

RESEARCH COMMUNICATION

Diagnostic and Prognostic Significance of Prostate Specific Antigen and Serum Interleukin 18 and 10 in Patients with Locally Advanced Prostate Cancer: A Prospective Study

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Abstract

Background: Prostate cancer is one of the most common cancers afflicting men today. Prostate biopsy, an invasive procedure is generally used for diagnoses but attempts are being made to find accurate and precise non-invasive biomarkers. Diagnostic accuracy of prostate specific antigen (PSA) has been well documented. Serum interleukin-18 (IL-18) and interleukin-10 (IL-10) have shown their diagnostic ability in other cancers but not investigated well in prostate cancer. This study, thus determines the diagnostic and prognostic significance of PSA, IL-18 and IL-10 prospectively in patients with carcinoma prostate. **Methods:** A total of 149 patients, aged 40-84 yrs were investigated during April 2007 to July 2010 and recruited for this study after Institutional ethical approval. Of the total of 149 patients, 71 had biopsy proven prostate cancers (TNM stage: T2=17, T3=26 and T4=28) and 78 clinical benign prostate hyperplasia (BPH). Peripheral blood samples of all patients and 71 age matched control subjects were obtained at baseline and estimation of PSA, IL-18 and IL-10 was done by enzyme linked immunosorbent assay (ELISA). Carcinoma prostate patients were followed for three years. Data were analyzed with ANOVA, ROC curve analysis and survival analysis. **Results:** The baseline levels of PSA, IL-18 and IL-10 in all groups of carcinoma prostate were found to be significantly ($p<0.01$) higher than both Control and BPH. The levels of IL-18 and IL-10 also found to be elevated significantly in stage T3 ($p<0.05$) and T4 ($p<0.01$) as compared to stage T2. The levels especially of IL-18 is found to be well associated with progression of the disease of various groups ($r=0.84$, $p<0.01$). In contrast, IL-10 showed significant direct association with progression of carcinoma ($r=0.84$, $p<0.01$) while inverse relation with survival duration ($r=-0.48$, $p<0.01$) and survival rate ($\chi^2=8.98$, $p=0.0027$; Hazard ratio=0.37, 95% CI=0.18-0.69). **Conclusions:** Study concluded that serum IL-18 has potential to be a better diagnostic marker with higher specificity and sensitivity and IL-10 may be valuable as a prognostic marker than PSA in carcinoma prostate.

Keywords: PSA - IL-18 - IL-10 - prostate cancer BPH - ROC curves - sensitivity - specificity

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Introduction

World-wide, prostate cancer is one of the commonest cancers in men affecting 33% of global burden. The prostate biopsy remains invasive method for detecting this cancer. Currently, serum prostate specific antigen (PSA) is considered to be the best tumor marker for detecting early prostate cancer which also has prognostic value. However, there are certain drawbacks of PSA. The most important drawback is that it is prostate-specific and not cancer-specific. The serum levels of PSA rise in many conditions like benign enlargement and prostatitis. The sensitivity and specificity of PSA is also controversial which varied from 0.78 to 1.00 and 0.06 to 0.66, respectively (Philip H et al., 2009).

With better understanding of the molecular mechanisms of carcinogenesis many newer molecules are being evaluated especially in cases of invasive procedures. Various cytokines have been found to participate in the steps of prostate carcinogenesis. This study was started with consideration of prostate volume, PSA velocity, expression of IL-12, IL-10 and IL-18 but two such molecules of this study as interleukin-18 (IL-18) a pro-inflammatory cytokine and interleukin-10 (IL-10) an anti-inflammatory cytokine has potency to be proved as better biomarkers. These cytokines have been reported to give some diagnostic and prognostic information in other cancers. Animal models and in-vivo study have demonstrated the anti-tumor activity of IL-18 (Fukuhara

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H et al., 2005; Cao DY et al., 2007).

