DNA damage and oxidative stress is higher in high-risk prostate cancer subjects

Eswar Shankar[1] Shiv Verma[1], Dr. Janmejai K Srivastava[1], Dr. Lee E Ponsky[1], Dr.Gregory T. MacLennan[2], Dr. Sanjay Gupta[1]

Department of Urology[1],Pathology[2], Case Western Reserve University, Cleveland, OH 44106, USA

SUMMARY

Background: Prostate cancer is a growing health issue in the Western nations as its incidence increases in the aging population. A major risk factor that contribute to the development of prostate cancer is oxidative stress and oxidant/antioxidant balance. Our objective is to investigate the oxidative DNA damage and changes in antioxidant status in high-risk prostate cancer subjects.

Methods: A total of 40 men in the age range of 52–84 years without any prior drug or treatment involvement were included in the study. Patients were recruited from the Urology clinic of the University Hospitals Cleveland Medical Center between January 2008 and May 2011. Twenty subjects were selected who were diagnosed having precursor high-grade intraepithelial neoplasia lesions confirmed by needle biopsy and serum PSA ≥ 4.0 ng/mL and abnormality observed in the prostate during digital rectal exam or transrectal ultrasonography. Twenty age-matched men within the same age group designated as controls were recruited in the study without any history of cancer, benign prostatic hyperplasia or prostatitis. Blood samples were drawn, buffy coat was separated for DNA isolation. The plasma samples and erythrocyte fraction were stored at −80 °C until assayed. We performed the profiling of 8-hydroxydeoxyguanosine (8-OHdG) in leukocytes, plasma antioxidant capacity, guanosine 3',5'-cyclic monophosphate (cyclic GMP), nitrite and nitrate levels, followed by glutathione S-transferase P (GSTP1) and O-6-Methylguanine-DNA Methyltransferase (MGMT) using assay kits and the patients' samples were repeated twice in triplicates. All biochemical assays were performed as mentioned in manufacturer's protocol.

Results: Levels of 8-OHdG, cGMP, nitrite and nitrate were significantly increased ($p < 0.0001$) in the buffy coat and plasma samples whereas the levels of GSTP1 and antioxidant capacity were significantly decreased in high risk subjects, compared to control subjects. Simultaneously, an increase in MGMT activity was also noted in the plasma of high-risk subjects, compared to control subjects.

Conclusion: The significant changes observed with an increase in the levels of 8-OHdG, cGMP, MGMT, nitrite and nitrate with a concomitant decrease in the levels of GSTP1 and antioxidant levels in the blood is indicative of increased oxidative stress and changes in antioxidant status susceptible to development of prostate cancer.

MATERIALS AND METHODS

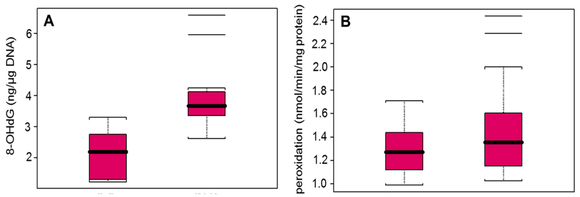
Study Subjects: A total of 40 men were included in the study in the age range of 52–84 years without any prior drug or treatment involvement. Patients were recruited from the Urology clinic of the University Hospitals Cleveland Medical Center between January 2008 and May 2011. Twenty subjects were selected who were diagnosed having precursor high-grade intraepithelial neoplasia (HGPIN) lesions confirmed by needle biopsy and serum PSA > 4.0 ng/mL and abnormality observed in the prostate during digital rectal exam or transrectal ultrasonography. 20 age-matched men within the same age group designated as controls were recruited in the study having serum PSA < 4 ng/mL, normal digital rectal exam without urinary symptoms and diagnosis of benign prostatic hyperplasia (BPH) or prostatitis. Written informed consent was acquired from all participants in the study before the collection of blood specimens. The study was approved on August 28, 2008 by the Institutional Review Board of Case Comprehensive Cancer Center (CASE11807) and clinicaltrials.gov identifier NCT00898274.

