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Prediagnostic Serum Hormones and the Risk of Prostate Cancer[1]

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ABSTRACT

Serum samples were obtained from 6860 men during their study examination from 1971 to 1975. After a surveillance period of about 14 years, 98 incident cases of prostate cancer were identified. Their stored sera and that of 98 matched controls from the study population were tested for the following: testosterone, dihydrotestosterone, estrone, estradiol, and sex hormone globulin. There was a suggestion that serum dihydrotestosterone levels were lower and the testosterone/dihydrotestosterone ratios were higher in the prostate cancer cases compared with their controls. However, none of these associations or that of the other hormones was strongly significant. Further work is needed to clarify the relationship between sex hormones and prostate cancer risk.

INTRODUCTION

There is a high index of suspicion that sex hormones have a role in the development of prostate cancer. Androgens are required for the growth, maintenance, and functional activity of the prostate gland. Eunuchs, whose testes have been removed or never developed, have not been clinically observed to develop prostate cancer or even benign prostatic hypertrophy (1, 2). Furthermore, castration and estrogen therapy can produce a palliative effect against prostate cancer (3). Because of some of these observations, researchers suspect that the risk of prostate cancer is determined by the exposure over time of prostate tissue to elevated levels of androgens (1).

Case-control investigations in men have found equivocal results in studying the association of serum hormone levels with prostate cancer. Serum testosterone levels in cases were observed to be elevated (4, 5), depressed (4, 6–8), or similar (9) as that of controls. Dihydrotestosterone was also measured in some studies and was found to be similar (4, 5, 7) or lower (8) in cases than in controls. Researchers have observed that serum estrone was similar (7) or higher (4, 6, 8) in cases than in controls. Serum estradiol levels were also higher in cases than in controls in one study (6), but no significant differences were noted in three other investigations (4, 7, 9).

Because blood was obtained after diagnosis in these case-control studies, it is uncertain to what extent the presence of prostate cancer affected the hormone results. In the present study, we had the opportunity to investigate the association of serum hormones with prostate cancer in serum obtained before the cancer was diagnosed.

Another advantage in this study is that it is focused on men of Japanese ancestry in the United States who have recently experienced a marked change in prostate cancer risk. The Japanese men in Japan have about the lowest incidence rate of prostate cancer (4.9/100,000/year in Miyagi Prefecture) among different population groups in the world (10). Their Japanese counterparts living in Hawaii, United States of America, have experienced a greater risk for this disease with an annual incidence rate of 35.9/100,000 over the same period of time. As such, studying this population provides us with an opportunity to identify hormonal factors related to prostate cancer risk in a transition group for this disease.

MATERIALS AND METHODS

From 1965 to 1968, 8006 men of Japanese ancestry, born between 1900 and 1919, were examined by the Honolulu Heart Program on the Hawaiian island of Oahu. Approximately 6 years later, from 1971 to 1975, 6860 of these men returned for another round of examinations. At that time, a nonfasting venous blood sample was obtained. The sera were stored at −75°C. The subsequent clinical diagnosis of prostate cancer among the 6860 men was recorded by continuous surveillance of all general hospitals on Oahu for a period of about 14 years. Each clinical case of adenocarcinoma was confirmed by histological examination of tissue obtained by biopsy or surgery. A computer linkage file was established with the Hawaii Tumor Registry to reduce the possibility of missing incident cases during the surveillance period. Based on a 19-year follow-up survey of the study subjects since their 1965-1968 examination, it was determined that only 1.6% of the men could not be found on Oahu. As a result, the surveillance for incident cases of prostate cancer should be nearly complete.

A stored serum sample was available from 98 of the 103 incident cases of prostate cancer. Each case was matched with one control from the study population. The controls were selected so that each case-control pair had the same age except for one pair (1 year difference), the same month and year of examination except for 10 pairs (up to 6 months' difference) and the same hour of day of blood sampling. The control subjects were alive and did not have any cancer diagnosis at the time of the diagnosis of the matched case. Therefore, death was not a competing risk in this study.

