Spectroscopic Analysis of Bladder Cancer Tissues Using Laser Raman Spectroscopy

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ABSTRACT:

The present study investigated the spectroscopic changes in bladder cancer tissues, compared with normal bladder tissues, using laser Raman spectroscopy.

FT Raman spectrometer, supplied with Nd: YAG laser as an excitation light source, was used to investigate fourteen bladder samples, collected from seven patients during surgery from different hospitals without any pre-treatment. Seven samples were normal, according to histopathology examination, while the others were cancerous samples.

Significant spectroscopic differences between the normal and cancerous bladder tissues were found around 1650 cm^{-1}, 1440 $cm^{-1,}$ 1270 cm^{-1} and 1080 cm^{-1}.

The results showed that laser Raman spectroscopy is a rapid, accurate, powerful, nondestructive, and easy to use as an alternative diagnosis method for bladder cancerous tissues.

Keywords: laser Raman spectroscopy, laser spectroscopy, bladder malignancy diagnosis.

1. INTRODUCTION:

Tumors are of two basic types: benign and malignant. By definition, benign tumors do not invade adjacent tissue borders nor do they metastasize to other sites. The primary descriptor of any tumor is its cell or tissue of origin. Most human malignancies arise from epithelial tissue and are called carcinomas. The “gold standard” in most cancer diagnostics is microscopic evaluation, by a pathologist, of a stained tissue obtained from biopsy of a particular organ (this procedure is called pathologic histology or histopathology).

This analysis is somewhat subjective. Additionally, in some cases (about 10%), a pathologic examination may not produce a firm diagnosis, either because certain tumors are histologically similar or because cells are so poorly differentiated that their tissue of origin cannot be determined. In these cases, other diagnostic procedures might be useful and include, but are not limited to, electron

microscopy, immunohistochemistry, cytogenesis, and levels of various tumor markers in the patient's serum or urine – and may be someday vibrational spectroscopy [1].

Raman spectroscopy is an optical technique that utilizes molecular specific, inelastic scattering of light photons to interrogate biological tissues. When tissue is illuminated with light, photons interact with intermolecular bonds present within the tissue. When occurs, the photon donates energy to or receives energy from the bond, producing a change in the bond's vibrational state. When it subsequently exits the tissue, the photon has an altered energy level and, therefore, has a different wavelength compared to the original light. This change in the photon's energy is known as the 'Raman shift' and is measured in wavenumbers. Photons interacting with different biochemical bonds within the tissue, undergo different Raman shifts, which taken together, form the 'Raman spectrum'. The Raman spectrum is a plot of intensity against Raman shift, in wavenumbers, and it is a direct function of the molecular composition of the tissue and can therefore give a truly objective picture of the pathology [2]. In this sense, Raman technique would be able to perform quantitative and qualitative analysis, since it shows high sensitivity to small biochemical changes in biological tissues [3].

To determine whether Raman spectroscopy can be used to differentiate between normal, inflammatory and malignant bladder pathologies in vitro, and secondly if it can be used to grade and stage transitional cell carcinoma (TCC), 1525 Raman spectra were measured from 75 bladder samples comprising normal bladder, cystitis, carcinoma in situ (CIS), TCC and adenocarcinoma. The results showed that Raman spectroscopy can be used to accurately identify and grade/stage TCC in vitro. The technique also has potential to provide immediate pathological diagnoses during surgical procedures [4].

Raman microprobes also have been used for the characterization and identification of renal lithiasis. The future would see the development of optical fiber probes to incorporate them into catheters, endoscopes and laparoscopes that will enable the urologist to obtain information during the operation [5].

A novel approach to noninvasively probe the composition of endogenous materials concealed deeply within mammalian tissue was presented by Nicholas Stone and Pavel Matousek in 2008 [6]. The method relies upon transmission Raman spectroscopy and permits the detailed characterization of the chemical composition of the probed volume. Both calcium hydroxyapatite and calcium oxalate monohydrate have been chemically identified from depths of up to 2.7 cm within a breast phantom made up of porcine tissues. The technique has shown significant potential to provide complementary data in the early diagnosis of breast cancer [6].

