# SPECTROSCOPIC STUDY OF ORGANIC AND INORGANIC CONSTITUENTS OF MALIGNANT BLADDER'S TISSUES

BY

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Received: 15-12-1992

#### ABSTRACT

Infrared absorption and atomic emission spectroscopic techniques were used for the analysis of the organic and inorganic contents of normal and malignant tissues from the same bladders of nine patients. Examination of the spectra of the organic part of the normal tissues revealed that, among the nine samples, two consist mainly of protein, two samples consist of mixture of lipids and protein where as the rest consist of lipids. The spectra of the malignant part of the organic tissue showed that six samples consist mainly of proteins and three samples consist of a mixture of protein and hydroxy-apatite.

Analysis of the spectra of the inorganic part of the normal tissue revealed that they are composed of basic phosphate compound and the variation in their spectral features was attributed to the variation in the crystalline structure, presence or absence of hydrogen bonding together with the nature of cation. The atomic emission study clarifies that the elements Mg, Na, P and Si exist in all samples in a minor amount with different concentrations. Calcium

exists also in a minor amount in all samples except for one sample (sample No.5) in which its level decreases to trace amount. Also the concentration of each element is not similar in all samples.

Comparison of the infrared spectra of the inorganic part of the malignant tissue proved that their spectral features are not simillar. Five samples show the characteristic bands of pyrophosphate compound, while the infrared of the other *ş*ampl*es* (three samples) show diagonestic absorption band a mixture of both hydroxyapatite and pyrophosphate. Moreover it was found that the calcification of tissues results formation hydroxyapatite the of consequently increases in the percentage of the inorganic content. Calcification of such tissue exists in malignant tissues regardless of its grade.

Further-more the atomic emission technique showed that the concentration of the element Ca, Mg & P which are present in minor amount increased in the calcified tissues. The concentration of Ag, Al, Cu, Cr, K and Ni exist in trace amounts in both normal and tumer tissue with unequal concentration.

#### INTRODUCTION

In the past few years, spectroscopic techniques [1-6] have been used for the characterization of organic and inorganic constituents of tumer tissues. Mizuno [7] et al (1984) used the infrared spectroscopy for analysis of calcified epithelioma from the eyelid. Moharram et al [8,9] (1987) used infrared absorption and atomic emission spectroscopic methods for determination of molecular and

atomic compositions of normal and malignant tissues. The results of the previous researchers proved that these techniques can be conveniently used to characterize the structure of the organic and inorganic contents of unknown biological material.

#### MATERIALS AND NETHODS

The materials included nine samples from tumer bladder tissue and nine normal samples (free from tumer) from the same bladder taken from nine patients harbouring cancer bladder and two tumer samples taken by cystoscope. The samples were collected from patients who did not recieve any of blood or chemotheraputic agents or radiotherapy. The samples were taken immediatly posoperatively and without keeping in preserving solution. The bladder excised is taken opened and a whole thickness part is taken:

- i- Through the tumer mass and the bladder wall.
- ii- Through the bladder wall free from tumer.

These two parts were directly examined pathologically and then were kept in refrigerator till drying process.

#### Dryness:

The water was removed by heating each sample at 37°C under vacuum. The process was performed using an interrupted suction pump, in presence of phosphorous penta oxide as a high hydrophillic compound.

#### Experimental Work:

The infrared spectra were recorded on a Beckman spectrophotometer 4250 by using kBr disk technique. Atomic emission spectroscopy was used for detecting the constituents

elements semiquantitatively. Medium quartz UV Spectrograph (Carl Zeiss Q24) an interrupted arc generator Carl Zeiss ABR3 as a source of excitation, ORWO blue extra hart plate (9x24cm) and spectrum projector were used. The excitation was carried out by 220 V. DC arc anode excitation. The current was started at 2 ampere and increased to 8 ampere to prevent the scattering of the sample. The time of exposure was 60 seconds and the gap between the sample and counter electrode was continuously adjusted at 3 mm. The sample electrode was a needle electrode prepared in the laboratory from graphite rod. Twenty mgs. Sample mixed with graphite in the ratio (1:1) were sufficient to fill the electrode. The addition of graphite was necessary to obtain a steady arc and to increase the conductivity of the sample. On the same plate Spex G standrd with 0.1%, 0.01%, 0.001% and 0.0001% of 43 elements in graphite were also burnt following the same conditions as for samples. The concentration evaluation of the elements under study was performed by visual comparison with the spectra of G standards. Analysed lines were selected to be free from interference. Seidel et al [10] was consulted in such selection.

