Comparative study of serum levels of *Perforin* in Prostate Cancer and Benign Prostatic Hyperplasia patients

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ABSTRACT

Perforin(p) is the primary mediator of short term cytotoxicity, it is accumulated in response to proinflammatory cytokines and stored in T lymphocyte , NK cells and NKT cells are released upon activation. Perforin is a prototypical cytotoxic molecule involved in cell mediated immunity against various pathogens , alloantigens and particularly different tumors.The purpose of this study was to determine perforin level in prostate cancer (P.Ca) and Benign Prostatic Hyperplasia (BPH). This study was performed on 59 patients consisting of 28 patients with P.Ca and 31 patients with BPH. Perforin and PSA levels were measured in cancer and BPH patients using ELISA method. Mean Perforin value was significantly lower in P.Ca patients than in BPH patients ($p < 0.01$) where as mean serum PSA level was significantly higher in the cancer patients in comparison to the BPH group ($P < 0.01$). Our finding indicate probability of problem in expression of cytotoxic molecule ,perforin in and around the tumor.

Keywords: Prostate cancer(P.Ca); Benign Prostatic Hyperplasia (BPH); Perforin; PSA.

INTRODUCTION

Prostate cancer,a common malignant disease in males[1], is the second leading cause of death from cancer in men in both Europe and the United States[1].The Iranian Annual of National Cancer Registration Report   in 2006, the number of Prostate cancer registered is about 3733 cases with ASR 12.80 per 100,000 population.[2].In Iran ,prostate cancer is the 3th most common cancer in male population following gastric and bladder cancers and is the 5th leading cause of death due to cancer in men[3].Naturally human body using of the many mechanism for inhibition of foundation of cancer. Cytotoxic T lymphocyte and Natural killer cells are one of the primary mechanisms used by higher organisms to eliminate viruses and transformed cells.Apoptosis is an innate mechanism of programmed cell death that can be induced by two pathways:
 a) granuledependent exocytosis pathway
 b) Fas-FasL intercellular linkage-mediated pathway [4,5].This pathway is established through intracellular signaling after target cell recognition by a cytotoxic lymphocyte (NK or cytotoxicT cell). In exocytosis or degranulation, there is microtubules mobilization that leads the preformed granules or lysosomes of the cytotoxic cell towards the point of contact with the target cell, releasing stored lytic molecules[6,7].
The lytic granule contains a proteoglycan matrix that maintains protease enzymes in an inactive stage and avoiding from self-destruction [6,7].There is a novel perforin inhibitor associated with cytotoxic T cell granules and termed it Cytotoxic Regulatory Protein 2 (CxRP2). The protein disulfide isomerase A1 had CxRP2 activity and indicated that protein disulfide isomerases, in the ER or elsewhere, may protect T cells from their own perforin [8].
 The lytic molecules stored in granules that induce apoptosis are perforin, granzymes (Grzs) and granulysin[9].
In this pathway, cytoplasmic granule toxins — predominantly *perforin*— and a family of structurally related serine proteases (granzymes) with various substrate specificities are secreted

by exocytosis and together induce apoptosis of the target cell[9]. The granule-exocytosis pathway powerfully activates cell-death pathways that operate through the activation of apoptotic cysteine proteases (caspases).The second pathway involves the engagement and aggregation of target-cell death receptors, such as FAS (CD95), by their cognate ligands, such as FAS ligand (FASL),on the killer-cell membrane,which results in classical caspase-dependent apoptosis. The main function of the FAS–FASL pathway is to eliminate self-reactive lymphoid cells[9].

Perforin is found in a soluble monomer shape within granules and, after the cytotoxic-cell/target-cell junction, perforin is released by exocytosis. Once it is anchored, perforin begins the polymerization in the presence of Ca2+ to form cylindrical pores with an internal diameter of 5 to 20 nm[6,7]. The perforin pores can serve as passive conductos of granzymes and granulysin through the target cell membrane and could also allow an ionic exchange, which causes an osmotic unbalance and in consequence, the cell death[6].

It's a protein produced by cells of the prostate gland and the blood level of PSA is often elevated in men with prostate cancer. In addition to prostatitis (inflammation of the prostate) and benign prostatic hyperplasia (BPH) (enlargement of the prostate) cause an elevation in PSA level . PSA levels of 4.0 ng/mL and lower as normal[10,11,12].