Raman spectroscopy also was used by B. Brożek-Płuska et al. in 2008 to obtain new results for the normal, malignant and benign tissues. This was a statistically reliable research on Raman spectroscopy-based diagnosis of

breast cancers where 321 spectra were recorded from 44 patients among the world's women population. The paper demonstrates that Raman spectroscopy is a powerful medical diagnostic tool with the key advantage in breast cancer research [7].

Conventional Raman spectroscopic studies of normal and malignant stomach tissues were carried out by K. Kalyan Kumar et al. in 2007 [8]. The aim of that study was to explore the feasibility of discriminating these tissues. Raman spectra of normal and malignant tissues exhibited significant differences in amide I, CH_2 and amide III region. The major spectral features of normal tissue with respect to malignant tissue are: weak amide I and an intense band at 1303 cm^{-1} and a hump at 1276 cm^{-1}. The results obtained in that study indicated the feasibility of discriminating normal and malignant stomach mucosal tissues by Raman spectroscopy [8].

The objective of this work was to use laser Raman spectroscopy to investigate the spectroscopic differences between normal and cancerous bladder tissues.

2. MATERIALS AND METHODS:

In this work all the FT-Raman measurements were carried out using the Nexus 670 FT- Raman spectrometer (Nicolet, USA). The excitation source is Nd-YAG laser. This laser emits at wavelength of 1064 nm and has a maximum power of approximately 1.5W at the sample. The installed detector is InGaAs, which is an air-cooled detector. The used sample configuration is 180° reflective with fully motorized sample position adjustment, with an NMR tube sample holder.

Fourteen fresh tissues of bladder cancer and normal bladder tissues were obtained from seven patients in many hospitals. All the 7 patients were diagnosed by physicians as Squamous and Transitional cell carcinoma with endoscopic biopsy.

The tissues were preserved in 10% formaldehyde solution and sampled immediately after removing during the operations. Two pieces of the tissues, each of about 3 cm in diameter, were taken. One was cut off from the center of the lesion and the other was from the distant edge of the removed tissues. Normal and cancer tissues were cut into small sizes (approximately 2 mm) and put in vacuum container beside silica jell to absorb H_2O in order to make the tissues dry. A powder grinding was done after that with an agate mortar and pestle. Powder sample was put in NMR tube sample holder to be investigated by a Nexus 670 FT Raman spectrometer. A total of 64 scans were done at a resolution of 2 cm^{-1} in the region from 1800 to 200 cm^{-1}. A total of 14 Raman spectra were thus obtained from the normal and cancer samples.

The spectra were processed using the computer program "Omnic E.S.P. Software, Version 5.2" provided by Nicolet, USA.

3. RESULTS AND DISCUSSION:

Some of the spectra of normal and cancer bladder tissues are shown in figures (1) to (4).

