ORIGINAL PAPERS

Adv Clin Exp Med 2006, **15**, 4, 619–624
ISSN 1230-025X

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Electrical Impedance Measurements in Assessing Laryngeal Squamous Cell Carcinoma

Pomiary impedancji elektrycznej w diagnostyce raka płaskonabłonkowego krtani

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Abstract

Background. Tissue differentiation may plausibly be based on measurements of its dielectric properties. It has been noted that cancerous cells differ from healthy ones in their local dielectric properties.

Objectives. In this study, impedance analysis, involving the measurement of voltage in response to the application of an alternating current over a wide frequency range, was used to study laryngeal carcinoma.

Material and Methods. The measurements were done on 16 tissues obtained from patients treated surgically for laryngeal squamous cell carcinoma. The acquisition system consisted of an AutoLAB impedance analyzer and a set of electrodes. The real and imaginary parts of the impedance were measured over a frequency range of 100 Hz to 1 MHz in cancerous and adjacent healthy epithelium. The modulus of impedance in the frequency function was then calculated.

Results. The values of the modulus of impedance $|Z|$ were essentially higher in healthy mucosa than in cancerous epithelium ($p < 0.05$). The ratio of $|Z|$ between them varied from 2.1 to 15.0. The impedance of healthy tissue rose significantly with decreasing frequency of the applied current.

Conclusion. The results of the study indicate that the impedance spectrum is quantitatively related to the structure of the tissue. The differences in the impedance values between the investigated areas enable differentiating healthy from cancerous mucosa of the human larynx with great selectivity (**Adv Clin Exp Med 2006, 15, 4, 619–624**).

Key words: impedance analysis, laryngeal carcinoma, diagnostics.

Streszczenie

Wprowadzenie. Różnicowanie ludzkich tkanek może być przeprowadzone za pomocą pomiaru ich własności dielektrycznych. Dotyczy to w szczególności nowotworów złośliwych, których wskaźniki bioelektryczne różnią się zasadniczo od stwierdzanych w tkankach niezmienionych nowotworowo.

Cel pracy. Ocena wartości impedancji raków płaskonabłonkowych krtani na podstawie pomiarów napięcia uzyskanego w odpowiedzi na przyłożony do tkanek prąd zmienny w szerokim zakresie częstotliwości.

Materiał i metody. Pomiary przeprowadzono *in vitro* na 16 krtaniach uzyskanych od pacjentów po zabiegu laryngektomii całkowitej wykonanym w Klinice Otolaryngologii AM we Wrocławiu z powodu potwierdzonego histopatologicznie raka płaskonabłonkowego. System pomiarowy składał się z urządzenia AutoLab oraz zestawu specjalnie opracowanych elektrod igłowych. We wszystkich przypadkach dokonano oceny składowej rzeczywistej oraz urojonej impedancji w szerokim zakresie częstotliwości (100–1000 Hz) zarówno w tkance guza, jak i przyległym niezmienionym nabłonku krtani. Na podstawie uzyskanych danych obliczono moduł impedancji w funkcji częstotliwości.

Wyniki. Wartości modułu impedancji $|Z|$ były istotnie stystycznie większe w zdrowym nabłonku w porównaniu z rakami krtani ($p < 0.05$). Stosunek modułu impedancji $|Z|$ guzów krtani do niezmienionego nabłonka wynosił 2,1–15. Wartości impedancji w tkankach zdrowych wzrastały istotnie wraz ze spadkiem częstotliwości przyłożonego prądu elektrycznego.

Wnioski. Wyniki badań potwierdzają, że wartość impedancji bioelektrycznej jest ściśle zależna od struktury badanej tkanki. Uzyskane wyniki sugerują również możliwość wykorzystania analizowanych wskaźników w różnicowaniu raka płaskonabłonkowego krtani od nowotworowo niezmienionego nabłonka dróg oddechowych (**Adv Clin Exp Med 2006, 15, 4, 619–624**).

Słowa kluczowe: pomiary impedancji, rak krtani, diagnostyka.

