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Lung Cancer Detection by Native Fluorescence Spectra of Body Fluids—A Preliminary Study

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Abstract Lung cancer takes a heavy toll every year, since the survival rate is not more than 15%. In this paper, we present results of a novel technique based on the autofluorescence of body fluids like blood plasma, acetone extract of cellular components, sputa and urine of lung cancer patients (N=27). A set of ratio parameters based on the fluorescence peaks of tryptophan and elastin, in plasma and sputum; flavin, NADH (reduced nicotinamide adenine dinucleotide) and porphyrin in urine; porphyrin alone in acetone extract of formed elements, were all evaluated. Similar sets of ratios were obtained for age adjusted normal controls (N=27) and all these ratios were given as inputs to multivariate (principle component and discriminant) analyses, which showed that the two groups could be classified with an accuracy of about 90%. Since the instrumentation involved was an ordinary steady state Xe lamp based spectrofluorometer, the technique is of significant advantage in screening and early detection of lung cancer in high risk population such as heavy smokers.

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Introduction

Lung Cancer is the commonest cancer in the world today, accounting for 12% of all new cancers and 18% of all cancer deaths [1]. Lung cancer has poor prognosis and is the cause of death in more than 90% of affected individuals [2]. 5-year survival rates are found to be less than 15% on the average for all lung cancer patients [3]. Many techniques have been employed for screening, early detection, diagnosis and prognosis of lung cancer [4, 5]. However, the most commonly used imaging techniques such as CT scan or MRI are not cost effective and access to these instruments is difficult, particularly in developing countries. Also techniques based on biochemical methods to quantify biomarkers like carcino embryonic antigen (CEA), Tumor M2-Pyruvate Kinase, RNA binding protein hnRNPBI, etc. are inadequate, in terms of sensitivity and specificity [6, 7]. In practice a panel of biomarkers, rather than only one, has given significant clinical advantage particularly in monitoring relapses [4, 6].

Recently, research based on native fluorescence and Raman signals of malignant tissues, a technique known as optical biopsy, is gaining importance [8, 9]. The pioneering works done by Alfano et al. [10–12] on a variety of tumour tissue and the recent introduction of stokes shift spectroscopy (SSS), which is equivalent to the synchronous fluorescence excitation spectroscopy (SFXS), used throughout this paper; and by Rebecca et al. [13] have shown the thrust in new avenues of cancer diagnosis. From the level of laboratory investigations, it has come out into clinical use, particularly as fluorescence bronchoscopes [14, 15]. In

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spite of extensive work done on native fluorescence of malignant tissues, very little has been done on the native fluorescence of body fluids for the detection of lung cancer.

In our earlier reports, we had employed fluorescence emission spectra (FES) and SFXS, of body fluids to identify and quantify different fluorophores. Based on the ratio of intensities of spectral bands in the UV-Vis region, we could detect most common cancers with 80% sensitivity, by the above said spectral analysis of blood plasma [16–18].

We had also shown in our earlier publication that acetone extract of cellular components of blood alone could show sensitivity and specificity, in the range of 80% and above, even when cancer of different etiology were taken altogether [19]. This was based on elevation of certain forms of porphyrin. Subsequently, it was shown that tryptophan of blood plasma alone is again capable of discriminating cancer diseased plasma from normal, with 80% accuracy. Similar level of sensitivity, specificity and accuracy were shown by the differential native fluorescence of flavins, NADH and porphyrin found in urine [20].

In this study, native fluorescence of blood, urine and sputa of a set of lung cancer patients, prior to treatment, were analysed. This shows that native fluorescence spectral parameter of these body fluids could discriminate the diseased samples from the normals with an accuracy of greater than 90%.

As there were only 7 of 27 samples in the early stages of lung cancer, we have confined our investigation, in this preliminary study, only to demonstrate distinct discrimination between the lung cancer set (early and advanced) and normal control set.

Methods and Materials

The instrument used was a spectrofluorometer (Perkin Elmer LS45), capable of taking excitation, emission and synchronous spectra in the range of 200 to 800 nm. We have used excitation and emission slit width of 10 nm and a scan speed of 1000 nm/min. All fluorescence emission spectra (FES) were obtained by exciting the sample at 400 nm, and scanning the emission band at 425–675 nm. All Synchronous Fluorescence Excitation Spectra (SFXS), were obtained by rotating the two gratings synchronously, but with an offset of 70 nm ($\Delta\lambda$ 70); such SFXS gives the excitation spectrum of each fluorophore found in the tissues [12] and body fluids [17, 20, 21] with greater resolution and sensitivity.