In a previous study, some authors observed that IL-18 protein was expressed at various levels in breast cancers and was associated with the concomitant presence of distant metastases (Gunel N et al., 2002). These results suggested a role of IL-18 in the control of tumor spreading in humans, was in line with the antitumor properties of IL-18 also observed in murine models. Clinical and experimental approaches to the pathophysiology of IL-18 in human tumors progression are still to research.

Several preclinical models support the hypothesis that IL-10 might blunt the immune response against cancer. The function of IL-10 is to act on the macrophages, inhibiting synthesis and suppressing gene of other cytokines (Negrier S et al., 2004). Several authors hypothesized that IL-10 is an immunosuppressive molecule secreted by tumor cells to allow malignant cell to escape from immunosurveillance (Marincola et al., 2000; Yang et al., 2004; Mapara et al., 2004).

Conversely, other preclinical and clinical models suggest that IL-10 might favor immune mediated rejection of cancer. Therefore, the role of IL-10 remains a controversial subject. In carcinoma prostate, except PSA, the role of both IL-18 and especially of IL-10 is not well documented. The comparative significance of all three markers in carcinoma prostate is also not evaluated yet. This study thus aimed to investigate the diagnostic and prognostic significance of prostate specific antigen and serum interleukin 18 and 10 prospectively in patients with carcinoma prostate.

Materials and Methods

A total of 276 new patients were enrolled during April 2007 to July 2010 in the Department of Urology, Chhatrapati Sahuji Maharaj Medical University (Erstwhile KGMC), Lucknow, India and in the Department of Urology, Sanjay Gandhi Post Graduate Institute, Lucknow, India. Total 149 patients were found suitable and included for this study after obtaining the Institutional ethical approval and informed consent from the patients. Patients were also informed that their blood samples could be used for research purposes. The age of all patients ranged from 40-84 yrs. Of total 149 patients, 71 biopsy proven prostate cancer patients (TNM stage: T2=17, T3=26 and T4=28) and 78 clinical benign prostate hyperplasia (BPH) patients. During the periods, blood samples of 71 age matched independent (of patients) healthy controls were also taken by organising various camps. The subjects/pat diabetes, arthritis, cardiovascular disease, hepatitis, AIDS and other inflammatory diseases including prostatitis were excluded as these patients also showed altered expression of IL-18. Thus, the blood samples of 220 subjects were taken at baseline (admission). Immediately after blood sampling, serum was obtained by centrifugation at 2000 r/min for 15 min at 4°C and stored at -80 °C until later analysis. Serum PSA, IL-18 (Bender Med Systems, ELISA kits Vienna, Austria) and IL-10 (R&D systems ELISA kits) levels were determined using ELISA kits as per standard protocol of manufacturers. All newly diagnosed men with carcinoma prostate received treatment according

to the stage of disease from the OPD. They were then followed 3-monthly for 3 years and other tests to document recurrence or progression.

Statistical analysis

Groups were compared by one way analysis of variance (ANOVA) and the significance of mean difference between the groups was done by Newman-Keuls post-hoc test. Pearson correlation analysis was used to assess association between the variables. Diagnostic (sensitivity and specificity) significance of variables was tested by receiver operating characteristic (ROC) curve analysis. Survival among groups was compared by Logrank test. The analyses were performed after transforming levels of PSA, IL-18 and IL-10 to Log10. A two-tailed ($\alpha=2$) probability (p), $p<0.05$ was considered statistically significant. STATISTICA (version 6.0) and Graph Pad Prism (version 3.0) were used for the analysis.