Studies on the electrical properties of tissues have been conducted since the end of the nineteenth century [1]. One of the methods, called electrical impedance spectroscopy (EIS), measures the electrical properties of tissue, i.e. conductivity (σ) and relative permittivity (ε), as functions of the frequency (ν) of the applied alternating current (ac). The work by Cole and Cole and later researchers led to techniques enabling the determination of the impedance properties of healthy, damaged, and pathologically changed tissue in a wide range of frequencies [2, 3]. Although electrical impedance measurements of malignant tissues have been investigated for years, it has only been in recent years that achievements in computer science and data processing have enabled progress in this field.

Head and neck squamous cell cancer comprises almost 5% of all malignant tumors diagnosed in Europe, making it the seventh most common malignancy. The majority of primary tumors in the head and neck region are located in the larynx. Although the diagnosis of head and neck cancer is established on the basis of the clinical and histological pictures, each attempt to reduce the time of diagnosis is worth undertaking. One of the most challenging problems of head and neck oncology, especially in developing countries, is the high local advancement of the tumors at the moment of diagnosis. This is why special attention should be directed to screening efforts and the diagnosis of suspicious changes. Electrical impedance measurement is a technique which seems to be an interesting basis for a clinically significant, fast, and cost-effective methodology for the detection of cancerous tissue. This study aimed to assess the electrical impedance spectra in cancerous and macroscopically healthy laryngeal mucosa.

Material and Methods

The material comprised larynxes obtained from patients with squamous cell carcinoma treated surgically at the Department of Otolaryngology, Silesian Piasts University of Medicine in Wrocław. All of the 16 patients included in the study underwent total laryngectomy. The diagnosis was made by histological examination pre- and postoperatively. Most of the patients (15) were males and one was female. Patient age ranged from 47 to 75 years old (mean: 56 years). The stage of the tumors assessed according to the TNM classification showed high local advancement, i.e. pT2 in 3 cases, pT3 in 11 cases, and pT4 in 2 cases.

The acquisition system consisted of an AutoLAB Analyser (Eco Chemie B.V., The Netherlands) serially coupled with a personal computer. Impedance data were obtained under the control of Frequency Response Analyzer (FRA) software for Windows (Eco Chemie). In each case, the larynx was positioned into a custom-built plastic chamber through which electrodes could be inserted to touch the surface of the mucosa. The intra-electrode distance was fixed in the plastic cover. Impedance measurements were made with needle electrodes 0.88 mm in diameter and 25 mm in length. The probes used in the study have the advantages of small size and the possibility of being placed in the structure to be studied. The probe was calibrated in saline of known electrical conductivity. The method involves application of a sinusoidally varying current and the measurement of the ensuing voltage. A current was passed between an adjacent pair of electrodes. Measurements of impedance were carried out over a wide range of frequencies from 100 Hz to 1 MHz. At least two measurements were made on each tissue: the cancerous and the adjacent, macroscopically normal epithelium. Electrical impedance was gauged from the center of the tumor. The regions of the tissues included in the impedance measurements were finally assessed histopathologically. The study had the approval of the local ethics committee.

Statistica 6.0 (StatSoft, Inc.) was used for statistical calculations and graphical presentations. The obtained data were shown to have a normal distribution with a statistical significance of $p = 0.05$. The association between the values of impedance |Z| in healthy and cancerous tissue was tested by the Wilcoxon test. Pairs of measurements conducted under similar conditions were compared. Differences with $p < 0.05$ were considered significant.

Results

In all cases, the real (Z’) and imaginary (Z”) parts of the impedance in the frequency (f) function were measured in healthy and cancerous tissue. The values of reactance (Z”) ranged from 144.89 Ω to 25.129 Ω (average: 3534.28 Ω, *SD*: 5176.91 Ω) in healthy epithelium and from 21.87 Ω to 886.67 Ω (average: 270.80 Ω, *SD*: 211.34 Ω) in cancerous tissues. The resistance (Z’) ranged from 727.82 Ω to 35.177 Ω (average: 5461.77 Ω, *SD*: 7012.64 Ω) in normal epithelium and from 314.18 Ω to 2340.50 Ω (average: 1060.55 Ω, *SD*: 617.34 Ω) in cancerous tissues. Then the modulus of impedance (|Z|) in the frequency function was calculated for healthy ($|Z^h|$) and cancerous epithelium ($|Z^c|$). $|Z^h|$ had values from 755.19 Ω to 43,230.64 Ω