Experimental samples were obtained from the following sets: 27 lung cancer patients (17 males and 10 females; median age of 55) and 27 age adjusted normal controls (17 males and 10 females; median age of 55). All the lung cancer patients were sampled after histopathological confirmation but before the onset of treatment. Since this is a preliminary report, all lung cancer patients were included irrespective of stage, type and grade. All subjects were from King Khalid University Hospital, Riyadh and King Saud Medical Complex, Riyadh. Informed consents were obtained from all the subjects after the approval of the ethical committees of the two institutions.

Exactly 5 ml of intravenous fasting blood from each subject was drawn into EDTA vial, gently rocked for even mixing of anticoagulants and then centrifuged at 4000 rpm for 15 min. The supernatant plasma was stored at 4 °C; and was taken out and allowed to reach room temperature before analysis. The maximum time lapse between sample collection and analyses was not more than 24 h. The cellular component, sedimented at the bottom, was taken in another sterile container and twice the amount of acetone was added; this was shaken for few minutes before centrifuging at 4000 rpm for 15 min. Then the supernatant, containing the dissolvable biofluorophores, was spectroscopically analyzed. All samples of sputa and urine were obtained in sterile vials, by standard procedures and stored refrigerated at 4 °C. Both the sputa and urine were analyzed without any further treatment. Each of these samples was taken in fused quartz cuvette (1 cm × 1 cm × 4 cm); and the sampling geometry was transverse. That is, sets of synchronous, excitation and emission spectra were collected at 90° to the incident beam. We never measured the absorbance of samples, as absorption—re-emission was not an issue for our experiment.

Thus, for the lung cancer and normal sets, we have four different samples: (1) blood plasma, (2) acetone extract of cellular components of blood, (3) sputa and (4) urine.

We have employed multivariate analysis for statistical evaluation of the experimental data thus acquired, using the standard SAS (Statistical Analysis System) 9.0 package.

Results

Figure 1(a) gives the FES of acetone extract of cellular component of blood of a typical normal control (55 M). This spectrum has a band around 470 nm due to the combination of the raman band of acetone (see the bump at 450 nm) and the fluorescence of NADH in residual plasma; another at 585 nm due to basic form of protoporphyrin and yet another at 630 nm due to neutral form of protoporphyrin [16, 22]. If we define a ratio $R_1=I_{630}/I_{585}$, i.e. the ratio of intensity of peak at 630 nm and that at 585 nm, this is about 1.12 for normal; similarly $R_2=I_{630}/I_{470}$ is about 0.4. In contrast R_1 and R_2 is about 2.9 and 2 for the lung cancer patient (70 M, Squamous Cell Carcinoma). That is, 630 nm peak is elevated more than twice for the diseased in comparison to the normal control, which has been shown in typical spectrum of a lung cancer patient (Fig. 1(b)). From Table 1, it can be seen that the mean for R_1 is 1.12

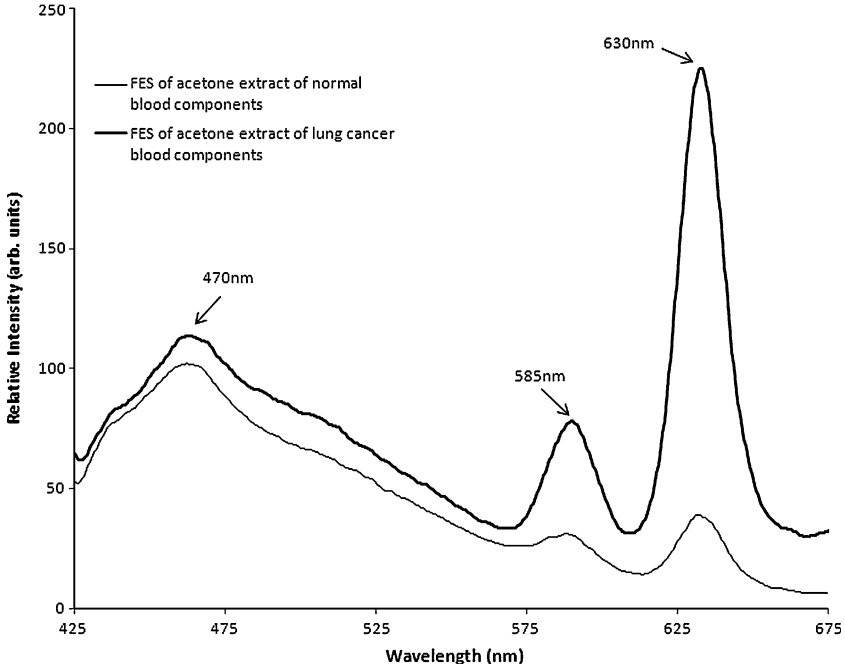

Fig. 1 FES of acetone extract of blood components for normal and lung cancer sample

and standard deviation (SD) is 34% and for R_2, the mean is 0.43 with SD of 50% for the normal control. In contrast, for the diseased, the mean for R_1 is 3.38 and SD is 59% and for R_2 the mean is 1.6 (with SD of 100%). That is, porphyrin has a wide variation in the diseased blood, depending upon the severity of disease. In any case, even the mildest diseased blood samples have porphyrin twice elevated than the average of normal controls.

