Preliminary study of bladder cancer patients using spectral techniques

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In the current study, we had obtained the spectral features of biomolecules of blood plasma, urine and bladder wash of a certain set of the patients of bladder cancer. The fluorescent biomarkers such as tryptophan, tyrosine, collagen, elastin, flavin and porphyrin, in the above body fluids are distinctly from those of normal controls. This study indicates the potential application of optical biopsy for bladder cancer detection.

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1. Introduction

Bladder cancer is more common in developed than developing countries [1-2]. In the United States, bladder cancer is the fourth most common cancer in males with an age-adjusted incidence rate of 37.3/100,000 person years [2]. In the kingdom of Saudi Arabia, bladder cancer ranked ninth among male population and twenty fourth among the female population and affected 81.1% males and 19.9% females with a male to female ratio of 4:1. But in the Gizan province of kingdom, bladder cancer was the fourth most common malignancy overall with a chronic renal failure three times higher than the lowest incidence regions. Due to the relative abundance of free-lying water and widespread agriculture Schistosomiasis is common in Gizan Province and may be responsible for the large number of bladder Squamous Cell Carcinomas cases in this region [3-4].

Urothelial cancer often behaves as a field change disease in which the entire urothelium from the renal pelvis to the urethra is susceptible to malignant transformation. The multiple occurrences and recurrences of urothelial tumors that are treated by local resection are highly characteristic of this disease. However, urothelial carcinoma cells can also implant and probably migrate to other sites of the urothelium, thus making it difficult to determine whether a recurrent tumor represents an inadequately treated initial tumor, tumor migration, or the effects on multi focal carcinogenesis. On the basis of clinical and experimental data, it is likely that all these factors are important.

The gold standard for diagnosing bladder cancer is biopsy obtained during cystoscopy. A number of different urine tests look for specific substances released by bladder cancer cells. These tests may be used along with urine cytology to help determine if a person has bladder cancer. They include the tests for NMP22, bladder tumor antigen (BTA), and the Immunocyt test. Fluorescence cystoscopy as an adjunct to white-light (WL) cystoscopy was shown to significantly enhance the detection of Urothelial carcinoma of the bladder (UCC) [5].

Recent studies Immuno Cyt/uCyt+ testing are encouraging , The test is intended to be used on voided urine specimens in conjunction with cytology analysis and increases the overall sensitivity for all grades of tumor while maintaining the high specificity of conventional cytology [6] Per-patient specificity for hexaminolevulinate (HAL) alone and targeted HAL was 62.5% and 87.5%, respectively. The limitation of optical coherence tomography (OCT) is due to poor visualization of flat lesions in white light, making the scanning a time-consuming procedure [7]. Bladder tumor antigen (BTA) cannot be detected in urine cytology but it has been found in bladder neoplasms [8].

In this study, we have selected a set of normal controls (N=35), and bladder cancer N = 35) of plasma, urine and bladder wash samples (only for malignant). The stokes shift spectra (SSS) for all the samples were

recorded and analyzed on the basis of the relative intensity of bio fluorophores like tryptophan, tyrosine, collagen elastin, flavin etc. The experimental results of the study provide significant discriminatory spectral features in the plasma, urine and bladder wash samples for the monitoring or detection of bladder cancer.

2. Methods and Materials

2.1. Instrument

The instrument used in this study was a spectrofluorometer (Perkin Elmer LS55) which is capable of taking excitation, emission, and stokes shift spectra (SSS) in the range of 200–800 nm. We used an excitation and emission slit width of 10 nm and a scan speed of 1000 nm/min. Light of a specified wavelength with a spectral width of 10 nm and a spot size of 3 by 2 mm target the sample in quartz cuvette. The stokes shift spectra (SSS) with $\Delta\lambda$ = 70 nm only were taken. In this case, the emission and excitation gratings were rotated synchronously with an offset of $\Delta\lambda$ =70 nm so that all the bio fluorophores were scanned in the range between 200-600 nm [9].

2.2. Patients

All the blood and urine sample of bladder cancer patients were collected before the onset of treatment. All subjects were from King Khalid University Hospital, Riyadh. Informed consents were obtained from all the subjects after the approval of the Institutional Review Board (IRB) (approval number12/3594/IRB)

2.3. Blood plasma, urine & bladder wash

The investigations were carried out on three different samples: (1) blood plasma (2) urine (3) bladder wash and the procedure for collecting the samples are described below.

Exactly 5 ml of intravenous 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 taken out for spectral analysis. 5 ml of first voided urine was collected in a sterile vial from each subject and was used as such in our experiment to take SSS. Both samples were analyzed by the same techniques as reported in our earlier studies [10-14]. Each of these samples was taken in fused quartz cuvette (1 cm×1 cm× 4 cm) and the sampling geometry was transverse to the incident beam. All the subjects (patients and the control normal) were required to be free from any medicine for 24 hours prior to collection. Samples of bladder wash (17 patients) were obtained by injecting water into the bladder of the patients and then the bladder wash was collected to take SSS in our experiment.