Specification of the samples under investigation and the ash percent of normal and tumer tissue are given in Tabble I .

#### RESULTS AND DISCUSSION

The infrared spectra of samples taken from the normal tissue of the bladder (far from the tumer mass) of nine patients were recorded. Examination of these spectra showed that their features vary from sample to another according to

the protein and lipid contents.

The spectra of the first group Fig. 1 exhibit absorption bands at the frequencies 3300, 3075, 2950, 2920, 2850, 1650, 1540, 1400 and 1240 cm<sup>-1</sup>. The band at 3300 (Amide A) and 3075 (Amide B) are assigned as N-H stretching vibrations. The three bands at 2950, 2920 and 2850 cm<sup>-1</sup> are assigned as asymetri and symetric vibrations. The two bands at 1650 and 1540 cm<sup>-1</sup> are assigned as C = 0 stretching (Amide I) and N-H bending (Amide II vibrations. The band at 1450 cm<sup>-1</sup> is assigned to characteristic deformation frequency of CH<sub>2</sub> and CH<sub>3</sub> groups. The band at 1400 cm<sup>-1</sup> is assigned to the lower COO absorption frequency. The 1240 cm<sup>-1</sup> band is assigned as N-H deformation frequency (Amide III).

Since the amide I peak appeared strongly at 1650 cm $^{-1}$  wavenumber, the main protein secondary structure was thought to be on  $\alpha$  -helix. The relatively wide peak of Amide II (1520 - 1540 cm $^{-1}$ ) suggested a small content of anti-parallel B-sheated and irregular protein structures.

The second group of spectra (Fig. 1) showed in addition to the characteristic bands of protein two peaks at 1750 and 2850 cm<sup>-1</sup>. These two bands belong to the absorption of c=o stretching vibration of saturated ester and c-H stretching vibration existing in lipids respectively. This means that the samples of this group consists of a major amount of protein in addition to minor amount of lipid.

The spectra of third group (Fig.  $1_c$ ) display absorption bands at the frequencies 2933, 2863, 1752, 1463, 1375, 1164 and 727 cm<sup>-1</sup> which are the diagnostic bands of lipids. The

presence of these bands indicates that these samples consists mainly of lipid [11].

The spectra of the samples taken from the tumer parts of the bladder of the same patients are classified into two group. The spectra of the first group (Fig. 2) of the malignant tissue show the characteristic absorption bands of protein at the frequencies 3300, 3075, 2934, 2867, 1660, 1543, 1454, 1303 and 1243 cm<sup>-1</sup>. Besides these spectra exhibit absorption bands at 1163, 613, and 1063 cm<sup>-1</sup> corresponding to the absorption of phosphate compound.

The spectra of the second group (Fig.  $2_{b}$ ) show the characteristic bands of protein and lipid.

The samples of the normal parts of the tissue were ignited in a muffle furnace adjusted at  $600^{\circ}$  C till maintaining constant weight of ash content. The IR spectra of the inorganic part showed the following:

Over the frequency region from  $1000-1200~{\rm cm}^{-1}$  each spectrum exhibits two strong stretching bands whose position vary from sample to another. These bands are assigned as the P-O stretching vibration of PO $_4^{3-}$ . The spectra of the first group (Fig. 3 $_4$ ) show definit shoulder at 995 cm $^{-1}$ . The spectra of second group (Fig. 3 $_b$ ) show band of medium intensity at 995 cm $^{-1}$  while the spectra of third group (Fig. 3 $_c$ ) show a weak band at 910 cm $^{-1}$ . These bands can be assigned as P-O stretching vibration of PO $_4^{3-}$ .

The spectral features of the samples over the region  $400-900~{\rm cm}^{-1}$ , are not simillar. The position and intensities of the absorption bands vary from spectra to another. However

these bands are in good correlation with the characteristic band reported in the literature of PO<sup>3</sup>. The variation in spectral features can be attributed to the variation in the crystalline structure, presence, or absence of hydrogen bonding together with the nature of cations.

