Cancer detection by native fluorescence of urine

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Abstract. Because cancer is a dreaded disease, a number of techniques such as biomarker evaluation, mammograms, colposcopy, and computed tomography scan are currently employed for early diagnosis. Many of these are specific to a particular site, invasive, and often expensive. Hence, there is a definite need for a simple, generic, noninvasive protocol for cancer detection, comparable to blood and urine tests for diabetes. Our objective is to show the results of a novel study in the diagnosis of several cancer types from the native or intrinsic fluorescence of urine. We use fluorescence emission spectra (FES) and stokes shift spectra (SSS) to analyze the native fluorescence of the first voided urine samples of healthy controls ($N=100$) and those of cancer patients ($N=50$) of different etiology. We show that flavoproteins and porphyrins released into urine can act as generic biomarkers of cancer with a specificity of 92%, a sensitivity of 76%, and an overall accuracy of 86.7%. We employ FES and SSS for rapid and cost-effective quantification of certain intrinsic biomarkers in urine for screening and diagnosis of most common cancer types with an overall accuracy of 86.7%.
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Keywords: biomarkers; cancer diagnosis; flavins; porphyrins; Stokes' shift spectra; urine.

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1 Introduction

As much as 15 to 20% of the population in every country is affected by cancer and the disease ranks next only to cardiovascular and infectious diseases in causing severe morbidity and mortality. This is a major global concern and any curative or palliative treatment is effective only when cancer is detected early. It is not uncommon that, by the time a patient comes to a doctor with noticeable symptoms of cancer, the disease has progressed to an advanced stage.

The three major diagnostic approaches are (1) biophysical imaging techniques such endoscopies, computed tomography (CT) scans, and magnetic resonance imaging (MRI); (2) biochemical techniques based on specific tumor markers such as prostatate-specific antigen (PSA) and alpha-fetoprotein (AFP); and (3) biopsy followed by histopathological or cytopathological confirmation. While biopsy is certainly regarded as the most reliable and direct method for disease evaluation, it is invasive, stressful to the patients, and beset with unexpected complications. Hence, often this is used as the final test for confirming and evaluating the disease condition and not considered suitable for a preliminary or routine checkup.

A diagnostic protocol for a routine checkup of symptomatic and asymptomatic subjects should be simple, noninvasive, inexpensive, and easily accessible in a large number of primary health centers. The protocol presented here meets most of these requirements reasonably well and is comparable to a Pap smear or acetic acid test for screening for cervical cancer; or more closely to the preprandial blood and urine test for diabetes mellitus.

Optical diagnosis of cancer is done based on the laser/light-induced fluorescence, laser Raman spectra, reflectance/scattering spectra, or a combination of these.[1–3] Out of these, analysis based on native or intrinsic fluorescence of tumor tissue has gained considerable importance[4] since 1924. Sev-

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eral groups have made important contributions along this line. Pioneering works were done by Alfano et al.[5,6] on Ar-ion-laser-induced fluorescence from cancer diseased rat models and on human tissues and on *in situ* lung cancer of human patients.[7] The work of another group[8] was based on 365-nm xenon-laser-induced fluorescence of human tumor tissues of different etiologies. They observed characteristic peaks of the different forms of porphyrin at 600, 630, and 690 nm and flavins at the 520- to 530-nm range in many tumors. A large number of similar results appeared subsequently, and among them, nitrogen-laser-induced fluorescence of oral carcinoma of 75 patients was one of the earliest reports, which showed[9] a sensitivity of 85%, and another report on gynecological malignancy of about 20,000 patients[10] gave abundant support to this line of approach. According to some of these groups, tumor tissues have distinguishable characteristic fluorescence around 630 nm due to endogenous porphyrin. A few more groups, particularly, Ramanujam[11] and Ramanujam et al.,[12] analyzed premalignant or early stages of cervical cancer tissue, mostly taking NADH, collagen, and flavin as biomarkers. They sometimes also employed a fluorescence emission-excitation matrix.

While most of the studies were based on fluorescence emission spectra, Alfano et al. came up with novel modifications in fluorescence spectroscopy. They employed Stokes shift spectroscopy (SSS) of a number of biofluorophores such as tryptophan, flavins, etc., and showed that normal and malignant breast tissues differ in the relative concentration of tryptophan and collagen.[13,14] Similar studies were extended to cervical cancer tissues by Ebenezer et al.[15]

Moving on from laboratory investigation, clinical trials, particularly in cancer of gastrointestinal tract[16] and urinary bladder cancer[17] have been done based on laser-induced fluorescence (LIF). Currently, LIF bronchoscopes and LIF endoscopes are available in the market to identify features of early stages of cancer that would eventually transform into carcinoma.

While fairly extensive studies were done on tissue characterization, only a few reports have concerned the spectral analysis of blood for cancer detection, perhaps because the complex composition of blood was believed to show a myriad of interfering signals. The native fluorescence spectra of blood sera as a function of different wavelength, as evaluated by Wolfbies et al.,[18] gave abundant importance to biofluorophores such as NADH, flavin, etc., which had overlapping emission bands. This group adopted a topographical representation to elucidate the differential components. Another group observed distinct differences in the UV region between the sera of healthy and cancer patients.[19]

A fairly detailed study was done by our group on the native fluorescence of the acetone extracts of blood of cancer diseased animal models and humans.[20–22] We observed that cancer patients had considerable elevation of porphyrin and flavins in comparison to healthy controls. In another study, based on analysis of 85 cancer patients of different etiology and 60 healthy controls,[23] we showed that tryptophan and flavins in the plasma of cancer patients were themselves sufficient to indicate malignancy of different organs.