Sample Preparation: Patients were first required to fast overnight, followed by blood samples drawn from the antecubital vein in EDTA glass tubes. These samples were centrifuged for 10 min at 4000× g at 4 °C. Plasma and buffy coats were removed. The remaining erythrocyte pellet was washed with isotonic saline and lysed with cold distilled water (1:4). Following lysis, the samples were stored in a 4 °C refrigerator for 15 min. Cell debris was removed by centrifugation (2000× g for 10 min). Plasma samples and erythrocyte fraction were stored at −80 °C until assayed. Plasma were used to assay the levels or activity 8-OHdG, lipid peroxidation, Anti-oxidant activity, Glutathione Pathway Assays, cGMP, MGMT, Nitrite and nitrate by ELISA using the manufacturer's protocol.

Statistical Analysis: Data was summarized as the mean, standard deviation (std. dev.). The difference of enzyme activities and DNA/lipid damage between healthy controls and high-risk patients was examined using a *t*-test. All tests were two-tailed and a *p*-value less than 0.05 was considered to be statistically significant.

RESULTS

High Risk patients exhibited increased levels of 8-OHdG & Lipid peroxidation

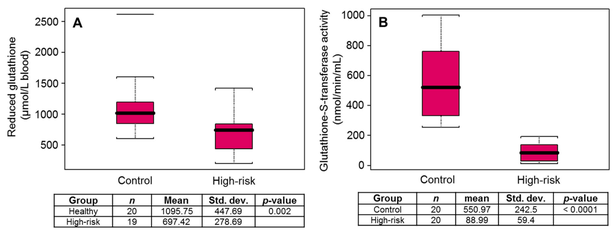


Group	*n*	mean	Std. dev.	*p*-value
Control	13	2.05	0.77	< 0.0001
High-risk	16	3.92	1.05	

Group	*n*	mean	Std. dev.	*p*-value
Control	20	1.29	0.22	0.129
High-risk	20	1.45	0.4	

Figure 1. Box plot for (**A**) 8-OHdG and (**B**) lipid peroxidation in healthy controls and high-risk subjects for prostate cancer. Black bar = median, red box = 25th to 75th percentiles, Bars = entire range. The horizontal lines beyond the bars are outliers or whiskers are drawn individually.

Levels of GST and reduced glutathione were decreased in High risk patients



Group	*n*	Mean	Std. dev.	*p*-value
Healthy	20	1095.75	447.69	0.002
High-risk	19	697.42	278.69	

Group	*n*	mean	Std. dev.	*p*-value
Control	20	550.97	242.5	< 0.0001
High-risk	20	88.99	59.4	

Figure 2. Box plot for (**A**) reduced glutathione levels, and (**B**) glutathione S-transferase activity in healthy controls and high-risk subjects for prostate cancer. Black bar = median, red box = 25th to 75th percentiles, Bars = entire range. The horizontal lines beyond the bars are outliers or whiskers are drawn individually.

High risk patients exhibited decreased total anti-oxidant capacity

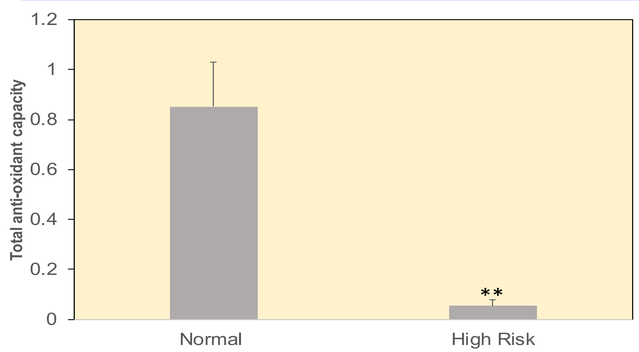


Figure 3: Bar Graph total antioxidant levels, in healthy controls and high-risk subjects for prostate cancer.

