

Changes in Serum Levels of Tumor Necrosis Factor–Alpha and Antioxidant status in Different Stages of Malignant Prostate Cancer Patients in Iraq

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Abstract

Chronic inflammation can induce proliferative events and posttranslational DNA modifications in prostate tissue through oxidative stress. The present study was designed to evaluate the changes in serum levels of TNF- α , malondialdehyde (MDA) and total antioxidant status (TAS) patients with different stages of malignant prostatic cancer (PCa) and benign prostatic hyperplasia (BPH). One hundred males (age range of 58-72 years) with different stages of malignant PCa were recruited from the Radiotherapy and Nuclear Medicine Teaching Hospital in Baghdad during the period from September 2010 to April 2011. The patients were categorized according to the 4 disease stages (I, II, III, and IV); 25 patients with benign prostatic hyperplasia (BPH) and 25 normal healthy subjects were considered as comparator groups. Blood samples were taken from all subjects for analysis of TNF- α , TAS and MDA levels. The results showed significant differences between the four stages of PCa patients in all parameters; however, highly significant difference was observed in stage IV compared to control and BPH patients. In conclusion, TNF- α and total antioxidant status could be utilized for marking the advanced stages of malignant PCa.

Key words: Malignant Prostate cancer, Inflammation, TNF- α , Oxidative stress

التغيرات في مستوى TNF- α وحالة مضادات الأكسدة في مصل الدم لدى مرضى سرطان البروستات الخبيث العراقيين بمختلف مراحل

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الخلاصة

للالتهابات المزمنة دور فعال في تطور نمو وتخصص الأورام السرطانية من خلال زيادة تكوين أنواع مختلفة من الجذور الحرة. تم تصميم هذه الدراسة لتقييم التغيرات في مستوى عامل التنخر السرطاني-الفا، المألوندايالديهيد، الحالة الكلية لمضادات الأكسدة ومعامل حالة الأكسدة خلال المراحل المرضية المختلفة لمرضى سرطان البروستات الخبيث. أظهرت النتائج اختلافا واضحا بين المراحل الأربعة لمرضى سرطان البروستات وعلى أية حال أظهرت المرحلة الرابعة من المرض فرقا معنويا واضحا في مستوى المعايير التي تمت دراستها مقارنة بمجاميع السيطرة ومجاميع تنسج البروستات الحميد. ويمكن الاستنتاج بإمكانية استخدام هذه المعايير كمعامل مضافة في تحديد مستوى تطور المرض ومتابعة علاجه.

الكلمات المفتاحية: سرطان البروستات الخبيث، الالتهاب، TNF- α ، فرط الأكسدة .

Introduction

Chronic inflammation can induce proliferative events and posttranslational DNA modifications in prostate tissue through oxidative stress. In fact, repeated tissue damage and oxidative stress related to this event may provoke a compensatory cellular proliferation with the risk of hyper-plastic growth or neoplastic modifications⁽¹⁾. It is well accepted that regions of prostatic inflammation can generate free radicals and many reactive species of oxygen. In particular, macrophages and neutrophil infiltrations provide a source of

free radicals that can induce hyper-plastic or precancerous transformations through the oxidative stress to the tissue and DNA⁽²⁾. Cytokines can contribute to cancer development in several ways. TNF- α has been shown to enhance the formation of Reactive Oxygen Species (ROS) by inflammatory cells, and thereby increase the risk for DNA damage and inhibition of DNA repair in tumor cells⁽³⁾; other mechanisms include direct stimulation of cell growth, induction of angiogenesis, and recruitment of inflammatory neutrophils⁽⁴⁾.

