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Correlation between stage of prostate cancer and tyrosine and tryptophan in urine samples measured electrochemically

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ABSTRACT

Prostate cancer (PCa) is the second most common cancer in men and one of the leading causes of cancer-related deaths. Early detection is the key to successful treatment and provides the greatest chance to cure the patient. Currently, early detection involves screening for prostate-specific antigen levels in blood, which is not a tumor-specific biomarker. There is a critical need to develop clinically useful methods for screening for more reliable biomarkers. Here, we introduce an electrochemical biosensor that measures the concentrations of the amino acids tyrosine and tryptophan, and propose it as a possible diagnostic and prognostic tool for PCa. The limits of detection of tyrosine and tryptophan using the electrochemical sensors were 1.15 and 1.13 μmol/L in 1:10 urine: PBS, respectively. This study is the first to present electrochemical measurements of tyrosine and tryptophan directly in patient urine samples. We demonstrated an inverse correlation between the measured electrochemical signals and the severity of PCa. The most notable observation was a significant difference between controls and metastatic PCa patients ($P \leq 0.001$). This observation was further validated using Liquid-Chromatography-Mass Spectrometry. Our data provides the basis for further research with electrochemical measurements of tyrosine and tryptophan as potential biomarkers for PCa.

1. Introduction

Prostate cancer (PCa) is the second most common cancer in men with higher prevalence in developed countries [1]. Worldwide, there were more than 1.400.000 new PCa cases and 375.000 PCa related death cases in 2020, placing PCa as the fifth most common cause of cancer-related death in men [1,2] accounting for about 7% of all cancer deaths in men worldwide. In 1991, Catalona et al. proposed using prostate-specific antigen (PSA) as a screening tool for PCa. Their results suggested that PSA measurements in combination with digital rectal examination (DRE) would provide a better prediction for PCa compared to DRE alone [2]. PSA measurements has improved prognosis of PCa and resulted in a decrease in PCa-related deaths [3]. Currently, measurements of PSA levels in blood and DRE are established routine examinations in men with suspicion of PCa [3,4].Screening with PSA has contributed to a notable rise in diagnosis of early-stage PCa [5]. However, time has shown that PSA as a screening tool for PCa does have considerable limitations [6–8]. Firstly, conditions such as benign prostatic hyperplasia, prostatitis and prostate injury may also be associated with high PSA levels [9,10]. Secondly, there is no specific normal level of PSA in the blood, though PSA values < 4 μg/L are generally considered as normal [11]. Thus, there is a critical need to develop better non-invasive diagnostic methods with new biomarkers ideally providing both a high sensitivity and specificity for PCa.

Amino acids have several physiological roles, including serving as building blocks of proteins and components in energy metabolism [12]. Amino acid metabolism is upregulated in carcinomas due to energy requirements and protein synthesis [13–16]. The amino acids tyrosine and tryptophan have in few earlier studies been investigated in relation to PCa. Derezinski et al. and Heger et al. measured tyrosine and tryptophan in urine relative to creatinine, Derezinki et al. observed a statistically significant downregulation in concentration of both amino acids in PCa

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patients compared to controls, while Heger et al. reported the opposite [17,18].

The possibility to measure tyrosine and tryptophan by a cheap, fast and simple electrochemical method is alluring. Additionally, as the measurement time typically is within a minute, the handheld setup allows on-the-spot screening in the clinics with no need for centralized laboratories. Tyrosine and tryptophan can be identified and quantified by electrochemical sensing with no sample pretreatment, as these molecules are electroactive compounds [19,20]. Selectivity is exhibited by the specific potential at which electrons are exchanged with the electrode surface while the concentration of the target compound is reflected by the intensity of the measured current [21,22]. Several studies have demonstrated that electrochemical detection of tyrosine and tryptophan is possible but to the best of our knowledge no earlier studies have electrochemically investigated tyrosine or tryptophan in patient samples with similar methods [19,23–26].

As previous knowledge about the role of tyrosine and tryptophan in PCa development and progression is limited, we investigated electrochemical measurements of tyrosine and tryptophan as a biomarker for PCa. A new stand-alone method for rapid screening of tyrosine and tryptophan in urine was developed and validated by Liquid Chromatography – Mass spectrometry/Mass spectrometry (LC-MS/MS).