The frozen sera, which had never been thawed before, were sent in dry ice to Los Angeles for analysis. The laboratory technician could not distinguish sera of cases from that of controls and treated them identically in the analysis. Testosterone levels were measured by the method of Anderson *et al.* (11). Radioimmunoassay procedures were used to determine the serum levels of dihydrotestosterone (12). Estrone and estradiol were measured by the method of Devane *et al.* (13). Sex hormone-binding globulin activity was determined by the selective ammonium sulfate precipitation technique of Rosner (14), using tritiated dihydrotestosterone as ligand.

The serum hormone distributions were sufficiently skewed to indicate the use of nonparametric methods of statistical analysis. The 1:1 matching was preserved in all analyses. For the simple comparison of case *versus* control serum hormone levels, the Wilcoxon signed-rank test for paired samples was used (15). Relative risks of prostate cancer by levels of serum hormones were derived from CLR[3] models (16). The relative risk estimates the risk of disease in a particular group (*e.g.*, hormone level) relative to the risk in a referent group. Tertiles of serum hormones were determined based on the controls only, and the lowest hormone tertile was chosen as the referent group. The tertile cut points for the different hormone variables in Tables 2 and 3 were as follows: testosterone (pg/ml): ≤3300, 3301–4700, ≥4701; dihydrotestosterone (pg/ml): ≤350, 351–500, ≥501; testosterone/dihydrotestosterone ratio: ≤7.70, 7.71–11.50, ≥11.51; estrone (pg/ml): ≤25, 25–34, ≥35; estradial (pg/ml): ≤15, 16–22 ≥23; sex hormone globulin (10^{-8} M): ≤3.60, 3.61–4.60, ≥4.61.

Confidence intervals (95%) were used to determine whether relative

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[3] The abbreviation used is: CLR, conditional logistic regression.

risk was significantly different from 1.0. Tests for linear trend in the logit of risk was derived from CLR models by using a grouped serum hormone variable (coded 1, 2, 3). All CLR models were fitted by using iterative maximum likelihood methods and a special application of proportional hazards regression models (17).

RESULTS

The comparison of cases and controls by serum hormone levels is shown in Table 1. In each comparison, the mean levels of the cases were lower than that of the controls, but none of the differences was statistically significant. The most notable result was the low level of dihydrotestosterone in the prostate cancer cases.

The relative risks of prostate cancer by approximate tertiles of serum hormone levels are presented in Table 2. A hint of a negative trend of decreasing risk with increasing levels of dihydrotestosterone was observed, but the trend was not statistically significant. For the testosterone/dihydrotestosterone ratio, subjects in the second and third tertiles had significantly elevated risks for prostate cancer, but the relative risk was highest for the subjects in the middle tertile.

In order to study the time dependency of the relationship between selected serum hormone variables and prostate cancer risk, we separated the cases into three approximately equal groups based on time interval from blood sampling to diagnosis. The results for serum dihydrotestosterone and the testosterone/dihydrotestosterone ratio are presented in Table 3. The risk of being diagnosed with prostate cancer within 5.5 years of examination progressively decreases with increasing dihydrotestosterone levels. There were 32 cases of prostate cancer and their 32 matched controls in this particular group of subjects. The mean serum dihydrotestosterone levels were 384 pg/ml for the cases and 510 pg/ml for the controls ($P = 0.03$ from Wilcoxon signed-rank test). The relationship between the testosterone/dihydrotestosterone ratio and prostate cancer risk was not appreciably affected by the time interval from examination to diagnosis. Comparable analyses for the other serum hormones were not remarkable.