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In addition, an imbalance of pro- and anti-inflammatory cytokines, caused by point mutations or polymorphisms, may prevent the normal self-limiting nature of the immune response, leading to prolonged inflammation with chronic exposure to cytotoxic mediators⁽⁵⁾. In prostate cancer (PCa), many studies have related serum levels of TNF- α to the development of the disease, associating the serum levels of TNF- α , the disease development and presence of metastasis⁽⁶⁾. The expression and action of TNF- α and its receptors have been reported in several tumors such as esophageal⁽⁷⁾, follicular thyroid⁽⁸⁾, skin⁽⁹⁾, ovarian⁽¹⁰⁾ and breast cancers⁽¹¹⁾. While increased ROS generation has traditionally been associated with tissue injury or DNA damage, new and exciting information points to an essential role for its increased generation in several cellular processes associated with neoplastic transformation and aberrant growth and proliferation⁽¹²⁾. Thus, excessive production of ROS or inadequacy in a normal cell's antioxidant defense system (or both) can cause the cell to experience ROS oxidative stress and the increased may play a broader role in cellular processes associated with initiation and development of many cancers including PCa. The present study was designed to identify the expected correlation between TNF-alpha levels and oxidative stress markers in different stages of malignant PCa and BPH patients and reveal their possible importance as future diagnostic markers for stage classification.

Patients and Methods

After screening 132 patients, a prospective case control study was carried on 100 patients with malignant prostate cancer (PCa) at the Radiotherapy and Nuclear Medicine Teaching Hospital in Baghdad. Their age range was 58-72 years, and all of them are newly diagnosed with the disease. After careful clinical, biochemical and histopathological evaluation by oncology specialists, they were classified into four groups according to the clinical stage of the disease (stages I, II, III and IV) and each class include 25 patients. Other 25 patients, with the same age ranges, diagnosed for benign prostatic hyperplasia, were included and served as comparator with BPH. Twenty five healthy subjects, with the same age ranges, were selected and served as control group for comparison of the studied parameters, at the Department of Urology/Baquba Teaching Hospital. From all subjects, blood samples were collected by vein puncture. TNF- α enzyme linked immunosorbent assay (ELISA)

was utilized depending on a technique called quantitative sandwich immunoassay⁽¹³⁾. Total antioxidant status (TAS) was determined by using a kit supplied by Randox⁽¹⁴⁾. Malondialdehyde (MDA) was determined by using a kit supplied by Northwest⁽¹⁵⁾. The data were statistically evaluated using unpaired Student's t-test and multiple-way ANOVA; *P* values less than 0.05 were considered statistically significant.

Results

Table 1 indicated that TNF- α levels in all stages of malignant prostate patients were significantly higher compared to control (485.88%, 1152.04%, 2469.73%, and 3640.16%, respectively, *P*<0.05), and (126.67%, 384.39%, 894.19% and 1347% respectively, *P*<0.05) compared to BPH patients; significant differences were observed between the four stages of PCa patients; however, highly significant difference was shown in stage IV of malignant PCa patients compared to control and BPH patients (*P*<0.05) (Figure 1).

Table 1: Serum levels of TNF- α in healthy subjects, BPH patients and patients with different stages of malignant prostate cancer.

Patients groups	Serum TNF- α pg/ml
Healthy subjects	28.61 \pm 8.9 ^a
BPH patients	73.95 \pm 16.3 ^b
Stage I P. Cancer	167.62 \pm 38.5 ^c
Stage II P. Cancer	358.21 \pm 96.5 ^d
Stage III P. Cancer	735.2 \pm 126.0 ^e
Stage IV P. Cancer	1070.1 \pm 250.6 ^f

Values represent mean \pm SD; values with non-identical superscripts (a,b,c,d,e,f) indicated significant differences between groups (*P*<0.05).

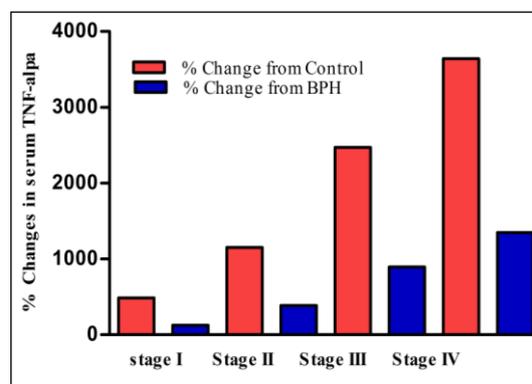


Figure 1: % differences in TNF- α in PCa patients with different stages of the disease compared to BPH patients and controls.

