Impact of Oxidative Stress Biomarkers and Carboxymethyllysine (an Advanced Glycation End Product) on Prostate Cancer: A Prospective Study

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Abstract

Advanced glycation end products have been linked to the development of prostate cancer. In a prospective nested case-control study (24 prostate cancer cases and 24 controls), we found that a high level of plasma carboxymethyllysine (a major advanced glycation end product) is a risk factor for prostate cancer. This suggests a potential new pathway for prostate cancer prediction and treatment.

Objective: Biomarkers of oxidative stress and advanced glycation end products (AGE) have been linked to the development of prostate cancer, but evidence from human studies is scarce or controversial. **Methods:** We conducted a prospective nested case-control study among 48 men (24 prostate cancer cases and 24 controls) aged 48 to 76 years at baseline. The participants of our study were a part of the Fernald Community Cohort. Prostate cancer cases and controls were matched individually on age (± 3 years) with a 1:1 ratio. Biomarkers included urine F2-isoprostanes (markers of lipid oxidation), plasma fluorescent oxidation products (markers of global oxidation), and carboxymethyllysine (CML) (a major end-stage AGE). **Results:** At baseline, cases had similar age, body mass index, proportion of family history of prostate cancer, history of benign prostatic hyperplasia, history of hypertension, history of diabetes, number of smokers, and plasma glucose levels compared with controls. Levels of plasma CML were significantly higher in cases than in controls (182 vs. 152 μg/mL, $P < .05$). In the conditional logistic regression model, an increase in CML equivalent to 1 standard deviation was associated with an increased risk of incident prostate cancer (relative risk, 1.79; 95% confidence interval, 1.00-3.21) and accounted for approximately 8% variance of prostate cancer liability. Urine F2-isoprostanes and plasma fluorescent oxidation products were not associated with prostate cancer incidence. **Conclusions:** Higher levels of plasma CML were associated with increased risk of prostate cancer. This suggests a potential new pathway for prostate cancer prediction and treatment.

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Introduction

Prostate cancer (PCa) is a frequently diagnosed cancer in men and remains the leading cause of cancer death in men worldwide.[1,2] However, novel biomarkers that are able to accurately predict the risk of PCa and that can be used as a treatment target for PCa are lacking.

One of the etiologic pathways related to PCa is oxidative stress. Oxidative stress occurs when the reactive oxygen species overwhelm the capacity of antioxidant defense system. Excessive reactive oxygen species cause DNA damage and mutation, cell and tissue damage, and further lead to cancer development.[3] There are several lines of evidence suggesting that oxidative stress is linked to the development of malignancy of the prostate.[4-7] However, the relationship between oxidative stress and PCa in human studies is

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still controversial.[8-12] Moreover, few human prospective studies have investigated the relationship between oxidative stress biomarkers and incident PCa.

The F2-isoprostanes are the most commonly used oxidation marker; however, they are a marker of lipid oxidation, not for oxidation from carbohydrate, DNA, and protein. Fluorescent oxidation products (FlOPs) are generated from many different oxidation pathways (lipid, protein, and DNA) and have been used as a marker of global oxidation in our studies[13-16] and other studies.[17-19] Compared with malondialdehyde measured by colorimetric thiobarbituric acid assay, the FlOP assay is 10 to 100 times more sensitive in measuring oxidative stress.[17] FlOPs have been found to be a significant predictor for coronary heart disease[13,16]; one type of FlOPs, FlOP_320, significantly predicted the risk of breast cancer.[14]

Another biomarker that may be associated with PCa is advanced glycation end products (AGE). AGE are constantly generated under a high level of glucose concentration (hyperglycemia) via a nonenzymatic pathway; this reaction is called "glycation." During the glycation process, glucose can bind with proteins, making cells stiffer, less pliable, and more subject to damage and premature aging. Heating and oxidative stress can accelerate the process of glycation. AGE are a type of marker for many chronic diseases, such as diabetes, atherosclerosis, and renal failure.[20,21] Ishiguro et al[22] recently found that the receptor of AGE (RAGE) has higher mRNA expression in PCa cell lines than normal prostate. Thus, higher levels of AGE are potentially involved in the development of PCa. However, the association between prediagnosis plasma AGE and PCa has not been examined in a prospective study. Carboxymethyllysine (CML), a major end-stage AGE, is the most commonly used AGE marker. Therefore, the aim of our current study was to investigate the associations of urine F2-isoprostanes, plasma FlOPs, and CML with the risk of PCa in a prospective study.