Results

Association of PSA, IL-18 and IL-10 levels among various groups: Diagnosis

The baseline (pre treatment) levels of PSA, IL-18 and IL-10 of five groups (Control, BPH, TNM stage T2, TNM stage T3 and TNM stage T4) were summarized in Table 1. Table 1 shows that as disease progresses (from control to TNM stage T4) the mean levels of PSA, IL-18 and IL-10 also increases. On comparing the levels of each PSA, IL-18 and IL-10 between the groups, the mean levels of PSA, IL-18 and IL-10 in Control and BPH did not differ significantly ($p>0.05$). However, the mean levels of PSA, IL-18 and IL-10 in all groups of carcinoma prostate (TNM stages) were found to be significantly ($p<0.01$) different and higher than both Control and BPH. Further, the mean levels of IL-18 and IL-10 in TNM stage T3 ($p<0.05$) and TNM stage T4 ($p<0.01$) were also found to be significantly higher than TNM stage T2. In contrast, the mean level of PSA, IL-18 and IL-10 in TNM stage T3 and TNM stage T4 was found to be statistically the same ($p>0.05$).

Correlation analysis (Table 2) also showed significant ($p<0.01$) and direct association (positive correlation) between disease progression (disease severity from Control to TNM stage T4) and baseline levels of PSA, IL-18 and IL-10. Further, the progression of disease was found to be associated most with IL-18 ($r=0.84$, $p<0.01$) followed by IL-10 ($r=0.77$, $p<0.01$) and PSA ($r=0.76$, $p<0.01$). The levels of PSA, IL-18 and IL-10 also correlated significantly ($p<0.01$) with each other.

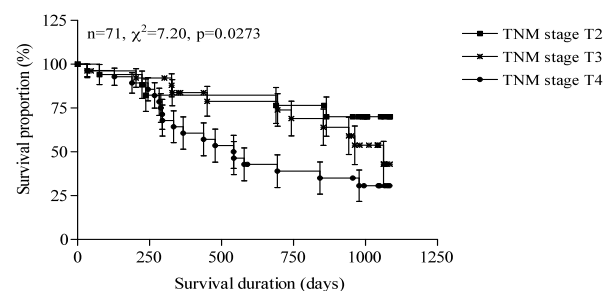


Figure 1. TNM Stage-wise 3-year Overall Survival Proportions (%) in Carcinoma Prostate Patients

Table 1. Baseline Summary (Mean \pm SE) of PSA, IL-18 and IL-10 of Five Groups

Levels	Control (n=71)	BPH (n=78)	TNM stage T2 (n=17)	TNM stage T3 (n=26)	TNM stage T4 (n=28)
PSA	1.96 \pm 0.18	2.37 \pm 0.17	5.94 \pm 0.75ab	13.35 \pm 1.07ab	17.28 \pm 1.01ab
IL-18	143.97 \pm 2.29	145.88 \pm 1.93	229.54 \pm 3.30ab	245.66 \pm 4.05abc	251.64 \pm 4.12abc
IL-10	2.08 \pm 0.27	2.15 \pm 0.18	8.03 \pm 0.49ab	13.06 \pm 0.45abc	16.32 \pm 0.29abc

^ap<0.05 or ^ap<0.01- in comparison with Control, ^bp<0.05 or ^bp<0.01- in comparison with BPH, ^cp<0.05 or ^cp<0.01- in comparison with TNM stage T2

Table 2. Correlation between Severity of Disease and Baseline Levels of PSA, IL-18 and IL-10 of All Subjects (n=220)

Variables	Severity	PSA	IL-18	IL-10
Severity	1.00			
PSA	0.76**	1.00		
IL-18	0.84**	0.81**	1.00	
IL-10	0.77**	0.68**	0.71**	1.00

**- p<0.01

Association with patients survival: Prognosis

Survival- TNM stage wise: The end point of treatment i.e. survival of all 71 prostate carcinoma patients according to TNM stage was summarised graphically in Fig. 1. Fig. 1 showed that, 3-year overall survival differed significantly between TNM stages ($\chi^2=7.20$, p=0.0273). Logrank test

