Research Article

Profiling Testosterone, Androstenedione, DHEA, and 17α-Hydroxyprogesterone in Prostate Cancer Patients Using LC–MS/MS

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Abstract: *Prostate cancer is one of the leading causes of cancer-related mortality in men and is often associated with alterations in androgen hormone levels and increased concentrations of Prostate-Specific Antigen (PSA). The relationship between androgen levels and PSA concentrations in prostate cancer patients remains to be further investigated to better understand its role in disease progression. This study aimed to analyze the profiles of testosterone, androstenedione, dehydroepiandrosterone (DHEA), and 17a-hydroxyprogesterone in prostate cancer patients using liquid chromatography–tandem mass spectrometry (LC-MS/MS) and to evaluate their correlations with PSA levels. Frozen serum samples from healthy men and prostate cancer patients aged 57–86 years were selected based on PSA concentrations ranging from 0.08–1.29 ng/dL in the healthy group and 21.42–801.18 ng/dL in the prostate cancer group. Prostate cancer patients exhibited lower levels of testosterone, androstenedione, and DHEA, but higher levels of 17a-hydroxyprogesterone compared with healthy controls. PSA levels showed a significant negative correlation with DHEA concentrations ($r = -0.416$; $p = 0.022$), whereas no significant correlations were observed between PSA and the other steroid hormones analyzed.*

Keywords: Prostate Cancer, PSA, Steroid Profiling, LC-MS/MS, DHEA

Abstrak: Kanker prostat merupakan salah satu penyebab kematian akibat kanker tertinggi pada pria dan sering dikaitkan dengan perubahan kadar hormon androgen serta peningkatan konsentrasi *Prostate Specific Antigen* (PSA). Hubungan antara kadar hormon androgen dan konsentrasi PSA pada pasien kanker prostat masih perlu dikaji lebih lanjut untuk memahami perannya dalam perkembangan penyakit. Penelitian ini bertujuan menganalisis profil testosteron, androstenedion, DHEA, dan 17α-hidroksiprogesteron pada pasien kanker prostat menggunakan LC-MS/MS serta mengevaluasi korelasinya dengan kadar PSA. Penelitian ini menggunakan sampel serum beku dari pria normal dan pasien kanker prostat berusia 57–86 tahun yang dipilih berdasarkan rentang konsentrasi PSA, yaitu 0,08–1,29 ng/dL pada kelompok normal dan 21,42–801,18 ng/dL pada kelompok kanker prostat. Pasien kanker prostat menunjukkan kecenderungan penurunan kadar testosteron, androstenedion, dan DHEA serta peningkatan kadar 17α-hidroksiprogesteron dibandingkan kelompok normal. Kadar PSA berkorelasi negatif signifikan dengan DHEA (r = -0,416; p = 0,022), namun tidak menunjukkan korelasi yang bermakna dengan hormon steroid lainnya.

Kata kunci: Kanker Prostat, PSA, Profiling Steroid, LC-MS/MS, DHEA

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1. Introduction

Steroid hormones are synthesized from cholesterol in steroidogenic tissues, including the adrenal glands, gonads, and placenta, through a series of enzymatic reactions collectively known as steroidogenesis (Bassi *et al.*, 2021). Cholesterol required for steroid hormone biosynthesis is mainly supplied by circulating low-density lipoproteins (LDL) through receptor-mediated uptake and intracellular trafficking pathways. These hormones regulate numerous physiological processes, with androgens and estrogens playing essential roles in sexual development and the maintenance of secondary sexual characteristics in males and females, respectively (Chatuphonprasert *et al.*, 2018; Cornejo *et al.*, 2024).

