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Original article

Furan and p-xylene as candidate biomarkers for prostate cancer

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Abstract

Background: Prostate cancer (PCa) is the most frequently diagnosed noncutaneous malignant tumor among males in the Western world. Prostate-specific antigen has been considered the most important biomarker for PCa detection; however, it lacks specificity, leading to the search for alternative biomarkers. Volatile organic compounds (VOCs) are released during cell metabolism and can be found in exhaled breath, urine, and other fluids. VOCs have been used in the diagnosis of lung, breast, ovarian, and colorectal cancers, among others. The objective of this study was to identify urinary VOCs that may be sensitive and specific biomarkers for PCa.

Methods: The study included 29 patients with PCa and 21 with benign prostatic hyperplasia. Urine samples were obtained from all participants before and after prostate massage. VOCs were identified by gas chromatography-mass spectrometry. IBM SPSS Statistics v.20 was used for statistical analysis. Sample normality and homogeneity of variances were studied and, according to the distribution normality, ANOVA or the Kruskal-Wallis test was applied to evaluate significant differences between groups. The Pearson test was used to establish correlations.

Results: Fifty-seven VOCs were identified. Samples gathered before prostate massage showed significant between-group differences in urinary levels of furan ($P \leq 0.001$), 2-ethylhexanol ($P = 0.032$), 3,5-dimethylbenzaldehyde ($P = 0.027$), santolin triene ($P = 0.032$), and 2,6-dimethyl-7-octen-2-ol ($P = 0.003$). Samples gathered after prostate massage showed significant differences in urinary levels of furan ($P \leq 0.001$), 3- methylphenol ($P = 0.014$), p-xylene ($P = 0.002$), phenol ($P \leq 0.001$), and 2-butanone ($P = 0.001$).

Conclusions: Significant differences between PCa and BPH patients were found in urinary levels of certain VOCs both before and after prostate massage, supporting the proposal that VOCs may serve as PCa-specific biomarkers.

Keywords: Organic volatile compounds; Prostatic massage; PCa; BPH; Gas chromatography-mass spectrometry

1. Introduction

Prostate cancer (PCa) is the most frequent noncutaneous malignant tumor among males in the Western world. It is the second cause of death by cancer in American men (32,050 deaths/y) after lung cancer [1], and the third in the European Union (89,300 deaths/y) after lung and colorectal cancer [2]. PCa is the most frequently diagnosed malignant cancer in Spain (32,641 cases in 2014), followed by colorectal, lung, and bladder cancers [3]. A Spanish study found that around 4% of PCa patients are diagnosed with disseminated disease and 90% with clinically localized disease (stage T1 or T2) and that 37%

[1] A. Jiménez-Pacheco sought funding, designed experiments, obtained samples, analyzed data, and prepared initial drafts of the manuscript.

[2] M. Salinero Bachiller was responsible for HPLC analysis of samples, conducted statistical analyses, and participated in preparing initial drafts of the manuscript.

[3] M.C. Iribar collaborated in data analysis, preparation of parts of the manuscript, and the final revision.

[4] López Luque and Miján-Ortiz assisted in sample gathering and in preparing substantial parts of the manuscript.

[5] J.M. Peinado, principal investigator of the project, sought funding, designed experiments, analyzed data, and prepared the final version.

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of tumors are classified as low risk, 23% as intermediate risk, and 28% as high risk [4].

Prostate-specific antigen (PSA) is considered the most useful biomarker for PCa detection and monitoring, but its routine clinical use generates a large number of unnecessary biopsies owing to its low specificity. Only marginal improvements in specificity have been obtained using alternative biomarkers such as the density (PSAD), velocity (PSAV), and free fraction (PSA_F) of PSA and its isoforms (e.g., [-2] pPSA) [5]. Besides offering high specificity and sensitivity, the ideal biomarker should be quantifiable in an accessible biological fluid (e.g., plasma, urine, or prostate liquid), reducing the delay in obtaining results and the economic costs, and it should be easy to interpret by clinicians. There should also be minimal variations in results among individuals [6]. Urinary biomarkers have been proposed based on: DNA, including hypermethylation, GSTP1, genes (RASSF, ARF); RNA, including PCa antigen gene 3 (PCA3), fusion genes (TMPRSS2-ERG), AMACR, GOLM1; and proteins, including PSA, annexin 3, matrix metalloproteinases, telomerase, and sarcosine activity [6,7]. The PCA3 test (Progensa PCA3, Hologic Inc, Marlborough, MA) is the only procedure approved by the US Food and Drug Administration for PCa detection in urine samples [5]. This test may also be useful for other purposes, including: assessment of the need for rebiopsy in patients with high risk of PCa and first negative biopsy; decision-making on the need for an initial biopsy; detection of recurrence after radical prostatectomy or radiotherapy; and the follow-up of patients receiving drugs that influence PSA levels, e.g., 5-alpha-reductase inhibitors [8]. However, the relationship of PAC3 test findings with tumor aggressiveness and the prognostic value of this test remain controversial [9].