Urine is a common diagnostic body fluid for a few maladies, and the tests are based on biochemical analysis and colorimetry. A few researchers have worked on fluorescence emission spectra[24] (FES) and SSS for diagnostic monitoring of multicomponent systems such as urine. Particular mention can be made of laser-induced fluorescence detection of pteridine in urine,[25] which measured biomolecules indicative of malignancy and *ex vivo* fluorescence cytology for bladder cancer,[26] which measured exfoliated cancer cells.

In a double-grating spectrofluorometer, such as the one used here, the excitation grating is kept fixed to choose one wavelength of excitation for the sample. The broadband fluorescence is scanned by the emission grating to obtain FES. In fluorescence excitation spectra (FXS), we select one particular wavelength, at the peak of the emission band, and rotate the excitation grating to get the excitation spectra (which often coincides with the absorption spectra). To obtain SSS, both gratings are scanned with a constant offset, i.e., $\Delta\lambda = 20$ or 70 nm, depending on the experimental requirements. An SSS is more complex than the FES, but gives better resolution and identification of weakly fluorescing molecules. For example, if $\Delta\lambda = 70$ nm and the scanning range is 200 to 800 nm, the excitation grating could be set at 280 nm and the emission grating at 350 nm and a fluorophore (in this case tryptophan) with an excitation peak at 280 nm would get detected. As both gratings rotate, different fluorophores would be preferentially excited and the spectrum of each would be recorded.[14] In the experimental conditions reported in this paper, we obtain excitation spectra of different fluorophores, since the Stokes shift is around 70 nm for most of the fluorophores of interest for this paper.

Following our analysis of blood components for cancer detection, we assumed that some of the fluorophores carried by the bloodstream[21,23] could be excreted through urine as well. The results of the study presented here give strong support for our expectation. We collected first voided urine samples of 100 healthy volunteers and 50 confirmed cancer patients. The FES and SSS spectra of these cases were obtained. A set of ratio parameters were defined based on the excitation and emission of the biomolecules. These ratio parameters were fed into a discriminant analysis, which gave a classification accuracy of 86.7%.

2 Materials and Methods

2.1 *Materials*

The fluorophores, porphyrins, riboflavin, NADH, and bilirubin were obtained in their purified forms from Sigma Aldrich (United States). Phosphate-buffered saline (PBS) was obtained from the pharmacy.

2.2 *Instrumentation*

The FES and SSS were obtained using the spectrofluorometers Elico SL174 (India), and Perkin Elmer LS45 (United States). Both instruments have the feature to choose an excitation/emission/SSS scan ranging from 200 to 800 nm. The spectral resolution and scan speed were kept constant at 10 nm and 500 nm/min, respectively. The spectra (excitation, emission, and synchronous) were all calibrated with the standards such as tryptophan and rhodamine supplied by the manufacturers.

2.3 *Patients*

Investigation was carried out on the first voided urine collected from 100 healthy volunteers (controls) and 50 cancer patients (of different etiologies); the healthy and diseased subjects consisted of both sexes, in equal number in an age group from 25 to 65 (median age was 50). Of the cancer subjects, 8 cancers were of breast, 10 of cervix, 5 of colon, 5 of leukemia, 6 of esophagus, 10 of liver, and 6 of bladder. All patients were waiting for treatment either at GVN Hospital, Trichy, India, or Riyadh Medical Complex, Riyadh, Saudi Arabia. They had cancer of different stages, as confirmed by histopathologists. Cancer of different etiology is classified differently, since our intent in this paper is mainly to discriminate the diseased from the normal urine, all cancer patients were required to be grouped by histopathologist as early and advanced only. Informed consents were obtained from the participants under the guidance of ethical committee of these two hospitals. All the participants, both the controls and the patients, were advised to refrain from medicines and spicy foods for 24 h prior to sample collection.

2.4 *Experimental*

Urine samples of 20 ml were collected in sterile plastic containers, kept at 10 °C, and analyzed within 4 h. There were no problems with any photodecomposition of the samples during handling because the fluorescence of urine analyzed after 20 min and after 4 h gave almost identical results. The 2 ml of urine was drawn into the quartz fluorescence cuvette for analysis. Since PBS has the closest background to urine, we have performed background subtraction for all our spectra with reference to PBS, which was almost nonfluorescent.

3 Results

To bring to focus the difference in the spectral features urine from healthy and diseased individuals, the corresponding spectra are given in one figure or one below the other. Wherever required, spectra were normalized with reference to the most prominent band. All FES were obtained with the excitation wavelength at 400 nm with all other operational parameters such as slit width, photomultiplier tube (PMT) voltage, etc. kept constant; similarly all SSS were with $\Delta\lambda = 70$ nm.