Figure 2(a) gives the typical SFXS of normal blood plasma. This spectrum has a band at 280 nm (due to tryptophan) and another at 360 nm (due to NADH), yet another at 440 nm (due to flavin). Note that, in our case, SFXS gives the excitation, equivalently, the absorption bands of fluorophores. It can be seen that $R_3=I_{280max}/I_{min}$ (maximum intensity of tryptophan band which occurred at 280 nm, over the local minimum of the same band which occurred around 310–335 nm) is 6 and $R_4=I_{327}/I_{360}$ is 0.6 for normal; we do not consider other bands in this paper. Fig. 2(b) is the typical SFXS of plasma of lung cancer patient. The remarkable elevation of 280 nm band and also the skew of this band up to 295 nm are unmistakable. The ratio R_3 is 12.6 and R_4 is 0.8 for the plasma of the cancer patients, which means R_3 and R_4 are elevated 200% and 30% respectively, for the plasma of the cancer patients. Figure 2(c) gives SFXS of plasma of another lung cancer patient (70-year-old female, squamous cell carcinoma). This particular plasma is marked by the distinct (excitation) band at 327 nm, due to the elevation of structural protein, elastin, about which details has been given in our earlier report [21]. Note that, this band also has a shoulder at 295 nm. The ratio $R_4=I_{327}/I_{360}$ is about 1.1 for the diseased but is only about 0.6 for normal. For the case of 2(c), the new distinct band has occurred at 327 nm, making the ratio $R_4=1.1$, and since this fills the minimum of 280 nm band, R_3 is only 2.45. Table 1 shows the wide variation of R_3 and R_4 particularly for the plasma of the cancer patients (SD≈ 93% for R_3 and 32% for R_4).

Figure 3(a) gives the SFXS of sputum of normal control. There are three bands of interest, one at 275 nm, another at 360 nm and yet another at 440 nm, similar to the SFXS of normal plasma, except that the band at 280 nm is blue shifted to 275 nm, for this sputum. Figure 3(b) gives the SFXS of sputum of cancer patient (65-year-old male, adenocarcinoma of lungs). It can be seen that there is a distinct band at 327 nm in the case of lung cancer patient's sputum. The presence of 327 nm band is more persistent and prominent in lung cancer sputum than in the blood plasma. We define the ratio R_5 as I_{327}/I_{360} for sputum and listed out in Table 1 for the normal and cancer patients. This is 34% elevated for the cancer patients

Figure 4(a) gives the FES, excited at 400 nm, of typical normal urine. There are three bands of interest, one at 475 nm (due to NADH), another at 515 nm (due to flavin) and yet another at 615 nm (due to porphyrin). Another set of ratio parameters $R_6=I_{515}/I_{475}$, $R_7=I_{555}/I_{475}$ and $R_8=I_{615}/I_{475}$ were evaluated and this is about 0.9, 0.56 and 0.21 for the normal control. The longer wavelength part of NADH has strong overlap with the short wavelength region of flavin. In order to avoid or reduce some overlap, the intensity at 555 nm is compared with the intensity at 475 nm. In fact, this ratio has proved to have very good sensitivity for cancer detection by native fluorescence of

Table 1 Group statistics of all 9 ratio parameters

	Variable	Mean	Std Dev	Std Error	Minimum value	Maximum value	95% Confidence Low limit	95% Confidence Up Limit	
Normal	R1	1.13	0.38	0.07	0.63	2.17	0.97	1.28	
	R2	0.44	0.20	0.04	0.17	3.00	0.33	0.75	
	R3	4.13	1.48	0.28	2.61	9.09	3.55	4.72	
	R4	0.77	0.10	0.02	0.55	0.98	0.73	0.52	
	R6	0.90	0.14	0.03	0.64	1.24	0.85	0.96	
	R7	0.56	0.13	0.02	0.28	0.79	0.51	0.61	
	R8	0.21	0.06	0.01	0.08	0.34	0.18	0.24	
	R9	0.23	0.09	0.02	0.07	0.44	0.19	0.26	
	Variable	Mean	Std Dev	Std Error	Minimum value	Maximum value	95% LW limit	Confidence UpLimit	Contrast
Lung Cancer	R1	338	2.00	0.38	0.96	7.80	2.55	4.18	3.00
	R2	1.60	1.72	0.33	0.34	8.35	0.92	2.28	2.97
	R3	16.87	15.71	3.02	2.45	69.4	10.66	23.09	4.08
	R4	0.96	0.32	0.06	0.38	1.91	0.84	1.09	1.25
	R6	1.51	0.42	0.09	0.86	4.54	1.32	1.91	1.78
	R7	1.09	0.44	0.08	0.43	4.01	0.91	1.47	2.12
	R8	0.54	0.21	0.04	0.26	1.30	0.46	0.67	2.67
	R9	1.58	1.23	0.24	0.08	10.16	1.13	2.79	8.60