Prostate cancer is one of the most prevalent malignancies and a leading cause of cancer-related mortality among men worldwide, with incidence increasing markedly with advancing age, particularly after 50 years (James *et al.*, 2024). Serum prostate-specific antigen (PSA), a kallikrein-related serine protease produced by both normal and malignant prostatic epithelial cells, remains the most widely used biomarker for prostate cancer screening, diagnosis, and disease monitoring (Kumar *et al.*, 2024). Aging in men is accompanied by progressive alterations in androgen homeostasis, including declines in circulating testosterone and dehydroepiandrosterone (DHEA), which may influence prostate physiology and disease progression (Welen & Damber, 2022). However, the relationship between PSA levels and circulating androgen profiles remains incompletely understood, warranting further investigation into steroid hormone dynamics in patients with prostate cancer.

This study aims to analyze the profiles of testosterone, androstenedione, DHEA, and 17α-hydroxyprogesterone (17α-OHPG) in prostate cancer patients using Liquid Chromatography-Mass Spectrometry/Mass Spectrometry (LC-MS/MS). By employing statistical analyses, including correlation tests and T-tests, this research seeks to clarify the hormonal landscape of the disease. Such findings are significant for improving diagnostic accuracy and understanding the endocrine dynamics within the broader field of urological oncology.

2. Materials and Methods

2.1 Materials

Standard stocks of testosterone (625 ng/mL), 17α-OH progesterone (10,000 ng/mL), androstenedione (11,868 ng/mL), and DHEA (10,000 ng/mL) were utilized, alongside internal standards (ISTD) for testosterone (10 ng/mL) and 17α-OH progesterone (25 ng/mL). Analytical grade reagents included methanol (MeOH), acetonitrile (ACN), isopropanol (IPA), formic acid (FA), and 8.9% $ZnSO_4$ Ultrapure water (18.2 MΩ) was used for all aqueous preparations. Analytical instrumentation consisted of a Waters Xevo TQ-XS LC-MS/MS system equipped with a Sample Manager FTN, utilizing Phree™ Phospholipid Removal and Strata® C18-E (55 µm, 70A) SPE cartridges (Phenomenex) for sample cleanup.

2.2 Sample Criteria and Preparation

This study utilized stored biological materials (SBM) comprising serum samples from normal male controls and prostate cancer patients, previously screened for Prostate Specific Antigen (PSA) levels at Prodia Clinical Laboratory. Subjects were selected based on age (57–86 years) and PSA concentrations. The control group exhibited PSA levels between 0.08–1.29 ng/dL, while the prostate cancer group ranged from 21.42–801.18 ng/dL. All samples were stored at -20 °C for five days post-collection.

Sample diluent was prepared by mixing 5.55 mL MeOH with 5.55 mL 8.9% $ZnSO_4$, spiked with 450 µL each of testosterone and 17α-hydroxyprogesterone internal standards (Liu *et al.*, 2021). Protein precipitation was performed by mixing 150 µL of serum with 300 µL of diluent, followed by vortexing and centrifugation for 10 minutes and 14,000 rpm (Owen *et al.*, 2016). The resulting supernatant was purified using C18 solid-phase extraction (SPE), a widely used approach for reducing matrix effects and improving analytical sensitivity in steroid profiling (Evangelista *et al.,* 2024). The extraction procedure was adapted from Owen *et al.* (2016) with minor modifications, involved conditioning with MeOH (2× 200 µL) and H_2O (200 µL). After loading 400 µL of supernatant, the column was washed sequentially with 40% MeOH, 2% FA in 40% MeOH, and 40% MeOH. Analytes were eluted with 80% MeOH (3 × 150 µL) to a final volume of 450 µL before LC-MS/MS analysis (Keevil, 2016).

2.3 Standard Preparation

A master mix (Level 6) was prepared by combining high-concentration stocks of 17α-OH progesterone, testosterone, androstenedione, and DHEA in MeOH (Boggs *et al.*, 2016). A six-point calibration curve was generated through serial dilution (1:4 ratio) in MeOH. Standard working solutions were prepared by mixing 75 µL of each level with 150 µL of a dedicated standard diluent (containing ISTDs in 50% MeOH). All preparations were vortexed and transferred to vials for LC-MS/MS quantification via TargetLynx software (He *et al.*, 2025; Fraissinet *et al.*, 2023).

