

## Bioimpedance Assessment of Oral Squamous Cell Carcinoma with Clinicopathological Correlation

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### ABSTRACT

**Aim:** Molecular alterations at membrane, cytosol and nuclear level in cancer cells/tissue show variations in bioimpedance measure. In the present study, bioimpedance assessment and comparison was investigated between oral squamous cell carcinoma (OSCC) and normal tissue. Study further involves clinicopathological correlation of bioimpedance values in OSCC.

**Materials and methods:** The present study is comprised of 50 OSCC cases and 50 healthy control subjects. Four electrical properties of OSCC were measured: Impedance (Z); Phase angle ( $\theta$ ); Real part of impedance (R); and Imaginary part of impedance (X) at six frequencies: 20 Hz; 50 kHz; 1.3 MHz; 2.5 MHz; 3.7 MHz; and 5 MHz with the amplitude of the applied voltage limited to 200 mV.

**Results:** The bioimpedance of OSCC as well as control group decreased as the measurement frequency increased from 20 Hz to 5 MHz. The bioimpedance of OSCC was generally smaller than that of control group. The mean bioimpedance of OSCC was found to be  $4493 \pm 216.9 \Omega$  and  $370.0 \pm 26.45 \Omega$  and that of control group was  $15490 \pm 287.2 \Omega$  and  $817.1 \pm 7.227 \Omega$  at frequencies of 20 Hz and 50 MHz respectively which is statistically significant ( $p < 0.0001$ ). The values of phase angle, real and imaginary part of impedance of OSCC group were found to be significantly larger than that of control group ( $p < 0.0001$ ) at 20 Hz and 50 MHz frequency. Impedance values of OSCC were seen to decrease from stages I to IV. Statistically significant differences in values of impedance were observed between stage I ( $4881 \pm 262.5 \Omega$ ) and IV ( $4500 \pm 181.6 \Omega$ )

( $p = 0.0060$ ) and also between stage I ( $4881 \pm 262.5 \Omega$ ) and III ( $4376 \pm 121.3 \Omega$ ) at frequency of 20 Hz ( $p$ -value 0.0005). Statistically significant differences in values of impedance were also observed between well differentiated ( $4557 \pm 260.8$ ) and poorly differentiated OSCC ( $4347 \pm 76.12$ ) ( $p = 0.0004$ ) but only at 20 Hz frequency.

**Conclusion:** Bioimpedance at a particular frequency showed significant alteration in OSCC tissue as compared to control. Hence, it can be potentially promising detection technique for OSCC.

**Clinical significance:** It is a low-cost real time method, which requires little training, and hence can be easily used in primary care centers or in developing countries where multiple challenges limit national screening programs.

**Keywords:** Bioimpedance, Detection, Early detection, Electrical impedance, Non-invasive, Oral cancer.

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### INTRODUCTION

Oral squamous cell carcinoma (OSCC) is an aggressive epithelial malignancy with more than 200,000 new cases occurring annually. Moreover, the Indian subcontinent accounts for one-third of the total cases in the world.<sup>1,2</sup> Oral squamous cell carcinoma has one of the lowest survival rates and remains unaffected despite recent therapeutic advances. Diagnostic delay has been shown to be a significant factor in disease progression. The time between diagnosis of premalignant lesion and malignant transformation is relatively short. Studies have demonstrated transformation occurred as soon as 6 months from the time of biopsy to definitive OSCC

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diagnosis.<sup>3</sup> Early diagnosis and referral is, thus, a cornerstone to improve survival and to reduce diagnostic delay. Unfortunately, almost one half of the OSCC cases are diagnosed at advanced stages (III or IV), with 5-year survival rates ranging from 20 to 50% depending on the site involved.<sup>4</sup>