3. Results

Here below we present the spectral features of plasma, urine, and bladder wash obtained from the known cases of patients of bladder cancer, most of them diagnosed with Urothelial cell carcinoma (UCC) (or papillary cell carcinoma). For comparison, spectral features of typical normal control and many other cancers are shown to high light the characteristic fluorescent biomarkers of UCC.

As shown by our earlier papers, it is the synchronous excitation spectra, more commonly known as stokes shift spectra (SSS) which give features of UCC distinctly different from others. Fig 1 (a) represents the SSS of plasma of a normal control (55 years, male). This shows that there are three well defined peaks namely at 290 nm (due to amino acid tryptophan), 360 nm due to co enzyme Nicotinamide adenine dinucleotide (NADH), and 450 nm due to metabolite flavin adenine dinucleotide (FAD). The identification of the above peaks had been established well in our earlier reports [10-14]. The intensity of 360 nm peak is about half of 290 nm and that of 450 nm is half of 360 nm. In contrast, for many common cancers, say, cancer of breast or liver, the spectral features are different, with the 450 nm and 290 nm peaks being significantly about twice higher in comparison to 360 nm band as shown in fig 1 (b) The above two figures could be compared with the high grade UCC shown in Fig. 1(c). The characteristic features of UCC are the appearance of a new band at 315 nm (most likely due to structural protein, collagen and elastin). Such elevation of 315 nm in SSS spectra had been observed in cancer of cervix too [15].

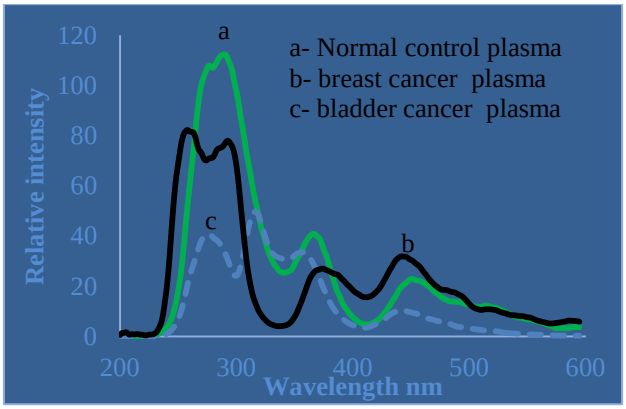

Fig. 1. (a) represents the SSS of plasma of a normal control (b) breast cancer plasma (c) bladder cancer plasma.

What gave us more important, clinically potentially useful result was the SSS of urine. Fig. 2(a) shows the SSS of normal urine; it could be seen that there is one to one correspondence between the SSS of plasma and SSS of urine, with the one exception that the characteristic peak at 290 nm due to amino acid tryptophan is totally absent in urine. (This is quite understandable because tryptophan is an essential amino acid and is restored back in the blood circulation by the kidney which excretes mostly metabolites found in blood). Fig. 2(b) shows the SSS of breast cancer urine. Here the peak at 400 nm due to FAD is 150% higher than the peak at 350 nm (due to NADH). This is because FAD is metabolite and this is excreted in abundance, as it is produced in abundance by the abnormal cell proliferation. This is characteristics of most common cancers. Fig. 2(c) shows the SSS of bladder cancer urine. The prominent peaks are at 290 nm (due to tryptophan), next at 315 nm (most likely due to structural protein). These are unique presently only in urine of bladder cancer patients.

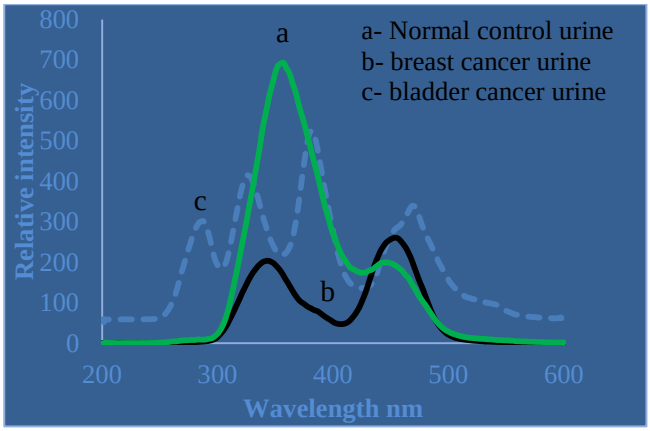

Fig. 2. (a) shows the SSS of normal urine (b) SSS of breast cancer urine (c) SSS of bladder cancer urine.

Fig. 3(a) shows the SSS of bladder wash of UCC. Here again there are three prominent peaks, one at 290 nm, next at 315 nm, then at 360 nm. In the bladder cancer urine also as shown in Fig. 3(b), there are four peaks one at 290 nm, second at 315 nm, third at 360 nm and fourth one at 450 nm. There is close similarity between the spectral features of plasma of Fig. 3(c), urine of Fig. 3(b) and bladder wash of Fig. 3(a) of UCC patients, but the major difference is that the 450 nm peak found in plasma & urine is largely unnoticeable in bladder wash as shown in Fig. 3(a).