The atomic emission spectroscopic technique was used to determine semiquantitatively the elements in both normal and tumer part of the samples. Evaluated concentration of the studied elements are given in table II.

The present clarifies that the elements Mg, Na, P and Si exist in all samples in minor amounts with different concentrations. Calcium exists also in minor amount in all samples except for sample number 5 in which its level decreases to trace amount. The concentration of aluminium varies from minor to trace amount. The concentration of other remainder elements are either trace or faint trace. The concentration of each element is not similar in all samples.

Based on the foroing survey, it can be concluded that the inorganic constituents of the analysed samples vary from sample to another and is composed mainly of basic phosphate compound. Hence one can suggest that the most likely cations are Na, Mg, Ca and P. In addition the percentage of ash content displays different values for different samples. As an example sample one shows the highest percentage of ash content (12.5 %). It notes worthy that pathology examination proved that the sample was mixed with tumer tissue.

The infrared spectra of the inorganic part of malignant

tissue were recorded. Comparison of these spectra proved that their spectral features are not simillar. The spectra can be classified according to their bulk constituents into three groups. The first group spectra (Fig.4) exhibits absorption bands at the frequencies 570, 600, 980, 1030, 1100 and 3400  ${\tt cm}^{-1}$  . These absorption bands are characteristic bands of calcium phosphate in the crystallographic structure of the mineral hydroxyapatite. This means that these samples consists mainly of hydroxyapatite. The percentage of ash content of these samples (Table I ) shows that the calcification of tissues result in the formation of hydroxyapatite and consequently explains the increase in the percentage of the inorganic content. It can be mentioned out that calcification of such tissues exists in malignant tissues regardless of its grade. Calcium phosphate in biological materials is thought to exist as a form of hydroxyapatite in the calcified structures such as: bone, dentine or abnormally calcified tissues. Hydroxyapatite is very complex substance showing multiformity of structures with different degree of crystalinity [12,13].

The spectra of the second group (Fig. 4) exhibit absorption bands at the frequencies 569, 732, 912, 1133, 1200 and 3450 cm<sup>-1</sup>, In addition to two weak absorption bands at about 980 and 1050 cm<sup>-1</sup>. The absorption bands at the frequencies 569, 732, 912 and 1133 cm<sup>-1</sup> are due to the absorption of Na<sub>3</sub>PO<sub>4</sub>. The presence of the two weak bands at 980 and 1030 cm<sup>-1</sup> indicates the presence of trace amounts of calcium phosphate. The absorption bands at 725 and 900 cm<sup>-1</sup> are due to the absorption of P-O-P vibrations of pyrophosphate

group. It has been accepted that pyrophosphate contains more band than the original phosphate and these are near 900 cm<sup>-1</sup> and 700 cm<sup>-1</sup> [11,14]. These assignments lead to a suggestion that the inorganic part of the second group consists mainly of pyrophosphate. On the basis of these findings it could be concluded that sample 2 and 9 are composed entirely of phosphate compounds other than hydroxyapatite.

The spectra of third group (Fig. 4) display the characteristic bands of mixtures of calcium phosphate and Na<sub>3</sub>PO<sub>4</sub>. The presence of calcium phosphate is indicated by the presence of the bands at 1163 and 613 cm<sup>-1</sup>. However one can come to conclusion that the malignancy produces marked alteration in the minerological structure and changes the concentration of inorganic elements constituents of these tissues. The extent of change in either minerological structure or inorganic elements depond upon biological structure of the person.

The atomic emission technique revealed that the concentration of the elements Ca, Mg and P which are present in minor amounts increased in the calcified tissues. On the other hand the concentration of the elements Ag, Al, Cu, Cr, K and Ni exist in trace amount in both normal and corresponding tumer tissues with unequal concentration.

The atomic emission tools could be considered as a new criterian increasing our ability to distinguish between normal and malignant tissues. The high concentration of Ca, P & Mg in tissues can be used to distinguish malignant tissues from the normal counter parts.

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The atomic emission tools could be considered as a new criterian increasing our ability to distinguish between normal and malignant tissues. The high concentration of Ca, P & Mg in tissues can be used to distinguish malignant tissues from the normal counter parts.

Examining the elements present in bladder tissues (normal and tumer) necessitates handling of two important and effective points.