urine [20]. Figure 4(b) gives the typical FES of urine of a lung cancer patient. The distinct differences are obvious. For urine of a cancer patient, the band at 515 nm is well elevated about 70%. That is, the concentration ratio between flavin and NADH is 1.7 times more for urine of cancer patient. Similar contrasts in ratios are given in Table 1.

In order to improve the sensitivity, for urine, SFXS was also taken. Figure 5(a) shows SFXS of urine of a normal control and 5(b) shows that of a cancer patient. Here 350 nm band is the fluorescence excitation band of NADH and that at 450 nm is for flavin. It is easily seen that the ratio $R_9=I_{450}/I_{350}$ is only about 0.14 for normal, but for the urine of cancer patient this ratio is 2.8. This ratio R_9 has a wider variation for the cancer patients than for normal controls (SD of 80% versus 40%). In any case, the mean R_9 of the of cancer patients is 7 times more than that of normal controls.

The data for sputa were available only for 15 patients (as there were technical difficulties to get sputa from old patients). Hence, ratio parameter R_5, which is the ratio of elastin and NADH in sputa, was eliminated. The ratio parameters R_1, R_2; R_3, R_4; R_6, R_7, R_8 and R_9 were taken for further statistical analysis.

Statistical Evaluation

The two sets of data with 8 variables, one for the normal (code 0) and the other for lung cancer diseased (code 1) were put through three statistical analyses, employing SAS package.

In the first step, the mean, standard deviation (SD), standard errors and 95% confidence interval (CI) were calculated individually for each variable from R_1 through R_9, as shown in Table 1. (Standard error represents SD/√N, where N is 27, in our case, the number of normal or cancer samples; this gives a measure of overall deviation of particular data from the mean. 95% CI means that 95% of the data, for example, for R_1 falls within the small range of 0.97 to 1.28). It can be seen that the range of variation in each variable is smaller for the normal (for R_1, minimum value is 0.63 and maximum value is 2.17) than for the cancer patients (0.96 and 7.8 respectively) and the contrast is best for R_1, R_3 and R_9.

We have obtained Pearson Correlation coefficient, which establishes possible correlation between two variables (when two variables are positively correlated, both increase together with a constant ratio; when negatively correlated, one increases while the other decreases; when close to zero, they are independent). Out of these variables only for those which have correlation coefficient close to 1 and level of significance of less than 0.05 are considered further. As seen in Table 2, R_6 and R_7 are highly and significantly correlated (i.e. correlation is >0.98 and level of significance

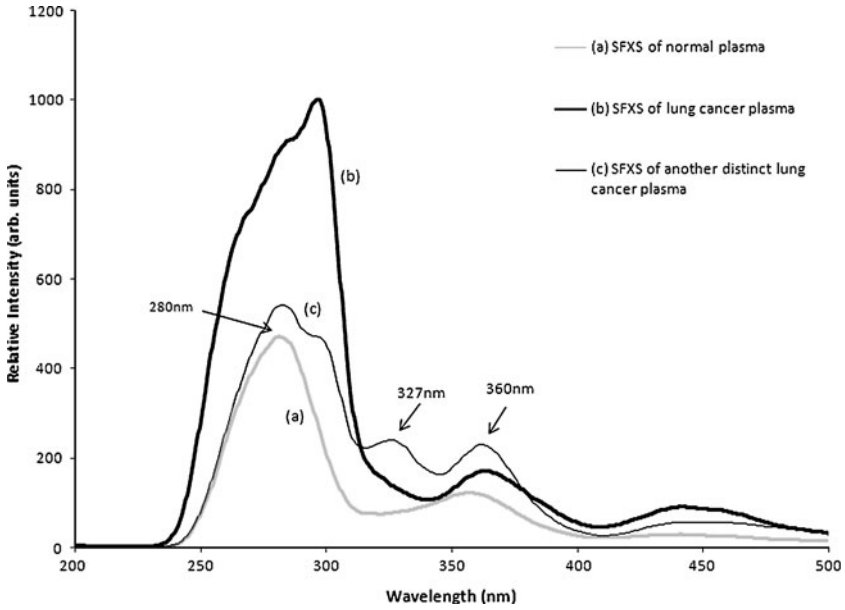

Fig. 2 SFXS of plasma for normal, a lung cancer sample and another distinct lung cancer sample with a peak at 327 nm

