MDA as A Bio-Marker for Benign Prostatic Hyperplasia in Pakistani Population

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Received: 13 August 2019; **Accepted:** 20 August 2019

ABSTRACT

Background: Serum malondialdehyde (MDA) is a convenient in vivo index of lipid peroxidation. It is a non-invasive biomarker of oxidative stress. Reactive oxygen species (ROS) could activate some specific signaling pathways that contribute to tumor development by regulating cell proliferation, angiogenesis and metastasis processes. Current study was designed to determine MDA levels in Benign Prostatic Hyperplasia (BPH) as compared to normal control. The study was a Cross-sectional analytical study, which was conducted at Department of Urology, Department of Biochemistry & Molecular Biology Rawalpindi, National University of Science and Technology, Islamabad over a period of one year. Study included sixty eight samples. MDA levels were determined by ELISA (enzyme linked immuno sorbent assay) technique.

Results: We have compared mean value of MDA in BPH and control group, the difference was statistically significant (p=0.002). Conclusions: MDA may be used as bio-marker to determine progression of BPH. Moreover, it is required that additional studies should be carried out to find the pathway involved serum MDA in BPH.

Keywords: *Benign Prostatic Hyperplasia (BPH); Malondialdehyde (MDA); Gleason Scoring*

Introduction and Review of Literature

Prostate cancer is an early onset disease (Dvoracek, 1998). According to a study, oxidant-antioxidant imbalance may be one of the major factor responsible for the development of prostate cancer and benign prostate hyperplasia (Srivastava and Mittal, 2005). One of the source of prostate cancer is heterogeneous, possibly involving both genetic and environmental factor (Zhang *et al*., 2011).

Reactive oxygen species (ROS) could active some specific signaling pathway that contribute to tumor development by regulating cell proliferation, angiogenesis and metastasis processes (Rebillard *et al*., 2013). ROS can also induce genetic instability (Rebillard *et al*., 2013). High fat Western diet explains the fact there is any correlation with benign prostatic hyperplasia (Dvoracek, 1998).

BPH is a major cause of morbidity and mortality in men in Western society (Perryman *et al*., 2006). Highly reactive aldehydes, products of lipid peroxidation, are capable of modifying both DNA and proteins, resulting in mutagnic, genotoxic and cytotoxic events (Merendino *et al*., 2003).

No significant changes in lipid peroxidation product malondialdehyde were observed in prostate tissue and plasma, as a result of lycopene administration (van Breemen *et al*., 2011). Although patients with advanced prostate cancer are subjective to high oxidative stress, as determined by increased susceptibility of serum lipid to peroxidation yet such association was not detected in the patients with localized cancer (Yossepowitch *et al*., 2007) yet it is confirmed that there is an imbalance between oxidative stress and antioxidant status in CaP patients (Arsova-Sarafinovska *et al*., 2009). A strong intensification of lipid peroxidation and accumulation of its final products occurred in cancer patients (Zibzibadze *et al*., 2009). The levels of MDA in plasma and tissue were significantly high in malignant carcinoma as compare to control (Guzel *et al*., 2012, Ozmen *et al*., 2006). Serum MDA were also high in CaP (Pande *et al*., 2012) as well as in patients of benign prostatic hyperplasia (BPH) as compare to control in both Macedonian and Turkish population (Arsova-Sarafinovska *et al*., 2009) Oxidative stress is associated with prostate carcinoma (Paschos *et al*., 2013). There was a strong correlation between blood and tissue levels of MDA (Dillioglugil *et al*., 2012). There was also a significant relationship ($p<0.05$) between prognostic indicator of CaP and blood and tissue MDA levels (Dillioglugil *et al*., 2012). In Italian population, BPH patients revealed an increase MDA levels and have a positive correlation between PSA and MDA levels (Merendino *et al*., 2003). Serum MDA emphasizes a convenient in vivo index of lipid peroxidation and a non-invasive biomarker of oxidative stress (Merendino *et al*., 2003). In a study, it indicated that MDA may be used for prognostic assessment of localized BPH (Dillioglugil *et al*., 2012). Serum MDA may be used as an important biomarker in BPH.

Material and Methods

Research has been conducted after the approval of Ethics Committee, Army Medical College, Rawalpindi. A consent was taken from each patient by the physician. The study was conducted at the department of biochemistry & molecular biology, Army Medical College, Rawalpindi, at the Center for Research in Experimental and Applied Medicine (CREAM). Blood samples (3 ml) were collected from Sixty eight subjects, thirty four (50%) were normal healthy control and thirty four (50%) were patients of benign prostatic hyperplasia, between ages of 55 – 85 years. Blood samples were transported in yellow top vacutainer on ice to prevent the degradation of MDA and to obtain maximum results. Serum was separated at 5000 rpm for 5 minutes after the samples were clotted. The serum samples were stored at -80^{o} C till further experimentation. Inclusion Criteria: Diagnosed patients for benign prostatic hyperplasia upon clinical interpretation and diagnosed in department of urology, Military Hospital, Rawalpindi. Exclusion Criteria: All those patients, who are diagnosed prostate carcinoma were excluded from the study.