2. Material and methods

2.1. Study population and sample collection

The study was approved by the Regional Committee on Health Research Ethics for Southern Denmark (VEK jr.nr: S-20210085) and based on urine samples collected from individuals diagnosed with PCa and from healthy individuals as controls. Urine samples were collected at any time of the day before any type of manipulation from non-fasting individuals. Patient samples and samples from healthy individuals were handled the same. Both groups were enrolled from the PerPros biobank (jr.nr: 18/11174), Department of Urology, Vejle Hospital, sampling urine and blood from men referred with suspicion of PCa before biopsy. A total number of 40 individuals were included. All men had prostate biopsies performed subsequent to blood and urine sampling. The control group had negative biopsies, and in a mean post-biopsy observation period of 69 months (range 67–71 month) none developed PCa. All samples were collected and subsequently stored at −80 °*C* until analysis.

2.2. Electrochemical detection of tyrosine and tryptophan

Tyrosine (#42H0656, Sigma-Aldrich) and tryptophan (#102K0370, Sigma-Aldrich) were diluted in 1:10 Urine: (PBS) (#RNBJ7479, Sigma-Aldrich) to get the desired concentrations between 1 and 150 μmol/L for tyrosine and tryptophan. We measured a control sample consisting of 1:10 urine: PBS and afterwards measured the increasing concentration of tyrosine in triplicates to generate the calibration curve. The same procedure was performed for tryptophan. Solutions containing both amino acids of 100 μmol/L concentrations each were also prepared in PBS and urine, respectively.

The urine patient samples were split into several volumetric flasks to avoid contamination during repeated use. All patient samples were diluted 1:10 by adding PBS with 0.1 M KCl to each flask. The electrochemical measurement of tyrosine and tryptophan takes 45 s. This means that the time from sample to result is less than 2 min including the time it takes to pipette the sample on the sensor.

The measurements of tyrosine and tryptophan were performed using single-use screen-printed carbon sensors (#PreD-Ca, PreDiagnose, Karlslunde, Denmark) with a three-electrode configuration design; a carbon working electrode (WE), counter electrode (CE) and a silver reference electrode (RE). The reaction of interest occurs at WE. The CE closes the circuit and the WE potential is measured against the RE [27]. The measurements of these two amino acids were performed with the software PS-trace 5.8 (PalmSens BV, Houten, The Netherlands) and potentiostat BlueStat3 (PalmSens BV, Houten, The Netherlands).

2.3. Characterization of carbon electrochemical sensors

The electrode requires no functionalization due to direct identification of the redox potential of tyrosine and tryptophan. Characterization of the carbon electrode was accomplished by cyclic voltammetry (CV). The CVs were performed from −0.65 to 1.0 V with scan rates between 0.05 and 0.25 V/s. The electrochemical measurements were performed using a solution consisting of the 2.5 mM ferriferrocyanide in PBS with 0.1 M KCL and measured against the reference electrode. The electrochemical detection of tyrosine and tryptophan was performed using Square Wave Voltammetry (SWV). SWV was conducted with the following settings: amplitude: 0.1V; frequency 40.0 Hz and the current ranges from 100 nA–1 mA, E_{begin} 0.2V and E_{end} 1.2V. The sample size was 70 μL pipetted on top of the electrodes. The measurement time was 45 s. A new sensor was used for each measurement and all the measurements were conducted against the reference electrode.

A schematic representation of the electrode with the three-electrode configuration and the electrochemical setup with the potentiostat connected to a computer are shown in Fig. 1.

2.4. Data analysis

The signal induced by the redox activity of the amino acids was obtained by subtracting the background signal from the analyte signal in PS. Trace 5.8. When measuring the samples, a spectrum with two peaks is obtained. After subtraction of the background, the accumulated charge was found by marking peak start and peak end for both peaks. The average sum charge for each patient was presented. The Limit of Detection (LOD) was calculated as 3 times the standard deviation of lowest concentration divided by the slope of the calibration curve. Unpaired T-tests with Welch's test were performed using Graphpad Prism 7.

2.5. Stability of tyrosine and tryptophan

To investigate the degradation pattern of tyrosine and tryptophan, stability tests were performed for each compound, which included electrochemical measurement of a solution with constant concentrations at 7 time points: Day 0, day 1, day 2, day 6, day 9, day 14 and day 30 (30 days timepoint is only for PBS). The amino acids were stored under five different temperatures: room temperature (21°*C*), −80 °*C*, −20 °*C*, 4 °*C* and37 °*C*, Samples in urine: PBS (1:10) and in only PBS containing 100 μmol/L tyrosine or tryptophan were prepared in triplicates. The obtained signals from stability experiment were normalized to zero-point measurement.

