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Research article

Spectrofluorimetric investigations of urine in cats diagnosed with renal disorders

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Abstract: Laboratory investigations are essential for establishing an accurate diagnosis in veterinary medicine; however, urinalysis in feline urinary tract pathology is often underestimated, despite its important role in determining etiology and monitoring therapy. This study reemphasizes the importance of urinalysis in cats with renal disorders and proposes urine spectrofluorometry as an innovative method for etiological diagnosis. The aim of this study was to reemphasize the importance of urinalysis in cats diagnosed with renal disorders and to propose urine spectrofluorometry as a novel laboratory method for the etiological diagnosis of these.
In the spectrofluorimetric examination, emission spectra were obtained by exciting urine samples at wavelengths of 280 nm and 400 nm, focusing on the presence of tryptophan fluorophore metabolites. Comparative analysis of emission band intensities revealed significant differences between urine from healthy cats and urine from cats diagnosed with urinary tract disorders. Spectrofluorimetric analysis of urine allows the identification of specific renal biochemical alterations by observing variations in the emission band intensities characteristic of tryptophan metabolites. The results indicate a decrease in fluorescence intensity at λ_ex = 280 nm in healthy cats and an increase at λ_ex = 400 nm in cats with urinary infections and renal impairment, thus confirming the potential of this laboratory method as a rapid, non-invasive, and sensitive diagnostic tool for establishing the etiology of the disease.

Keywords: spectrofluorimetry, urine, cats, renal disorders, tryptophan, fluorophores

1. Introduction

In veterinary medicine, urinalysis is sometimes treated superficially or, in many cases, even ignored, even though this examination can provide crucial information for determining disease etiology or monitoring instituted therapy. A major focus of researchers worldwide has been, and continues to be, the development of non-invasive, reliable, and cost-effective methods for investigating renal diseases and monitoring health status in both animals and humans [1,2]. Fluorescence spectroscopy is considered a potential tool for investigating various systemic disorders, and due to qualities such as sensitivity, specificity, and non-invasiveness, it can be regarded as a complementary technique for diagnosing multiple conditions, including renal diseases [3]. This technique allows the investigation of numerous tissues and biological fluids. In this study, urine was used as the biological fluid of interest, primarily due to its ease of collection and high content of metabolites, including tryptophan metabolites and native fluorophores [4,5]. Because of these metabolites, urine fluorescence can serve as a laboratory method for diagnosing a wide range of conditions, such as proteinuria, nephritic syndrome, hepatopathy, lipofuscinosis, or neuronal ceroid lipofuscinosis [6]. Renal disorders and urinary tract infections in cats represent a common problem in veterinary medicine, significantly affecting quality of life and the prognosis of various pathologies.

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Classical paraclinical diagnostic methods, based on biochemical and microscopic urine analysis, can be complemented by modern spectroscopic techniques. Spectrofluorimetry is a sensitive laboratory technique based on the property of certain biological compounds to emit fluorescent radiation upon excitation with ultraviolet or visible light. In biological fluids, the most important endogenous fluorophores are derivatives of tryptophan, tyrosine, and NADH (Reduced Nicotinamide Adenine Dinucleotide) [7,8]. The objective of this study was to evaluate the spectral differences between urine samples from healthy cats and those from cats with renal disorders, by highlighting variations in emission band intensities specific to tryptophan metabolites.

Fluorescence spectroscopy is a highly sensitive analytical technique used to characterize the biochemical composition of tissues and biological fluids, providing semi-quantitative information on the distribution of endogenous or exogenous fluorophores [7]. The principle of the method is based on the interaction between electromagnetic radiation and matter, particularly within the UV–VIS (<700 nm) and NIR (700–900 nm) ranges, generating phenomena such as absorption, emission, and scattering [9].