Materials and Methods

Study Setting and Participants

The participants and their biospecimens for the present study were obtained from the Fernald Community Cohort (FCC), which has been reported.[23] The FCC is a result of the Fernald Medical Monitoring Program, which was a voluntary ongoing medical surveillance program for 9782 community residents living within 5 miles from the perimeter of a former US Department of Energy uranium-processing site, located near Cincinnati, Ohio. Members of the cohort (N = 9778) received medical screening examinations every 2 or 3 years, over an 18-year period, from 1990 to 2008 when the program ended. Some of the members of this cohort were exposed to emissions from the Fernald plant, but others were not. Extensive uranium dose reconstruction using the methods developed by the Centers for Disease Control and Prevention demonstrated that more than 60% of the cohort had such minimal exposure to uranium and radon that their cumulative ionizing radiation exposure was less than 3.2% over lifetime background levels.[24]

At baseline, 8496 adult participants provided blood and urine samples between 1990 and 1993. Detailed questionnaires, administered at baseline and yearly, collected information on age, smoking status (current and past smoker, or never smoked), family history of PCa, history of benign prostatic hyperplasia, history of hypertension, history of diabetes, and other demographic characteristics (www.uc.edu/fmmp). Height and weight were measured at interview, and body mass index was calculated. After the initial visit, a regular follow-up examination was performed every 2 or 3 years by physicians board certified in internal medicine or environmental/occupational medicine. Diagnoses for each FCC participant, including those of diabetes and major cancers (ie, breast, prostate, lung, colon, skin and urinary system cancers, leukemia, and lymphoma), were based on International Classification of Diseases, 9th Revision codes assigned by a medical record coder during the yearly review of each medical chart.

Among members of the FCC who had extremely minimal amounts of uranium exposure (< 2% over lifetime background level), we conducted a prospective nested case-control study of PCa. All cases and controls were Caucasian, because 99.5% of the FCC members were Caucasian. In this study, 24 eligible incident PCa cases were randomly selected from eligible incident PCa cases (n = 86) diagnosed between 1990 and 2006. All selected PCa cases were confirmed via medical records. The average duration from baseline sample to the diagnosis of PCa was 11 years (range, 6-14 years). With the use of risk set sampling, controls who never had PCa during the whole study period of the FCC were selected from the remaining eligible male FCC cohort members and were matched individually with cases (1:1) on age (± 3 years at case diagnosis). All participants provided written consent to participate in the FCC and to have their data and samples used for research studies.

Blood and Urine Collection and Storage

All participants in our study had at least 8 hours of food fasting (except water) before blood draw. Blood samples were collected in tubes that contain liquid sodium heparin. All collected blood samples were processed within 8 hours and stored in freezers (−70°C) before measurement. Urine samples were also collected in clean tubes and stored in −70°C freezer before assay.

Laboratory Assay

All markers in matched case and control set were measured within the same run.

Measurement of Plasma Glucose. The level of plasma glucose was measured with the Beckman Synchron CX3 Clinical Chemistry Analyzer (Beckman Instruments Inc, Brea, CA). The overall precision of the assay expressed as percentage of coefficient of variation (CV) for the measurement was < 3.5%.

Measurement of Urine F2-Isoprostanes. Urine F2-isoprostanes were measured by gas chromatography/negative ion chemical ionization mass spectrometry. The method has been reported.[25] Briefly, gas chromatography used a 15-m, 0.25-mm diameter, 0.25-m film thickness, DB 1701 fused silica capillary column (Fisons, Folsom, CA). The column temperature was programmed from 190°C to 300°C at 15°C per minute. The metabolite was chemically synthesized and converted to an 18 O_2-labled derivative, which was used as an internal standard. Precision of the assay is 4%, and accuracy is 97%.[25]

Measurement of Plasma FlOPs. Measurement of FlOPs was performed with previously described procedures.[26] Briefly, we extracted