1- Dealing with in vivo samples necessitates that original available samples are of relatively little amount. To evaluate element one has to get rid of organic matter. This means that the sample must be ashed. Naturally the resulting ash sample is less than the starting sample. Hence the most suitable technique for evaluating elements present to apply atomic emission technique.

2- Investigated elements handle diverse volatilities, examples are alkalies such as K and Na. volatiles such as Cu and involatile such as P and Si. Hence it was not possible to find a precise quantitative spectroscopic technique which can cover all these in one exposure and with one internal standard. Therefore the natural behaviour was to follow visual semiquantitative technique. Yet it is note worthy that the use and the burning of spex standards together with samples on the same let plate the used technique can be considered approximately close to quantitative technique.

Finally, it is worth mentioning that success in presenting these results opened the door for similar studies for other tumers in human body.

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Table I.

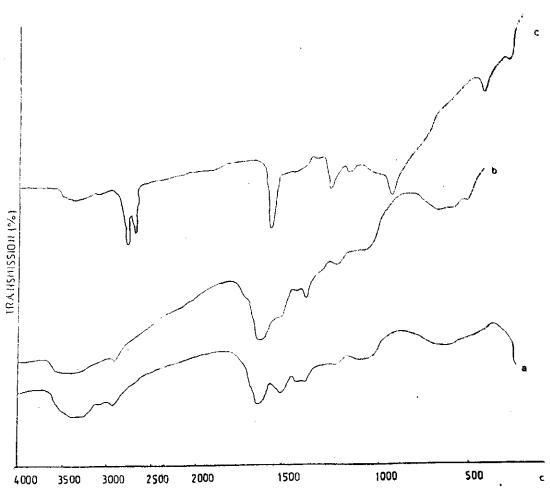
Grade and ash percent of normal and tumer tissue.

Sample	Grade	Ash content	
ио.	GIAGE	normal tissue	tumer tiesue
1	I aq.	12.5 🐔	15.4 %
2	I Tr.	0.1 %	4.6 %
3	II Sq.	3.8 %	33.4 %
4	II Tr.	1.2 %	11.4 %
5	III Sq.	3.3 %	5.7 <b>%</b>
6	<b>v</b> erreous	4.5 %	13.6 %
7	III Tr.	3.7 %	3.6 🐔
8*	villous		10.4 🛪
9	med.diff.	2.2 🞋	5.6 %
10	II Sq.	3.6 ≴	0.1 %
11*	II Sq.		29.2 %

<sup>\*</sup> Samples taken by cystoscope from the tumer part only.

Table II
The elements and the concentration in the normal and huma: tissue.

Ju	•	-	1 1 1	2	1	•				5	9			7		8	-	6		유	7	
G energy	*	<b>+</b>	×	£-	2	<b>H</b>	Z	t <del>-</del>	z	£m	2:	<b>L</b>	2	Ļ	2	<b>L</b>	2	-	2	1	=	þe
Ą	,	,	1	0.001	0.001 0.00		0.003	-	ı	0.001	1 1%	0.00	0.001 0.00L	XI	ı	ì	ı	0.001 0.001 0.001	0.001	0.001	٠,	•
7	볼	펐	1	ĸ	ĸ	0.03	ĸ	7	0.03	m	1	0.1	0.1%	ĸ	t	0.1%	ĸ	Ħ	Ħ	片	1	r.
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8	0.08	3.003	ļ	0.1	0.3	9.003	0.1	60.03	0.033	0.1	0.081		0.003 0.008 0.11	0,11	1	0.01%	0.1	0.1%	0.1	0.1	1	0.003
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c) third group.

