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Evaluation of Plasma Interleukin-8 Concentration in Patients with Prostate Cancer and Benign Prostate Hyperplasia

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ABSTRACT

Background: Prostate specific antigen (PSA) has been used as a screening test for the early detection of prostate cancer (PC) for many years. Although the introduction of PSA test led to a considerable increase in reported prostate cancer cases, there is still some controversy over the sensitivity and specificity of this marker in distinguishing PC patients from those with benign prostate hyperplasia (BPH), the most common benign prostate condition. **Objective:** An attempt is made to elucidate if the plasma level of Interleukin 8 (IL-8) could be used effectively as a marker for the detection of prostate cancer. **Methods:** Plasma levels of IL-8 and PSA were measured in two groups of 40 BPH and PC patients using enzyme-linked immunosorbent (ELISA) and radioimmunoassay (RIA) techniques, respectively. In addition IL-8 levels in PC3 and DU145 cell line supernatants were measured by ELISA technique. **Results:** The concentration of IL-8 in the plasma of PC patients was not significantly higher than the BPH subjects. Although, a correlation between plasma IL-8 concentration and the Gleason score of PC patients was found, no indicated correlation was detected between the concentration of IL-8 or PSA and age of the patients in both groups. DU145 and PC3 cell lines produced and secreted IL-8 in the media. **Conclusion:** Data of this investigation collectively conclude no correlation between IL-8 concentration in PC and BPH patients.

Keywords: BPH, Prostate Cancer, IL-8, PSA

INTRODUCTION

Prostate cancer (PC) is one of the most frequently diagnosed and the second leading cause of cancer related deaths in men (1). Over the course of decades, the development of the prostate-specific antigen (PSA) has allowed for the detection of PC in its early stages. Despite its evident increase in the detection of PC, controversy regarding the efficacy of PSA as a tumor marker exists. Although PSA test has shown positive results as a predictive tool for identifying PC, it remains uncertain as to whether it is effective in

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improving patient survival rates. PSA levels by themselves do not provide sufficient information needed to make a distinction between cancer and benign prostate hyperplasia. As a result of its limitations, PSA may not ultimately be a precise enough marker to be used as a screening tool (2). Therefore, finding a more reliable tumor marker for the early detection of PC is needed.

There is little information concerning the role of CXC chemokines in PC. CXCL8 (IL-8) is elevated in PC patients and may serve as an indicator of tumor growth. IL-8, a major member of the CXC chemokine family, is a pro-inflammatory and pro-angiogenic cytokine, and can promote tumor-cell proliferation and affect metastasis (3). IL-8 is secreted by a variety of stromal cells, e.g. endothelial cells, fibroblasts, and tumor cells. It binds with a high specificity to CXCR-1 and with a less specificity to CXCR-2 (4).

A tumor promoting role for IL-8 has been suggested for a wide variety of human solid tumors including non-small-cell lung cancer; malignant mesothelioma; head and neck squamous carcinoma; epithelial ovarian carcinoma; gastric, pancreatic, and colorectal carcinoma; prostate adenocarcinoma, renal cell carcinoma, and breast cancer (5-14).

The over expression of IL-8 is associated with angiogenesis, tumorigenesis and lymph node metastasis of androgen-independent PC (AIPC) in athymic nude mice. Elevated serum levels of IL-8 were reported in patients with the localized disease and AIPC. Elevated expression of IL-8 and each of its G-protein-coupled receptors [CXC chemokine receptor (CXCR-1 and CXCR-2)] in tumor cells of human prostate biopsy sections compared with normal epithelial cells has been reported as well. Expression of IL-8, CXCR1 and CXCR2 increases with the disease stage in prostatic intraepithelial neoplasia, but reaches the highest level in androgen-independent disease. Furthermore, elevated IL-8 gene expression in prostate biopsy tissue associated with both increased Gleason score and the pathologic stage of the tumors has been reported using colorimetric *in situ* hybridization techniques (3).

The primary objective of our study was to investigate whether plasma levels of IL-8 in PC patients are significantly different from individuals suffering from BPH. Therefore, we try to elucidate the diagnostic value of IL-8 as a marker of PC.