Figure 1 (line a) is the average FES of the urine of healthy controls ($N = 100$). This has a peak at 485 nm, a shoulder at around 444 nm, and a weak band at around 615 nm. Figure 1 (line b) is the corresponding average FES of the urine of early cancer patients ($N = 16$) of different etiologies. We can see in Fig. 1 (line b) that there are four bands, namely. 444, 485, 515, and 615 nm. (Note the weak band at 675 nm is henceforth ignored as it is inconsistent.) In contrast, in Fig. 1 (line a), the band around 515 nm is almost absent. The distinct difference between the two spectra is unmistakable. Figure 1 (line c) is the average FES spectrum of the urine samples of patients with advanced cancers of different etiologies ($N = 34$). One can see that the band around 515 nm gets largely shifted to 530 nm due to excessive concentration of flavin and bilirubin to the level that the bands at 444 and 485 nm are almost swamped. The spectra of urine samples from cancer patients of different etiologies are presented here to show that the FES depend more on the severity of cancer than on etiology. That is, FES of urine of all early stage cancer looks similar to what is shown in Fig. 1 (line b). In the same manner, all advanced cancer patient samples of any etiology look alike, as shown in Fig. 1 (line c).

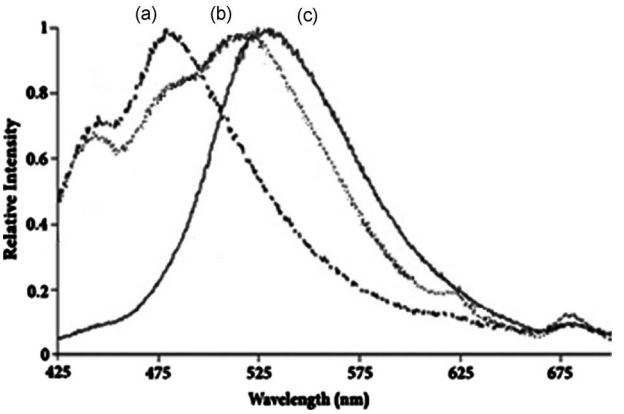

Fig. 1 Average FES of the urine of healthy controls (a), of urine of early cancer cases (b), and of urine of advanced cancers of different etiologies (c).

It is well known from many reports on biofluorescence that the 444-nm band is due to NADH bound to a protein and the 485-nm band is due to unbound, free NADH, 515 nm is due to flavins and the band at 615 nm is due to porphyrin.[18] We also confirmed this by spiking these fluorophores to the healthy urine and recording the increase in fluorescence at that mentioned wavelengths. Figure 2 (line a) is the spectrum of a typical healthy urine sample and Fig. 2 (line b) is the spectrum taken, for the same urine sample, under identical conditions, but with a small speck of additional riboflavin, which is spectroscopically identical to the flavoproteins, FMN (flavin mononucleotide) and FAD (flavin adenine dinucleotide), as shown by Koenig and Schneckenburger.[27]

Having identified the different fluorescence bands in urine (444 nm, bound NADH and 485 nm, free NADH; 515 nm, flavins; 555 nm, bilirubin; 615 nm, porphyrins), we could see that flavoproteins (possibly bilirubins) and porphyrins are elevated but both NADH are depressed in the urine of cancer patients.

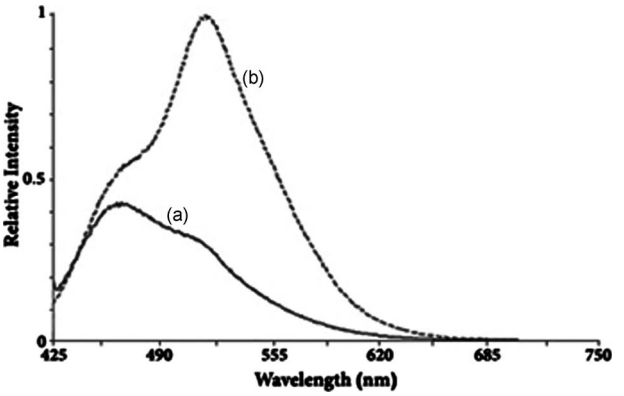

Fig. 2 FES of the urine of a healthy control (a) and the same sample, with a speck of riboflavin added (b).

To quantify these elevations, we have defined a set of fluorescence ratio parameters as following: PK/444, R515/444, R555/444, and R615/444. For instance, the ratio of fluorescence intensity at the peak to the fluorescence intensity at 444 nm is denoted by PK/444. We have taken the intensity at 444 nm, found consistently in healthy as well as diseased urine, as the reference point for all our future discussions. We define R515/444 as a ratio between flavin and bound NADH, R555/444 as the ratio between bilirubin and bound NADH, and R615/444 as the ratio between porphyrin and bound NADH.

Flavin and bilirubin have overlapping bands and since flavin is more fluorescent than bilirubin, the contribution of bilirubin is not distinct and prominent in Fig. 1 (lines b and c), yet it could be identified in synchronous spectra of the preceding sample with $\Delta\lambda = 10$ nm (not shown).

We can see that the concentrations of some of these fluorophores go out of proportion as malignancy sets in. Note also that the shift in the peak wavelength from 485 to 525 nm as we move from the samples of healthy urine to that of those with advanced cancer. One can see that all the three cases exhibit distinct features. The most striking difference is the marked enhancement of a peak at 515 nm due to flavin and the dramatic decrease in the peak at 444 nm. When the disease is advanced, the peaks at 485 and 444 nm are almost absent.

The contrast between the healthy and diseased urine can be seen in SSS with greater clarity. Note that wavelength offset ($\Delta\lambda$) should be carefully chosen to elucidate different components. Though we had tried other wavelengths of offsets ($\Delta\lambda = 10$, 20, 30, 50, 70, and 100 nm) in this study, we report with $\Delta\lambda = 70$ nm, as this gave the clearest distinction. As mentioned earlier the SSS with $\Delta\lambda = 70$ nm gave the excitation peaks of individual components in a multicomponent system such as urine.