Levels of serum MDA were determined by commercially available quantitative sandwich enzyme immuno-assay kit from ORGENTEC diagnostic. Samples were run in duplicate and test was performed according to instructions of manufacturer. Microtiter plates coated with anti-MDA monoclonal antibodies bound with MDA present in patient's sera or standards. A secondary anti-MDA monoclonal antibody conjugated with enzyme horse reddish peroxidase (HRP) specifically bound to antigen-antibody complex, if present. Addition of substrate solution reacted with HRP enzyme and turned its color which was proportional to amount of MDA present in samples or standards. The reaction was stopped by adding stop solution which was acidic in nature. Before starting the assay, reagents and samples were brought to room temperature and mixed gently. ELISA work sheet was prepared and wells were specified for standards, controls and samples. All the reagents including MDA standards (0.1µg/ml – 40 µg/ml); standard 1 (0.1 µg/ml), standard 2 (0.4 µg/ml), standard 3 (2.0 µg/ml), standard 4 (10.0 µg/ml) standard 5 (40.0 µg/ml), were brought to the room temperature. Wash buffer and sample buffer solutions were prepared according to manufacturer's instructions. Both standards and controls were in the ready to use focus according to manufacturer's instructions. 50µl of each diluted sample, standard and control was added to appropriate well using calibrated micropipettes. Blank wells contained only distilled water and there was no sample in it. 50µl of enzyme HRP-conjugated solution was dispensed in all wells except blank. Mixed well and plate was incubated for 1hour at 37^0C. Each well was aspirated and washed properly by wash buffer, which has been prepared according to the manufacturer's instruction. The steps were repeated for three times as performed by the using of 200 µl wash buffer in each well. By the use of multi-channel micropipette, 50 µl of substrate A and 50 µl of substrate B were added to all wells and mixed well. The plate was incubated in dark for 15 minutes at 37^0C. The color of each well was turned to visible yellow. 50µl of stop reagent was added to each well, gently tap the plate to ensure through mixing. The optical density of each well was determined within 10 minutes by using a microplate reader (Mod # 2100 Stat fax USA) set to 450 nm. Amount of MDA was calculated using regression analysis from optical density of standard wells using micro plate manager software. Standard curve report and regression analysis of MDA is given in Fig 1.

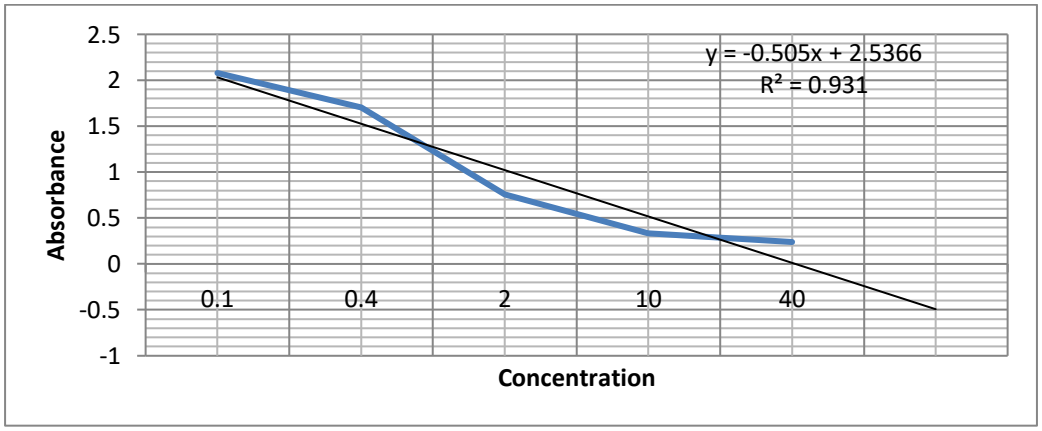

Figure 1: MDA ELISA standard curve report
(Correlation Coefficient (R value): 0.931)

Results

The data obtained from patients were entered and analyzed using standard SPSS software version 20.0 (SPSS, Inc, Chicago, IL, U.S.A.). For quantitative variables such as serum MDA levels mean + SD was determined. For qualitative variables such as gender and age groups, frequencies and percentages were calculated. Independent sample t test was applied to observe group mean differences. A p-value of <0.05 was considered as statistically significant.

Out of Sixty-eight subjects, thirty-four (50.0%) were Normal control while thirty-four (50.0%) were Benign Prostatic Hyperplasia (BPH) patients. Age range of the subjects was fifty-five years to eighty-five years. Mean age of subjects was 71.69 ± 6.04 years. Range of PSA was 7ng/ml to 187ng/ml. Mean & standard error of mean of PSA was 106.02 ± 9.43 ng/ml. In the study, mean value of MDA in BPH patient was 19.27 ± 4.84 µg/ml and normal control was 0.72 ± 0.02 µg/ml. The difference between BPH patients as compared to normal control was statistically significant (p=0.002).

Table 1: p-value of MDA in different prostatic carcinoma patients

Cancer groups		Mean ± SEM (µg/ml)	p-value
Subjects (n=68)	BPH (n=34)	19.27 ± 4.84	0.002*
	Normal control (n=34)	0.72 ± 0.02	

*Statistically significant difference

Conclusion

We concluded that MDA may be utilized as future a biological marker of BPH. After some more research, it is also appears that MDA may have some role in progression of disease. Our study also illustrated a significant increase in MDA in Benign Prostatic Hyperplasia.

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