

Short Communication

Androgens in Serum and the Risk of Prostate Cancer: A Nested Case-Control Study from the Janus Serum Bank in Norway

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Abstract

We tested the hypothesis that serum levels of testosterone (T), dihydrotestosterone (DHT), and the DHT metabolite 3 α ,17 β -androstane-3 α ,17 β -diol glucuronide are positively associated with the risk of prostate cancer. This nested case-control study was based on the cohort of men who donated blood to the Janus serum bank at Oslo University Hospital (Oslo, Norway) between 1973 and 1994. Cancer incidence was ascertained through linkage with the Norwegian Cancer Registry. The study included sera from 59 men who developed prostate cancer (cases) subsequent to blood donation and 180 men who were free of any diagnosed cancer (controls) in 1994 and were of similar age (± 1 year) and had similar blood storage time (± 6 months) to the cases.

Neither T, DHT, nor the ratio T:DHT was associated with risk of developing prostate cancer. Compared to the bottom quartile, the odds ratio (OR) associated with the top quartile of T was 0.83 [95% confidence interval (CI), 0.36-1.93]; the OR for the top (compared to the bottom) quartile of DHT was 0.83 (95% CI, 0.36-1.94), and the equivalent OR for T:DHT was 1.31 (95% CI, 0.58-2.97). Similarly, 3 α ,17 β -androstane-3 α ,17 β -diol glucuronide showed no association with prostate cancer risk; the OR for the top (compared to the bottom) quartile was 1.10 (95% CI, 0.41-2.90).

These results showed no association, positive or negative, between androgens measured in serum and the subsequent risk of developing prostate cancer.

Introduction

Normal prostatic growth is mediated by androgens, and treatment of established prostate cancer with surgical or chemical

castration results in tumor regression. T² is produced in large amounts by the testes and circulates primarily bound to sex hormone-binding globulin and, to a lesser extent, to albumin (1). T is metabolized to DHT by the enzyme 5- α reductase, which consists of two isoforms, type I and type II. Compared to T, DHT has a 5-fold higher affinity to the intraprostatic androgen receptor, and one important role of DHT is to control mitotic activity of prostate cells (1, 2). Because the type II isoform of 5- α reductase is expressed in high levels in the prostate, it has been suggested that type II, but not type I, is associated with prostate cancer risk.

Epidemiological studies, using both case-control and prospective designs, have studied the association between serum T, DHT, and the risk of prostate cancer, but the results have been inconsistent (3-9). Recently, it has been suggested that differences in 5- α reductase activity may explain the difference in prostate cancer incidence between men of Western and Asian origin (10, 11), so biomarkers of 5- α reductase activity might be expected to predict prostate cancer risk. The metabolite A-diol-g, which is the glucuronide product after reduction of DHT by the enzyme 3 α -hydroxysteroid oxidoreductase, is thought to be the most specific biomarker of 5- α reductase activity presently available (12, 13). Three recent nested case-control studies have measured A-diol-g in serum, but there was no clear association with prostate cancer in any of them (9-11).

We conducted a nested case-control study within a cohort of Norwegian men who donated blood before the onset of prostate cancer to answer the question of whether markers of 5- α reductase activity are associated with prostate cancer risk.

Patients and Methods

Subjects. This study was based on the cohort of approximately 28,000 men who donated blood samples to the Janus serum bank in Oslo, Norway (14). The objective of this serum bank is to study serological markers (chemical, biochemical, and immunological) that may predict preclinical cancer. The sera have been kept frozen at -25°C and constitute, on average, four (range, 2-16) consecutive samples from each subject. Median length of serum storage was 17 years.

Linkage to the Norwegian National Cancer Registry was conducted as follows. All Norwegians are given a unique identification number at birth. Linkage with this number enabled us to identify incident cases of prostate cancer among male blood donors. From the initiation of the serum bank in 1973 until October 1994, a total of 60 cases have been identified among cohort members. The case sera were obtained from men who developed prostate cancer 1-19 years after the blood sample was drawn (mean, 10 years). This minimized the likelihood that

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² The abbreviations used are: T, testosterone; DHT, dihydrotestosterone; A-diol-g, 3 α ,17 β -androstane-3 α ,17 β -diol glucuronide; OR, odds ratio.