2.4 LC-MS/MS Analysis and Method Validation

Steroid hormone analysis was performed using an LC–MS/MS method adapted from Owen *et al.* (2016), with modifications to the sample preparation, chromatographic conditions, and mass spectrometric parameters based on optimization studies conducted in our laboratory. Chromatographic separation was performed on an ACQUITY UPLC® BEH C18 column (1.7 µm) maintained at 40°C, using a 10 µL injection volume and a constant flow rate of 0.20 mL/min. The mobile phase consisted of 0.1% FA in ultrapure water (A) and 0.1% FA in ACN (B), both filtered (2 µm) and sonicated for 15 minutes prior to use. A 6.00-minute gradient program was employed: 0.0–0.5 min (10% B), 4.0–4.5 min (90% B), and 5.0–6.0 min (10% B). Mass spectrometry was conducted in electrospray positive ionization (ESI+) mode using Multiple Reaction Monitoring (MRM). Source and desolvation temperatures were set at 150°C and 400°C, respectively, with a capillary voltage of 3.00 kV. Gas flow rates were optimized at 200 L/Hr (cone), 800 L/Hr (desolvation), and 0.15 mL/min (collision). Specific MRM transitions, including parent/daughter ions (m/z), cone voltage (CV), and collision energy (CE), are detailed in Table 1.

Method validation was performed to ensure robustness, assessing precision and accuracy across three batches for standards (n=15) and seven batches for samples via quintuplicate injections. Recovery was evaluated using spiked matrices across six levels, while parallelism was confirmed by comparing linearity between non-spiked and spiked standard curves. All data were processed using TargetLynx and analyzed statistically via SPSS 24 (Rakete *et al.,* 2023).

Table 1. Optimized MRM Parameters for Steroid Profiling

Analyte	Parent (m/z)	Daughter (m/z)	CV (V)	CE (eV)
DHEA	271.0319	253.0363	4	12
Testosterone	289.0000	96.9000	30	20
Androstenedione	287.2000	97.0000	30	20
17-α-OHPG	331.2000	97.0000	30	20

3. Results

3.1 Analytical Method Validation

The LC–MS/MS method demonstrated excellent linearity for testosterone, androstenedione, DHEA, and 17α-hydroxyprogesterone, with coefficients of determination (R^2) ranging from 0.998 to 0.999 (Table 2). Inter-assay precision was acceptable for all analytes, with coefficients of variation (CVs) below 15%, while accuracy ranged from 89% to 114%. Recovery values ranged from 87% to 114%, except for the highest androstenedione concentration level, which showed a recovery of 76%. Parallelism analysis revealed high agreement between spiked and non-spiked calibration curves, with R^2 values exceeding 0.98 for all analytes, indicating minimal matrix interference.

Table 2. Method Validation Summary

Analyte	Linearity (R^2)	Precision CV (%)	Accuracy (%)	Recovery (%)
DHEA	0.999	<12.79	93–110	99–115
Testosterone	0.999	<10.68	91–113	92–109
Androstenedione	0.998	<13.30	89–109	76–104
17-α-OHPG	0.998	<13.38	91–112	96–108

3.2 Characteristics of Subjects and Hormone Concentrations

A total of 30 serum samples were analyzed, consisting of 15 healthy men and 15 patients with prostate cancer (Table 3). The age of participants ranged from 57 to 86 years. The prostate cancer group was significantly older than the control group (72 vs. 66 years, $p < 0.05$).

PSA concentrations ranged from 0.08–1.29 ng/mL in the control group and 21.42–801.18 ng/mL in the prostate cancer group. As expected, PSA concentrations were markedly higher in patients with prostate cancer than in healthy controls (184.10 ng/mL vs. 0.66 ng/mL, $p < 0.05$).

Among the steroid hormones analyzed, DHEA concentrations were significantly lower in prostate cancer patients than in healthy controls (1.24 ng/mL vs. 2.59 ng/mL, $p = 0.001$). Testosterone concentrations also tended to be lower in the prostate cancer group; however, the difference did not reach statistical significance ($p > 0.05$). No significant differences were observed for androstenedione or 17α-hydroxyprogesterone concentrations between groups ($p > 0.05$).