MATERIAL AND METHODS

Patients and Controls. A prospective analysis of 40 patients with untreated PC (age, 50–83 years, median 71) and 40 patients with benign prostatic hyperplasia (BPH) (age, 54–82 years, median 70) were carried out between 2005 and 2007 at Shiraz University of Medical Sciences hospitals, also 34 normal plasma samples were evaluated in this study. Neither the patients nor the controls had apparent severe infections or autoimmune diseases. Diagnosis was confirmed pathologically by transrectal ultrasonography (TRUS) and guided systematic biopsy. The histological grade was determined by the Gleason score system. Studied samples were scored from 4 to 10 (mean 7.1 ± 1.77) (Table 1). PC patients were further divided into three subgroups.

Cell Culture. PC3 and DU145 human prostate cancer cells were obtained from the Pasteur National Cell Bank of Iran (Tehran-Iran). Cells were maintained in RPMI 1640 with 10% fetal bovine serum (FBS), 100 units/ml penicillin, and 100 ng/ml streptomycin.

Blood Samples. Blood samples from patients were collected in EDTA containing tubes prior to prostate biopsy, and plasma was immediately separated by centrifugation at

1000xg for 20 minutes and kept at -70 °C until analyzed. Informed consent was obtained from all patients for measuring plasma IL-8 concentration.

Cell Culture Supernatants. For the determination of IL-8 level in the medium, PC cell lines were plated. When the cells reached to 70% confluence, media were changed and replaced with serum-free fresh ones. After 24 hours when the cells were almost confluent and each flask contained nearly the same number of cells, supernatants were collected. After removing the particles by centrifugation, they were either assayed immediately or aliquoted and stored at -20° C.

Measurement of Plasma IL-8 and PSA Concentration. IL-8 in cell culture supernatants and in plasma was measured by Quantikine Human CXCL8/IL-8 Immunoassay kit (R&D systems, Minneapolis, USA) according to the manufacturer's instructions. PSA levels were also measured by radioimmunoassay (Spectra, Finland) using manufacturer's instructions. Analyses and calibrations were performed in duplicate and intra- and inter-assay variations were within the range given by the manufacturer.

Statistical Analysis. All statistical analyses were carried out with SPSS version 16.0 and Prism graph pad 5.0 softwares. Differences in the plasma levels of IL-8 between BPH and PC patients were calculated by Independent T-test. A Spearman test was used to analyse the correlation between IL-8 levels and the Gleason score. The correlation between IL-8 level and age or PSA level was calculated using Pearson test. In addition, a one-way ANOVA test was used to analyse the differences in IL-8 concentration among various PC subgroups. A statistical difference with a $p<0.05$ was considered significant.

Table 1: Frequency and percentage of PC patients with different Gleason scores

Gleason Score	Frequency	Percent
4	4	10
5	2	5.0
6	10	25
7	8	20
8	7	17.5
9	4	10
10	5	12.5
Total	40	100

RESULTS

Patients and Disease Score. As mentioned before, 40 PC patients were divided into three subgroups according to their Gleason scores: severe PC with a Gleason score of 8-10 (40%), moderate PC with a score of 6-7 (45%) and mild PC with scores less than 5 (15%).

Plasma IL-8 and PSA Concentration. The plasma IL-8 concentration of patients measured by the ELISA method was not significantly higher in PC patients (52.3 ± 7.1 pg/ml) relative to BPH patients (49.1 ± 11.3 pg/ml, $p>0.05$) (Figure 1). IL-8 in PC and BPH patients ranged between 40 to 72.7 pg/ml and 19.78 to 67.3 pg/ml, respectively. Plasma PSA concentration of PC and BPH patients were 5.8 to 100.0 ng/ml and 3.0 to 75.0 ng/ml, respectively (Figure 2). All normal samples measured less than the lowest

IL-8 standard, 31.2 pg/ml. Mean PSA level in PC patients was significantly higher than that of BPH patients ($p<0.03$).

