong to a large group of cellular messenger molecules, the cytokines, which let cells of the immune system to associate with each other and produce a particular response to a target antigen. In tumorigenesis, these cytokines enhance tumor cell recognition by cytotoxic effector cells [11]. IL-2, IL-7, IL-15, and IL-21 are of particular interest in cancer immunotherapy [12]. IL-2 has been ratified for the melanoma and renal cell carcinoma therapy, whereas IL-7 has been applied with promising outcomes in clinical oncology. There is a little knowledge about the expression of IL-2, IL-7, IL-15, and IL-21 genes and secretion of protein in prostate cancer. In fact, IL-2 gene expression was detected in benign prostatic hyperplasia tissues [13, 14].

Moreover, IL-7 gene has been shown to be expressed in prostate cancer tissues [15]. Considering that few studies have been conducted on the role of IL in prostate cancer in an Iranian population, we selected two of interleukins which received less attention in prostate cancer patients. Thus, the purpose of the current investigation was to evaluate the expression level of two specific interleukins, interleukin-2 (IL-2) and interleukin-7 (IL-7), for early prostate cancer detection and their potential as prognostic factors in this cancer.

Materials and Methods

Participants

In this case-control investigation, 80 samples, including 40 patients with prostate cancer, diagnosed in the Isa Ibn Maryam Hospital (Isfahan, Iran) and 40 healthy subjects, were chosen for the present case-control investigation. Prostate cancer patients were diagnosed by an expert according to the Prostate Cancer Antigen (PCA) test. Four ml of peripheral blood was collected into EDTA-containing tubes and was quickly transferred to the laboratory on ice. Isa Ibn Maryam Hospital's ethics committee (Isfahan, Iran) approved the protocol of the current investigation. Before taking a sample of Participants, a written informed consent was taken from each person.

RNA extraction and cDNA synthesis

The RNeasy Kit (Qiagen, Germany) was applied to extract RNA from peripheral whole blood under RNase-free condition according to the manufacturer's instruction. The quality of total RNAs was investigated at a 260/280 nm wavelength ratio using NanoDrop spectrometer (WPA Biowave II, Biochrom, USA). The cDNA was synthesized with the QIAGEN kit (Qiagen, Germany). For cDNA synthesis, 4 µl of RT buffer, 1 µl of RT enzyme and 14 µl of RNA extracted sample was mixed in the final volume of 20 µl. PCR was performed at 42°C for 15 min and finally at 95°C for 3 min to inactivate the enzyme. cDNA was stored at -20°C until the next step.

Real-time PCR

Relative expression of IL-2 and IL-7 was examined using real-time PCR analysis on the base of SYBR Green (TaKaRa, Kusatsu, Japan) exploration with ABI Prism 7500 (Applied Biosystems, USA). For the normalization of expression levels, GAPDH was selected. The sequences of primer for quantitative real-time PCR reactions for IL-7 were: forward 5′ CTGGGTGAA GCCCAACCA 3′ and reverse 5′ TTCAGTGTTCTTTAGTGCCCATCA 3′ [16], for IL-2 were forward 5′ AACTCACCAGGATGCTCACATTTA 3′ and reverse 5′ TCCCTGGGTCTTAAGTGAAAGTTT 3′ [17], and for GAPDH were forward 5′ CCACTCCTCCACCTTTGACG 3′ and reverse 5′ CCACCACCCTGTTGCTGTAG 3′.

(PCR reaction: in a total volume of 10 µl, cDNA produced was added to a master mix including 0.5 µl of forward primer, 0.5 µl of reverse primer, 3 µl of DEPC-treated water, and 5 µl of SYBR premix ExTaq II). The protocol was as follows: 95°C for 10 min, followed by 40 cycles at 95°C for 10 s, 60°C for 20 s (annealing) and 72°C for 25 s (extension). Triplicate experiments were applied to each sample. Also, $2^{-\Delta\Delta C}$t method was applied to evaluate relative gene expression levels.

Statistical analysis

Data were analyzed using Graph Pad Prism statistical software, version 5.01 (Graph Pad, San Diego, CA, USA). The normality of data was assessed by Kolmogorov-Smirnov.

The independent samples T-test were used to analyze the data among groups. The amount of P<0.05 was meaningful.

Results

Biological features of participants

In this study, 40 patients with prostate cancer (average age: 67.05±1.85, range: 44-80 years, average PSA free: 6.61±1.67 ng/ml and average PSA total: 22.26±2.62 ng/ml) were studied. The same number of persons was selected as control group. Those in the control group had no history of the genetics of prostate cancer and did not use any particular drug. The biological features of prostate cancer patients and healthy subjects are indicated in Table 1.

Expression of IL-2

In order to identify the role of IL-2 in prostate cancer, the relative expression of IL-2 was investigated in both groups of prostate cancer (n=40) and healthy subjects (n=40). The efficiency of the primer was investigated and it was about 95.47 for different dilutions of real-time PCR products of the IL2. Relative Quantitation (RQ) for the IL-2 was not statistically significant in patients with prostate cancer nor in control group (P=0.12). However, the average relative expression was slightly higher in prostate cancer compared to healthy subjects (Figure 1).

Expression of IL-7

In this study, the average relative expression of IL-7 expression was investigated between two groups studied. The efficiency of the primer was investigated and it was shown about 94.58 for different dilutions of real-time PCR products of the IL7. We observed that the level of IL-7 expression increased significantly in patients with prostate cancer compared to controls (P=0.0001) (Figure 2).