Table 3. Results of T-test for Age, PSA, Testosterone, Androstenedione, DHEA, and 17α-OH Progesterone (Mean ± SD)

Variable	Healthy Controls (n=15)	Prostate Cancer (n=15)	Mean Difference	p-value
Age (years)	66.12 ± 4.10	72.07 ± 9.23	5.95	0.034*
PSA (ng/mL)	0.66 ± 0.43	184.10 ± 203.11	183.44	0.004**
Testosterone (ng/mL)	3.96 ± 1.77	2.62 ± 2.37	-1.34	0.090
Androstenedione (ng/mL)	0.70 ± 0.32	0.62 ± 0.64	-0.08	0.653
DHEA (ng/mL)	2.59 ± 1.09	1.24 ± 0.90	-1.35	0.001**
17α-OHPG (ng/mL)	0.76 ± 0.57	0.88 ± 0.54	0.12	0.549

* = Correlation is significant at the 0.05 level
** = Correlation is significant at the 0.01 level

3.3 Correlation Between PSA and Steroid Hormones

Pearson correlation analysis revealed a significant negative correlation between PSA and DHEA concentrations ($r = -0.416$, $p = 0.022$). No significant correlations were observed between PSA and testosterone, androstenedione, or 17α-hydroxyprogesterone (Table 4).

Table 4. Correlation between PSA with age and hormones

Variable	PSA				
	Age	Testosterone	Androstenedione	DHEA	17α-OHPG
P *value*	0.091	0.645	0.388	0.022	0.153
r	0.314	-0.088	0.164	-0.416*	0.267
N	30	30	30	30	30

* = Correlation is significant at the 0.05 level

4. Discussion

The present study evaluated the serum concentrations of testosterone, androstenedione, dehydroepiandrosterone (DHEA), and 17α-hydroxyprogesterone (17α-OHP) in patients with prostate cancer using a validated LC–MS/MS method and investigated their associations with prostate-specific antigen (PSA) levels. The analytical method demonstrated excellent linearity, precision, accuracy, recovery, and parallelism, indicating that the developed LC–MS/MS platform was suitable for quantitative steroid hormone profiling in human serum. These findings are consistent with previous studies identifying LC–MS/MS as the preferred analytical approach for steroid hormone measurement because of its high specificity, sensitivity, and reduced susceptibility to cross-reactivity compared with conventional immunoassays (Keevil, 2016; Taylor *et al*., 2017).

A significant finding of this study was the markedly lower serum DHEA concentration observed in prostate cancer patients compared with healthy controls. DHEA is the most abundant circulating adrenal androgen in humans and serves as a precursor for androstenedione and testosterone biosynthesis. Previous studies have demonstrated that circulating DHEA concentrations progressively decline with advancing age, a phenomenon commonly referred to as adrenopause (Lin *et al*., 2025; Nawata *et al*., 2026). Since the prostate cancer group in the present study was significantly older than the control group, age-related reductions in adrenal androgen production may have contributed to the lower DHEA concentrations observed. However, the magnitude of the decrease suggests that factors beyond physiological aging may also be involved.

The reduction in DHEA observed in prostate cancer patients may reflect alterations in steroidogenic pathways associated with prostate carcinogenesis. Although DHEA itself possesses relatively weak androgenic activity, it serves as an important substrate for intracrine

androgen synthesis within peripheral tissues, including the prostate. Emerging evidence suggests that prostate cancer cells can modify steroidogenic pathways to maintain androgen receptor signaling even under conditions of reduced circulating androgen concentrations (Gopi *et al.*, 2025; Cho *et al.*, 2014). Consequently, decreased circulating DHEA levels may indicate increased utilization of adrenal androgen precursors or dysregulation of systemic steroid metabolism during disease progression.