In our case, we could get one clear, distinct excitation band at 365 nm and another double band at 440 nm and around 475 nm, as shown in Fig. 3. This figure is to be compared with SSS of NADH, flavin and bilirubin, shown in Figs. 4(a)–4(c), respectively. Note especially that flavin is many times more fluorescent than bilirubin, though both have overlapping bands at 475 nm. These values of excitation bands of NADH, flavins, and bilirubin are in good agreement with those reported earlier.[11,12,18] Also note that the long-wavelength bands at 430 and 470 nm of a normal urine sample are red shifted to 450 and 475 nm for urine from a cancer patient. This is to be compared with the red shift mentioned in Fig. 1.

One can see that the ratio parameter $R460/365 \approx 0.34$ and $R475/365 \approx 0.25$ for healthy [Fig. 3(a)]; and 0.58 and 0.64 for early cancer [Fig. 3(b)], and 1.35 and 1.6 for advanced cancer [Fig. 3(c)]. That is, flavins and bilirubin both are elevated in cancer (in comparison to NADH), as seen from the SSS spectra shown for early and advanced cancer.

Having seen that FES with excitation at 400 nm and SSS with $\Delta\lambda = 70$ nm showed clear distinction between the normal and the diseased urine, we wanted to test the similarity in the spectral features of blood plasma.

Figure 5 shows the close similarity between the biomarkers in plasma [Fig. 5(a)] and urine [Fig. 5(b)] through SSS,

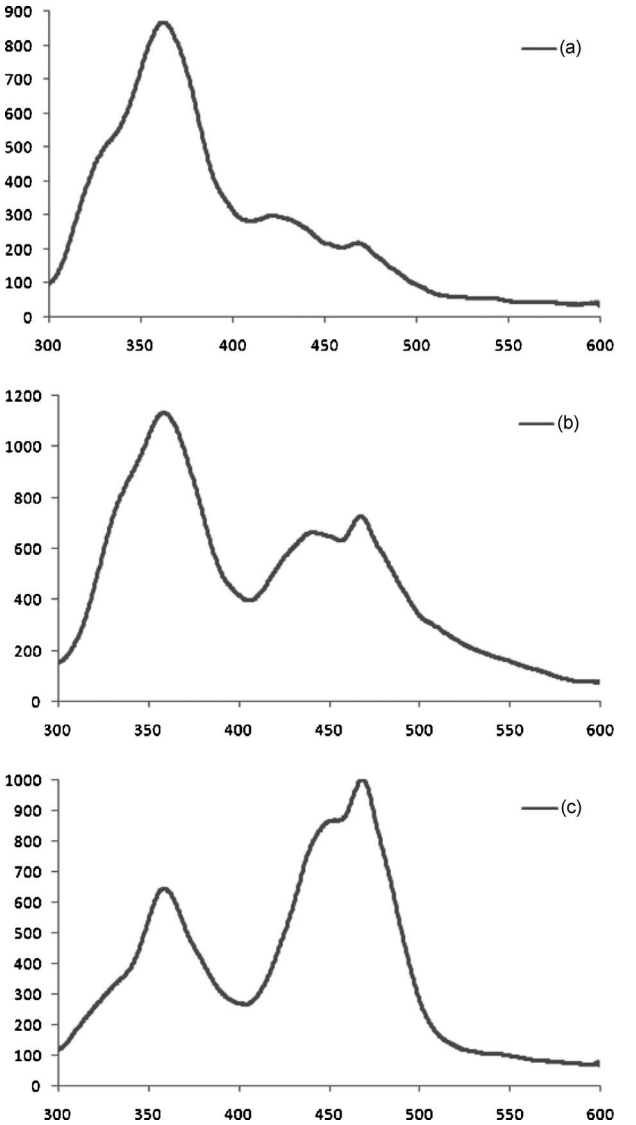

Fig. 3 SSS spectra of urine of (a) healthy control, (b) early cancer sample, and (c) advanced cancer sample.

with $\Delta\lambda = 70$ nm; this set is from a hepatoma patient (48 F). In both the spectra, flavin (460 nm) and bilirubin (475 nm) are elevated more than twice in comparison with NADH (365 nm). In contrast, in the plasma of a normal control, as shown in Fig. 5(c), the bands at 460 and at 475 nm both are only half the intensity of the band at 365 nm. Similar arguments hold for the SSS of the urine of normal control, as shown in Fig. 3(a). (Note also that this set of spectra was started from 325 nm to avoid confusion with the SSS of tryptophan, which is very strong in plasma and very weak in urine.)