Table 1 *Comparison of prostate cancer cases and controls by serum hormone levels*

Serum hormone	Cases ($n = 98$) Median	Cases ($n = 98$) Mean ± SD	Controls ($n = 98$) Median	Controls ($n = 98$) Mean ± SD	P[a]
Testosterone (pg/ml)	3960.0	4205.5 ± 1695.6	4104.0	4308.8 ± 1784.7	0.84
Dihydrotestosterone (pg/ml)	388.0	416.8 ± 182.2	407.0	450.3 ± 191.5	0.30
Estrone (pg/ml)	28.0	29.5 ± 9.3	30.0	31.3 ± 10.9	0.35
Estradiol (pg/ml)	18.0	18.0 ± 4.7	17.0	18.9 ± 6.2	0.33
Sex hormone binding globulin (10^{-8} M)	4.0	3.9 ± 1.2	4.1	4.0 ± 1.3	0.80

[a] For paired case-control differences by Wilcoxon signed-ranks test.

Table 2 *Relative risk of prostate cancer by tertile of serum hormone levels*

Serum hormone	Tertile 1 (low)	Tertile 2	Tertile 3 (high)	P value for trend
Testosterone	1.00	0.82	0.99	1.00
Dihydrotestosterone	1.00	0.75	0.66	0.23
Testosterone/dihydrotestosterone ratio	1.00	4.92[a]	2.69[a]	0.06
Estrone	1.00	1.09	0.89	0.79
Estradiol	1.00	1.57	0.57	0.32
Sex hormone binding globulin	1.00	1.12	0.85	0.60

[a] Relative risk is significantly different from unity, with $P < 0.05$.

Table 3 *Relative risk of prostate cancer by tertile of serum hormone levels and by time interval from examination to diagnosis*

Serum hormone and time interval (yr)	Tertile 1 (low)	Tertile 2	Tertile 3 (high)	P value for trend
Dihydrotestosterone				
≤5.5 ($n = 32$)[a]	1.00	0.48	0.24[b]	0.03
>5.5–8.25 ($n = 33$)	1.00	0.89	1.25	0.76
>8.5 ($n = 33$)	1.00	1.00	1.00	1.00
Testosterone/dihydrotestosterone ratio				
≤5.5 ($n = 32$)	1.00	4.65	3.73	0.06
>5.5–8.25 ($n = 33$)	1.00	2.51	0.73	0.62
>8.25 ($n = 33$)	1.00	6.06[b]	3.13	0.20

[a] n, number of case/control pairs.
[b] Relative risk is significantly different from unity, with $P < 0.05$.

DISCUSSION

Because of the marked increase in prostate cancer risk among the Japanese in Hawaii and because of compelling evidence indicating the presence of a hormonal influence on the course of prostate cancer, we studied the association of serum hormone levels with the risk of this disease. Serum dihydrotestosterone levels tended to be lower in cases than in controls, but the magnitude of the differences was not impressive. When the cases were separated by the time interval from examination to diagnosis, the cases diagnosed within 5.5 years of examination had a significantly lower serum dihydrotestosterone level than their matched controls. This could be a chance finding or it could be that there is a reduction in circulating levels of dihydrotestosterone that can occur within a few years of the diagnosis of prostate cancer. In either case, it is clear from the data that a low serum dihydrotestosterone level does not predict an increased risk for prostate cancer many years after the serum specimen is obtained.

One case-control study in the past noted that prostate cancer cases had lower levels of serum dihydrotestosterone than controls (8), while three others did not (4, 5, 7). It is difficult to compare the results of our study with that of case-control studies in which blood samples were obtained after the diagnosis of prostate cancer. Many of the past studies collected the blood before the patients underwent treatment, but more likely than not the patients were already symptomatic from their condition. In our study, the mean time interval from examination to diagnosis was 7.2 years with a range of 2 months to 14 years. On this basis, it is likely that many of the subjects were not yet experiencing the effects of their cancer.

Another problem in past case-control studies rests on the selection of the control subjects. Oftentimes, information is not provided on the source of controls or how they were selected. In epidemiological studies, the controls should be representative of the population from which the cases were identified and should have an equal opportunity to be exposed to the variables under study. It is not certain that these conditions prevailed in past studies, as they did in this study.