The fluorescence phenomenon is characterized by a red shift of the emission wavelength relative to the excitation wavelength, caused by non-radiative transitions, as well as by the independence between the emission and excitation wavelengths. The fluorescence spectrum of a molecule is generally the mirror image of its absorption spectrum [10]. The main parameters that define the fluorescent properties of a molecule are the quantum yield, the ratio between emitted and absorbed photons, and the fluorescence lifetime, which represents the average time a molecule remains in the excited state [10]. This method allows the analysis of fluorescence differences between amino acids within protein structures and the evaluation of urinary metabolites, proving useful in identifying biochemical changes associated with renal pathological processes [11]. Therefore, fluorescence spectroscopy represents a valuable tool in metabolomics, capable of highlighting alterations in metabolite profiles under physiological or pathological conditions [10,12].

The fluorescence process involves three successive stages: excitation, relaxation, and emission, which reflect the energetic transformations of fluorophores following interaction with electromagnetic radiation [12]. Emission spectra were obtained using a PerkinElmer LS 55 spectrofluorometer, with sample excitation at two distinct wavelengths: 280 nm for tryptophan metabolites and 400 nm for oxidized aromatic compounds. Spectra were recorded within the 300–600 nm range, and results were expressed according to the maximum height of the emission bands (Imax). Statistical analysis was performed by comparing the mean intensity values between the two groups.

2. Materials and Methods

The study was conducted on 12 adult cats divided into two groups: a control group (n = 6), consisting of clinically healthy cats, and an experimental group (n = 6), including cats diagnosed with renal disorders or urinary tract infections. A total of 12 urine samples (one per animal) were analyzed.

Diagnosis was established based on clinical examination and complementary paraclinical investigations, including hematology, serum biochemistry, and urinalysis.

Urine samples were collected aseptically using sterile 60 mL urine collection containers (manufacturer specified by supplier). Depending on the clinical situation, three collection methods were used: manual expression (abdominal massage), performed by transabdominal palpation in the ventral abdominal region or at the pelvic floor level. Gentle pressure was applied to the caudal pole of the urinary bladder to induce micturition, and urine was collected directly into sterile containers. Litter box collection, a commercial urine collection kit with non-absorbent beads (manufacturer specified by supplier) was used. The litter box was thoroughly cleaned and disinfected before placement of the beads. Urine was collected using a sterile pipette and transferred into sterile containers. Urethral catheterization, performed under general anesthesia using sterile urinary catheters adapted to patient size and sex (manufacturer specified by supplier). The catheter was inserted into the urinary bladder through the urethra, and urine was collected directly into sterile containers. All samples were protected from light exposure and stored at 4°C until analysis.

Analyses were performed immediately or within a short time after collection to minimize biochemical and fluorescent degradation. Routine urine examination was performed using Laboquick Urinalysis Reagent Strips (Laboquick®, manufacturer according to product packaging).

The parameters assessed included pH, specific gravity, protein, glucose, ketones, bilirubin, urobilinogen, nitrites, and leukocytes. Urinary sediment examination was carried out after centrifugation (centrifuge

model and manufacturer to be specified), followed by microscopic evaluation using a light microscope (model and manufacturer to be specified).

Fluorescence analysis was performed using a Jasco FP-8200 (JASCO International Co., Ltd., Tokyo, Japan). Before measurements, the instrument was equilibrated for approximately 30 minutes to allow stabilization of the xenon lamp and electronic components. Wavelength calibration and system verification were performed according to the manufacturer's protocol using certified reference standards. Urine samples were placed in quartz cuvettes with a 1 cm optical path length (manufacturer specified by supplier). Spectral acquisition was conducted at room temperature. Excitation and emission wavelength ranges were selected to detect endogenous fluorophores (e.g., aromatic amino acids and NADH). Each sample was analyzed in duplicate to ensure reproducibility. Background correction was performed using distilled water blanks measured under identical conditions. All procedures were conducted under standardized laboratory conditions to ensure methodological consistency, reliability, and reproducibility of the results.

3. Results

The analysis of fluorescence spectra obtained by exciting the urine samples at 280 nm revealed, in most cases, the presence of three main emission bands located between 390 nm and 620 nm, with variations correlated to the clinical status of each examined cat (Table 1).

Table 1. Analysis of fluorescence spectra obtained by excitation of urine samples at 280 nm.