2.6. LC-MS/MS analysis for tyrosine and tryptophan

Patient samples were analyzed using a Xevo TQ-S tandem mass spectrometer (Waters) connected to an Acquity UPLC system (Waters). The Liquid Chromatography separation was performed at 40 °*C* using Acquity BEH C_{18} column (100 Å, 1.7 *μM*, Waters). The high-performance liquid chromatography (HPLC) was done by gradient system using solvent A (0.1% formic acid) and solvent B (Methanol). The gradient program was set up as follows: Initial (A99%; B1%). 0.5 min (A99%; B1%), 4 min (A60%; B40%) 4.10 min (A0%; B100%), 5 min (A0%; B100%), 5.10 min (A99%; B1%), 6.50 min (A99%; B1%). A summary of multiple reaction monitoring (MRM) transitions and settings such as collision energy and cone for mass spectrometer are listed in Table 1. Moreover, capillary voltage was set to 0.75 kV (positive ionization mode), desolvation temperature to 500 °*C* and desolvation gasflow to 750 L h^{-1}. Calibrators were prepared with concentrations of resp. 1, 10, 25, 50, 150 and 250 μmol/L and controls were prepared with

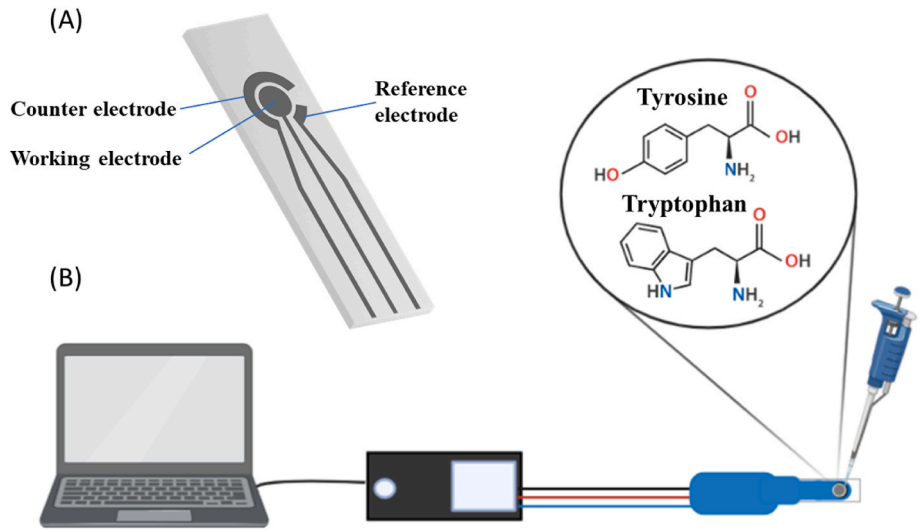

Fig. 1. Schematic representation of the electrochemical setup. (A) The three-electrode configuration setup consisting of a working electrode, a counter electrode and a reference electrode. (B) The experimental setup where the sensor is connected to a potentiostat, which is connected to a computer for recording the obtained data. One measurement takes 45 s and 70 μL (equivalent to one drop) of urine is used for each measurement.

Table 1
Settings for urinary tyrosine and tryptophan analyses by LC-MS/MS.

Sample	Q1	Q3	Collision energy (eV)	Cone (volt)
Tyrosine	182.10	136.09	12	5
[$^{13}C_9^{15}N$] Tyrosine	192.10	145.09	8	5
Tryptophan	205.20	146.10	15	5
[$^{13}C_9^{15}N$] Tryptophan	218.20	156.11	23	5

the concentrations of resp. 4, 5 and 250 μmol/Lol/L. The prepared controls and calibrators were measured repeatedly, and it was found that the analysis evaluated by measuring on controls was reproducible. Calculation of the concentrations of tyrosine and tryptophan was done by TargetLynx (Waters) by the use of the internal standard method.

3. Results

3.1. Characterization of carbon sensors for electrochemical detection of tyrosine and tryptophan

The performance and reversibility of the screen-printed carbon sensor was evaluated by cyclic voltammograms using a solution consisting of 2.5 mM ferriferrocyanide in PBS with 0.1 M KCl (Fig. S1). Symmetrical voltammograms were observed. The half-wave potential for a reversible system (each voltammogram) were calculated according to the standard equation ($E_0 = (E_{pa} + E_{pc})/2$), yielding a value of 0.94 ± 0.005 V vs. the reference electrode. The Randle-Sevcik plot demonstrates that anodic peak current (Ipa) and cathodic peak current (Ipc) are proportional to the square root of the scan rates.