A number of novel techniques to aid in the early detection of OSCC are available like vital staining (Toluidine blue), chemiluminescence (ViziLite Plus; Microlux/DL, Orascope—DK), tissue fluorescence imaging (VELscope), Tissue fluorescence spectroscopy, Laser capture microdissection, oral brush biopsy (OralCDx), light-based detection systems, spectral cytopathology, optical coherence tomography, etc.<sup>5</sup> All these methods have their own merits and demerits but have failed in their practical implication in the community setup (patients are still reporting in advanced stages). Furthermore, with some methods a lot of subjectivity is associated with the interpretation of results.<sup>6</sup>

Bioimpedance indicates the impedance signal obtained when a low-level sinusoidal current is passed through the tissue and detecting the voltage drop generated by the bioimpedance. It gives information about electrochemical processes at tissue level, and thus can be used for detecting any physiological changes in the tissue. The electrical properties of tissue vary with the frequency of the applied electric field as seen from  $\alpha$ -,  $\beta$ - and  $\gamma$ -dispersion.<sup>7</sup> The  $\alpha$ -dispersion is obtained at lower frequencies (10 Hz–10 kHz) and is related to the ionic environment surrounding the cells. The  $\beta$ -dispersion (10 kHz–10 MHz) shows structure relaxation. The  $\gamma$ -dispersion is released at higher frequencies is related to water molecules. The  $\alpha$ - and  $\beta$ -dispersion regions are meant for medical implications, since most of the changes between pathological and normal tissue occur in this region.<sup>8</sup> In this study, the electrical properties of OSCC tissue and normal mucosa were measured at  $\alpha$ - and  $\beta$ -dispersion regions.

Bioimpedance is a well established technique and has been introduced into clinical investigation of breast cancer<sup>9-12</sup> and cervical cancer.<sup>13-15</sup> In 1999, TransScan TS2000 (TransScan Medical Ltd., Sweden), an impedance-imaging device for breast cancer detection has been approved by the American Food and Drug Administration as an adjunct to mammography for the diagnosis of breast lesions.<sup>16</sup> At present, no studies using bioimpedance for the screening or detection of cancerous changes in the oral cavity have been reported in the literature. Many *in vitro*<sup>17-20</sup> and *in vivo*<sup>9-12</sup> studies showed that there are significant differences in bioimpedance between normal and malignant human breast tissues. Malignant breast lesions have low bioimpedance than

normal tissues. The change in the bioimpedance of malignant tissue as compared to the normal tissue are attributed to increased cellular water and salt content, altered membrane permeability, changed packing density, and orientation of cells.<sup>21</sup> Other studies have also demonstrated that there are significant differences in bioimpedance between benign and malignant breast tumors.<sup>9,11,18,22</sup>

Over the years, bioimpedance has emerged as a better screening tool over the current screening methods since it is a relatively low-cost; real-time method; requires little training; and therefore, can be easily used in primary care centers or in developing countries where the organizational structure and economical factors limit national screening programs. The potential advantages of real-time screening tests include: a reduction in patient anxiety; improved patient compliance; and the ability to repeat inadequate tests immediately.

With this view in mind, the present study was designed to assess and compare the electrical bioimpedance of OSCC and healthy controls. The bioimpedance property was also compared with the clinical stages and histopathological grades of OSCC.

## MATERIALS AND METHODS

The present study is comprised of 50 OSCC cases and 50 (age and sex matched) control cases. Local scientific and ethics committee of Dr DY Patil Dental College and Hospital approved the study. Subjects with pacemakers were not included in the study.

### Impedance Analyzer (Precision Impedance Analyzer AD5934, Analog Devices, USA)

The electrical circuit design made for the present study (Fig. 1). The AD5934 is a high precision impedance converter system solution. An external complex impedance is excited with a known frequency. The produced signal from the impedance is analyzed by the on-board analog-to-digital converter (ADC) and a discrete fourier transform (DFT) is processed by an on-board DSP engine. At each output frequency, the DFT algorithm returns a real (R) and imaginary (I) data-word. The machine is calibrated and all the parameters are calculated (Fig. 2). A disposable probe with four 1 mm diameter silver electrodes (2 mm between electrode centers) mounted in square configuration on a wooden spatula was used (Fig. 3).