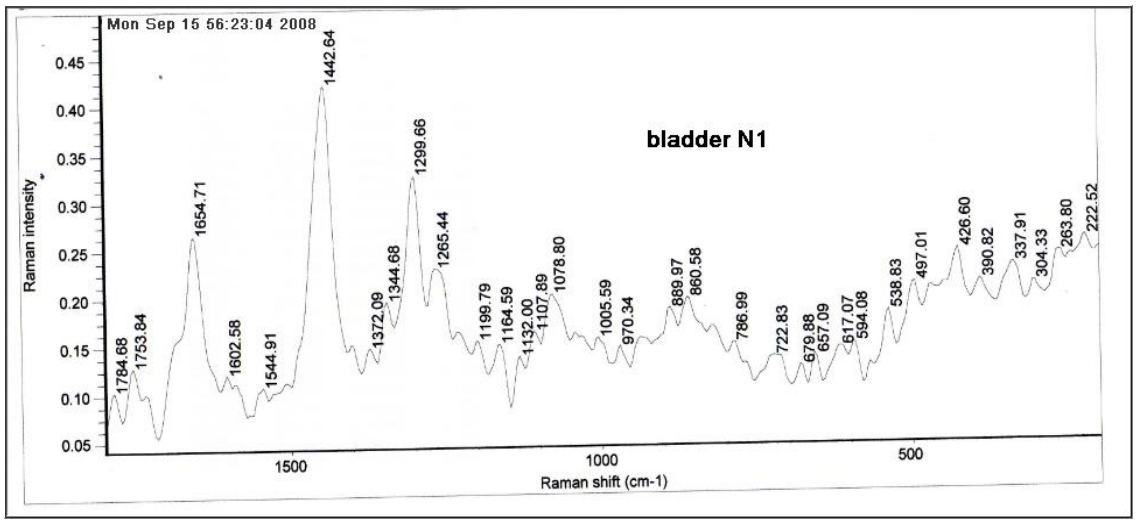

Figure (1-a): Raman Spectrum of Normal Bladder (tissue 1)