Whereas blood is a circulating fluid and urine is a localized excretory fluid, pleural effusion is a localized fluid in close contact with the affected organ, the lungs. If the ratio of concentration of flavin and NADH is a reliable indicator of cancer, a similar trend could be expected in pleural effusion and also in the tumor tissue, the latter being the reservoir from which all the biomarkers are carried through. Figure 6(a) shows the FES of lung cancer tumour tissue (75-year-old fe-

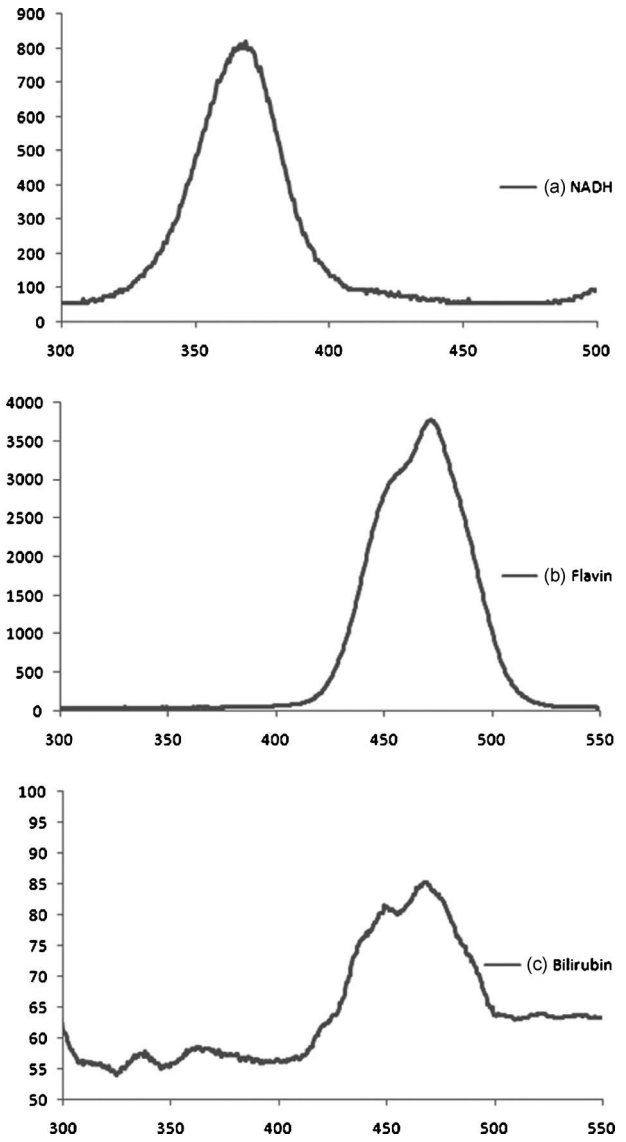

Fig. 4 SSS of (a) NADH, (b) Flavin, and (c) bilirubin.

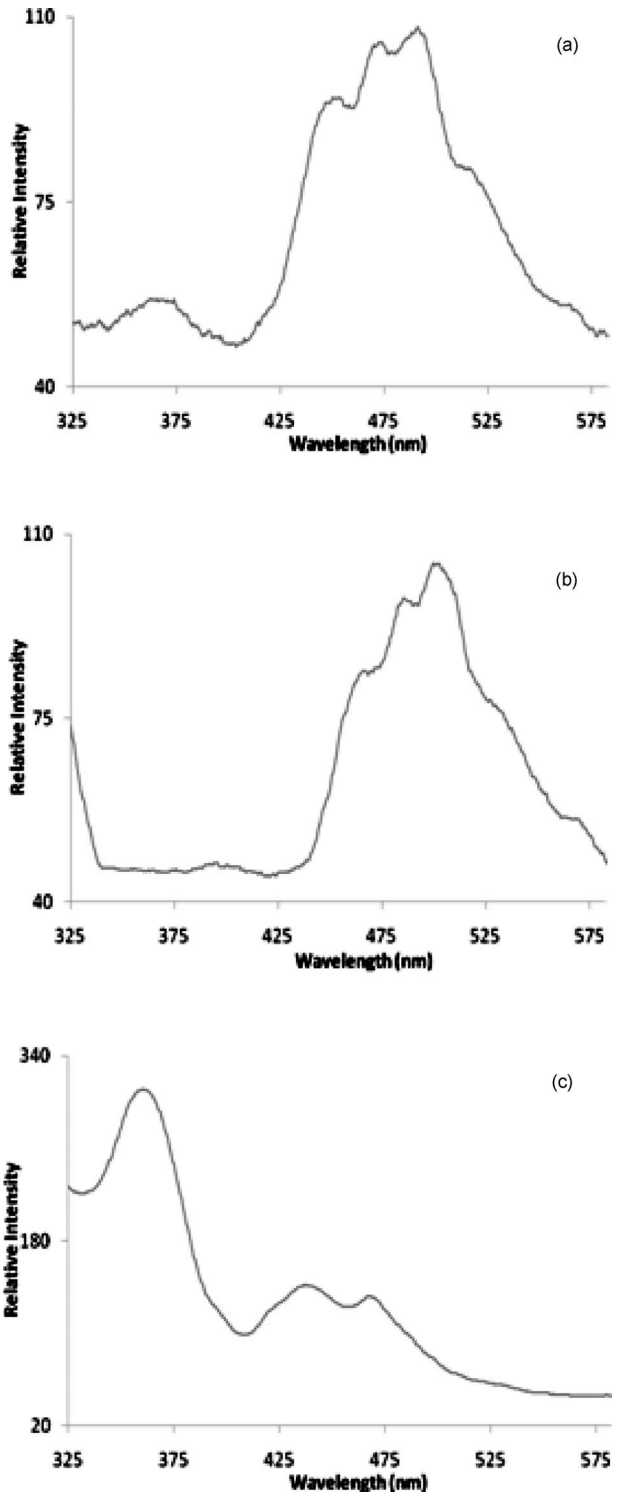

Fig. 5 SSS spectra of (a) the plasma of a hepatoma patient, (b) the urine of a hepatoma patient, and (c) the plasma of a normal control.

male, adenocarcinoma). Figure 6(b) is the pleural effusion, Fig. 6(c) is the plasma, and Fig. 6(d) is the urine of the same lung cancer patient. Here again, the one-to-one correspondence among the four samples of the same cancer patient is quite obvious. Note that the FES of lung cancer tissue has the same features and shape of the urine FES, but the whole spectrum is slightly blue shifted by 15 nm, since the local environment of the tissue is different. Note also the band at 615 nm in the urine of the lung cancer patient is as distinct as in the tumor tissue [Figs. 6(d) and 6(a)].