For quantitative analysis of tyrosine and tryptophan, the signal curves were obtained using SWV. The well-defined oxidation peak for tyrosine was obtained at 0.7 V while the potential for tryptophan was at 0.8 V. In redox reactions, protons are removed with the electrons from

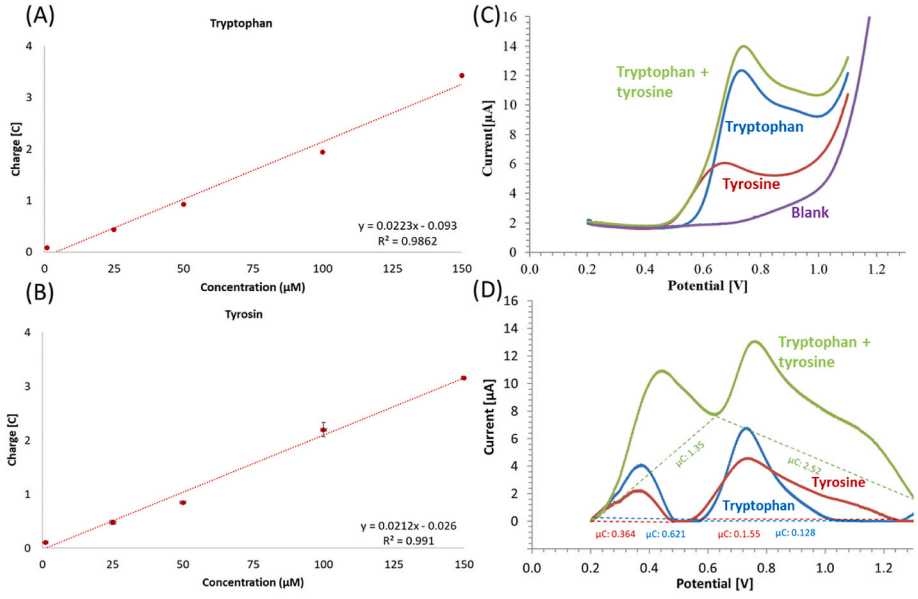

Fig. 2. Detection of tryptophan and tyrosine with screen-printed carbon sensors. (A) Calibration curve with a slope of 0.0223 μA/μmol/L and R^2 of 0.986 for tryptophan. **(B)** Calibration curve with a slope of 0.0212 μA/μmol/L and R^2 of 0.991 for tyrosine. Both calibration curves were obtained by measuring the increasing concentration of the two amino acids in 1:10 urine: PBS using square wave voltammetry. **(C)** Voltammograms of the background, tyrosine, tryptophan and tyrosine and tryptophane in PBS. **(D)** Voltammograms showing the detection of tyrosine and tryptophan spiked in a urine patient sample.

the molecule during oxidation and returned during reduction. Oxidation and reduction of tyrosine occur on the phenyl group and on the phenyl ring for tryptophan [28] (See molecular structure in Fig. 1). The increasing concentrations of tryptophan and tyrosine (0–150 μmol/L) were measured in 1:10 urine: PBS and followed a linear correlation with a slope of 0.0223 μA/μmol/L and a regression coefficient of 0.986 for tryptophan (Fig. 2A) and slope of 0.0212 μA/μmol/L and a regression coefficient of 0.991 for tyrosine (Fig. 2B). The LOD was calculated to 1.15 and 1.13 μmol/L in 1:10 urine:PBS and 1.92 and 2.87 μmol/L for tyrosine and tryptophan in PBS, respectively. To evaluate the ability of the screen-printed carbon sensors to distinguish between tyrosine and tryptophan, we measured a 100 μmol/L of tyrosine sample, a 100 μmol/L tryptophan sample, and a mixed sample containing 100 μmol/L tyrosine and 100 μmol/L tryptophan (Fig. 2C). The obtained peaks from separate spiking with tyrosine and tryptophan appear close to each other and distinguishing between the two individual amino acids is difficult due to overlap. Thus, the charge was found for each peak and the sum of the charges for tyrosine (0.667 μAV) and tryptophan (2.05 μAV) was approximately equal to the measured charge of the mixture of tyrosine and tryptophan (2.61 μAV). The same experiment was performed with a patient control sample. When measuring the patient samples, a spectrum with two peaks is obtained (Fig. 2D). Therefore, the sum of accumulated charge was measured to account for the total signal contribution from tyrosine and tryptophan and provide a measure for the total concentration of the two, as both peaks have an impact on the concentration value. The charge for tyrosine peaks (0.257 μAV) plus charge for tryptophan peaks (1.116 μAV) was approximately equal to the charge of sum tyrosine and tryptophan peaks (1.322 μAV).