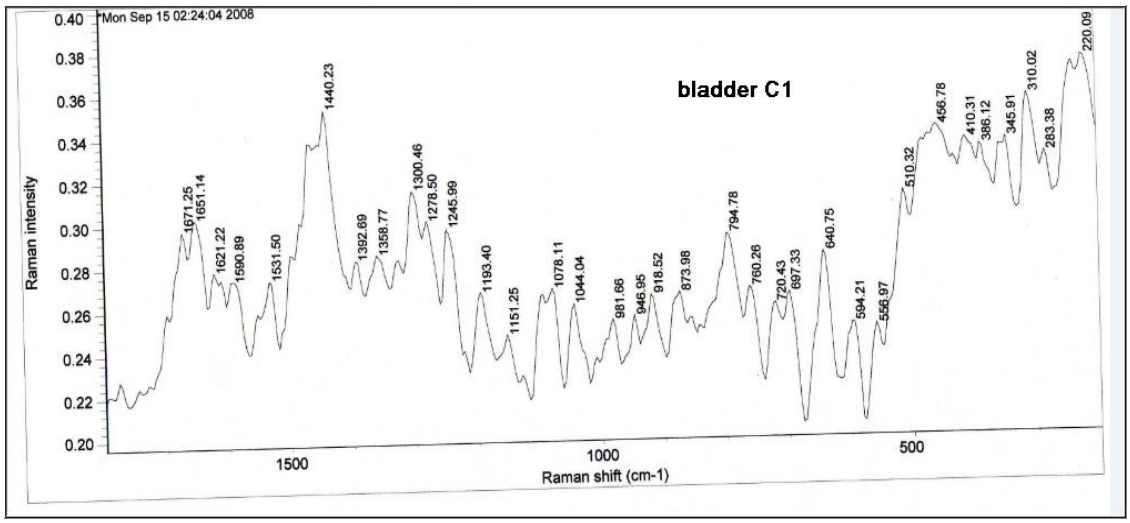

***Figure (1-b): Raman Spectrum of Cancerous Bladder* (tissue 1)**

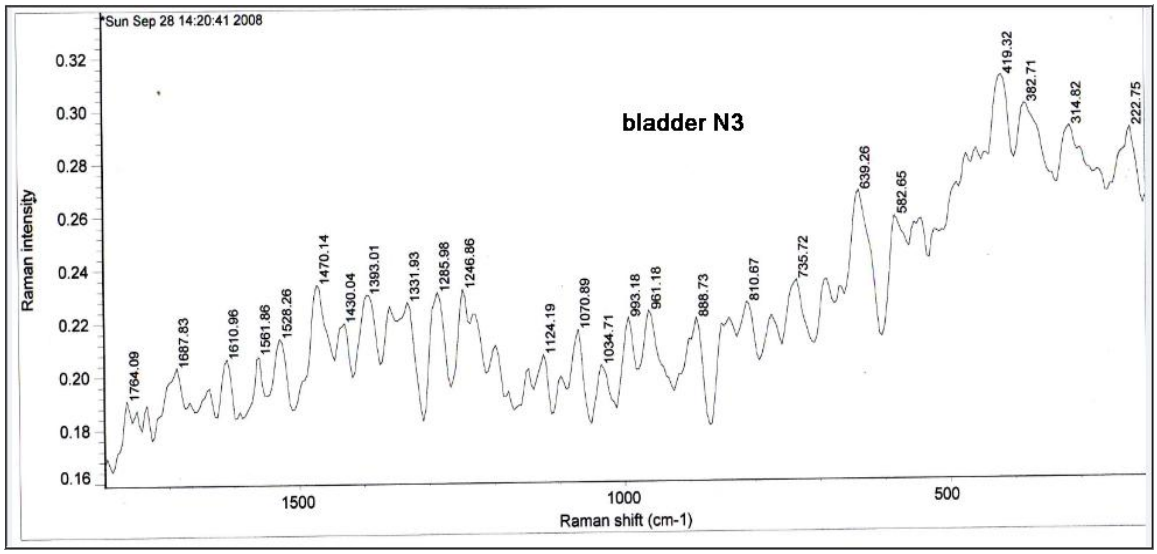

Figure (2- a): Raman Spectrum of Normal Bladder (tissue 3)

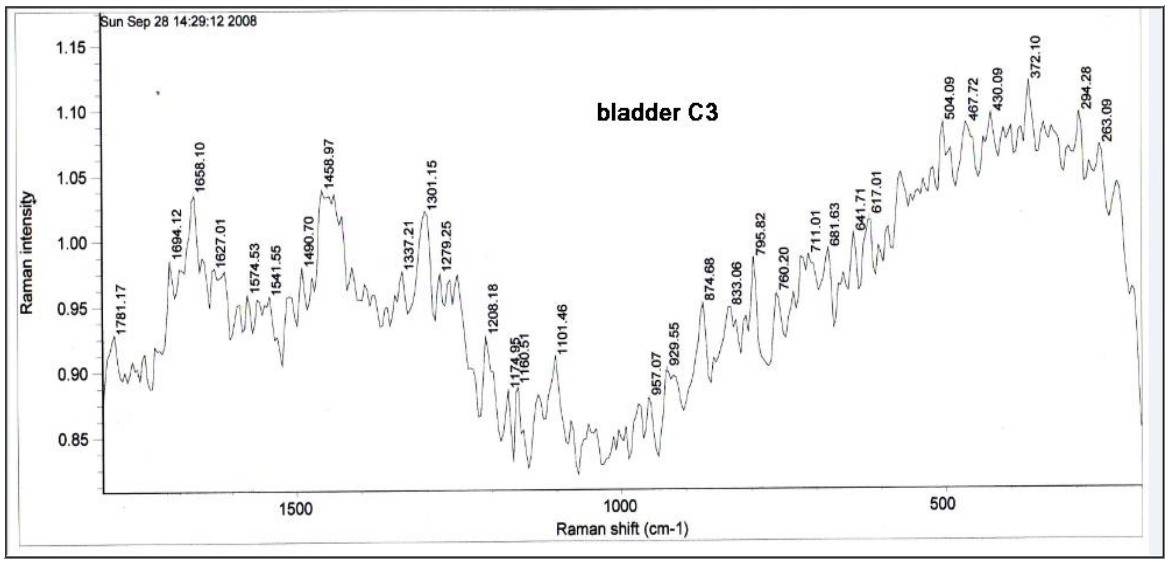

Figure (2- b): Raman Spectrum of Cancerous Bladder (tissue 3)