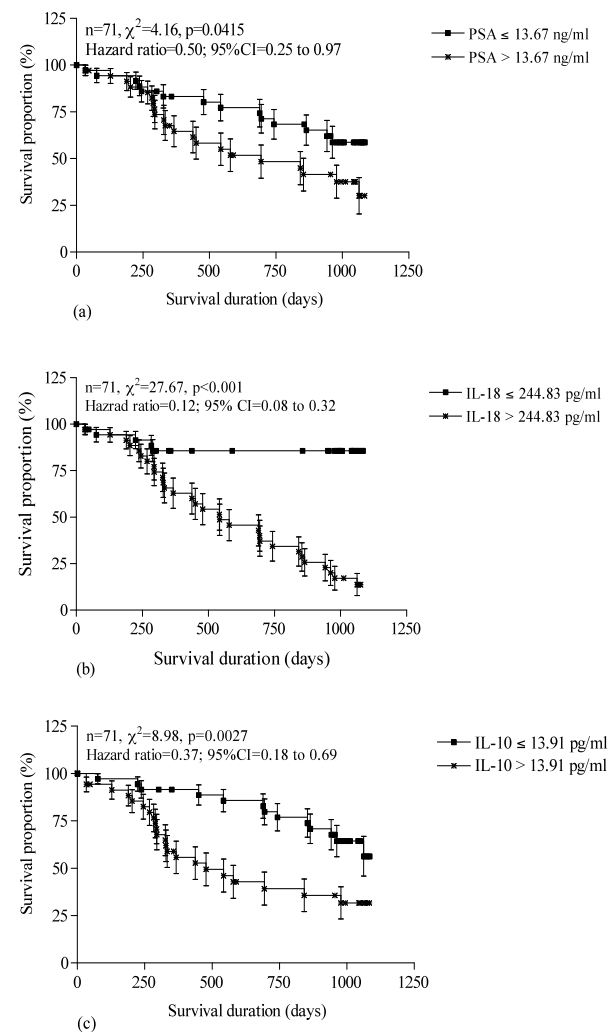


Figure 2. 3-year overall survival proportion (%) on the basis of low and high levels of PSA (a), IL-18 (b) and IL-10 (c) in patients with carcinoma prostate.

Table 3. Correlation between TNM Stage, Survival duration and Baseline Levels of PSA, IL-18 and IL-10 of All Subjects (n=220)

Variables	TNM	Duration	PSA	IL-18	IL-10
TNM	1.00				
Duration	-0.26*	1.00			
PSA	0.65**	-0.11ns	1.00		
IL-18	0.39**	-0.43**	0.32**	1.00	
IL-10	0.84**	-0.48**	0.55**	0.43**	1.00

ns- p>0.05, *- p<0.05, **- p<0.01

for trend also revealed significant and inverse association between survival and TNM stages ($\chi^2=6.81$, p=0.0091) i.e. survival lowered more in TNM stage 4 (30.7%) followed by TNM stage 3 (43.0%) and TNM stage 2 (70.1%).

Survival- according to low and high levels of PSA, IL-18 and IL-10: The levels of each PSA, IL-18 and IL-10 of all 71 prostate carcinoma patients were further sub grouped into two groups (low and high) according to respective median values and respective 3-year overall survival were summarised graphically in Fig. 2. Fig. 2 showed that patients with high levels of PSA, IL-18 and IL-10 had a poor survival than those with low levels PSA, IL-18 and IL-10.

On comparing, the patients with higher PSA (PSA > 13.67 ng/ml) had significantly lower survival (58.7% vs. 30.2%, $\chi^2=4.16$, p=0.0415) as compared to lower PSA (PSA \leq 13.67 ng/ml) (Fig. 2a). Similarly, survival in patients having higher IL-18 (IL-18 > 244.83 pg/ml) had significantly (85.8% vs. 13.7%, $\chi^2=27.67$, p<0.001) lower survival than those having lower IL-18 (IL-18 \leq 244.83 pg/ml) (Fig. 2b). Further, the survival in patients with higher IL-10 (IL-10 > 13.91 pg/ml) also lowered significantly (56.3% vs. 31.7%, $\chi^2=8.98$, p=0.0027) as compared to lower IL-10 (IL-10 \leq 13.91 pg/ml) (Fig. 2c).