Table 1. The real (Z’) and imaginary (Z”) parts of impedance and the modulus of impedance |Z| in six cases of cancerous and healthy laryngeal mucosa as a function of the frequency (f) of the applied current

Tabela 1. Wartości pomiarów części rzeczywistej (Z’), urojonej (Z”) i modułu impedancji |Z| w paśmie częstotliwości (f) uzyskane w tkance zdrowej i zmienionej nowotworowo

n	f [kHz]	Healthy epithelium (Tkanka zdrowa)						Cancerous tissue (Tkanka zmieniona nowotworowo)					
		Z’ [Ω]	Z” [Ω]	$\|Z^h\|$ [Ω]	Z’ [Ω]	Z” [Ω]	$\|Z^h\|$ [Ω]	Z’ [Ω]	Z” [Ω]	$\|Z^c\|$ [Ω]	Z’ [Ω]	Z” [Ω]	$\|Z^c\|$ [Ω]
1	100	2212.4	1492.4	2668.70	2038.4	1288.7	2411.60	723.47	181.18	745.811	915.07	267.70	953.42
	10	5795.2	4049.7	7069.96	5372.5	3555.3	6442.35	1207.6	396.02	1270.87	1553.4	444.03	1615.61
	1	13923	9636.4	16932.52	12372	6756.5	14096.69	1739.8	486.46	1806.52	2120.4	627.21	2211.21
2	100	1785.6	874.3	1988.15	1091.7	480.11	1192.60	745.85	75.565	749.66	584.12	46.66	585.98
	10	3614.6	1292.2	3838.63	2257.8	1102.1	2512.42	856.67	82.16	860.60	654.53	62.96	657.55
	1	5430.8	1587.9	5658.18	4176.8	1682.6	4502.97	972.16	206.47	993.84	753.54	220.84	785.23
3	100	2337	2236.1	3234.45	1589.6	1516.5	2196.95	1249.9	410.75	1315.66	1212.1	420.40	1282.93
	10	8084.5	10187	13005.16	5519.1	6438.7	8480.40	1911.2	373.77	1947.40	1926	401.29	1967.36
	1	35177	25129	43230.64	22596	15770	27554.89	2302.5	454.13	2346.85	2340.5	438.62	2381.24
4	100	1462.6	471.67	1536.77	1168.8	311.49	1209.59	697.16	78.186	701.53	542.69	67.38	546.85
	10	2356.2	541.31	2417.58	1777.3	387.6	1819.07	816.94	114.84	824.97	652.15	83.49	657.47
	1	3074.5	809.93	3179.39	2376.6	661.75	2467.01	1020.8	359.54	1082.26	783.05	236.80	818.07
5	100	2212.4	1492.4	2668.70	2038.4	1288.7	2411.60	915.07	267.7	953.42	488.08	149.46	510.45
	10	5795.2	4049.7	7069.96	5372.5	3555.3	6442.35	1553.4	444.03	1615.61	898.21	492.04	1024.15
	1	13923	9636.4	16932.52	12372	6756.5	14096.69	2120.4	627.21	2211.21	1804.6	886.67	2010.66
6	100	927.07	144.89	938.32	727.82	201.47	755.19	323.45	22.4	324.22	314.18	21.87	314.94
	10	1144.9	196.1	1161.57	1243.5	433.84	1317.00	355.23	32.975	356.75	347.1	31.87	348.56
	1	1460.3	561.16	1564.40	1816.7	658.34	1932.30	393.07	121.6	411.44	385.52	114.64	402.20

(average: 6581.59 Ω; *SD*: 8657.59 Ω), while $|Z^c|$ ranged from 314.94 Ω to 2381.24 Ω (average: 1099.79 Ω, *SD* 643.43 Ω). Examples of the magnitudes of these parameters for the two different measurements for cancerous and healthy epithelium are shown in Table 1. Readings from the tissues showed that the values of |Z| differed significantly between the two groups. The values of $|Z^h|$ were statistically higher than those of $|Z^c|$ ($p < 0.05$) (Fig. 1). The ratio of $|Z^h|$ to $|Z^c|$ varied from 2.1 to 15.0. The impedance of healthy tissue clearly rose with decreasing frequency of the applied current. The same values did not increase significantly in cancerous epithelium (Fig. 2). This difference enables one to differentiate healthy from cancerous areas of the human larynx with great selectivity. Despite the presence of some variations in |Z| in both groups, the impedance in healthy epithelium was always higher.