is <0.0001); similarly are R_7 with R_8; R_8 with R_9. This means that all these four variables (R_6, R_7, R_8 and R_9) are inter-related. Out of these four variables, we took only R_9 for further analysis, since this has much better contrast. Similarly, we have good correlation between R_1 and R_2; out of this only R_1 was preferred, since contrast between normal and diseased is better and SD is less. Similarly a good correlation was found between R_3 and R_9, and R_3 was preferred. Thus we have three independent variables, R_1 (for cellular components), R_3 (for plasma) and R_9 (for urine).

In order to understand the relative significance of each of these variables and dispersion of the data, principal component analysis (PCA) was done. Each PC is a linear combination of eight original variables (eigenvectors), so that the purport of the data could be gauged with few parameters. The coefficients of this linear combination are shown in Table 3. For example, principal component, PC1=0.26R1 + 0.21R2 + …… + 0.38R9. These three principal components are capable of giving approximately 81% of essential features of the data.

Figure 6 shows the scatter plot of the normal set (code 0) and diseased set (code 1) in the two dimensional perspective, with each dimension being represented by the principal components, 1 and 3, with PC1 along y-axis and PC3 along x-axis. One could notice that: a) the two sets are well separated out, b) R1 and R2 belong to one cluster,

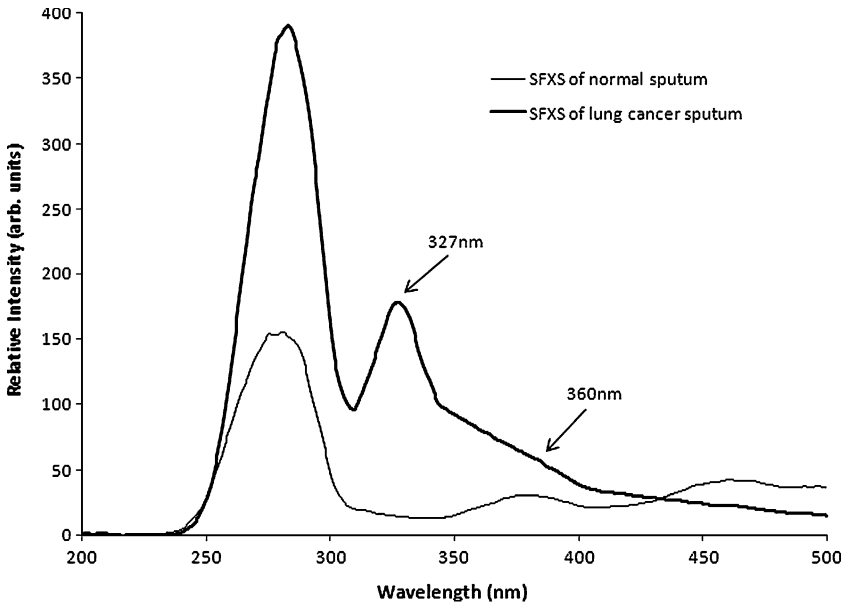

Fig. 3 SFXS of sputum for normal and lung cancer sample

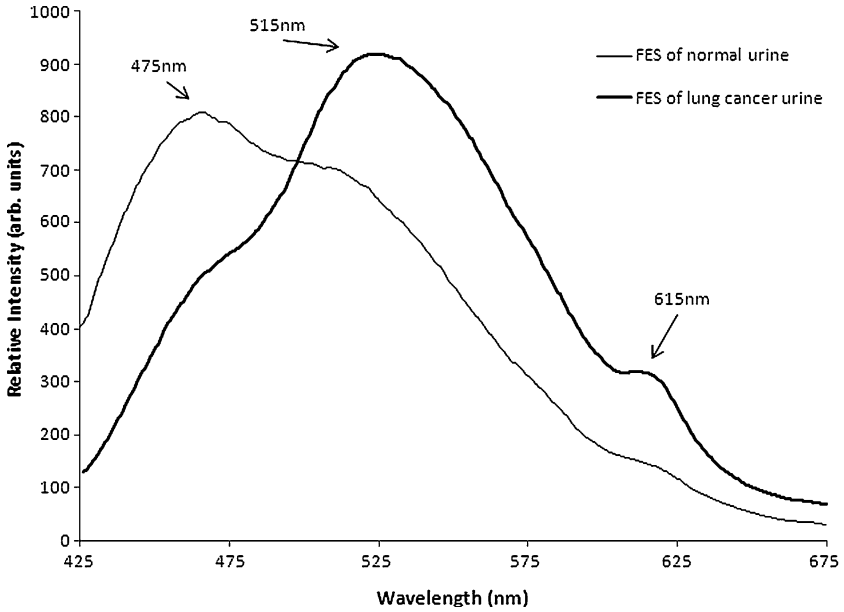

Fig. 4 FES of urine for normal and lung cancer sample

c) similarly R6, R7 and R8 belong to another cluster and, d) R3 and R9 belong to yet another cluster. These are in good agreement with Pearson correlation coefficients obtained.