Interestingly, testosterone concentrations tended to be lower in prostate cancer patients than in healthy controls; however, the difference did not reach statistical significance. This finding is consistent with recent evidence suggesting that circulating testosterone concentrations are not always directly associated with prostate cancer risk or severity. While androgen receptor signaling plays a central role in prostate cancer biology, intraprostatic androgen concentrations may be maintained independently of serum testosterone levels through local steroidogenesis and intracrine conversion of adrenal precursors (Quistini *et al.*, 2025; Schiffer *et al.*, 2018). Therefore, serum testosterone may not adequately reflect androgen activity within the tumor microenvironment.

Similarly, no significant differences were observed in serum androstenedione or 17α-hydroxyprogesterone concentrations between the two groups. Androstenedione functions as an intermediate precursor in androgen biosynthesis, whereas 17α-OHP occupies an upstream position in the steroidogenic pathway (Zhang *et al.,* 2022). The absence of significant differences in these hormones may indicate that systemic concentrations remain relatively stable despite the presence of prostate cancer. Alternatively, alterations in steroid metabolism may occur predominantly within prostate tissue rather than in the circulation, thereby limiting the ability of serum measurements to capture disease-associated changes (Deb *et al.,* 2021).

The most notable finding of this study was the significant negative correlation between PSA and DHEA concentrations. Patients with higher PSA levels tended to exhibit lower circulating DHEA concentrations. PSA is a well-established biomarker of prostate epithelial activity and is widely used for prostate cancer diagnosis and monitoring. Previous studies have reported age-dependent increases in PSA concentrations accompanied by reductions in circulating adrenal and gonadal androgens (Welén & Damber, 2022; Putra *et al.*, 2016). The inverse relationship observed in the present study is therefore biologically plausible and may reflect concurrent age-related endocrine changes and disease-associated alterations in androgen metabolism.

Several mechanisms may explain the observed association between PSA and DHEA. First, reduced DHEA concentrations may decrease the availability of androgen precursors required for maintaining endocrine homeostasis (Lin *et al.*, 2025). Second, enhanced intracrine conversion of DHEA within prostate tissue may contribute to depletion of circulating DHEA while simultaneously sustaining androgen receptor activation in tumor cells (Schiffer *et al.*, 2018). Third, lower DHEA levels have been associated with increased inflammatory activity and metabolic dysregulation, both of which have been implicated in prostate cancer progression (Vickman *et al.*, 2020; Archer *et al.,* 2020). Nevertheless, the moderate correlation coefficient observed in this study suggests that PSA regulation is multifactorial and cannot be explained solely by changes in circulating DHEA concentrations.

In contrast, PSA showed no significant correlations with testosterone, androstenedione, or 17α-OHP. This finding supports the concept that serum PSA is influenced by multiple biological processes beyond circulating steroid hormone concentrations (Hsieh *et al.*, 2024). Tumor heterogeneity, androgen receptor sensitivity, local steroid metabolism, inflammation, and genetic alterations have all been reported to affect PSA expression independently of systemic androgen status (Tang, 2022; Saranyutanon *et al.*, 2019; Ge *et al.*, 2022). Therefore, circulating hormone concentrations alone may have limited value for predicting PSA levels in prostate cancer patients.

This study has several limitations. First, the sample size was relatively small, which may have reduced the statistical power to detect subtle hormonal differences. Second, the prostate cancer group was significantly older than the control group, introducing a potential confounding effect because androgen concentrations naturally decline with age. Third, only circulating hormone concentrations were evaluated, whereas intraprostatic steroid concentrations and androgen receptor activity were not assessed. Future studies involving larger age-matched cohorts and comprehensive steroidomic analyses are warranted to clarify the role of adrenal androgen metabolism in prostate cancer progression.

In conclusion, the validated LC–MS/MS method provided reliable quantification of steroid hormones in human serum. Among the hormones analyzed, DHEA was significantly lower in prostate cancer patients and exhibited a significant inverse correlation with PSA levels. These findings suggest that DHEA may serve as a potential biomarker of hormonal alterations associated with prostate cancer and support further investigation of adrenal androgen metabolism in prostate cancer pathophysiology.

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