Group	λex (nm)	λem (nm) – maxim	Mean intensity (Imax ± SD)	Observations
Control	280	340	1520 ± 110	Typical tryptophan spectrum, high intensity
Experimental	280	340	890 ± 95	Significant decrease in intensity ($p < 0.05$)
Control	400	460	720 ± 80	Weak band, oxidized aromatic compounds
Experimental	400	460	1180 ± 105	Marked increase in intensity ($p < 0.01$)

The results obtained highlight clear changes in the fluorescent characteristics of urine in cats with renal disorders. For example, the sample from a cat diagnosed with renal colic (Case 1) exhibited three emission bands at 410 nm, 450 nm, and 520 nm. In the case of the cat diagnosed with a bacterial urinary tract infection (Case 2), the fluorescence spectrum showed three emission bands at 415 nm, 450 nm, and 520 nm (Figure 1). This profile is similar to that of Case 1, but with a slight red shift of the emission maximum, indicating changes in the composition of fluorophores, most likely due to the presence of leukocytes and hemoglobin degradation products in the urine.

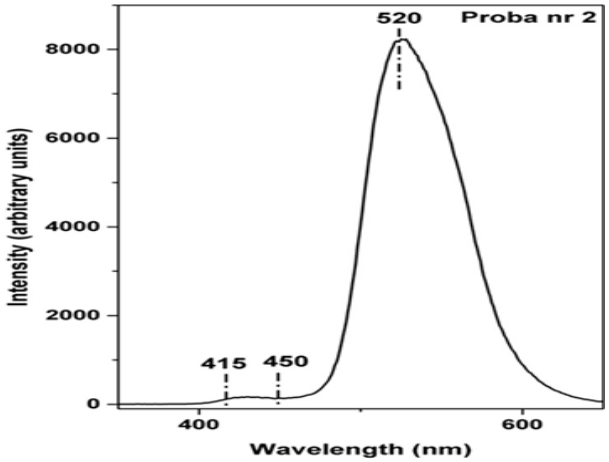

Figure 1. Fluorescence spectrum of the urine sample collected from Case 2

The spectrum of the sample from the cat diagnosed with lower urinary tract disease (Case 3) exhibited emission bands at 390 nm, 450 nm, and 520 nm (Figure 2). This result confirms the involvement of a bacterial infectious process and supports the hypothesis that indolic metabolites can be used as urinary biomarkers for renal disorders and urinary tract infections.

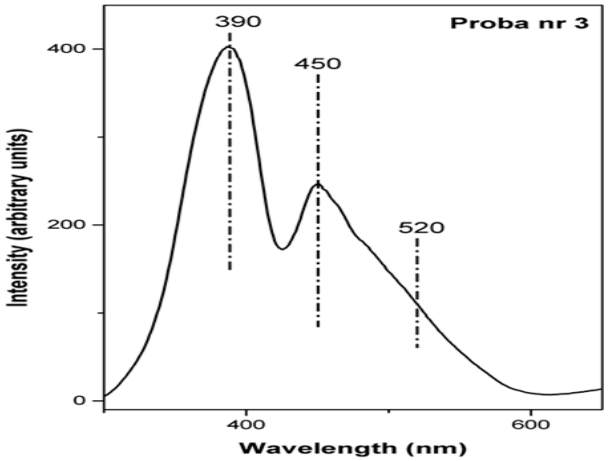

Figure 2. Fluorescence spectrum of the urine sample collected from Case 3

Regarding the cat whose urine was collected by abdominal massage (Case 4), the sample spectrum exhibited emission maxima at 405 nm, 450 nm, and 520 nm (Figure 3). The slight shift of the first band toward longer wavelengths compared to Case 3, suggests a lower concentration of indoxyl compounds, while simultaneously indicating a more pronounced presence of urinary proteins, possibly albumin or cellular enzymes released following renal epithelial damage.