To find out prognostic significance of PSA, IL-18 and IL-10, correlation analysis was further subjected between TNM stages, survival duration (days) and baseline levels of PSA, IL-18 and IL-10 in patients of carcinoma prostate and summarised in Table 3. Survival duration was inversely correlated with TNM stages (r=-0.26, p<0.05). Further, a direct association was evident between baseline PSA (r=0.65, p<0.01), IL-18 (r=0.39, p<0.01) and IL-10 (r=0.84, p<0.01) and TNM stages. The duration of survival did not correlate well PSA (r=0.05, p>0.05) while significant and inversely correlated with IL-18 (r=-0.43, p<0.01) and IL-10 (r=-0.48, p<0.01).

Discussion

This study examined the role of serum PSA, IL-18 and IL-10 in carcinoma prostate and tried to correlate them

with progression of the disease (diagnosis) and patient's survival (prognosis). For diagnosis and prognosis, study we found that serum IL-18 and IL-10 was more valuable than PSA. For diagnosis IL-18 was better while for prognosis it was IL-10. IL-18 at value > 192.05 pg/ml (criterion or cut-off value), discriminating the non-cancerous and cancerous cases with 100.0% sensitivity and specificity. In the present study, the prevalence of carcinoma prostate was 32.3%. The 3-year overall survival proportion in patients were in well accordance with the TNM stages i.e. low stage having higher survival than high stages. In patients of TNM stage T2, T3 and T4 the survival proportion was found to be 70.1%, 43.0% and 30.7%, respectively.

The diagnostic accuracy of prostate specific antigen was well documented (Philip H et al., 2009). This showed that PSA has a role to play as one of several indicators for invasive prostate biopsy but having high false positive and significant false negative rate. Further, in this systematic review it was also well documented that the sensitivities and specificities of PSA also varied from 0.78 to 1.00 and 0.06 to 0.66, respectively. It was also well documented that PSA is a prostate-specific, not cancer-specific.

In the present, we found that the levels of serum IL-18 was well correlated with disease progression of various groups and elevated significantly more as compared to PSA and IL-10 in patients of carcinoma prostate as compared to both controls and benign prostate hyperplasia patients and its expression levels also increases more with progression of the disease. It may be due to up-regulation of IL-18 secretion which may reflect the influence of prostate tumors on systemic immune responses. It can also be speculated that IL-18 production by the normal adjacent prostate cells may reflect the degree of defense mechanism against tumor growth and dissemination of prostate carcinoma (Pages et al., 1999).

For IL-18, our results are in well accordance with the study of Shaojun Nong et al., 2007. The higher expression of IL-18 is also documented in various other carcinoma as gastric (Kawabata et al., 2001), breast (Gunel et al., 2002) and oesophageal carcinoma (Tsuboi et al., 2004). Our results were similar to above discussed findings. The pathways for IL-18 production and its mechanisms for anti tumor effect are well documented but its clear mode of action in patients with prostate carcinoma is not well documented.

The IL-18 performs its various biological activities via its capacity of stimulating innate immunity and both Th1 and Th2-mediated responses. It also exerts anti-tumor effects that are mediated by enhancement of NK cell activity, reduction of tumorigenesis, induction of apoptosis and inhibition of angiogenesis in tumor cells (Tanaka et al., 2004). In addition, recent data suggest that an inappropriate production of IL-18 contributes to the pathogenesis of cancers and may influence the clinical outcome of patients (Lebel-Binay et al., 2000).