It has been found that trained dogs can identify the presence of PCa by sniffing the urine of individuals, leading to the proposal that urinary levels of volatile organic compounds (VOCs) might serve as biomarkers of PCa, with the advantage of being a low-cost, noninvasive approach [10–12]. It has been observed that the growth of lung, breast, ovarian, colorectal, hematological, and prostate tumors is accompanied by genetic or protein changes that lead to membrane peroxidation and therefore the emission of VOCs [13].

The objective of this study was to compare VOC levels between urine samples from patients with PCa and benign prostatic hypertrophy (BPH) and to evaluate the correlation of VOC levels with PSA levels and Gleason scores.

2. Materials and methods

2.1. Patient population

The eligible study population comprised all patients in the Departments of Urology of 2 referral centers in Southern Spain with lower urinary tract symptoms (LUTS) and suspicion of PCa who underwent echo-directed transrectal biopsy between April 2016 and January 2017. Study inclusion criteria were: (1) the presence of LUTS or PSA of 3–10 ng/ml with PSAf/PSAt ratio $<20\%$, or PSAf/PSAt ratio $>20\%$ but PSA density >0.15 ng/ml/g or the doubling time is <3 years or PSAt growth rate is >0.75 ng/ml/year; (2) PSA >10 ng/ml; (3) age between 40 and 50 years and first-degree family history of PCa with PSAt >2.5 ng/ml (4) suspicious rectal examination (RE), regardless of PSA value; and (5) PCA3 >35 in a second biopsy (after a first negative biopsy). Exclusion criteria were: (1) LUTS with predominant filling symptoms secondary to obstructive disease, vesical hyperactivity, or other condition impairing the ability to delay micturition for ≥ 1 hour; (2) pathological diagnosis of atypical small acinar proliferation or low- or high-grade prostatic intraepithelial neoplasia; (3) inflammatory process in prostate biopsy; (4) lower urinary tract infection confirmed by urine culture; (5) alcohol or tobacco consumption during the 12-hour period before sample collection; (6) previous treatment for PC; (7) the presence of cancer at any other site at the time of sample collection; (8) treatment with 5-alpha-reductase inhibitors; or (9) moderate or severe kidney failure.

Eligible patients with a pathologic diagnosis of PCa were assigned to a test group and those with a pathologic diagnosis of BPH were assigned to a control group. All participants signed informed consent to participation in the study, which was approved by the local research ethics committee. Data were also gathered on the participants' diet, workplace, and any smoking habit.

2.2. Urine sampling and analysis

Patients were instructed to reduce their liquid intake during the afternoon/evening before their appointment and to avoid urinating later than 2 AM, enabling collection of their first-morning micturition at the clinic; a second sample was taken 2 hours later, after prostate massage (3 strokes). Two aliquots were taken from each sample. One aliquot (11 ml) was used for urine culture to rule out possible infection. The other (25 ml) was frozen at −22ºC until VOCs analysis; then, after defrosting, samples of 5 ml were drawn and a pH of 1–2 was obtained by using 5.0 M hydrochloric acid (HCl), adding 1.85 g sodium chloride (NaCl) to saturate these sample [14].

VOCs were analyzed, identified, and semiquantified following the method of Silva et al. [14], using gas chromatography-mass spectrometry (GC-MS). After dynamic head space-solid phase micro-extraction, samples were injected using an automatic injector with head space (Palo Alto, CA). Extraction time was 40 minutes at 50ºC. The space-solid phase micro-extraction fiber with absorbed VOCs was inserted at 250ºC for 6 minutes in the injection port of an Agilent 7890 gas chromatographer equipped with HP-innowax fused silica capillary column (30 m × 0.25 mm