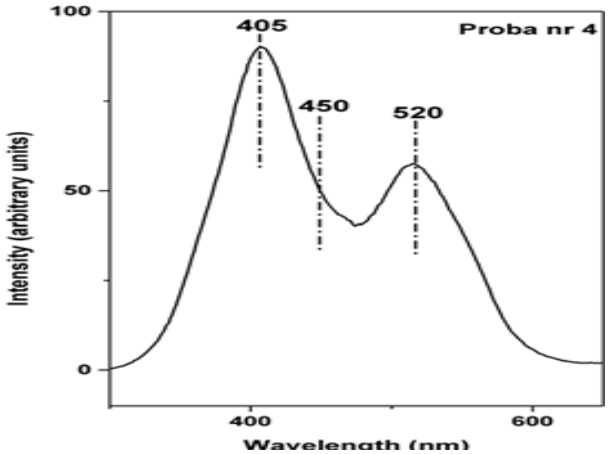

Figure 3. Fluorescence spectrum of the urine sample collected from Case 4

The sample collected from the cat diagnosed with dysuria (Case 5) exhibited three emission bands at 420 nm, 455 nm, and 512 nm (Figure 4). These results suggest the predominance of protein fluorophores, associated with moderate inflammatory activity and the presence of proteinuria. The lower emission wavelengths indicate a simpler composition of fluorophores, with a possible contribution from flavin compounds.

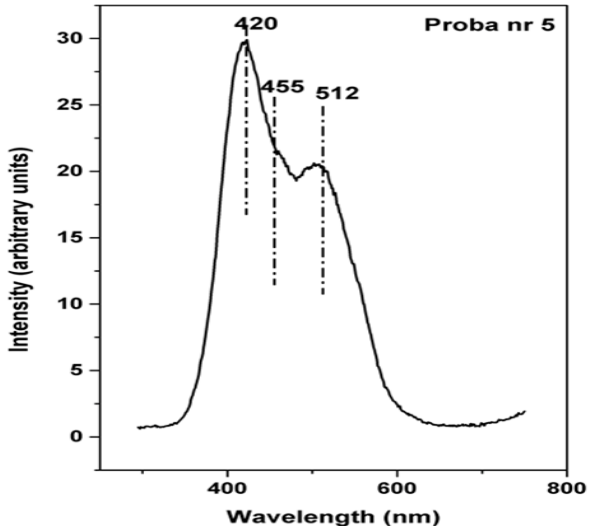

Figure 4. Fluorescence spectrum of the urine sample collected from Case 5

The urine sample from the cat diagnosed with a renal disorder (Case 6) exhibited emission maxima at 474 nm, 560 nm, and 619 nm respectively, indicating a significant red shift of the fluorescence bands.

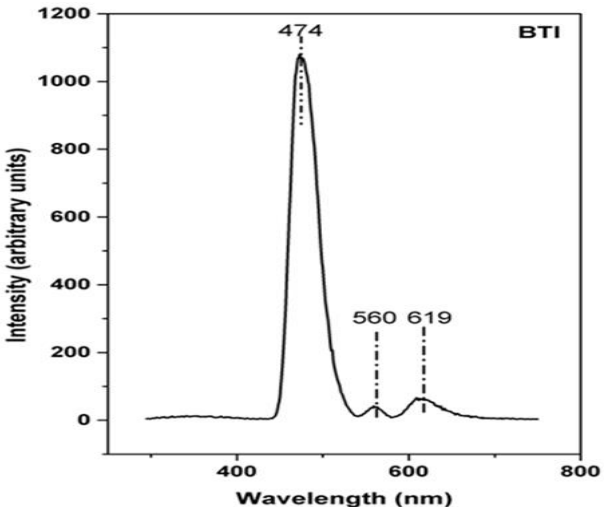

Figure 5. Fluorescence spectrum of the urine sample collected from Case 6

In future studies, the combined analysis of emission spectra with chromatographic and metabolomic methods could allow the precise identification of the compounds responsible for the observed bands and the establishment of specific urinary biomarkers for the early diagnosis of renal diseases in cats. The decrease in the intensity of bands associated with tryptophan (λ ex = 280 nm) may be correlated with reduced excretion of protein metabolites or degradation of fluorescent compounds during pathological processes. In contrast, the increase in intensity at λ_ex = 400 nm suggests the accumulation of oxidized aromatic products, associated with oxidative stress and impaired renal function. These observations confirm the usefulness of spectrofluorimetry as a complementary diagnostic and monitoring tool for renal disorders in cats.