Discussion

In this study, the association of IL-2 and IL-7 expression with prostate cancer was examined by real-time PCR in prostate cancer patients in an Iranian population. We observed a higher mRNA level of IL-7 in patients with prostate cancer than healthy subjects. On the other hand, our data indicated that there was no significant difference between the expression level of IL-2 in prostate cancer and healthy subjects. Prostate cancer is dependent on androgen metabolism; therefore, it seems sensible to assume that genetic changes in active genes can affect the risk of developing prostate cancer [18].

Since the type of underlying genetic and environmental risk factors involved in prostate cancer could be different in various populations, further investigation of IL expression should be warranted in prostate cancer. Thus, we investigated the association of IL-2 and IL-7 expression with prostate cancer in an Iranian population.

Cytokines associated with cancer are significantly increased in cancer [19]. In our study, even though the average relative expression of IL-2 was slightly higher in prostate cancer compared to healthy subjects, it was not statistically significant in patients and controls. However, the average of the relative expression of IL-7 was

Table 1. Biological features of patients with prostate cancer and controls

Features		Prostate Cancer		Controls	
		No.	Mean±SD	No.	Mean±SD
Number of subjects	-	40	-		40
Age	Range (y) (44-80)	-	67.05±1.85	40-78	59.37±1.95
PSA (ng/ml)	Average PSA free	-	6.61±1.67	-	1.83±2.15
	Average PSA total		22.26±2.62		10.37±1.52
Number of family history	-	13	-	-	-
Smoker	-	26	-	17	-
Disease duration (y)	-	-	5.42±0.67	-	-
Stage	Stage I	18	-	-	-
	Stage II	13			
	Stage III	9			

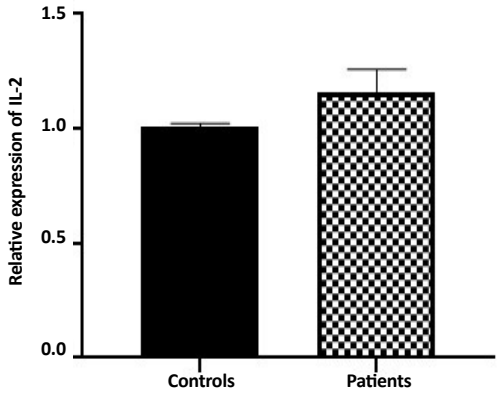

Figure 1. The relative mRNA expression level of IL-2 in patients and controls

P<0.12; Error bars display SE

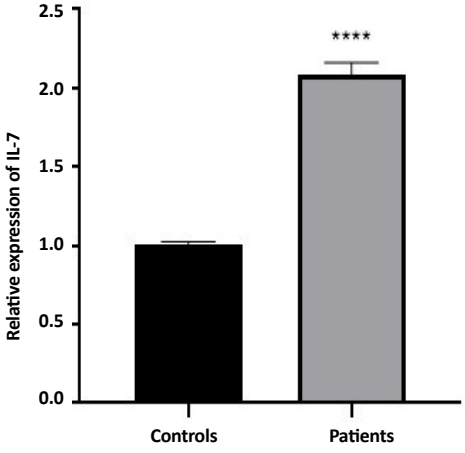

Figure 2. The relative mRNA expression level of IL-7 in patients and controls

P<0.0001; Error bars display SE

significantly higher in patients with prostate cancer versus controls. Mengus et al have indicated that in prostatic tissues from patients bearing early-stage prostate cancer, the expression of IL-7 gene significantly increased, while no such significant differences were observed in IL-2 and IL-21 genes expression [15].

The results of this study were similar to our findings. Roato et al measured IL-7 serum levels in bone-forming metastases of prostate cancer patients and controls. IL-7 levels were significantly upper in bone metastatic patients compared to controls [20]. It has proposed that IL-7 serum levels were high in Hodgkin illness and in ovarian cancers and also IL-7 was made by breast and colorectal cancer cells [15].

On the other hand, IL-2 was a primary selection for immunotherapy of cancer. Recently, realization of IL-2 in regulating lymphocytes has led to novel directions in immunotherapy of cancer. It has been shown that improved IL-2 formulations might be probably onsidered as monotherapies [1]. IL-2 complexes indicate promising impacts in various models of immunopathology and cancer [21]. Thus, progressing the knowledge of IL-2 in different cancers is warranting. The findings of the previous investigation confirmed the results of this investigation. The use of Interleukins as therapeutic factors is a new strategy for restoring prostate cancer. We investigated the correlation of the expression of two Interleukins (IL-2 and IL-7) in prostate cancer. It can be concluded that Interleukins probably have an important role in cancers such as prostate cancer.

In conclusion, IL-7 represents high-level in prostate cancer, but our results showed no association between IL-2 expression and prostate cancer. Further investigations should be included to appraise the exact relevance of these genes to the development of various cancers such as prostate cancer.

Ethical considerations

Compliance with ethical guidelines

Isa Ibn Maryam Hospital's ethics committee approved the protocol of this study.

Funding

This research was financial supported by Dr Mansour Moghimi (Associate Professor of Clinical and Surgical Pathology) in Shahid Sadoughi University of Medical Sciences.

Authors contribution

Contribution to design: Mansour Moghimi; Contributed to sample collection: Faezeh Namazi, Nasrin Hadi; Contributed to all experimental work and molecular experiments, statistical analysis, interpretation of data and discuss the findings: All authors

Conflict of interest

The authors declared no conflict of interests.

Acknowledgements

This study was kindly supported by Mansour Moghimi (Associate Professor of Clinical and Surgical Pathology) in Shahid Sadoughi University of Medical Sciences.

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