### Measurement of Electrical Impedance

Four electrical properties of OSCC tissue were measured: Impedance (Z); Phase angle ( $\theta$ ); Real part of impedance

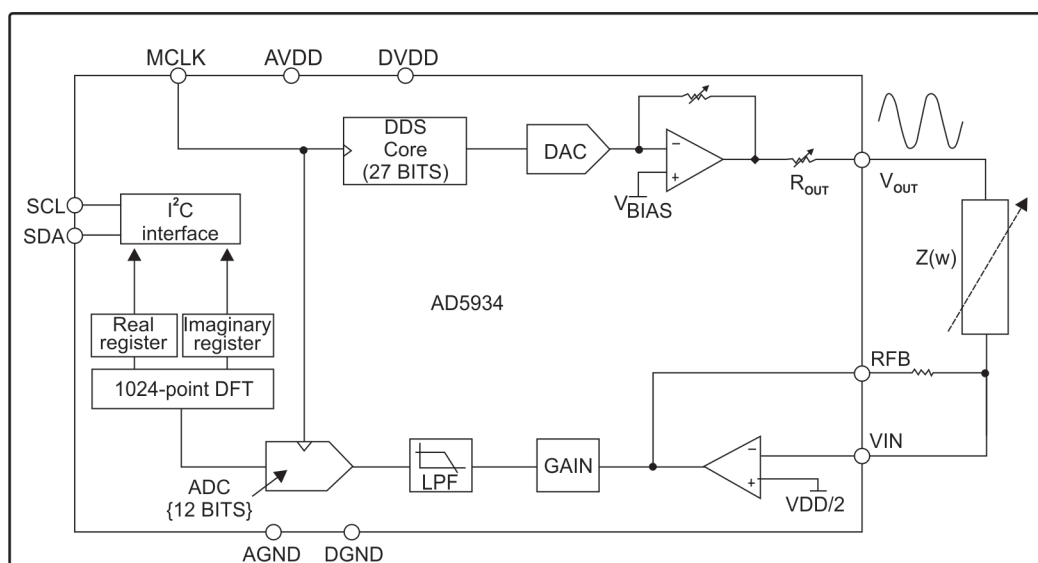


Fig. 1: Functional block diagram for AD5934: Impedance analyzer

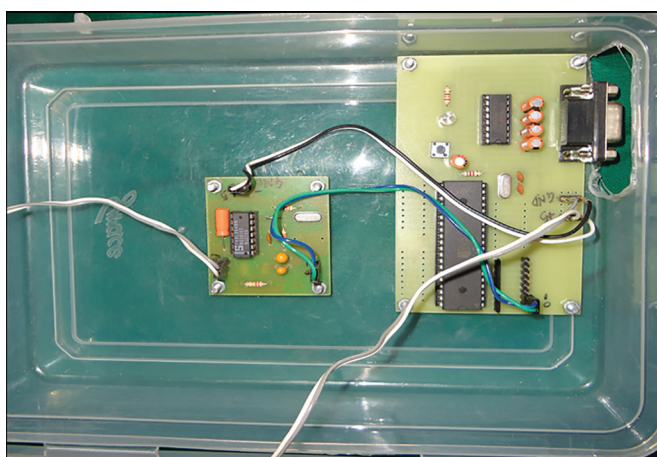


Fig. 2: AD5934 impedance analyzer: Hardware



Fig. 3: Disposable probe made up of four 1-mm-diameter silver electrodes (2 mm distance between electrode centers) mounted in square configuration on a wooden spatula

(R); and Imaginary part of impedance (X). The disposable probe was connected to an impedance analyzer (Precision Impedance Analyzer AD5934, Analog Devices, USA) for all measurements. The probe was evaluated in saline of known electrical conductivity with an accuracy of  $\pm 0.2 \Omega$  on measuring resistance. Pre- and postexperimental oral temperatures were orally measured using body-temperature thermometer.