In the present study we found that the levels of anti-inflammatory (IL-10) also aggravated with progression of the disease as of pro-inflammatory (IL-18). The most probable reason of higher IL-10 expression is may be due to secretion of IL-10 by own tumor cells and thus

regulate immunosuppressive environment by involving cross talk between different players of immunity (Yang L et al., 2004). Further, they reported that IL-10 can impair tumor-associated antigen (TAA) cross-presentation by Dendritic cell (DC) thus potentially preventing T cells from mounting an effective immune response against malignant cells (Yang L et al., 2004). Furthermore, in cancer, IL-10 expression at early tumor sites promotes the generation and activation of TGF- β -T reg, which in turn, leads to the systemic suppression of anti-tumor immunity in mice (Seo N et al., 2001). These may be the reasons that we have found elevated levels with progression of the disease.

Several researchers have found poor prognosis of IL-10 in cancers like lymphoma (Herling M et al., 2003), non-small cell lung cancer (De Vita F et al., 2000), and colon cancer (Galizia G et al., 2002). In contrast, our results showed high prognosis of IL-10 in prostate carcinoma while correlating it with patient's survival. In the present study, IL-10 showed more inverse relation as compared to both IL-18 and PSA with survival duration and survival rate. It may be due to because of elevated serum IL-10 levels decreases after patient's treatment (Galizia et al., 2002); therefore survival increases as we found inverse relation between IL-10 levels and survival.

Several preclinical models support the hypothesis that IL-10 might blunt the immune response against cancer. IL-10 can act as a negative mediator in the cross-talk between innate and adaptive antitumor immunity. Investigators have reported that TCR-bearing T cells and TCR-intermediate T cells suppress NK and NKT cells by elaborating IL-10 and TGF- β , which ultimately, leads to impaired activation of CTL (Cytotoxic T Lymphocyte), Th1 CD4 T cells, and tumor immune privilege (Seo N et al., 2002). Moreover, IL-10 expression by tumor cells has been associated with increased expression of the nonclassical HLA class Ib molecule (HLA-G), which may inhibit the cytolytic activity of NK cells and CTL (Urosevic et al., 2003). Moreover, IL-10-producing monocytes, which inhibit T cell proliferation, have been isolated from the ascites of patients with ovarian carcinoma (Loercher et al., 1999). CTLA-4 is critical in several experimental settings, where its blockade promotes antitumor immunity (Abrams et al., 2004). In a mouse model of plasmacytoma it has been demonstrated that a large part of the immunosuppressive effects of CTLA-4 can be attributed to IL-10. In fact inhibition of IFN- γ secretion induced by CTLA-4 is blocked using an anti-IL-10 antibody, and in vivo treatments with anti CTLA-4 and anti IL-10 antibody are equally effective in inducing tumor responses without any addictive effect (Jovasevic et al., 2004).

Other investigators have also shown that the immunosuppressive effects of COX-2, over expressed by some tumor cells, at least in part, depend on IL-10 up regulation (Huang et al., 1998; Stolina et al., 2000; Sharma et al., 1999) nevertheless, opposite findings have been also reported (Specht et al., 2001; Enk et al., 1997).

It is important that inhibition of IL-10 production by T cells or malignant cells using low-dose cyclophosphamide (Matar Pet et al., 2001), anti-IL-10/IL-10 R-blocking

antibodies (Curiel et al.,2004), or anti-IL-10 antisense oligonucleotides improves cancer-specific immune response in some periclinical tumor models, which suggest the use of IL-10-neutralizing agents as immunological adjuvants in the design of anticancer vaccines.

The shortcoming of the present study was that we failed to find T1stage patients and we also excluded distant metastasis patient as they have some other medical complications that may gives some confounding and biases, thus more T1 stage patients and larger sample size may be more helpful in predicting better outcome and we can achieve more accurate and precise findings.

In conclusion, the serum prostate specific antigen, interleukin-18 and interleukin 10 all showed high diagnostic and prognostic significance in patients with carcinoma prostate but the efficacy of interleukin-18 and interleukin-10 was found more valuable than the prostate specific antigen generally used biomarker. The findings of this study may be helpful in the management of carcinoma prostate, but inclusion of our drawbacks may be more imperative.

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