3.2. Stability of tyrosine and tryptophan under different conditions

To ensure that tyrosine and tryptophan are stable despite storage of samples under different temperatures, a stability test was performed in urine: PBS (1:10) by measuring the signal of a constant concentration at fixed temperatures for a period of 14 days. Fig. 3A and B shows the recovered concentration as a function of time for samples with 100 μmol/L of each amino acid. After 14 days no significant degradation was observed for any sample. Figs. S2A and S2B illustrate that both amino acids were stable in PBS for the whole period of 30 days.

3.3. Measurement of tyrosine and tryptophan concentrations using liquid chromatography – Mass spectrometry

To validate the new electrochemical method, the patient samples were analyzed with LC-MS/MS. Interestingly, the results showed that the urinary concentration of tyrosine and tryptophan are strongly correlated with a concentration change of 0.6 μM of tryptophan for each μM of tyrosine (Fig. 4A). This means that roughly 40% of the contribution of tryptophan and tyrosine to the electrochemical signal comes from tryptophan. The EC measurements were compared to those from LC-MS/MS, the average sum accumulated charge for both amino acids are plotted against sum concentration of both amino acids for each patient in Fig. 4B. The results showed a linear correlation with a slope of 0.0185 μA/μmol/L and a regression coefficient of 0,51. This is not impressing, but the results make very good sense when compared with calibrations shown in Fig. 2. The population weighted average of the tryptophan and tyrosine responses of 0.0216 μA/μmol/L is shown in red in Fig. 4B. As can be seen in the plot, the red line coincides with the lowest charge values for each concentration value. This agrees well with the fact that we only measure free tryptophan and tyrosine with LC-MS/MS, while we expect contributions from other molecules with similar ring structures as well as tryptophan and tyrosine in short peptide segments in the EC measurements.

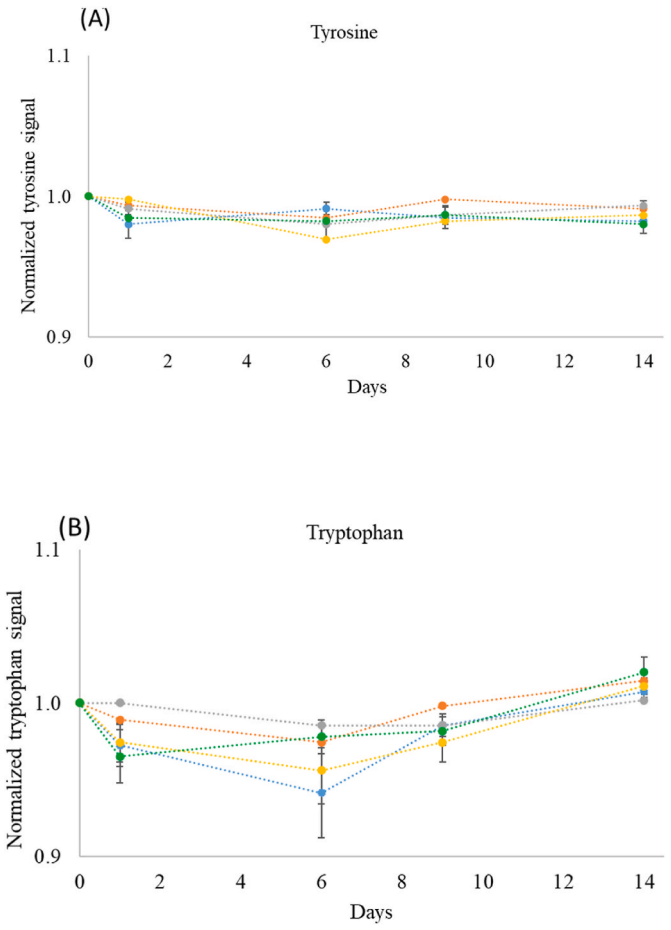

Fig. 3. Stability test of tyrosine and tryptophan. The two amino acids in urine: PBS (1:10) were stored under different temperature conditions for a period of 14 days. The graphs show the recovery of tyrosine (A) and tryptophan (B) concentration from the initial value of 100 μmol/L. The test demonstrated that tyrosine and tryptophan were stable under all conditions during the whole test period. ● Room temperature ● 4° ● 37° ● −20° ● −80 degree