We then applied multivariate step-wise discriminant analysis (MVSDA) to find out Fisher's function, which is a linear combination of the best original variables that could separate two groups distinctively. It showed that R_1, R_3 and R_9 are the best, reinforcing the results of principal component analysis with level of significance of 0.05. This also showed that out of 27 histopathologically confirmed diseased samples, 24 were correctly classified as diseased by spectral analysis, leading to a sensitivity of 89%; all the clinically found normals were correctly classified by spectral analysis as normal (specificity of 100%). The apparent error rate is 3/54=5.5%. The above error rate is calculated with a priori weightage of 50% for normal and 50% for the diseased (see Table 4).

Discussion

Any cancer biomarker study is based on the fact that malignancy, with abnormal cell proliferation and degeneration upsets the biochemistry inside and outside the cellular matrix. Many of the amino acids, proteins, metabolites go out of proportion when malignancy sets in, and out of them some are detectable by fluorescence techniques. The

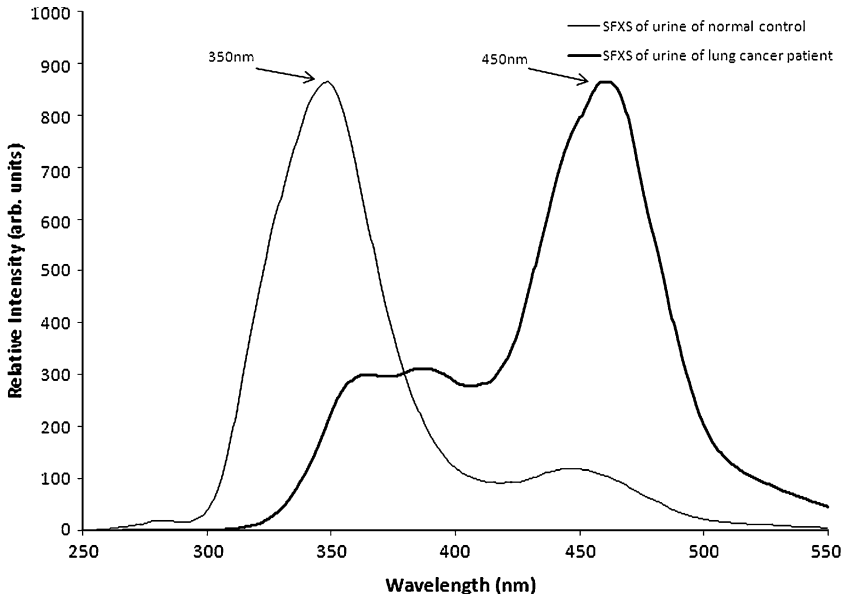

Fig. 5 SFXS of urine for normal and lung cancer sample

Table 2 Pearson correlation coefficients between all 9 variables

Pearson Correlation Coefficients, N=54								
Prob > |r| under H0: Rho=0								
	R1	R2	R3	R4	R6	R7	R8	R9
R1	1	0.67465	0.31718	0.15266	0.25911	0.24864	0.34345	0.21694
R1		<.0001	0.0194	0.2704	0.0585	0.0698	0.011	0.1151
R2		1	0.28232	0.14866	0.17055	0.20133	0.2293	0.12333
R2			0.0386	0.2834	0.2176	0.1443	0.0953	0.3743
R3			1	0.25848	0.1414	0.16009	0.36618	0.56438
R3				0.0591	0.3078	0.2475	0.0065	<.0001
R4				1	0.33973	0.31978	0.40725	0.44041
R4					0.012	0.0184	0.0022	0.0009
R6					1	0.9815	0.8386	0.46505
R6						<.0001	<.0001	0.0004
R7						1	0.824	0.49828
R7							<.0001	0.0001
R8							1	0.68425
R8								<.0001
R9								1
R9								

earliest report in this line is by Picard, who found the red fluorescence associated with tumour tissue. Subsequently research by a host of workers has identified number of fluorescent biomarkers of cancers [8–16].