Electrical properties of OSCC tissue were measured with patients sitting on a chair. The disposable probe was placed directly on the tissue of interest. At each position, electrical properties of oral tissue measurements were made at six frequencies: 20 Hz; 50 kHz; 1.3 MHz; 2.5 MHz; 3.7 MHz; and 5 MHz, with the amplitude of the applied voltage limited to 200 mV. In all cases, three separate sets of measurement of a position were made in succession in order to check reliability of the measurements. The same operator and experimental design was used throughout the study.

## STATISTICAL ANALYSIS

Unpaired 't' test was used to determine whether there were significant differences between the values of bioimpedance of OSCC and control group for the test parameters of Z,  $\theta$ , R and X at each frequency. All statistical analyses were carried out using statistical package for social sciences (SPSS) software with the level of statistical significance set at 0.05.

## RESULTS AND OBSERVATIONS

### Demographic Data

A wide variation in the ages of subjects was noted. Age of OSCC patients ranged from 28 to 80 years with an average age of  $53.8 \pm 14.71$  years whereas control subjects were in the age range of 25 to 77 years with the mean age of  $53.07 \pm 15.04$  years. There was no significant difference between the control and patients group with



respect to the mean age. The majority of OSCC cases were noted in the 5th decade of life. Both OSCC as well as control group consisted of 32 (64%) males and 18 (36%) females. The male to female ratio was found to be 1.7:1. Clinically, exophytic growth was seen in 22 cases (44%), 13 cases (26%) presented as an ulcerated growth, seven cases (14%) were seen as ulcers, four of the cases (8%) presented as endophytic lesions, three cases (6%) were of speckled type of leukoplakia and one case (2%) was of erythroleukoplakia.

After TNM staging six cases (12%) were found to be in stage I, three cases (6%) in stage II, 17 cases (34%) in stage III, 16 cases (32%) in stage IVa, five cases (10%) in stage IVb and 3 cases (6%) in stage IVc. In all the three cases of stage IVc, distant metastasis had taken place to the lungs. On histopathological examination, 26 cases (52%) were found to be well differentiated, 15 cases (30%) were moderately differentiated and nine cases (18%) were poorly differentiated.

### Measurement of Oral Temperature

Patients' oral temperatures ranged from 35.8 to 39.6°C. No statistical significant difference was found in the pre- and post-experimental oral temperatures in both the study group and control group (Table 1).

### Impedance at Different Frequencies: OSCC Patients vs Controls

The impedance of OSCC group as well as control group decreased as the measurement frequency increased from 20 Hz to 5 MHz. The impedance of OSCC group was generally smaller than that of control group. Moreover, at 20 Hz the impedance of study group ( $4493 \pm 216.9$ ) was found to be significantly smaller than that of control group ( $15490 \pm 287.2$ ) ( $p < 0.0001$ ). Similarly at 50 kHz, the impedance of study group ( $370.0 \pm 26.45$ ) was found to be significantly smaller than that of control group ( $817.1 \pm 7.227$ ) ( $p < 0.0001$ ) (Table 2).

### Phase Angle at Different Frequencies: OSCC Patients vs Controls

The phase angle of study group as well as control group decreased as the measurement frequency increased from 20 Hz to 5 MHz. The phase angle of study group was generally larger than that of control group. Moreover, at 20 Hz the phase angle of study group ( $-21.81 \pm 2.092$ ) was found to be significantly larger than that of control group ( $-14.56 \pm 0.6917$ ) ( $p < 0.0001$ ). Similarly at 50 kHz also, the phase angle of study group ( $-37.24 \pm 2.614$ ) was found to be significantly larger than that of control group ( $-50.35 \pm 1.787$ ) ( $p < 0.0001$ ).