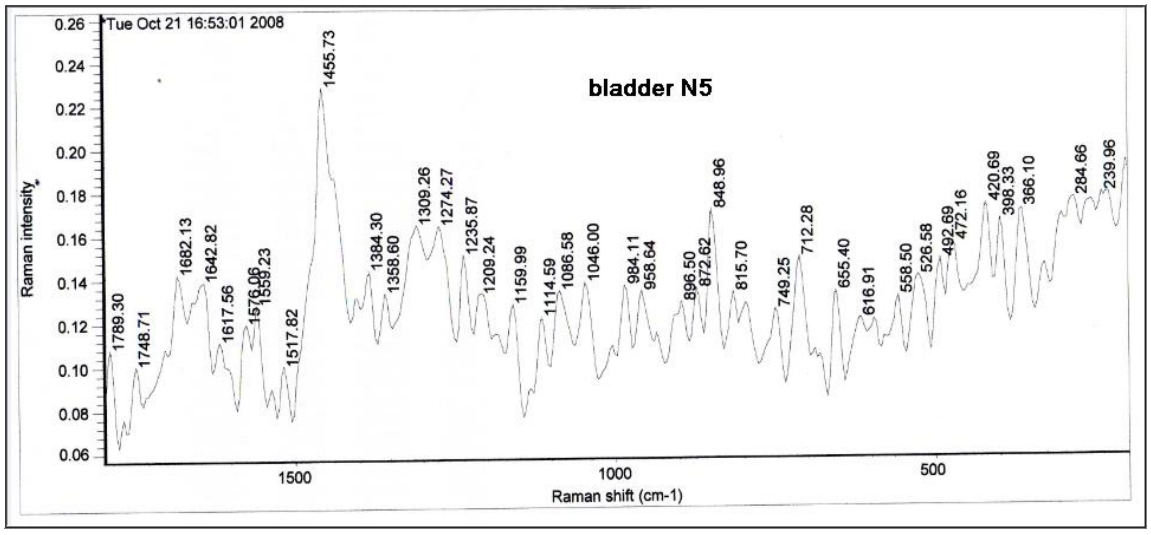

Figure (3-a): Raman Spectrum of Normal Bladder (tissue 5)

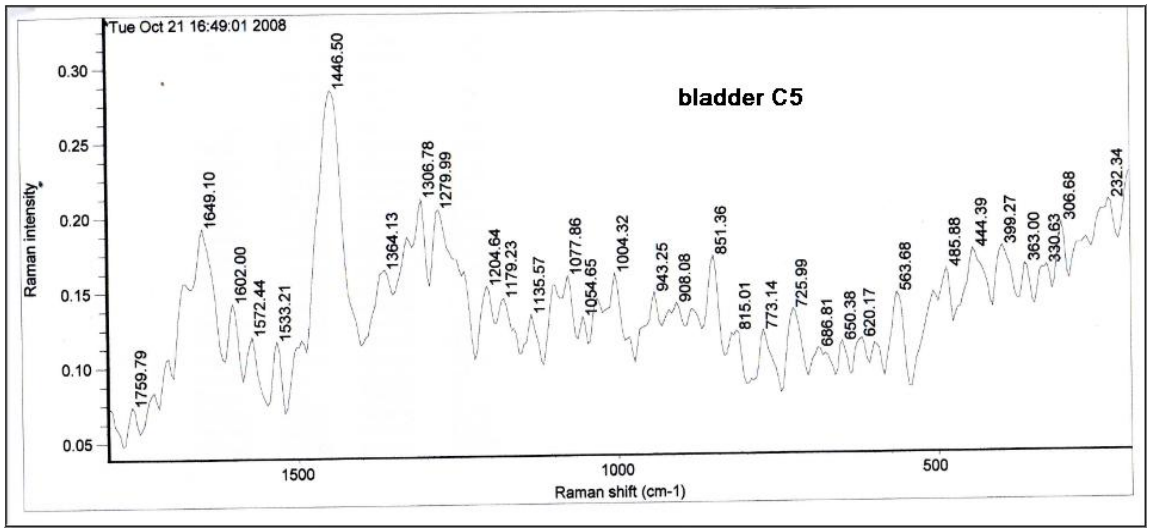

Figure (3- b): Raman Spectrum of Cancerous Bladder (tissue 5)