The common feature for all the preceding samples from cancer patients is the elevation of bands around 515 (flavin), 555 (flavin and bilirubin), and 615 nm (porphyrin) in comparison to 485 or 444 nm, due to the two forms of NADH.

4 Statistical Analysis

We performed statistical analysis to discriminate diseased patients from healthy patients using seven predictors: six of them being ratios from fluorescence intensity of different spectra and the seventh one, the peak wavelength shift in FES. All seven predictors were used as input for statistical analysis using SPSS/PC 10.0.1 software. Though our spectra show a clear distinction between early and advanced stages of cancer, we put all stages of the diseased patients into one group and the healthy controls into another group for statistical analysis,

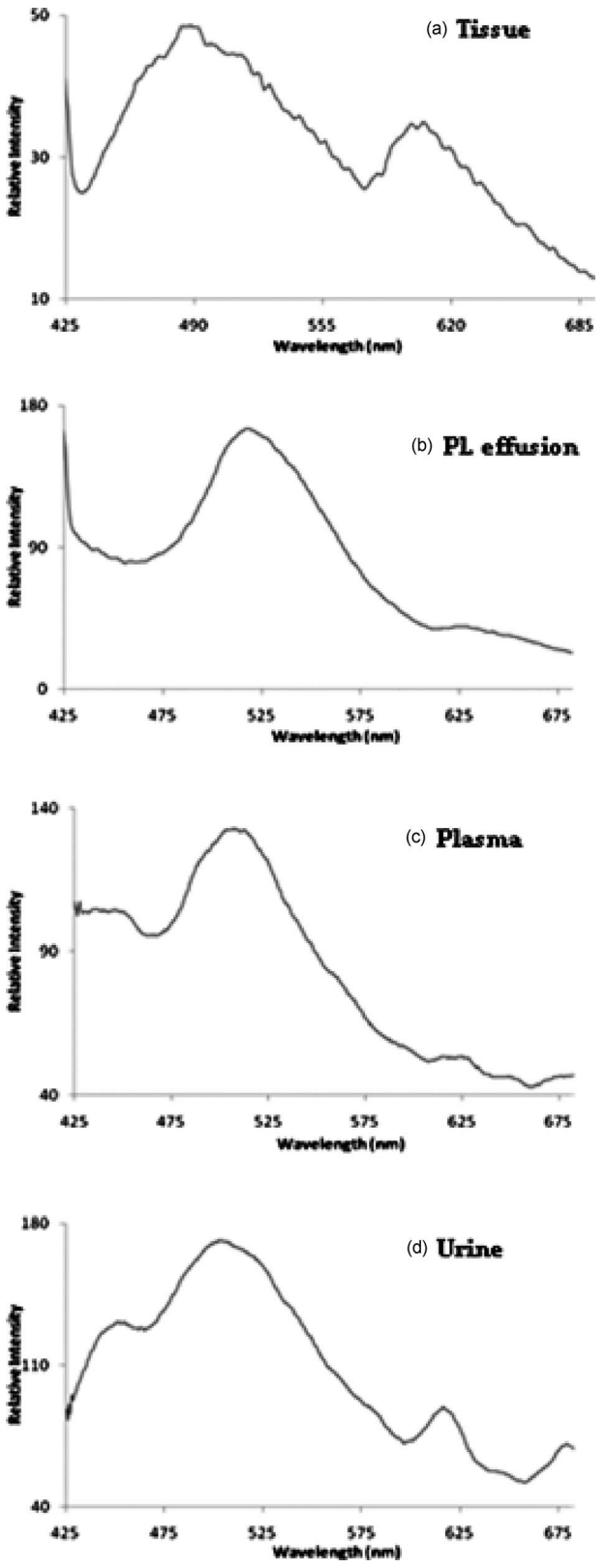

Fig. 6 FES of (a) lung cancer tumor tissue, (b) lung cancer pleural effusion of the same patient, and (c) lung cancer plasma of the same patient, and (d) lung cancer urine of the same patient.

since the number of early cancer samples were only a few ($N=16$).

4.1 *Groupwise Statistics and* t *Test*

The groupwise statistics are shown in Table 1. It can be seen that the mean of each predictor of diseased urine is well separated from that of the healthy. Note that the cancer patients were in different stages, with varying severity of the disease, so the spread of data is wider for cancer patients when compared to healthy subjects [see the standard deviations (SD) for peak wavelength, R4 or R5].