In this line of research, the results of the present study, based on spectral analysis of blood, urine and sputa samples show that porphyrin is elevated thrice in the blood of lung cancer patients in comparison to the age adjusted normal controls (Fig. 1). It is important to mention that this abnormal kind of elevation is common to most of the bulk or diffused cancers. This elevation is only about 50 to 100% for certain low grade cancers (of prostate, and bladder); but 200 to 500% for high grade cancers (like pancreas). From this point of view, the results of this study with 3-fold elevation put lung cancers in high grade, virulent cancers category. Such elevation of porphyrin in blood is reflected in the urine too (R_8). Comparing the spectra of urine of normal control (Fig. 4a) and diseased (Fig. 4b), it can be seen that R_8, a measure of porphyrin elevation, is 2.6 times higher for diseased than for the normal urine. It is well known that porphyrin accumulates on the tumour tissue either due to inherent biochemical imbalance initiated by malignancy or due to microbial activity on the necrotic sites of tissue or both [22, 23]; hence the circulating blood could carry the excessive porphyrin and because of the same reason, this porphyrin metabolites might be excreted (as uro or copro or proto porphyrin with band at 615 nm) in urine. There could be another reason for the elevation of porphyrin: some of the

Table 3 Principal Components and dataset reduction

Eigenvectors/Variables	Prin1	Prin2	Prin3
R1	0.26	0.57	−0.27
R2	0.21	0.61	−0.29
R3	0.26	0.32	0.57
R4	0.28	−0.04	0.35
R6	0.43	−0.30	−0.31
R7	0.44	−0.28	−0.30
R8	0.47	−0.16	−0.04
R9	0.38	−0.05	0.45

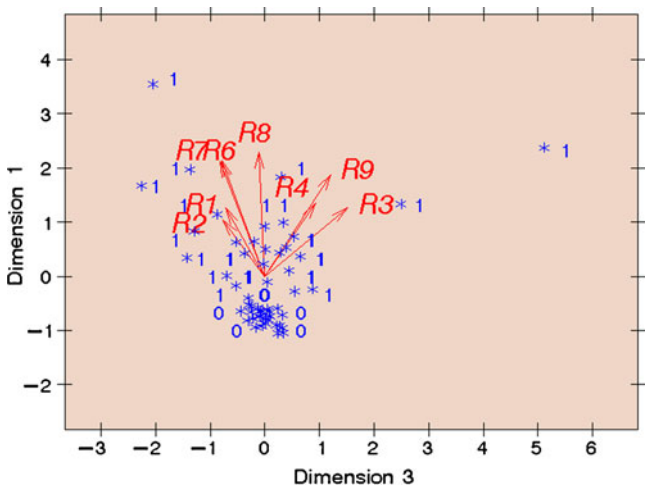

Fig. 6 Representation of eigenvectors in two dimensions (of principal components 1 and 3)

Table 4 Results of discriminant analysis

Number of Observations and Percent Classified into Group			
From Group	LUNG_CA	Normal	Total
LUNG_CA	24.00	3.00	27.00
	88.89	11.11	100.00
Normal	0.00	27.00	27.00
	0.00	100.00	100.00

enzymes released by the malignant cells may degrade hemoglobin of blood, releasing more porphyrin in the blood stream. Our investigation on spectral analysis of blood of pregnant women and people suffering from sickle cell anemia and tuberculosis indicate that porphyrin elevation is more likely due to cellular biochemical imbalance [24].

The close correlation by R_1 and R_2 from the statistical point of view is testified by the Pearson correlation coefficient (Table 2) and the principal component analysis (Fig. 6) which clearly show that R_1 and R_2 cluster together. Out of them R_1 was shown to be more important variable than R_2.

Tryptophan and tyrosine are essential amino acids involved in cell growth. It is no surprise that cancer cells have excess of tryptophan, as shown by stoke's shift spectroscopy of breast cancers [12] and cervical cancers [25]. Particular interest, for this work, is the spectra of normal breast cancer tissue [12], where ratio of intensity of tryptophan band is 3 for normal breast tissue but is 5 for the malignant breast tissue. That is, tryptophan level is elevated about 1.6 times when malignancy set in. This is very similar to what has been indicated in our results, as shown in Fig. 2, where R_3 for normal is about 6, but varies from 7 upwards to as high as 57.5 (for 22 out of 27 lung cancer patients). In other words, there is one-to-one correlation between the SFXS of tissue and plasma. Another important observation is that tyrosine (275 nm) and tryptophan (280 nm) have overlapping excitation bands, though their emission bands are well separated (315 and 360 nm respectively). As can be seen in Fig. 2, the 280 nm band get red shifted to 295 nm for cancers. If we take a ratio of I_{295}/I_{275} it varies from 0.9 to 1.5 for most cancer plasma; but is 0.7 for most normals (see Fig. 2). This would indicate that malignancy sets an imbalance in the concentration between tyrosine and tryptophan or even in conversion from tyrosine to tryptophan as they are structurally closely related molecules.