3.4. Correlation between stages of PCa and tyrosine and tryptophan measurements

As the electrochemical detection method was validated, the method was applied to investigate the correlation between the tryptophan and tyrosine signal with the stage of PCa. The average sum of accumulated charge of the peaks at 0.4 V and 1.0 V was found for all the patients in the specific stage of PCa. The average sum of accumulated charge for every stage of PCa were plotted and compared (Fig. 5A). The data showed significant differences between the control group and local advanced PCa ($P = 0.0296$) and metastatic PCa ($P = 0.0010$). Also, a significant difference between local PCa and mPCa was observed ($P = 0.0207$). The relation between the sum of the concentrations of these two amino acids obtained from LC-MS/MS and disease progression was also investigated and plotted in Fig. 5B. The average concentration decreases with the severity of PCa. LC-MS/MS only indicated a significant difference between the control group and mPCa ($P = 0.0321$). This validation supports the results obtained by EC, that there is an inverse correlation between tyrosine and tryptophan concentration and PCa progression. The large error bars are likely attributed to the sampling conditions. Hence, sampling was not restricted to e.g. morning urine, specific diets prior to sample collection; and further, information regarding smoking status was not available. However, the clear trend with statistically significant decreases in concentration of both amino acids in PCa patients compared to controls observed by two independent

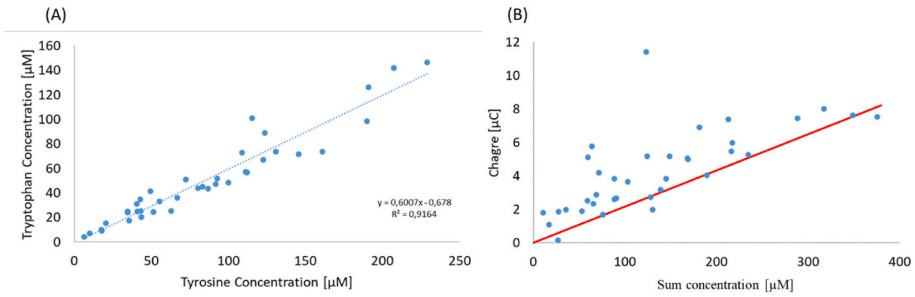

Fig. 4. Tyrosine and tryptophan quantified by Liquid Chromatography – Mass spectrometry and Square Wave Voltammetry. (A) The urinary concentration of tyrosine and tryptophan measured by LC-MS/MS are strongly correlated with an R^2 of 0.916 and a change of 0.6 µM of tryptophan for each µM of tyrosine. (B) Comparison of the readings using the two analytical techniques. The red line corresponds to population weighted average of the calibration curves for tryptophan and tyrosine shown in Fig. 2. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

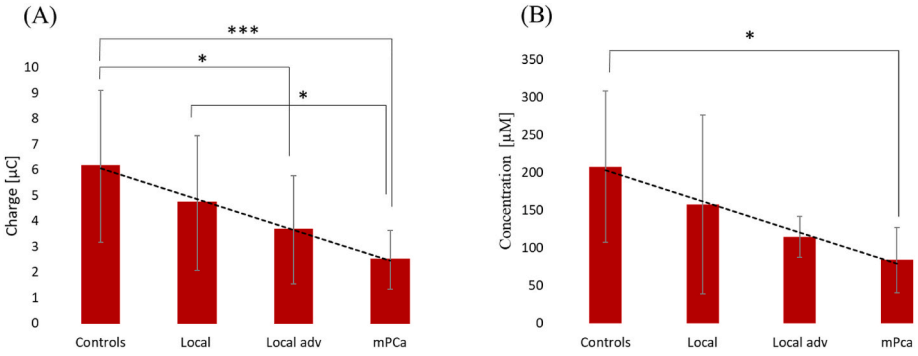

Fig. 5. Correlation of tyrosine and tryptophan measurements with disease progression. Tryptophan and tyrosine correlation with the stage of PCa was investigated by using SWV (A) and LC-MS/MS (B). Both analyses demonstrated that there is a correlation and showed that the concentration of these two amino acids decreases with the disease severity. *P $\leq$ 0.05, ***P $\leq$ 0.001.

techniques justify that this observation should be further validated in a larger patient cohort.