Table 2: Serum levels of MDA, total antioxidant status (TAS) and the oxidative stress index (OSI) in healthy subjects, BPH patients and patients with different stages of malignant prostate cancer.

Patients groups	Serum MDA ($\mu\text{mol/L}$)	Serum TAS (mmol/L)	OSI
Healthy subjects	2.44 \pm 0.6 ^a	1.84 \pm 0.2 ^a	0.14 \pm 0.04 ^a
BPH patients	6.21 \pm 1.7 ^b	1.49 \pm 0.1 ^b	0.41 \pm 0.13 ^b
Stage I PCa	10.49 \pm 0.8 ^c	1.28 \pm 0.1 ^c	0.82 \pm 0.1 ^c
Stage II PCa	13.61 \pm 2.6 ^d	1.15 \pm 0.1 ^d	1.19 \pm 0.24 ^d
Stage III PCa	17.49 \pm 2.4 ^e	1.09 \pm 0.2 ^e	1.68 \pm 0.5 ^e
Stage IV PCa	20.64 \pm 3.1 ^f	0.81 \pm 0.25 ^f	2.93 \pm 1.4 ^f

Values represent mean \pm SD; values with non-identical superscripts (a,b,c,d,e,f) indicated significant differences between groups ($P < 0.05$).

Table 2 clearly demonstrated that MDA levels in all stages of PCa patients were significantly higher compared to control (329.92%, 457.79%, 616.8%, and 745.9% respectively), and 68.92%, 119.16%, 181.64%, and 232.37%, respectively compared to BPH patients, and significant differences were observed between the four stages of PCa; however, highly significant difference was observed in stage IV of malignant PCa patients compared to control and BPH patients ($P < 0.05$) (Figure 2). Table 2 also indicated that TAS levels in all stages were significantly lower compared to control (-30.43%, -37.5%, -40.76%, and -55.98%, respectively) and -13.5%, -22.3%, -26.35% and 45.27%, respectively compared to BPH patients, and there is significant difference reported between the four stages of PCa; however, lower significant difference was observed in stage IV of malignant PCa patients compared to control and BPH patients ($P < 0.05$) (Figure 3).

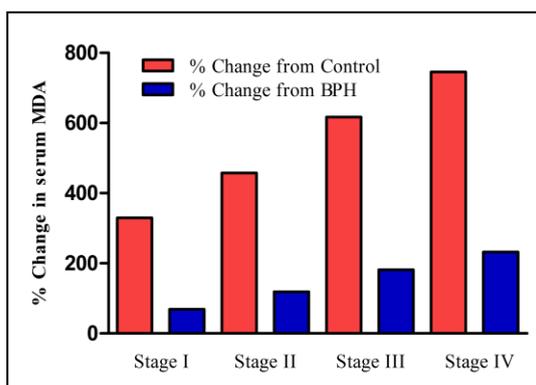


Figure 2: % differences in serum MDA levels in PCa patients with different stages of the disease compared to BPH patients and controls.

Moreover, oxidative stress index (OSI) values in all stages were significantly higher compared to control (485.71%, 750%, 1100%, and 1992.86%, respectively), and 100%, 190.24%, 309.76%, and 614-63%, respectively compared to BPH patients; significant

differences were observed between the four stages of PCa; however, highly significant difference was observed in stage IV of malignant PCa patients compared to control and BPH patients ($P < 0.05$) (Figure 4).