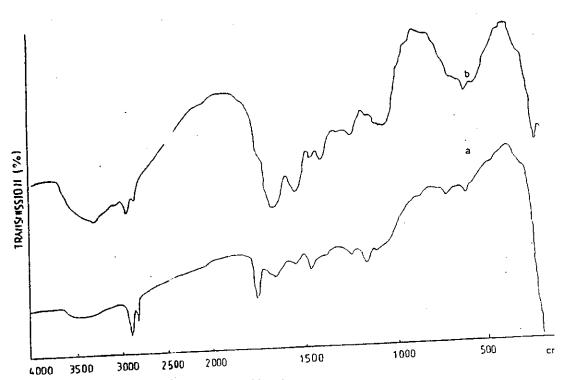
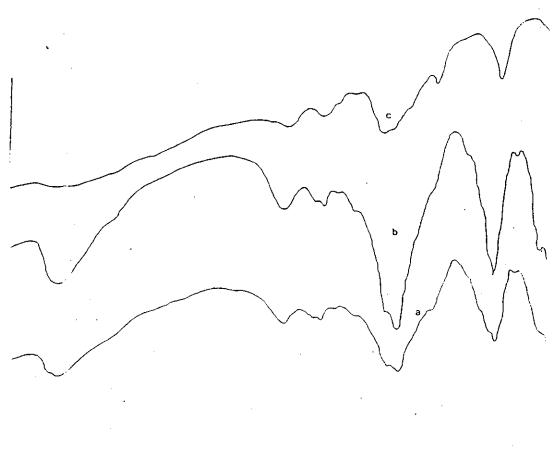


Fig. 2: I.R spectra of organic tumer tissue

a) first group

b) second group

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10 3500 3000 2500 2000 1500 1000 500 c:

a) first group

b) second group

c) third group

<sup>;- 3:</sup> I.R spectra of inorganic normal tissue.

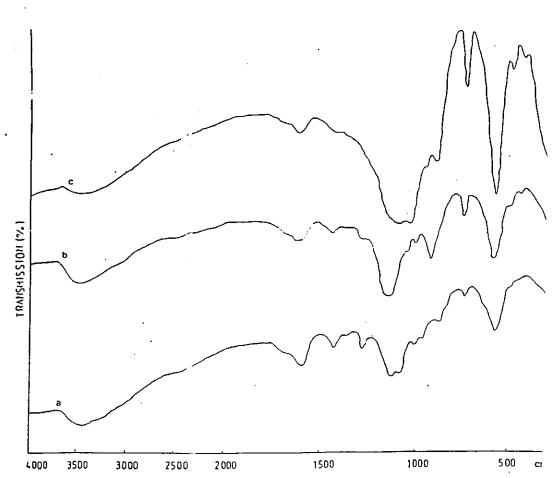


Fig. 4: I.R spectra of inorganic tumer tissue

a) first group
b) second group
c) third group

# عراسه طيفيه للمكونات العصويه والغير غصويه لأنسجة المثانيه السرطانيه

اً، كَ مَهِم عَبِهُ القاهر مهرم - كا، بنائيت ميهائيل إبراهيم. قسم الطيف - شعبة الغزياء - عمركز القومي للبحوث

عبية الطسب - جامعية القامسره على جسنى أو غبط الرجيم عبط اللطيف محرم

تم إستخدام طرق طيف الأشعه تحت الحمراء وطيف الإنبعاث الذرى لتحليل المكونات العضويه والغير عضويه للأنسجه السليمه والسرطانيه لنفس المثانه لعدد من المرضى ٠

أوضح تحليل المواد العضويه لعينات الأنسجه السليمه ؛ أن بعض العينات تتكون من تتكون أساسا من البروتين وبعضها خليط من البروتين والدهون والأخير يتكون من دهون فقط ، أما الأنسجه السرطانيه فوجد أن معظم العينات تتكون من بروتين والباقى خليط من البروتين وفوسفات الكالسيوم في صوره معدن Hydroxy-apatite ،

أما فى حالة المواد الفير عضويه فى الأنسجه السليمه تبين من التحليل أنها تتكون أساسا من فوسفات قاعدى وأعزى الإختلاف فى أشكال أطياف الأشعه تحت الحمراء إلى الإختلاف فى درجة تبلور العينات ووجود أو عدم وجود الرابطه الهيدروجينيه •

علاوه على ذلك وجد أن الجزء الغير عضوى فى الأنسجه المصابه لايتشابه ومنها ما يتكون من خليط من المعدد hydroxy-apatite and pyro-phosphate

أوضح إستخدام الطيف الذرى الإنبعاثي أن الماغنسيوم ؟ الصوديوم ؟ الغوسفور ؟ السليكون والكالسيوم تزيد نسبته بطريقه ملحوظه في الأنسجه السرطانيه عنها في الأنسجه السليمه • بينما المواد مثل الفضه ؛ الألومنيوم ؛ النحاس ؛ الكرميوم ؛ البوتاسيوم والنيكل توجد في جميع العينات السليمه والمصابه ولكن بنسب غير متساويه •