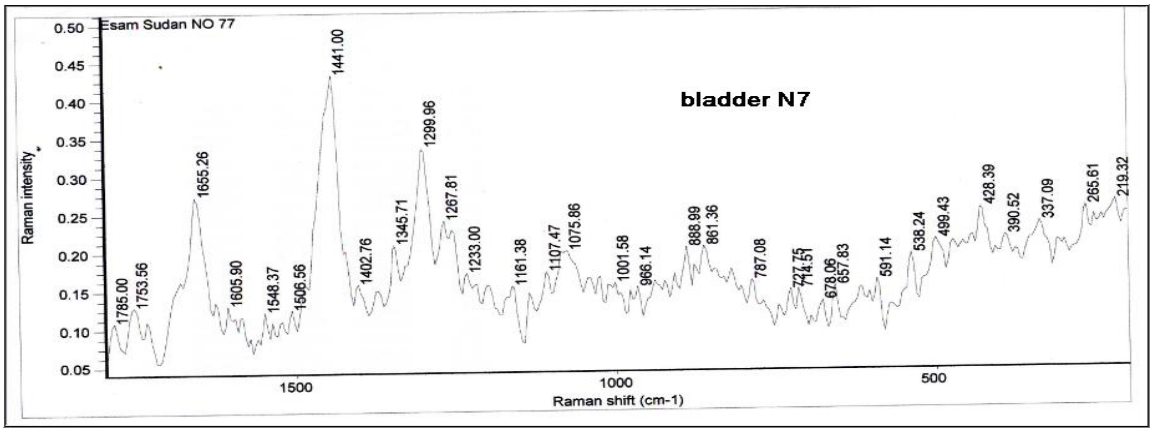

Figure (4-a): Raman Spectrum of Normal Bladder (tissue 7)

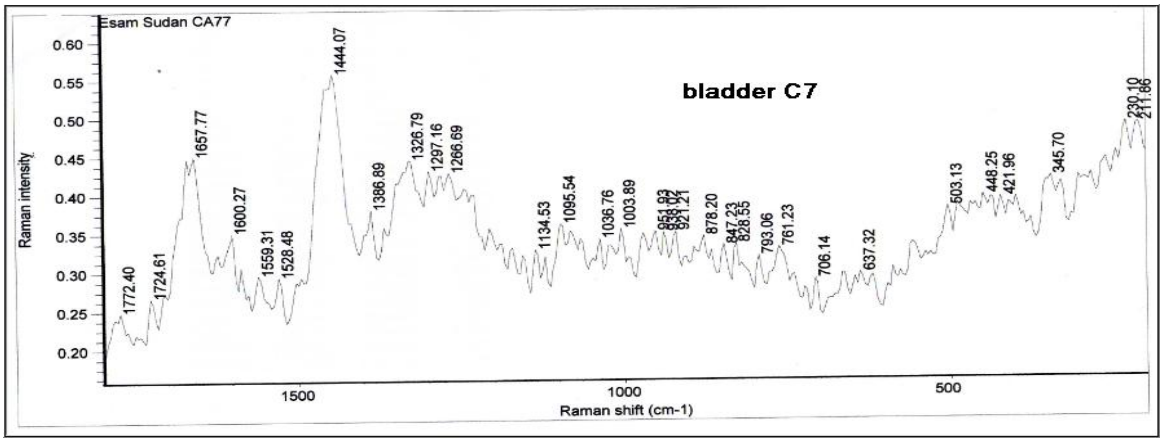

***Figure (4-b): Raman Spectrum of Cancerous Bladder* (tissue 7)**

These figures illustrate the significant differences in the spectra of the two cases.

Raman spectra, for malignant and normal bladder tissues, showed similar vibrational bands that were dominated by several prominent Raman peaks. For instance, the Raman bands in both tumor and normal tissues around 1270 cm^{-1} and 1650 cm^{-1} are presumably attributed to the amide III and amide I bands of proteins in the α-helix conformation, respectively. The Raman bands around 1300 cm^{-1} and 1440 cm^{-1} are characteristic of the CH_2CH_3 bending modes of collagen and phospholipids, respectively [3, 9, 10]. The Raman bands in both normal and tumor bladder tissues around (750, 1200, 1550 and 1600 cm^{-1}) are attributed to the tryptophan, and phenylalanine shown around the Raman peaks (1000, 1580 and 1600 cm^{-1}) in both tissues. The peaks around 1080 cm^{-1} are due to the C–C or C–O stretching mode of phospholipids.