High risk patients exhibited decreased Glutathione S-transferase Pi (GSTPi)

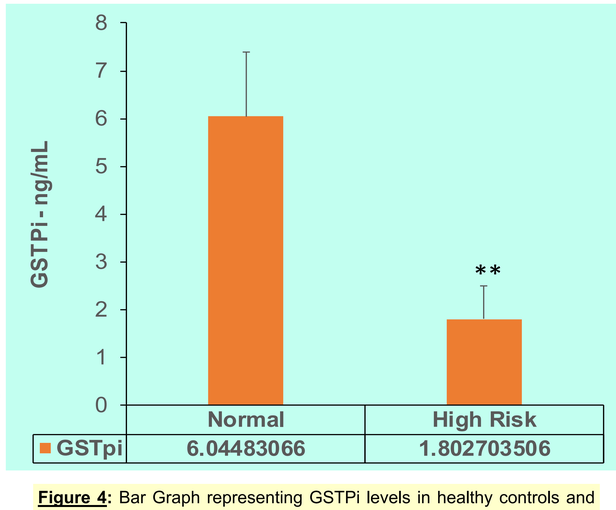


Figure 4: Bar Graph representing GSTPi levels in healthy controls and high risk subjects for prostate cancer.

Levels of MGMT significantly increased in high risk patients

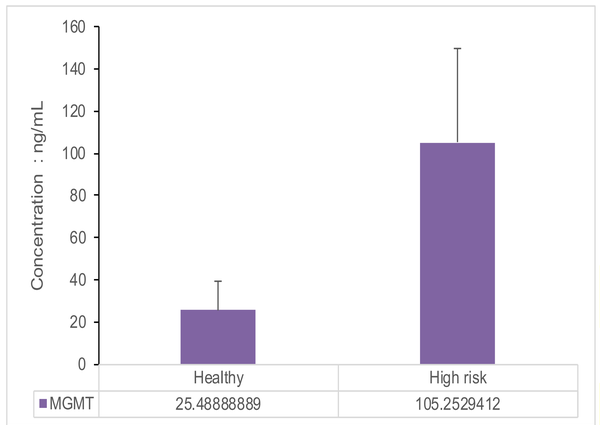


Figure 5: Bar Graph representing MGMT levels in healthy controls and high risk subjects for prostate cancer.

cGMP levels increased in the plasma of High risk patients

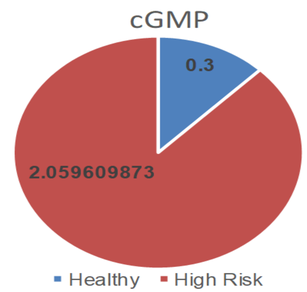


Figure 6: **ELISA determination of cGMP levels between healthy and high risk patients for prostate cancer reveals a significant increased levels.**

Comparison of Nitrite, Nitrate and Nitrite+Nitrate plasma levels between Healthy versus High Risk Patients

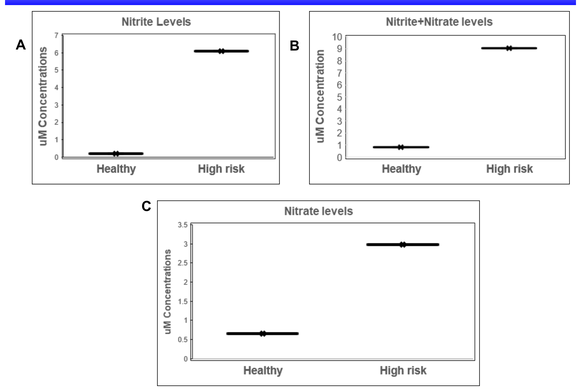


Figure 7: **ELISA determination of Nitrite, Nitrate and Nitrite+Nitrate levels between healthy and high risk patients for prostate cancer reveals significant increased levels.**

CONCLUSION

The significant changes observed with an increase in the levels of 8-OHdG, cGMP, MGMT, nitrite and nitrate with a concomitant decrease in the levels of GSTP1 and antioxidant levels in the blood is indicative of increased oxidative stress and changes in antioxidant status susceptible to development of prostate cancer.

ACKNOWLEDGEMENTS

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