In the case of urine samples, FES and SFXS of normal and cancer sets show distinct differences, in terms of ratios R_6, R_7, R_8 and R_9. Out of this R_6 and R_7 are essentially the concentration ratio between flavins (520 nm) and NADH (emission band at 475 nm). Both are elevated about twice for cancers. The only difference between them is that R_7 is taken as a ratio between intensities at I_{555}/I_{470}, away from the residual overlap of NADH which has a long tail till 500 nm. In any case, this ratio is only marginally better than R_6. The ratio R_9, which gives the ratio of flavins (450 nm) with NADH (350 nm), is same as R_6 in principle, but the sensitivity is quite high. That is, the contrast ratio for R_6 is 1.7, but for R_9 is 7 (see Figs. 4 and 5). This would mean that SFXS is atleast 4 times more sensitive than the FES of the same sample containing different fluorophores. This is in good agreement with the observations of Alfano et al. [12].

Flavins, particularly FMN (flavin mononucleotide) and FAD (flavin adenine dinucleotide) are cofactors of many cellular metabolic activities. They are involved in oxidation-reduction processes in the mitochondria [10]. Hence, excessive cell proliferation and degeneration could lead to enhancement of these flavins and out of those FMN is 8-fold more fluorescent than FAD [23]. Perhaps, some enzymes of abnormal cells could convert FAD into FMN.

In all these ratios, R_6 to R_9, there is some significant contribution by bilirubin and biliverdin; since they have excitation band at 475 nm (which overlap with the 450 nm excitation band of flavins) and emission band at 555 nm (which overlap with the 515 nm emission band of flavin). However, the relative contribution of the two could not be quantified at this stage.

All the above discussed features are common for most of the solid or diffused tumours [19]. In fact, a host of cancers could be detected by spectral analysis of blood and urine with 80% sensitivity and specificity.

The distinguishing features of lung cancer are in the band at 327 nm in the blood plasma and sputa of lung cancer patients only. Out of a few thousand plasma samples that have been analysed for spectral diagnosis of cancer, this band does not appear in the plasma or sputa of normals or other cancers, or for other lung pathologies [21]. This biomarker is more persistent and more elevated in the lung cancer sputa than in the plasma. Inspite of all these, the 327 nm band, attributed most likely to the structural protein elastin and collagen, is present only about 75% of all lung cancer cases. Since structural protein elastin and collagen are abundant in lungs, when malignancy sets in, this protein may be more easily and copiously released into the blood stream [21].

When all the ratios from R_1 through R_9 were fed into PCA algorithm, only three variables R_1, R_3 and R_9 came out as independent variables, one for each; R_1 for acetone extract of cellular components of blood which measures porphyrin content, R_3 for plasma which measures tryptophan level and R9 which measure flavin. However multivariate analysis favoured R_8 instead of R_9, both of which are found to be inter-related in PCA.

In any case, with the three most important variables (R_1, R_3 and R_9), the sensitivity and specificity were found to be

around 90%. This would mean that in any future detection protocol in an assembly of normals and lung cancer patients, the latter would be separated out with an accuracy of 90%.

Conclusion

In this paper, on spectral diagnosis of lung cancers, a set of samples (blood, urine and sputa) of 27 lung cancer patients and equal number of age adjusted normal controls were analysed. Employing FES and SSS, a set of ratio parameters were obtained. These were given as inputs for multivariate statistical analysis, PCA and MVDA. It was found that porphyrin, elastin, tryptophan and flavins taken collectively could detect lung cancer samples with an accuracy of 90%, since all these biofluorophores were elevated 2 to 3 fold for lung cancer patients. The results of this study have important and significant impact in the future lung cancer detection and screening protocols. For example, when a high risk population such as men or women, above the age of 55, with 20 pack year (that is, with the habit of smoking 20 cigarettes a day for 20 years) is taken through screening or early detection procedure, low dose CT (computed tomography) scan is the only accepted technique, as of now. But this is expensive, involves considerable radiation risk, and may not be accessible to large segment of people, particularly in developing countries. In all these segments, this body fluid based spectral analysis, with an accuracy of 90%, could be of great value to complement and supplement the existing procedures.

In this study, different kind of lung cancers (squamous cell carcinoma, adenocarcinoma, small cell or non-small cell) and of different sites (bronchial tree, right or left lobe) were all taken together, mostly because limitation of getting enough number of samples of each category. As this is a preliminary study, our objective was limited to distinguish the features of lung cancer in toto from the normal controls. We plan to extend the study for lung cancer of different sites, grades and different categories.

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