4.2 *Receiver Operator Characteristic Analysis*

A receiver operator characteristic (ROC) curve was plotted for each of the parameters to determine the optimal cutoff value that would give the maximum sensitivity and specificity (Fig. 7). The area under the curve (AUC) for each curve is shown in Table 2. The AUC must be closest to 1 for maximum discrimination efficiency. Along with AUC, the 95% confidence interval range (lower and upper bound) is also considered in selecting the parameters that best discriminate samples from healthy from those from cancer diseased patients. We can see that the parameters, peak wavelength, R515_444, R555_444, R615_444 (representing FES), and R470_365 (representing SSS) have high AUC and hence higher sensitivity and specificity. The cutoff values for each parameter and the corresponding sensitivity and specificity are shown in Table 3.

4.3 *Discriminant Analysis*

Using the discriminant analysis (DCA) function of the SPSS software, we tried different combinations of parameters to see if a combination of these parameters could discriminate the cases better than any single parameter alone. As expected, the five parameters with highest AUC and 95% confidence interval, in the ROC curve, when combined, gave the best discrimination (Table 4). Out of the 100 healthy cases and 50 diseased cases which were included in the DCA, 92 healthy cases and 38 diseased cases were correctly identified. This gave a sensitivity of 76% and a specificity of 92%. Overall, 86.7% of the total cases were correctly classified. Hence, we can conclude that 86.7% of the unknown cases can be classified correctly.

5 Discussion

The basis of disease detection and diagnosis based on tumor markers is that there are definite changes in the biomolecular composition within the tumor cell or in the matrix environment. In our earlier papers, we showed that autofluorescence of the blood components themselves could be used for disease diagnosis since porphyrin in formed elements[21] and flavins and tryptophan in plasma of cancer patients are elevated twice or even thrice.[23] A comparison between the urine of the healthy control and that of the cancer diseased subject shows distinct features as viewed through FES and SSS.

The difference comes in the form of relative increase in the concentration of three biomolecules (flavins, bilirubin, and porphyrins) with respect to the concentration of another biomolecule (bound NADH). The change in the concentration is not 10 to 20%, but in a significant range of 50 to 200%.

Table 1 Group statistics.

	Healthy Control		Cancer Cases		
Parameters	*N*	Mean (SD)	*N*	Mean (SD)	Significance
Peak wavelength	100	484.06 (6.21)	50	502.40 (12.13)	0.000
R1=R515_444	100	1.73 (0.73)	50	2.52 (0.90)	0.001
R2=PK_444	100	1.38 (0.74)	50	2.27 (0.74)	0.045
R3=R555_444	100	0.737 (0.41)	50	1.46 (0.60)	0.000
R4=R615_444	100	0.316 (0.15)	50	0.656 (0.32)	0.000
R5=R460_365	100	0.283 (0.46)	50	0.860 (1.14)	0.000
R6=R475_365	100	0.727 (0.27)	50	0.938 (0.23)	0.048

Table 2 AUC of the ROC.

				95% Confidence Interval	
Parameters	Area	Std. Error	Significance	Lower Bound	Upper Bound
Peak wavelength	0.932	0.019	0.000	0.894	0.969
R1	0.842	0.035	0.000	0.773	0.910
R2	0.900	0.025	0.000	0.851	0.948
R3	0.908	0.023	0.000	0.862	0.953
R4	0.874	0.031	0.000	0.813	0.935
R5	0.791	0.041	0.000	0.711	0.871
R6	0.713	0.044	0.000	0.627	0.799

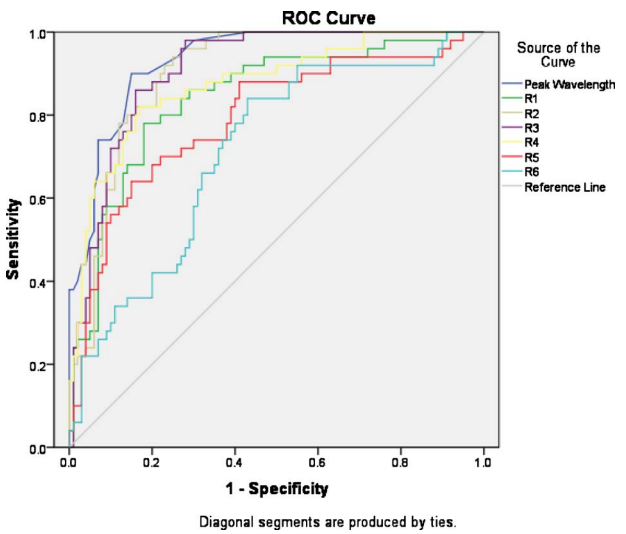

Fig. 7 ROC curves for each of the parameters.

Table 3 Sensitivity and specificity of predictor parameters.

Parameters	Cutoff Value	Sensitivity (%)	Specificity (%)
Peak wavelength	487.5	90	85
R1	1.85	78	82
R2	1.49	94	75
R3	0.916	86	84
R4	0.397	82	83
R5	0.390	64	85
R6	0.779	84	57

Table 4 DCA classification summary.

		Predicted Group Membership		
	Group	Healthy Control	Cancer Cases	Total
Original count	Healthy control	92	8	100
	Cancer cases	12	38	50
%	Healthy control	92.0	8.0	100.0
	Cancer cases	24.0	76.0	100.0

Note that 86.7% of original grouped cases were correctly classified.