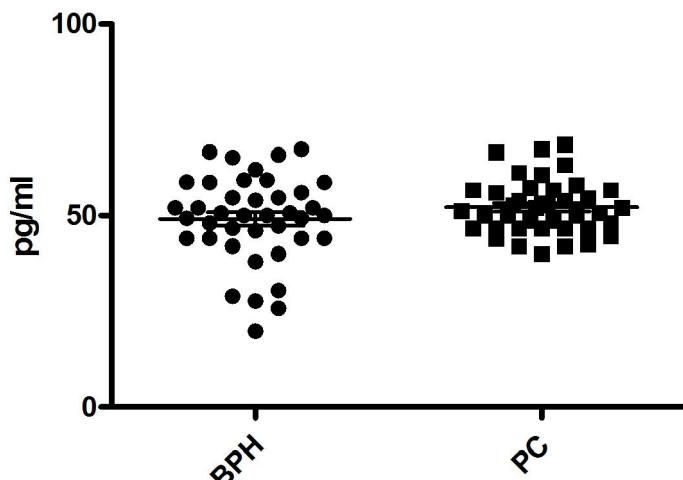


Figure 1. Distribution of IL-8 concentration measured by ELISA method in different samples. There is no significant difference between Plasma IL-8 of PC and BPH patients.

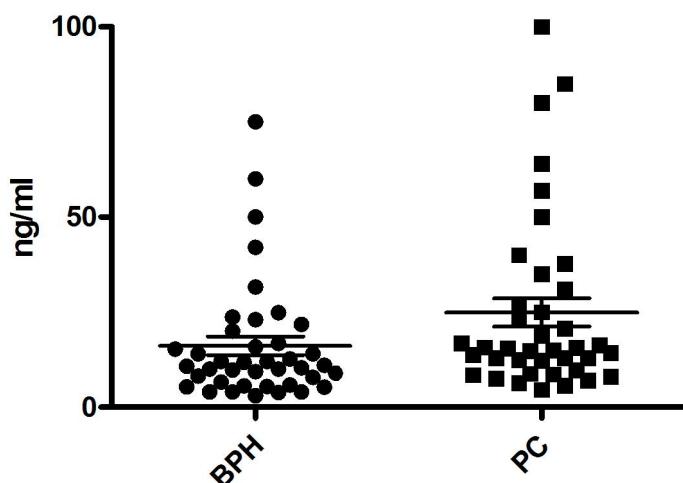


Figure 2. Distribution of PSA concentration measured by RIA technique in different samples. There is a significant difference between plasma PSA level of PC and BPH patients.

There was no correlation between IL-8 level and either PSA concentration or the age of both PC and BPH patients. However, a correlation was found between plasma IL-8 level and the Gleason scores of PC patients using the Spearman test ($p<0.001$). When using one-way ANOVA test specifically, a significant difference was noticed between the IL-8 concentration of severe PC and the mild PC patients ($p<0.003$), but not with the moderate PC patients (Figure 3).

IL-8 Concentration of Cell Culture Supernatants. IL-8 concentration of PC3 and DU145 cell line supernatants measured by an ELISA method were 2650.6 and 443.5 pg/ml, respectively.

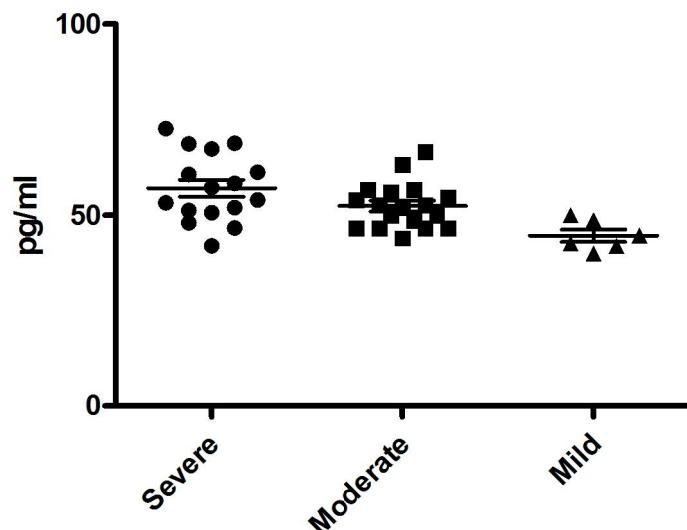


Figure 3. Distribution of IL-8 concentration measured by ELISA method in different subgroups of PC patients. There is only a significant difference in plasma IL-8 concentration of severe and mild PC individuals.