The purpose of this study was to investigate serum level of perforin in patients with (P.Ca) and BPH.

MATERIALS AND METHODS

In this study 59 patients consisting of 28 patients with P.Ca and 31 patients with BPH who preferred to Tajrish Shohada of Hospital were recruited. P.Ca and BPH patients were chosen based upon their serum PSA level and Digital rectal examinatrion(DRE).

The exclusion criteria for all subjects enrolled in this study were presence of immunological disorder , acute/chronic inflammatory disease and history on immune suppressive or radiation therapy. All participants gave their written informed consent and agreed to proceed with study protocol. 5 cc blood was collected from the subjects , serum was isolated and stored of

-20c. The concentration of serum PSA of all study subjects was measured using the ELISA method (Diaplus Diagnostic, U.S.A). Serum perforin level was measured using the ELISA method(CUSABIO Company, china).

TNM system was used for staging cancer in the prostate cancer group.

Statistical Analysis

Result were expressed as mean±SD of experiments. Student's *t*-test was used for comparison of the means between two groups, and one-way ANOVA for comparison between multiple groups. To assess correlation between GranzymeH and Estrogen,age,cancer stage correlation bivariate test was performed. For all tests, *P*<0.05 was considered statistically significant. Data were analyzed using SPSS software version 16.

RESULT

In this study the mean age of the patients was 68 ± 5.8 years (ages 56-85 years). As shown in Table 1 serum perforin level was significantly lower in P.Ca patients compared to the BPH patients(p < 0.01).

Where as serum PSA level was significantly higher in cancer patients in comparison to the BPH group (p < 0.01).

Table1:Perforin in the P.Ca and BPH group

Perforin	Group	mean	SD	Min	max	
	BPH	10.05	5.95	1.73	26.03	***p* value <0.01**
	P.cancer	6.74	4.15	1.92	16.22	

Table 2: PSA in the P.Ca and BPH groups

PSA	Group	mean	SD	min	max	
	BPH	4.015	2.22	1.9	14.2	***p* value <0.01**
	P.cancer	18.38	8.59	6.7	42	

Table3:Perfoin levels in three stage of prostate cancer

Stage	N	Mean	SD	min	max
1	17	6.87	4.27	1.92	16.22
2	9	6.30	3.68	2.26	11.61
3	2	7.58	7.90	1.99	13.17

Pearson correlation analysis between PSA ,Perforin and Age indicates a weak negative ,non significant correlation between perforin and PSA in both P.Ca and BPH groups. In BPH group a positive correlation was observed between PSA and Age (r = 0.34 , p value < 0.05). We performed one way ANOVA test to compare perforin levels in different stages of cancer in P.Ca patients , with reference to the results given in Table 3 , there was no significant difference in perforin levels in the 3 different cancer stages (p < 0.9). However PSA levels showed a significant difference in the three stages (p < 0.05).

DISCUSSION

The purpose of this study was to investigate serum level of perforin in patients with (P.Ca) and BPH. P.Ca and BPH are both related to chronic inflammatory process and are recognized as result of altered immune response[13]. Perforin is the primary mediator of short term cytotoxicity , it is accumulated in response to proinflammatory cytokines and stored in T lymphocyte , NK cells and NKT cells are released upon activation. Studies on perforin deficient mice have confirmed the essential invivo role of perforin in target cell apoptosis induced by CTLs and NK cells , in immune responses to cancer and in certain infections[13].

In this study serum level of perforin was significantly lower in P.Ca patients in comparison to BPH patients. Ebelt et al reported that perforin expressing T lymphocytes are rare in BPH and particularly P.Ca tissue[14]. This finding is consistent with the observation of Takmadzic et al who also reported lower perforin expression in the BPH and P.Ca tissue although they did not find significant difference in total perforin expression in peripheral blood lymphocytes between P.Ca and BPH group[13-14]. Negligible expression of perforin in T lymphocytes , NKT , NK cells may be result of tumor activity leading to the development of a chemical barrier around the tumor that probably inhibits infiltration and activation.

In accordance to the result obtained by Takmadzic et al we did find a negative correlation between PSA and Perforin , [15],however this correlation was not statically significant ; which could be attributed to poor sample size . Poor sample size in this study could be also the reason we did not find a significant difference among perforin levels in the three cancer stage groups.

In conclusion our finding indicates the possibility of problem existing in expression of perforin in and around the tumor.

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