In studies investigating biomarkers in urine, urine - creatinine is often used as a laboratory marker of urine dilution [29]. Although no significant difference in urine creatinine concentrations between the four investigated groups were observed, the difference between the control and mPa group were lost when creatinine was used for normalization. The non-correlation between the amino acids and creatinine concentrations (Fig. S3B) implies that their occurrence is not mainly due to renal excretion raising the possibility that amino acids in the prostate fluid may contribute to what is found in the urine.

4. Discussion

Risk of relapse after radical management and survival from Pca are highly dependent on the extent of the disease at the time of diagnosis. There is about 50% reduction in 5-year overall survival in patients diagnosed with *de-novo* metastatic PCa [30]. The currently used diagnostic tools for PCa are quite limited in regard to distinguishing between indolent and aggressive disease [31]. With a lack of more specific diagnostic biomarkers, an aggressive approach to early-stage cancer can lead to overtreatment, which can cause inherent side effects or complications, some of which can be permanent [32,33]. In addition, biomarkers with a prognostic value will be helpful in guiding for management decision for PCa.

The urgent need for biomarkers and advances in molecular technologies resulted in numerous findings that might improve the diagnosis of PCa [17,34]. The proposed findings belong to various classes of biological compounds including proteins and metabolites [17]. The largest study of metabolic biomarkers for PCa (ProtecT) identified 35 potential biomarkers including four amino acids; valine, isoleucine, tyrosine, and leucine that all were strongly associated with PCa [17,34]. Derezinski et al. and Adams et al. demonstrated that the study of amino acids in body fluids may be a promising tool in search of diagnostic biomarkers for PCa [17,34].

In this respect, urine has emerged to be a valuable source of biomarkers in PCa [35–38]. Urine provides several advantages since the collection is non-invasive [35], and it can easily be collected in larger quantities than other types of samples. Furthermore, urine is enriched with material coming directly from the prostate gland, and prostate materials can be enhanced in the urine by manipulation of the gland during DRE [35]. Lastly, urine may be a better source of biomarkers for PCa compared to blood, as blood contains higher levels of markers from almost all body tissues [35,39], which results in high interference, and therefore may reduce detection ability [35]. Urine contains approximately 1000-fold lower protein concentrations compared to blood, and urinary proteins are less interactive compared to plasma proteins [40].

Several methods have been reported for detection of tyrosine and tryptophan in other biological samples than urine. These methods include LC-MS/MS, gas chromatography-mass spectrometry (GS/MS), spectrophotometry and fluorometry [41–43] and these methods have been proven to measure tyrosine and tryptophan with high sensitivity. Tyrosine as a marker for cancer has been studied in different cancer types. Miyagi et al. showed a significant decrease in tyrosine levels in plasma samples from gastric cancer patients compared to controls, and the same pattern was also apparent between early stage and late-stage gastric cancer [44]. Similarly, Zhang et al. showed decreased tyrosine levels in serum from patients with esophageal cancer compared to healthy controls [45]. Adams et al. found a strong association of tyrosine with PCa presence [34], however the role of tyrosine in PCa progression is still largely unknown.

Tryptophan is widely explored in cancer, and tryptophan catabolism is a known mechanism in immune system modulation [46]. Tryptophan is metabolized by indoleamine 2,3-dioxygenase (IDO), its split variant (IDO2) and tryptophan 2,3-dioxygenase (TDO) [46]. The ability of cancer to escape immune response is an important factor that drives the development of aggressive forms of tumors [47]. Numerous studies are pointing to consumption of tryptophan as a critical factor in cancer progression [48,49]. Studies indicate that TDO enzymatic activity is sufficient to sustain biologically relevant tryptophan catabolism that is

capable of suppressing antitumor immune response [50]. Opitz et al. also reported that the primary product of tryptophan catabolism by IDO, IDO2 and TDO, kynurenine, is a ligand of for the aryl hydrocarbon receptor (ARH), which mediates invasive tumor growth [47]. Zhang et al. demonstrated that aggressive breast cancer cell lines have higher contribution from tryptophan compared to non-aggressive breast cancer cell lines and normal cell lines [51]. In that way Zhang et al. could use tryptophan as the fingerprint for distinguishing between aggressive and non-aggressive breast cancer cell lines [51]. These results are consistent with the results obtained in our study. We demonstrated that tryptophan and tyrosine concentration correlates inversely with the stage of PCa. Earlier studies indicated that tryptophan catabolism may serve as a biomarker to monitor disease activity and response to therapy in patientsKlik eller tryk her for at skrive tekst.. Furthermore, strategies targeting tryptophan catabolism and ARH activity might be beneficial in tackling immunosuppression and cancer progression [48]. These findings support possible utilization of both tyrosine and tryptophan for diagnosis of PCa.