### Real Part of Impedance at Different Frequencies: OSCC Patients vs Controls

The real part of impedance of study group as well as control group was found to decline as the measurement frequency increased from 20 Hz to 5 MHz. The real part of impedance of study group was generally smaller than that of control group. Moreover, at 20 Hz, the real part of impedance of study group ( $4198 \pm 162.6$ ) was found to be significantly smaller than that of control group ( $14000 \pm 348.7$ ) ( $p < 0.0001$ ). Similarly at 50 kHz, the impedance of study group ( $315.0 \pm 3.666$ ) was found to be significantly smaller than that of control group ( $523.7 \pm 3.072$ ) ( $p < 0.0001$ ).

### Imaginary Part of Impedance at Different Frequencies: OSCC Patients vs Controls

The imaginary part of impedance of study group as well as control group was found to increase as the measurement frequency increased from 20 Hz to 5 MHz. The imaginary part of impedance of study group was generally larger than that of control group. Moreover, at 20 Hz, the imaginary part of impedance of study group ( $-1375 \pm 34.76$ ) was found to be significantly larger than that of

**Table 1:** Pre- and post-experimental oral temperatures in OSCC and control group

	Oral temperature		t-value	p-value	Statistical significance
	Pre-experimental	Postexperimental			
OSCC	$37.68 \pm 0.7403$	$37.69 \pm 0.7556$	0.08629	0.9315	No
CTRL	$37.48 \pm 1.297$	$37.48 \pm 1.302$	0.01987	0.9842	No

**Table 2:** Comparison of bioimpedance in OSCC and control group at different frequencies

Frequency (Hz)	OSCC Z (ohm)	Control Z (ohm)	t-value	p-value	Statistically significant
20 Hz	$4493 \pm 216.9$	$15490 \pm 287.2$	167.3	<0.0001	Yes
50 KHz	$370.0 \pm 26.45$	$817.1 \pm 7.227$	89.30	<0.0001	Yes
1.3 MHz	$112.8 \pm 151.6$	$85.60 \pm 3.611$	0.9820	0.3302	No
2.5 MHz	$45.00 \pm 3.015$	$45.10 \pm 2.506$	0.1304	0.8967	No
3.7 MHz	$40.47 \pm 1.839$	$41.61 \pm 1.793$	2.424	0.0185	No
5 MHz	$28.85 \pm 3.481$	$30.13 \pm 2.601$	1.609	0.1130	No

control group ( $-3552 \pm 211.2$ ) ( $p < 0.0001$ ). Similarly, at 50 kHz also, the imaginary part of impedance of study group ( $-185.3 \pm 2.877$ ) was found to be significantly larger than that of control group ( $-620.8 \pm 3.530$ ) ( $p < 0.0001$ ).

### Comparison of Electrical Parameters in Histopathological Grades of OSCC at Different Frequencies

Impedance values declined as the histological grade progressed from well to poor. Statistically significant differences in values of impedance were also observed between the grades, well ( $4557 \pm 260.8 \Omega$ ) and poor ( $4347 \pm 76.12 \Omega$ ) only at 20 Hz ( $p = 0.0004$ ) (Graph 1).

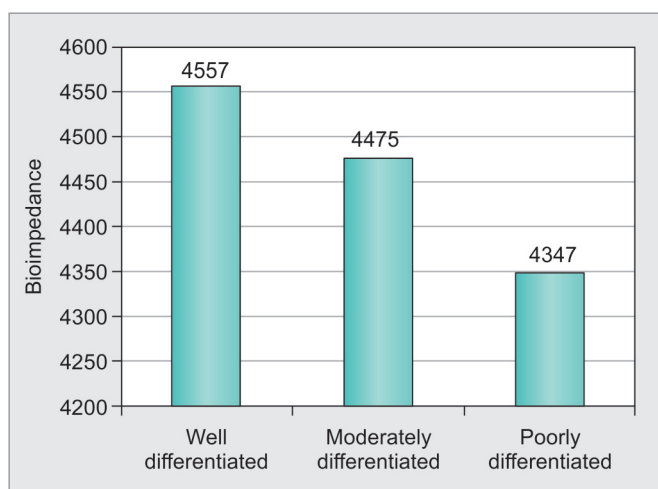
No statistically significant difference was obtained in the values of phase angle, real part of impedance and