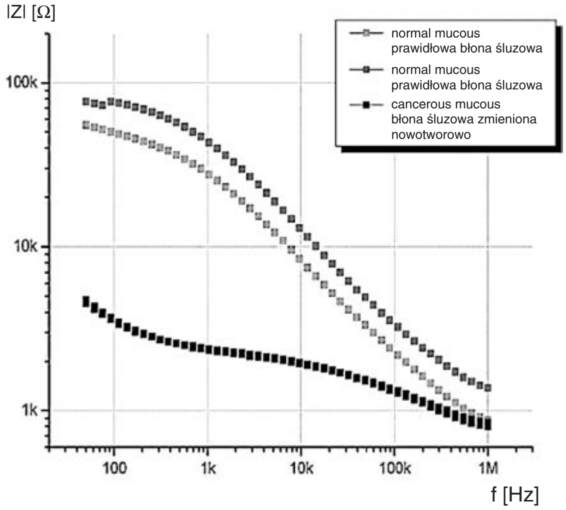

Fig. 2. Frequency (f) dependence of the modulus of the impedance |Z| in normal and cancerous laryngeal epithelium

Ryc. 2. Rozkład wartości modułu impedancji |Z| tkanki zdrowej i zmienionej nowotworowo w paśmie częstotliwości (f) 100 Hz–1 MHz

Discussion

The body offers two types of resistance (R) to an electrical current: capacitative R (reactance), and resistive R (simply called resistance). Each human tissue contains elements that have either resistive and capacitative properties. The capacitance arises from cell membranes and the resistance from extra- and intracellular fluid and the cell structure. Impedance is the term used to describe the combination of both. Measuring impedance in living tissues is difficult to achieve. Different internal components determine the electrical properties of tissue at different frequency bands within an impedance spectrum [4]. Molecular structure is the main factor at frequencies over 1 GHz, whereas at low frequencies (< 100 Hz) it is charge accumulation at the membranes. At frequencies of a few kHz to 1 MHz, also called the β dispersion region, the electrical current passes mainly through the extracellular space, so the shape of the cells, their intercellular relationships, and their arrangement in the tissue are the crucial elements determining impedance. With increasing frequency, current can penetrate the cell and flow through both the intracellular and extracellular space. In such a case, the intracellular volume and the size of the nucleus can influence the current [5]. Consequently, electrical impedance spectral measurements open the way to assessing tissue structure. The relationship between capacitance, resistance, and thus impedance, reflects different electrical properties of tissues that are affected by disease and other factors, e.g. nutrition and dehydration. Electrical impedance measurement has recently been investigated for monitoring tissue damage *in vivo*, e.g. the influence of ischemia on rat muscle [6], toxic injury of the liver [7], skin reaction after radiation [8], X-ray injury in rat muscle [9], and the detection of dental decay [10].