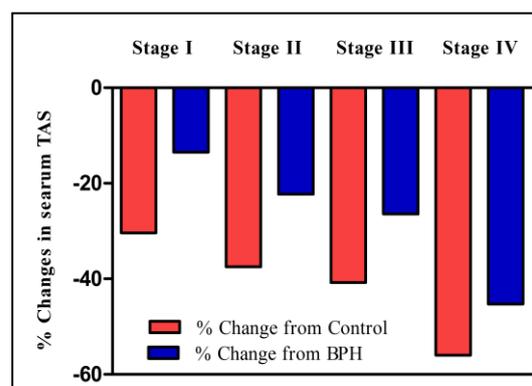


Figure 3: % differences in serum TAS levels in PCa patients with different stages of the disease compared to BPH patients and controls.

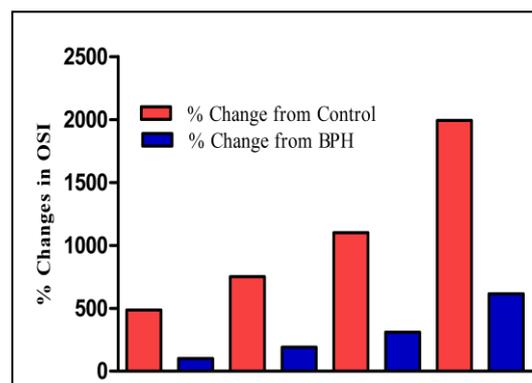


Figure 4: % differences in OSI value in PCa patients with different stages of the disease compared to BPH patients and controls.

Discussion

Chronic inflammation orchestrates a tumor supporting microenvironment that is an indispensable participant in the neoplastic process. In the present study, we reported significantly elevated serum levels of TNF- α in patients with PCa compared with control and BPH, and there was a strong association between the serum levels of the TNF- α , disease stage and the presence of metastatic disease (Table 1, figure 1). These results were compatible with those observed by others^(11,16). This clearly indicates that the role of TNF- α has been linked to all steps involved in tumorigenesis, including cellular transformation, promotion, survival, proliferation, invasion, angiogenesis, and metastasis. Prostate cancer is mostly a disease of elderly men. The progressive inherent or acquired changes in cellular metabolism occurring with aging may play an important role in the development of this disease. Reactive oxygen species (ROS) generated either endogenously or from external sources, due to decrease in intracellular ROS scavenging system plays a vital role in regulating several biological phenomena⁽¹²⁾. The involvement of oxidative stress as an early event in PCa development was suggested by Miyake *et al.*⁽¹⁷⁾ who showed that androgen suppression is capable of decreasing oxidative stress. Polybarchou *et al.* reported that over production of H₂O₂ plays a major role in androgen-independent cell proliferation and migration of LNCaP cells⁽¹⁸⁾. Thus in the present study, we have demonstrated the status of lipid peroxides and antioxidants in plasma of PCa patients in comparison with normal and BPH subjects; this result indicates that MDA in stage IV of PCa patients was significantly higher compared to other groups (Table 2, Figure 2), which means overproduction of free radicals by the inflammatory processes of PCa cause potential oxidative injury to erythrocytes and erythrocyte membranes and damage their antioxidant defense systems. Similar reports of higher MDA levels in PCa were observed by others^(19,20). In contrast to our finding, no significant change in lipid peroxidation in patients with PCa was reported by Dogru-Abba Soglus⁽²¹⁾. The data presented in table 2 also showed that TAS was significantly lower in patients with carcinoma of prostate when compared to controls and BPH; however, when TAS levels in all stages were compared significantly lower values observed in stage IV (Figure 3); the lower TAS levels may be due to increased turnover of TAS for preventing oxidative damage in those patients. This finding was compatible with those reported by Ealon *et al.*⁽²²⁾. Under certain conditions the

increases in oxidants and decrease in antioxidants cannot be prevented and the oxidative/antioxidative balance shifts towards the oxidative status, accordingly, table 2 and figure 4 clearly showed significant elevation in OSI values in stage IV PCa patients compared to other groups. In conclusion, TNF- α and total antioxidant status could be utilized for marking the advanced stages of malignant PCa.

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