Although conventional methods as LC-MS, spectrophotometry and fluorometry have a high sensivitity and selectivity, they dependent on multi-step sample purification procedures and are therefore expensive and time-consuming. In addition, they require advanced and expensive equipment and special expertise. Electrochemical biosensing is eligible to serve as a screening tool for PCa biomarkers and provides rapid, on-site monitoring with no sample pretreatment. Other benefits include low-cost, ease of operation as well as high sensitivity and selectivity [21, 52].

With the new electrochemical method, we have investigated the correlation between a measurement that depends on the concentration of tyrosine and tryptophane in urine of healthy individuals and patients diagnosed with different stages of PCa. Several studies have demonstrated that electrochemical detection of tyrosine and tryptophan are possible, but to the best of our knowledge, this study is the first to present electrochemical measurements of these two amino acids directly in urine samples from patients diagnosed with PCa and healthy men [19, 23–25]. The electrochemical method was validated by quantifying tyrosine and tryptophan using LC-MS/MS, but we do not know whether there are other metabolites that contribute to the signal in urine. However, it has earlier been demonstrated that it is possible to develop an electrochemical sensor for detection of tryptophan in presence of other amino acids, including tyrosine [26]. Majidi et al. demonstrated the development of gold -screen printed electrode modified with multiwall carbon nanotube (MWCNT-AuSPE) and a MWCNT-AuSPE modified with tryptophan aptamer molecules (Apt-MWCNT-AuSPE) for selective determination of tryptophan [26]. The two different modified sensors detected tryptophan down to an LOD of 3.6×10^{-4} μmol/L and 4.9×10^{-6} μmol/L in PBS, respectively [26]. Compared to our study, Majidi et al. developed a more sensitive electrode for determination of tryptophan, however they did no present the LOD in biological fluids [26].

Electrochemical biosensing of tyrosine and tryptophane has potential to be used as supplementary diagnostic tools to improve the diagnostic accuracy of PCa. New analytical methods for screening or detection of tumor specific metabolic biomarkers may aid early and precise diagnosis and support successful treatment strategies. However, there is a need for further studies to investigate the questions that this pilot study has given rise to regarding creatinine correlation, origin of amino acids and related pathways and impact of diet on the urinary secretion of the amino acids. Furthermore, the observed differences between the control group and the different clinical stages of PCa need further validation in larger cohort studies.

5. Conclusion

In summary, this work established a novel approach for electrochemical fast and direct detection of tyrosine and tryptophan sum in urine of patients. Measurements were validated using LC-MS/MS. The sensing method is suitable for screening of tyrosine and tryptophan sum on site, directly in urine samples without any pre-treatment procedures. We obtained quantifiable results from tyrosine and tryptophan detection in urine samples from healthy men and PCa patients and demonstrated inverse correlations between concentrations of the two amino acids and the severity of PCa. However, a larger sample size is required for validation of this demonstrated correlation. The role of tyrosine in cancer progression is not fully understood and further research is needed to shed light on the biochemical mechanisms of this observation.

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Contribution

All contributors meet the criteria for authorship. FAA and HN designed the study to detect tyrosine and tryptophan electrochemically. Further, data management and statistical analysis were performed by FAA and HN. AS set up the LC-MS/MS method. HN prepared the first draft of the manuscript. FAA, JSM, PJSO, AHZ, AM, AS and HN contributed to editing the manuscript. FAA, JSM and PJSO provided the resources for this study. All authors approved the manuscript for submission.

CRediT authorship contribution statement

Hashmatullah Nasimi: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Visualization, Writing – original draft, Writing – review & editing. **Jonna Skov Madsen:** Conceptualization, Resources, Funding acquisition, Supervision, Writing – review & editing. **Ahmed H. Zedan:** Supervision, Writing – review & editing. **Anne Vibeke Schmedes:** Validation, Writing – review & editing. **Anders Malmendal:** Formal analysis, Supervision, Writing – review & editing. **Palle Jörn Sloth Osther:** Conceptualization, Resources, Funding acquisition, Supervision, Writing – review & editing. **Fatima AlZahra'a Alatraktchi:** Conceptualization, Methodology, Formal analysis, Investigation, Resources, Funding acquisition, Supervision, Writing – review & editing, Project administration.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ab.2022.114698.

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