It is possible that the testosterone/dihydrotestosterone ratio is a reliable index of circulating androgens, since testosterone is metabolized to dihydrotestosterone. Ghanadian *et al.* (5) observed that a high testosterone/dihydrotestosterone ratio in serum discriminated prostate cancer cases from controls, but the work of others (4, 7) did not support this finding. In the present study, there was a weak suggestion that an elevated testosterone/dihydrotestosterone ratio in serum increased the risk for prostate cancer.

There was no indication that serum testosterone levels were higher or lower in cases than in controls. There is a circadian rhythm of testosterone in the blood with maximum levels noted

usually in the early morning with progressive decrease till the lowest levels are reached in the early evening (8). On this basis, it could be considered difficult for a single specimen to be a reliable indicator of a person's serum testosterone profile. We attempted to control for this factor by selecting a control subject seen at the same hour of the day at which time their matched case was examined. Dai *et al.* (19) have studied the epidemiology of testosterone in the blood and provide evidence to indicate that a single specimen is repeatable and reliable in individuals. The correlation coefficient of plasma testosterone taken 4 days apart was 0.81 and for 1 year apart, 0.51.

Past studies have found that testosterone levels of cases were either higher (4, 5), lower (2, 4, 6, 7), or similar (9) to that of controls. If one accepts the view that testosterone excess could increase the risk for prostate cancer (1), then the cases would be expected to have elevated serum testosterone levels. However, the situation may be a complex one. For example, if the metabolic clearance rate of testosterone in the blood is greater in cases than in controls as suggested in one study (7), then the serum testosterone levels would not be greater in cases. On the other hand, if our serum specimens were taken at much younger ages, it is conceivable that a difference could have been detected. Ross and colleagues found that United States black men, who have a high risk for prostate cancer, had elevated levels of serum testosterone compared with United States white men (20). This study, which was done in college students, indirectly supports the view that testosterone contributes to prostate cancer risk.

Although the mean serum levels of estrone, estradiol, and sex hormone globulin were lower in cases than in controls, there was no indication that subjects with low levels of these compounds are at a substantially elevated risk for prostate cancer. We also looked at the ratio of testosterone to the sum of estrone and estradiol. The highest tertile group had a relative risk of only 1.1 (95% confidence interval of 0.5 to 2.2) for prostate compared with the lowest tertile group. On this basis, it is unlikely that serum levels of these estrogens are related to prostate cancer risk, although it is well known that estrogens are used therapeutically against prostate cancer.

The 98 prostate cancer cases in this study could be divided into two clinical groups: patients with clinically overt prostate cancer and patients with latent prostate cancer diagnosed in tissue specimens usually obtained by means of transurethral resection for benign prostatic hyperplasia. Due to the concern that the latent cases might be different from the clinical cases, the analyses were repeated, excluding the 26 latent cases and their matched controls. The results are very similar to those with all cases included.

Overall, it would be worthwhile to conduct further studies to see if a low serum dihydrotestosterone level, or even an elevated testosterone/dihydrotestosterone ratio is a marker for a person who will develop prostate cancer in the near future. However, our data for now indicate that the levels of sex hormones in serum obtained before diagnosis are not strongly related to prostate cancer risk.