Table 1 Mean serum levels of T, DHT, and A-diol-g in 59 prostate cancer cases and 180 controls

	Cases		Controls		<i>P</i>
	Mean serum level	SD	Mean serum level	SD	
T (ng/ml)	5.60	1.71	5.77	2.16	0.88
DHT (ng/ml)	0.48	0.18	0.49	0.20	0.93
T:DHT ratio	12.3	3.24	12.4	3.51	0.97
A-diol-g (ng/ml)	6.75	2.96	6.61	3.16	0.73

a preclinical effect of the disease may have influenced the value of serum measurements.

Controls were randomly selected from the cohort as follows: three controls were matched to each case based on year of birth (± 1 year) and time of blood draw (± 6 months). All controls had to be free of any diagnosed cancer as of October 1994 and had to be born within 1 year of a case. One case had to be excluded because the serum had degraded and could not be properly analyzed. The study thus consisted of 59 cases of prostate cancer and 180 matched controls.

Serum containers were tightly capped to prevent evaporation. The sera were not removed from the freezers, and the temperature was kept as constant as possible throughout storage, with no thaw-freeze cycles. Under these conditions, we expected minimal deterioration of the analytes measured.

Laboratory Analyses. The following assay methods were used to measure the analytes in this study. T and DHT concentrations were measured by specific RIA following extraction with hexane:ethyl acetate (1:1) and Celite column partition chromatography (15–17). Sex hormone-binding globulin was determined by selective ammonium sulfate precipitation. The glucuronide conjugate of androstenediol, A-diol-g, was measured by RIA after β -glucuronidase hydrolysis (10). Following removal of unconjugated steroids from serum by extraction with diethyl ether, glucuronide conjugates were hydrolyzed at 37°C by addition of β -glucuronidase (type VIII, Sigma, St. Louis, MO) to serum, which was first adjusted to pH 6.8. After 16 h of hydrolysis, unconjugated hormones were extracted with diethyl ether, and A-diol-g and androstenediol were separated by Celite column chromatography; specific fractions were then measured by RIA. [3 H]A-diol-g was added to serum immediately after hydrolysis to allow for monitoring of procedural losses. For all assays, the only identifier was a code number; thus, the case status of a sample was blinded. The intra- and interassay coefficients of variation for the analytes measured were 5–10% and 10–15%, respectively.

Statistical Analyses. Steroid hormone measurements were expressed as natural logarithms to achieve approximately normal distributions. Pearson's correlation coefficients were estimated for the associations between hormone levels and age. The statistical significance of the differences between cases and controls was evaluated using *t* tests. The data were also analyzed by standard matched case-control study analyses (17), in which the ORs of prostate cancer associated with each exposure category beyond the reference category was estimated. Precision of the ORs was assessed by 95% confidence intervals. Departure from a linear trend across categories of each metabolite was evaluated using Mantel's extended test for trend (17).

The Janus serum bank does not collect information on covariates that may be of importance for specific diseases. Thus, adjustment for risk factors for prostate cancer other than age was not possible in this study.

Table 2 The association between serum androgens and prostate cancer risk in 59 cancer cases and 180 controls: results from conditional logistic regression

	Cases	Controls	OR	95% confidence interval	<i>P</i> trend
T (ng/ml)					
<4.41	17	45	1.0		
4.41–5.25	13	45	0.75	0.32–1.73	
5.26–6.95	14	45	0.79	0.34–1.82	
>6.95	15	45	0.83	0.36–1.93	0.70
DHT (ng/ml)					
<0.35	17	45	1.0		
0.35–0.45	11	45	0.59	0.24–1.48	
0.46–0.59	16	45	0.87	0.38–1.99	
>0.59	15	45	0.83	0.36–1.94	0.92
A-diol-g (ng/ml)					
<4.5	11	45	1.0		
4.5–5.8	18	45	1.52	0.62–3.72	
5.9–8.6	17	45	1.40	0.58–3.39	
>8.6	13	45	1.10	0.41–2.90	0.97
T:DHT ratio					
<10.1	14	45	1.0		
10.1–12.0	16	45	1.10	0.49–2.47	
12.1–13.9	11	45	0.79	0.33–1.89	
>13.9	18	45	1.31	0.58–2.97	0.68

Results

The median age at time of blood draw was 60 years (range, 43–66 years) among cases and 59 years (range, 42–66 years) among controls. Median age at diagnosis was 69 years (range, 56–83 years), and median time between the blood draw and onset of prostate cancer among the cases was 11 years (range, 1–19 years). Age at blood draw was inversely correlated with level of T (Pearson's correlation coefficient, $r = -0.21$; $P = 0.004$), DHT ($r = -0.12$; $P = 0.12$), and A-diol-g ($r = -0.20$; $P = 0.007$) among controls.