DISCUSSION

Although IL-8 does not serve as a diagnostic marker for the early detection of PC or for differentiating between BPH and PC, it may be used as a prognostic tool for monitoring PC. PC is a leading cause of sickness and death among men worldwide. Pathologically, investigators have unveiled small prostatic carcinoma in up to 29 percent in 30 to 40 year old men and 64 percent in 60 to 70 year old men. In addition, the incidence of PC is 1 in 6 and the risk of death from metastatic PC is 1 in 30 (15).

The new advances of PC epidemiology, is due to the discovery of prostate specific antigen (PSA). Utilizing this tumor marker for the past two decades, have dramatically improved the diagnosis as well as the pre- and post-treatment monitoring of PC. Despite this, there is a limitation in the use of PSA for the early diagnosis of PC, and controversy exists over its use. The lack of a cut-off point for a normal PSA level is the most disputed issue considering its inefficiency. Therefore, there is some question about the positive predictive value of this marker (16). Pathological studies on PC biopsies have revealed poor sensitivity at the PSA cut-off point of 4ng/ml, since clinically important PC was diagnosed at all PSA levels. It has also been suggested that the pre-diagnostic PSA level no longer adequately predicts key prognostic features, particularly at levels less than 10 ng/ml. A setback concerning the usage of PSA as a screening tool has been discerned due to the overall sensitivity, which may be too low to effectively reduce mortality (2). The requirement for new PC markers is clear. Recently some blood-based tumor markers, such as early PC antigen, insulin-like growth factor-1 (IGF-1) and its binding proteins (IGFBP-2 and IGFBP-3) have been compared; however, neither of these markers could completely discriminate PC patients (17).

The aim of our study was to evaluate the reliability of utilizing IL-8 as a tumor marker for diagnosis and monitoring of PC. IL-8 is widely expressed in tumor, stromal, and endothelial cells. Its autocrine and paracrine functions have been shown to play important roles in angiogenesis, tumor growth, and metastasis (18,19). Presently, a number of mechanisms such as transforming growth factor beta 1 (TGF-beta1) signalling pathway (20,21), thrombin/PAR-1 (protease-activated receptor1) system (22) and zinc deficiency (12) have been reported about the over expression of IL-8. In addition to the role of IL-8 in cancer biology, its over-expression is clearly presented and its role as a biochemical marker in diagnosis and/or monitoring of PC has been questioned. Our study showed that the plasma IL-8 concentration of patients measured by the ELISA assay was not significantly higher in PC patients (52.3 ± 7.1 pg/ml) relative to BPH patients (49.1 ± 11.3 pg/ml, $p>0.05$) (Figure 1). A study done by Veltri et al. (1999) found contradicting results. It was reported that the serum IL-8 concentration in clinically well-defined patients was independent of the free/total PSA ratio as a predictor of PC (23). Limited number of our samples may be the basis for the controversy. Studies on breast cancer patients have also revealed that elevated serum IL-8 levels strongly correlate to the clinical stage of the disease (24). In our research using the one-way ANOVA test, a significant difference was noticed only between the IL-8 concentration of severe PC and mild PC patients ($p<0.003$), but not with moderate PC patients (Figure 3). This agrees well with the work of Lehrer et al., who showed a significant elevation of serum IL-8 in men with bone metastases diagnosed by Tc-99 MDP bone scan when compared to men with the localized disease (25). In addition, IL-8 level in supernatants of PC3 and DU145 cell lines was measured (2650.6 vs. 443.5 pg/ml). It was found that these cells produce and secrete IL-8 in the media, showing that part of the increase in IL-8 concentration maybe due to its secretion in the medium. We also showed that PC3 cell lines which are responsible for bone metastasis secrete more IL-8, in agreement with the studies of Lehrer et al. (25).

Mean PSA level in PC patients were significantly higher than the level in BPH ones ($p<0.03$, Figure 2). No correlation was found between IL-8 and PSA level or the age of either PC or BPH patients. However, a correlation was found between plasma IL-8 level and Gleason scores of PC patients using the Spearman test ($p<0.001$). Therefore, plasma IL-8 level may increase with progression of tumor to a more malignant state, perhaps due to more cell proliferation and angiogenesis. To prove this claim, plasma IL-8 concentration could be measured in more PC patients.

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