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REFERENCES

1. Henderson, B. E., Ross, R. K., Pike, M. C., and Casagrande, J. T. Endogenous hormones as a major factor in human cancer. Cancer Res., *42:* 3232–3239, 1982.
2. Wilson, J. D. Recent studies on the mechanism of action of testosterone. N. Engl. J. Med., *287:* 1284–1291, 1972.
3. DeVita, V. T., Hellman, S., and Rosenberg, S. A. (eds.). Cancer: Principles and Practice of Oncology. Philadelphia: J. B. Lippencott Co., 1982.
4. Ahluwalia, B., Jackson, M. A., Jones, G. W., Williams, A. O., Rao, M. S., and Rajguru, S. Blood hormone profiles in prostate cancer patients in high-risk and low-risk populations. Cancer (Phila.), *48:* 2267–2273, 1981.
5. Ghanadian, R., Puah, C. M., and O'Donoghue, E. P. N. Serum testosterone and dihydrotestosterone in carcinoma of the prostate. Br. J. Cancer, *39:* 696–699, 1979.
6. Hill, P., Wynder, E. L., Garbaczewski, L., and Walker, A. R. P. Effect of diet on plasma and urinary hormones in South African Black men with prostatic cancer. Cancer Res., *42:* 3864–3869, 1982.
7. Meikle, A. W., Smith, J. A., and West, D. W. Familial factors affecting prostate cancer risk and plasma sex-steroid levels. Prostate, *6:* 121–128, 1985.
8. Zumoff, B., Levin, J., Strain, G. W., Rosenfeld, R. S., O'Connor, J., Freed, S. Z., Kream, J., Whitmore, W. S., Fukushima, D. K., and Hellman, L. Abnormal levels of plasma hormones in men with prostate cancer: evidence toward a "two-disease" theory. Prostate, *3:* 579–588, 1982.
9. Hammond, G. L., Kontturi, M., Vihko, P., and Vihko, R. Serum steroids in normal males and patients with prostatic diseases. Clin. Endocrinol., *9:* 113–121, 1978.
10. Cancer Incidence in Five Continents, Vol. 4. J. Waterhouse, C. Muir, K. Shanmugaratnam, and J. Powell (eds.). IARC Scientific Publications No. 42. Lyon, France: International Agency for Research on Cancer, 1982.
11. Anderson, D. C., Hopper, B. R., Lasley, B. L., and Yen, S. S. C. A simple method for the assay of eight steroids in small volumes of plasma. Steroids, *28:* 179–196, 1976.
12. Coyotupa, J., Parlow, A. F., and Abraham, G. E. Simultaneous radioimmunoassay of plasma testosterone and dihydrotestosterone. Anal. Lett., *5:* 329–340, 1972.
13. DeVane, G. W., Czekala, N. M., Judd, H. L., and Yen, S. S. C. Circulating gonadotropins, estrogens, and androgens in polycystic ovarian disease. Am. J. Obstet. Gynecol., *121:* 496–500, 1975.
14. Rosner, W. A simplified method for the quantitative determination of testosterone-estradiol binding activity in human plasma. J. Clin. Endocrinol. Metab., *34:* 983–988, 1972.
15. Gibbons, J. D. Nonparametric Statistical Inference, pp. 106–119. New York: McGraw-Hill Publications, 1971.
16. Breslow, N. E., and Day, N. E. Statistical Methods in Cancer Research, Vol. 1, pp. 247–276. Lyon, France: International Agency for Research on Cancer, 1980.
17. Harrell, F. The PHGLM procedure. *In:* SUGI Supplemental Library User's Guide, pp. 437–466. Cary, NC: SAS Institute, Inc., 1986.
18. deLacerda, L., Kowarski, A., Johanson, A. J., Athanasiou, R., and Migeon, C. J. Integrated concentration and circadian variation of plasma testosterone in normal men. J. Clin. Endocrinol. Metab., *37:* 366–371, 1973.
19. Dai, W. D., Kuller, L. H., LaPorte, R. E., Gutai, J. P., Falvo-Gerard, L., and Caggiula, A. The epidemiology of plasma testosterone levels in middle-aged men. Am. J. Epidemiol., *114:* 804–816, 1981.
20. Ross, R., Bernstein, L., Judd, H., Hanisch, R., Pike, M., and Henderson, B. Serum testosterone levels in healthy young black and white men. J. Natl. Cancer Inst., *76:* 45–48, 1986.