4. Discussion

Assessment of renal function in domestic carnivores typically involves a combination of complementary tests. However, the interpretation of these tests often remains uncertain due to the limited sensitivity of the equipment used, the high biological variability related to species, and the high costs involved. Urinalysis offers numerous benefits, namely: urine serves as a good indicator of certain conditions, making these analyses recommended for monitoring health status; this type of examination can be performed daily, as direct urine collection during micturition does not cause discomfort to the patient; and urinalysis or urine culture

can be performed annually, even in the absence of clinical symptoms, because urinary tract infections are often asymptomatic. Traditional diagnostic methods used in feline renal pathology remain fundamental, yet spectrofluorimetric examination, as a modern diagnostic approach, complements the methodological toolkit and may become a new tool for exploring and monitoring the health status of cats. Urine is recognized as one of the biological fluids with significant fluorescent properties, due to its high concentration of metabolites present in both physiological and pathological states [13]. Fluorescence intensity is not a strictly linear function of fluorophore concentration, being influenced by factors such as self-quenching, energy transfer, and interactions between compounds. Thus, changes in the composition and concentration of fluorophores result in significant variations in the shape and intensity of emission spectra [13]. The literature reports that excitation of urine samples at a wavelength of 290 ± 10 nm induces emission bands associated with fluorophores derived from tryptophan metabolism [14]. Moreover, in the presence of infectious processes, bacteria can produce indoxyl compounds, such as indoxyl sulfate and indoxyl-3-acetate, which exhibit emission maxima around 390 nm [15,16].

These wavelengths mainly correspond to the fluorescence of urinary proteins and tryptophan metabolites. The increased intensity around 450 nm suggests the accumulation of fluorescent aromatic compounds, possibly derivatives of NADH and flavins, which are indicators of oxidative stress and inflammation [10].

The band at 390 nm is particularly significant, as it is associated with the presence of indoxyl compounds, such as indoxyl sulfate and indoxyl-3-acetate, formed as a result of bacterial activity on tryptophan [14,15,17]. This variation is observed when there is an accumulation of bile pigments and oxidized aromatic compounds, suggesting severe renal impairment or an advanced lower urinary tract infection [10,14]. The results obtained clearly demonstrate that fluorescence spectroscopy can be used as a non-invasive method to evaluate biochemical changes in the urine of cats with renal and urinary disorders. Differences in the position and intensity of emission bands reflect variations in metabolic composition and may indicate the presence of infectious or inflammatory processes. The emission spectra obtained in this study are consistent with observations reported in the literature, which associate excitation in the 280–290 nm range with the detection of aromatic fluorophores such as tryptophan, tyrosine, NADH, and flavins, as well as indolic metabolites [10,14,15,17].

5. Conclusions

Fluorescence spectroscopy of urine samples from cats diagnosed with renal and urinary disorders revealed distinct variations in emission spectra, correlated with the nature and severity of the pathological process. Emission maxima between 390–450 nm are attributed to aromatic fluorophores, derivatives of tryptophan, whereas emission bands between 500–620 nm are associated with flavin compounds, bile pigments, and oxidation products. The presence of emission bands around 390 nm represents a potential spectral marker for bacterial urinary tract infections, due to specific indoxyl compounds. Spectrofluorimetric analysis of feline urine allows the identification of biochemical changes specific to renal disorders by examining intensity variations in the emission bands characteristic of tryptophan metabolites. The results indicate a decrease in fluorescence intensity at λ_ex = 280 nm and an increase at λ_ex = 400 nm in cats with urinary infections and renal impairment, confirming the potential of this method as a rapid, non-invasive, and sensitive tool for etiological diagnosis. This method proves to be a valuable instrument in veterinary metabolomic studies, providing rapid and non-invasive insights into kidney functional status. In future research, integrating fluorescence spectroscopy with chromatographic methods and multivariate statistical analysis could enable the identification of specific biomarkers for the early diagnosis of renal diseases in cats.

The study is limited by the small number of cases, the heterogeneity of the included conditions, the single time-point evaluation, and the variability of urine collection methods, factors that may affect the robustness and generalizability of the results.

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