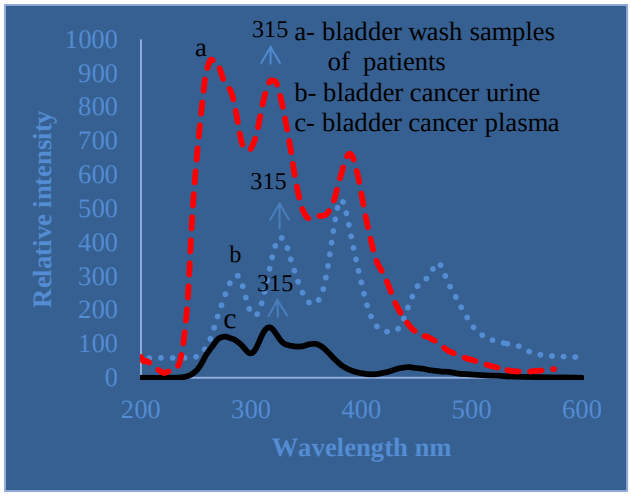

Fig. 3. (a) shows the SSS of bladder wash of UCC (b) SSS of urine (c) SSS of plasma.

There are few important points to which specific attention must be drawn.

(a) The 315 nm peak in plasma, or bladder wash or in urine appears only about 60 percent cases of bladder cancer (sensitivity about 60%).

(b) The peak at 315 nm appear in cervical cancer plasma too

(c) This peak at 315 nm appears only in the urine samples of UCC patients and never in normal or other cancers (specificity 100%).

(d) Though we could get the blood, urine samples of normal controls and many others cancer patients, we could not get bladder wash from any others for a more meaningful comparison of spectral features of bladder wash.

4. Discussion

Optical biopsy employs light as the probe for analyzing the healthy or diseased conditions of any person. This is an emerging field of non-invasive, sensitive, on line technique, particularly for detection of early stages of cancer. A variety of approaches have been employed and only one particular instrument, fluorescence based endoscope has come into clinical use.

In this line of research, our group has pioneered in the diagnosis of cancer [10-18] and hemoglobimnopathies [19] based on the spectral features of biofluorophores of blood and urine. This particular paper is one in that series which has highlighted the spectral features of urine of UCC.

Out of a host of biomolecules present in tissue and carried through in blood and urine, only a few such as tryptophan, NADH, FAD are fluorescent. They are present in normal as well as diseased condition. For a

serious malady like cancer, they go out of proportion. For example, the fluorescence peak at 450 nm is due to metabolite FAD is only 50% of the peak due to NADH and 25% of that of tryptophan in normal control plasma. But it is 150% of peak of NADH for cancer of HCC or pancreas [15-16]. Such abnormal elevation of FAD has been identified in tissue, plasma and urine, all because of the high level of metabolic activity associated with uncontrolled cellular proliferation of cancer. These features have been described in a number of our earlier papers.

However the following are the unique features of UCC

There is a new peak at 315 nm in about 60% of plasma, of cervical cancer and bladder cancer patients only. This peak is most likely due to the collagen present in abundance in soft tissues of bladder and cervix. Malignancy might erode the structural integrity and release structural protein into the blood plasma and get detected by the spectral analysis or another possibility is that 315 nm peak might correspond to the fluorescence due to circulating tumour marker (CTM), which is often-sought-after in cancer diagnosis.

The major difference between the samples of cervical and bladder cancer patients are in urine. Even though collagen is abnormally present in blood circulation of cervical cancer patients, it gets filtered in the kidney and restored in blood circulation. On the other hand, for bladder cancer patients, the urine carries the collagen available in abundance in the eroded lesions of malignancy. There is no way for these molecules to get filtered as bladder is the last reservoir of urine before excretion. The spectral feature of bladder wash corroborates this argument.

A bladder wash obtained by filling the bladder and stressing the bladder with plain water could induce mechanical pressure releasing the loosely bonded filamentary tissues and biomolecules. There was one to one correspondence between the urine and bladder wash with peaks at 290 nm (due to tryptophan) and 315 nm (due to collagen). These two peaks (290 nm, 315 nm) could be the spectral finger prints of CTM.

When malignancy sets in this corrodes and releases structural protein like elastin and collagen and these are our circulating tumour markers (CTM) captured in fluorescence spectroscopy. For all cancers the CTM is filtered by kidney but for bladder cancer there is no way of filtering because bladder is the last reservoir of urine and holds it for substantial time before excretion.

5. Conclusion

In this preliminary investigation with a very limited number of samples of plasma, urine, and bladder wash of patients of UCC, the potential of spectral diagnosis of body fluids of bladder cancer patients is indicated. This paper has given evidence for the first time about fluorescent circulating tumour markers. It is only a proof of concept. A more systematic study employing large number patients of different grades of UCC is planned in near future.

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Conflict of interest

The authors declare no conflict of interest.

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