The amide I and the amide III bands of proteins observed in the spectra are similar to those previously reported by Zhiwei Huang et al,

Basil Rigas and Anita Mahadevan Jansen et al. [11 - 13]. These assignments are summarized in table (1) below.

Table (1): Assignments of major peaks in the spectra of normal and cancer bladder tissues

Peaks position (cm^{-1})	Vibrational Mode	Major Assignments
1650 -1680	$\nu(C=O)$ of amide I, $\nu(C=C)$	proteins (α-helix), lipids
1440 – 1470	$\delta(CH_2)$	lipids, carbohydrates, proteins and pentose
1260 – 1275	$\nu(C—N)$ of amide III, $\nu(=C—H)$	proteins (α-helix), lipids
1040 -1100	$\nu(C—C)$ or $\nu(C—O)$, $\nu(C—C)$ or $\nu(PO_2)$, $\nu(C—N)$, $\nu(O—P—O)$	lipids, nucleic acids, proteins, carbohydrates

Significant intensity differences in Raman spectra for the cancer tissue can be observed, like the lower intensities around 880, 1000, 1100, 1200, and 1750 cm^{-1}, and the higher intensities around 1270, 1300, 1440, and 1650 cm^{-1} compared with normal tissues. This indicates that there is an increase or decrease in the percentage of a certain type of bimolecular relative to the total Raman-active constituents in cancer tissues. There are also obvious changes of Raman peak shifts and bandwidths in the spectral ranges of 1200–1500 cm^{-1} and 1600–1800 cm^{-1}, which are related to the amide III and amide I of proteins, CH_3CH_2 twisting of proteins/nucleic acids, and C=C stretching of phospholipids for cancer tissues [13].

To compare Raman shifts and Raman intensities in the spectra of normal and cancer tissues, the 1440 cm^{-1} band is chosen as a reference point, because it is fixed and it is the intense peak in all spectra. Raman peaks at 1265 and 1654 cm^{-1} in normal bladder tissue (sample 1) attributed to the amide III and amide I bands of proteins, respectively, are shifted to 1278 cm^{-1} and to 1651 cm^{-1} in malignant tissue. The peak at 1070 cm^{-1} in normal bladder tissue (sample 2 as example), due to the C–C or C–O stretching mode of phospholipids, is shifted to 1101 cm^{-1} in tumor tissue, reflecting a decreased vibrational stability of lipid chains in tumors. The Raman shifts in normal and cancer bladder tissues for samples 1-7 are illustrated in table (2) below.

Table (2): Comparison between Raman shifts in normal and cancer tissues for samples 1-7

Sample number	Cancer tissue	Normal Tissue
	Raman Shift (cm^{-1})	Raman Shift (cm^{-1})
1	1651	1654
	1440	14402
	1278	1265
	1078	1078
2	1651	1630
	1448	1440
	1272	1279
	1047	1064
3	1634	1658
	1470	1458
	1285	1279
	1070	1101
4	1660	1648
	1456	1449
	1298	1259
	1097	1097
5	1649	1642
	1446	1455
	1279	1274
	1077	1086
6	1662	1665
	1457	1448
	1287	1267
	1083	1102
7	1657	1655
	1444	1441
	1266	1267
	1075	1095

4. CONCLUSIONS:

From the results obtained in this work, one can conclude that:

- Significant spectroscopic differences between normal and malignant bladder tissues were recorded either in Raman shift or in the peaks intensities.
- Laser Raman spectroscopy has high resolution and it is a fast technique so it can be used to provide immediate results especially in surgery theaters.
- FT Raman technique does not require wide sample preparation like FTIR, as example.

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