Since urine is complex in composition, there are many major and minor fluorophores with overlapping bands.[18,24] The fluorescence band at 444 nm is most likely due to bound NADH and, to some extent, is due to collagen and pyridoxal phosphate. Similarly, the band at 485 nm is mostly due[28] to free NADH. The co-enzymes, NAD and NADH are commonly involved in cellular metabolism, with the former being nonfluorescent. The NADH bound to protein has an emission peak around 444 and unbound NADH at 485 nm, with the former being more fluorescent than the latter. The band at 515 nm is due to FMN and FAD, with the former dominating, since FMN was found to be more fluorescent than FAD.

Another important point is that the fluorescence peaks of these fluorophores are red shifted considerably in the urine of cancer patients, mostly due to the high concentration of flavins and also possibly due to the acidic microenvironment. Such a red shift could itself be a strong, reliable indicator for disease progression as it shifts from 450 to 530 nm for advanced stages of cancer.

In a series of papers on native fluorescence of cervical cancer tissue,[13,28] it has been shown that NADH with fluorescence at around 450 to 475 nm and structural proteins such as elastin and collagen (with emission around 420 to 440 nm and an excitation band around 320 to 340 nm) all decrease. This is because as healthy tissue gets transformed into malignant tissue, the extracellular matrix consisting of collagen and elastin is degraded.[28] There could be significant transformation of NADH from the bound state to the free state, when malignancy sets in, as shown by others from a similar spectral study on cancer tissues. The overall effect is a decrease in fluorescence around 444 nm in tumor tissue. Our investigation on tumor markers in urine strongly corroborates this observation and to make the discrimination unambiguous, we have taken the ratio of other fluorescence peaks with respect to the intensity at 444 nm.

Flavonucleotides such as FMN and FAD are essential molecules involved in redox reactions and electron transport in the mitochondria. Many researchers, like us, have observed enhancement of the fluorescence peak at 515 nm due to FMN in tissue in animal tumor models and cancer patients. The most direct support for our result is the work on rat tumors at different sites, native fluorescence of human neoplasm of the bronchial tree, light-induced fluorescence in human cervical cancer,[28] and native fluorescence of bronchial neoplastic lesions as excited with a 407-nm laser.[29] They all show almost the same spectra that we have reported in Figs. 1 and 2. In our experiment with lung tumor tissue [Fig. 6(a)], pleural effusion [Fig 6(b)], plasma [Fig. 6(c)], and urine [Fig. 6(d)] of the same patient, there is a significant one-to-one correlation among FES of all samples from cancer patients. In short, an out of proportion in intensity at 515 nm and a strong red shift from 500 to 530 nm (due to flavins) in urine, blood plasma, pleural effusion, and in tissue is a strong indication of cancer.

What is more striking is that in the case of the urine of advanced cancer patients there is a clear band at 615 nm, as distinct as in tumor tissue (at 620 nm). Observation of red fluorescence from the tumor tissue is well established and dates back to 1924 and is used for endoscopic inspection of a tumor prior to photodynamic therapy with a laser. The specific accumulation of porphyrin at a tumor site has been variously attributed to microbiological activity, heme degradation, or enzymatic imbalance set by cancer cells resulting in insufficient supply of ferrochelatase that could not keep up with the synthesis of porphyrin.[27] Nevertheless, the fact that a tumor site exhibits distinct red fluorescence both due to endogenous porphyrin (native fluorescence of the tumor) and due to an accumulation of exogenous hematoporphyrin derivative or 5-aminolevulinic acid (labeled fluorescence of tumor) is universally accepted.

Some of the endogenous porphyrins found in tumor tissue, particularly protoporphyrins, could be carried along by the bloodstream and could serve as a tumor marker, as we previously established.[21] The results presented in this paper show that porphyrin excreted most probably as uroporphyrin in urine could also do well as a tumor marker as it is considerably elevated in the urine of cancer patients. In a nutshell, what other researchers have identified as generic tumor markers on tissue, we have done in urine and blood.[23] Often urine has been considered to carry the biomarkers only of cancers of the urinary system (bladder, kidney, etc.). However, our study is as true for renal and bladder cancer as much as for cancer of a remote organ such as the lungs. All this is because certain fluorescent biomarkers are released in excess due to malignancy of any organ, which are carried through by the circulating blood and excreted in urine.

Our spectroscopic data indicate a marked increase in flavin, bilirubin, and porphyrin, but a decrease in NADH in the urine of cancer patients. This would strongly support the hypothesis that red fluorescence associated with tumor tissue is most likely due to disruption of heme biosynthesis and degradation. The origin of heme may be from the cytochrome of the cell or from the erythrocytes in blood. Some of the enzymes released by the cancer cells could degrade heme rapidly into bilirubin and porphyrin by simultaneously depleting the coenzyme NADH, because for one decayed heme molecule two NADH molecules are used up. More biochemical investigation is essential to confirm the preceding arguments.

6 Conclusion

We have reported the result of our investigation on the diagnosis of cancer from the spectral analysis of urine. By employing the FES and SSS techniques on untreated urine, we have shown the distinctive features of the urine of persons with cancer. Note that this technique is true for cancer of any etiology as we are targeting certain generic fluorescent bio-

molecules, produced by the excessive proliferation and degeneration of cells, and not the exfoliated cells of any organ.

In conclusion, we have demonstrated the feasibility of a simple, noninvasive, inexpensive protocol for the detection of cancer based on the spectral analysis of urine. This is comparable to the preprandial blood and urine analysis for diabetes mellitus (DM) and could be developed in the future to become part of general health checkups in symptomatic and asymptomatic subjects. It will also find application during or after cancer treatment for repeated monitoring of regression or recurrence.

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