There were no clear differences in mean values between cases and controls for any one of the three measured androgens (Table 1). The mean T level was 5.60 ng/ml in cases and 5.77 ng/ml in controls, and the mean DHT level was 0.48 ng/ml in cases and 0.49 ng/ml in controls. The mean level of the biomarker A-diol-g was 6.75 ng/ml in cases and 6.61 ng/ml in controls. The mean T:DHT ratio was also essentially identical in the two groups.

After subdividing cases and controls into categories according to quartile values among controls, there was no tendency for a trend in association with increasing (or decreasing) levels of the measured androgens and risk of prostate cancer (Table 2).

Excluding the one case who was diagnosed before age 45 or the 9 cases diagnosed after age 75 and their matched controls did not alter the results substantially. Excluding men diagnosed within 2 or 5 years after the blood draw also yielded essentially identical results. Finally, excluding men whose samples were obtained prior to 1980 yielded similar results.

Discussion

It has been suggested that different 5- α reductase activity may explain the difference in prostate cancer incidence between men of Western origin and men of Asian origin (10, 11). 5- α reductase biomarkers may therefore be useful predictors of prostate cancer risk (18). In this nested case-control study, there was no association between the risk of prostate cancer and

serum measurements of T and DHT. Nor was there any association with serum A-diol-g and prostate cancer.

To date, only six studies have been published in which prediagnostic sera were used to examine the relationship between serum hormones and subsequent risk of prostate cancer (4–9). The results have been inconsistent and have not indicated clear associations. The largest study (7) was the first to evaluate the role of 5- α reductase activity, by using plasma levels of A-diol-g as an approximate measure for androgen influence on the prostate. That study showed a risk increase associated with total T but only a weak and not statistically significant association between 5- α reductase (A-diol-g) and prostate cancer risk. Subsequent to that study, two recently published nested case-control studies (8, 9) did not show any clear association between androgens (including A-diol-g) and prostate cancer. Other case-control studies have compared serum androgens; in these studies, blood from cases was obtained after the diagnosis of prostate cancer (3). In these studies, the presence of disease might have affected the levels of androgens, and the validity of the results must therefore be questioned.

Serum DHT and A-diol-g levels do not completely reflect 5- α reductase activity within the prostate because there are at least two types of 5- α reductase enzymes that can lead to these hormonal byproducts (13). The type II isoenzyme is expressed in high levels in the prostate and is deficient in a syndrome of male pseudohermaphroditism associated with congenital underdevelopment of the prostate. The type I isoenzyme is expressed in low levels in the prostate and is not associated with underdevelopment of the gland. One would therefore expect type II but not type I activity to be associated with prostate cancer. The measured serum levels of DHT and A-diol-g are affected by both isoenzymes, and the contribution of each isoenzyme on an individual or even a population varies. Thus, by using total serum levels of DHT and A-diol-g as an index of the type II isoenzyme activity, extraneous measurement variability may be substantial, due to the contribution of the type I isoenzyme activity in determining the hormone level. This measurement error will most likely result in bias toward the null hypothesis (*i.e.*, it will tend to obscure any contribution by type II isoenzyme activity).

Individual variability in androgens may also dilute any association with prostate cancer. To fully characterize androgen profiles for an individual may require several specimens collected longitudinally over a long interval; this may achieve the necessary sensitivity to detect androgen differences between cases and controls (7).

It would be desirable to standardize blood draw to a certain time of day because of the circadian rhythm of several hormones in the blood (19). Although blood was typically drawn during working hours in this study, no attempt was made to match cases and controls with respect to exact time of day. This also contributes to a possible dilution of differences in hormone levels between cases and controls.

We had no data on risk factors for prostate cancer other than age. It is possible that unmeasured confounders may have obscured a true association between these hormones and prostate cancer risk.

In conclusion, we found no association between T, DHT, A-diol-g, and prostate cancer risk. The association with serum

T and DHT has varied across studies, and the failure of this study to detect an association with 5- α reductase activity (expressed in A-diol-g) may be due, in part, to our current inability to separate type I and type II 5- α reductase activity. To increase precision, it might be useful in future studies to use more than one serum specimen to characterize hormonal profiles and to control for diurnal variability of serum androgens.

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