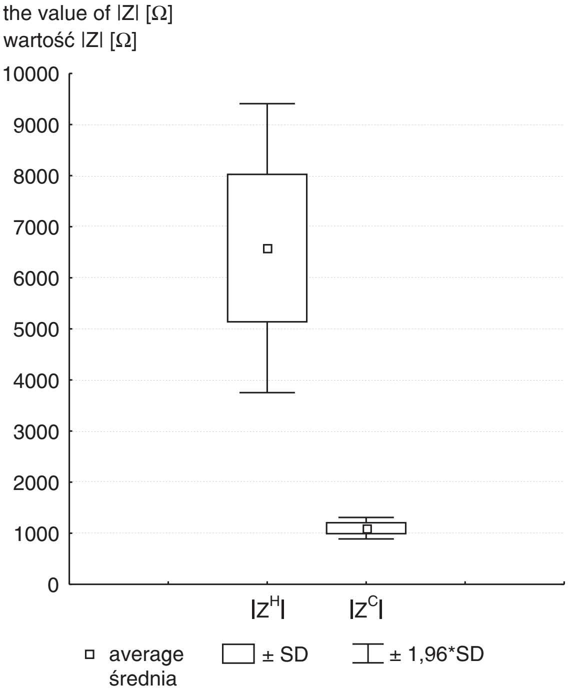

Fig. 1. Comparison of impedance in healthy ($|Z^h|$) and cancerous tissue ($|Z^c|$) ($p < 0.05$)

Ryc. 1. Porównanie wartości modułów impedancji tkanki zdrowej ($|Z^h|$) i zmienionej nowotworowo ($|Z^c|$) ($p < 0,05$)

The altered electrical properties of cancerous tissue compared with healthy tissue are attributed to increased cellular water and salt content, altered membrane permeability, and altered packing density and orientation of the cells [4]. In neoplastic tissues, the cell layering of normal squamous epithelium is destroyed, so current of low frequencies which does not penetrate the cell membrane does not have to track around the cell layers and the tissue impedance is expected to be lower. Due to enlargement of the cell nuclei in cancerous tissue, the conduction pathways through the intracellular space are smaller. Cancerous lesion is assumed to have higher conductivity than surrounding tissue. The dependence of lesion detectability on the conductivity ratio between lesion and the adjacent tissue is basic to EIS. Emtestam et al. [11] investigated the electrical impedance properties of nodular basal cell carcinoma (BCC). Among the set of the four indices they used (magnitude index, phase index, and the real and imaginary part index), only values of the modulus of magnitude and the imaginary part of the complex electrical impedance significantly decreased in BCC compare with normal skin, which is in accordance with presented results.

In presented study the reproducibility of the measurements in each case was high. In most of the tissues the shapes of the graph which represent measurements on the same tumor in different places were similar (Fig. 2). Disturbances in the form of the graphs could be caused by the electrode depth in the tissue, the presence of cartilage, and changes in mucous humidity [12, 13]. Increased electrode depth in the investigated tissues led to a decrease in impedance.

Described results are in accordance with observations obtain by Jossinet et al., who worked with freshly excised breast tissue and observed large differences in dielectric parameters between neoplastic and normal areas [14]. The potential application of this technique was also assessed in detecting neoplastic and precancerous changes in the cervix [5, 15] and bladder [16]. Current flow measurements can help in accurately diagnosing cervical neoplasia in patients with positive smear tests [7]. The clinical usefulness of these devices has not yet been fully reported.

Presented results indicate that the magnitude of impedance and its dependence on frequency are a function of the composition and structure of the tissue. The impedance method can be used to confirm the diagnosis of malignant tumors by endoscopy or directly before they are verified histologically. Such a procedure could facilitate taking a representative sample of mucous. Fortunately, some of the results of *in vivo* measurements can be simply predicted. It was shown that to obtain corresponding values of the bioelectrical parameters at the same frequency for *in vivo* and *ex vivo* measurements, a multiplying factor should be applied [13].

The future aim of authors' research is to develop a diagnostic aid based on a multiple-electrode array enabling non-invasive real-time measurement of the local distribution of tissue electrical impedance at various frequencies from the surface of the skin. On the basis of the collected regional impedance data, further reconstruction into 2D could be done. Significant progress has been made in recent years in the technique known as electrical impedance imaging or tomography (EIT). The scope of the medical application of EIT has also greatly widened. EIT has been applied to the monitoring of lung ventilation [17], brain activity [18], gastric emptying [19], and diastolic function of heart [20]. Further investigation must be undertaken to specify the role of this technique in the diagnostics of head and neck tumors.

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Conflict of interest: None declared

Received: 8.09.2005
Revised: 13.05.2006
Accepted: 13.05.2006

Praca wpłynęła do Redakcji: 8.09.2005 r.
Po recenzji: 13.05.2006 r.
Zaakceptowano do druku: 13.05.2006 r.