imaginary part of impedance between the three grades of OSCC at any frequency (Table 3).

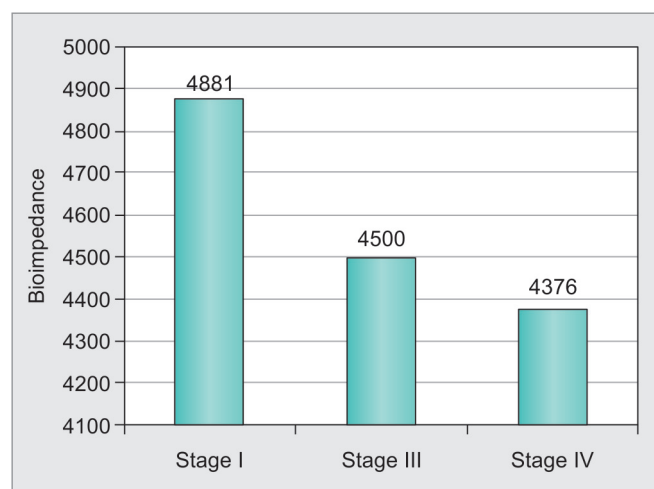
### Comparison of Bioimpedance with TNM Stages at Various Frequencies

Impedance values were seen to decrease from stages I to stage IV. Statistically significant differences in values of impedance were observed between stage I ( $4881 \pm 262.5 \Omega$ ) and stage IV ( $4500 \pm 181.6 \Omega$ ) at frequency of 20 Hz ( $p = 0.0060$ ) and also between stage I ( $4881 \pm 262.5 \Omega$ ) and stage III ( $4376 \pm 121.3 \Omega$ ) at frequency of 20 Hz ( $p = 0.0005$ ) (Graph 2).

No statistically significant difference was obtained in the values of phase angle, real part of impedance and imaginary part of impedance between the three clinical stages at all frequencies (Table 4).



**Graph 1:** Comparison of bioimpedance with histopathological grades of OSCC at 20Hz



**Graph 2:** Comparison of bioimpedance with TNM stages of OSCC at 20Hz

**Table 3:** Comparison of bioimpedance with histopathological grades at different frequencies

Frequency	Grade	Mean ± SD	f-value	p-value	R-square	Statistical significance
20 Hz	Well	4557 ± 260.8	2.267	0.0004	0.1438	Yes
	Moderate	4475 ± 130.4				
	Poor	4347 ± 76.12				
50 KHz	Well	366.2 ± 25.57	0.9829	0.3872	0.06787	No
	Moderate	367.6 ± 29.58				
	Poor	383.5 ± 24.44				
1.3 MHz	Well	135.7 ± 207.9	0.3755	0.6905	0.02706	No
	Moderate	88.24 ± 4.497				
	Poor	84.45 ± 3.253				
2.5 MHz	Well	44.86 ± 3.253	0.3481	0.7092	0.02514	No
	Moderate	44.6 ± 3.012				
	Poor	45.92 ± 2.634				
3.7 MHz	Well	40.41 ± 2.138	0.01848	0.9817	0.001367	No
	Moderate	40.56 ± 1.702				
	Poor	40.52 ± 1.350				
5 MHz	Well	28.37 ± 4.042	0.4409	0.6480	0.03163	No
	Moderate	28.99 ± 2.957				
	Poor	29.95 ± 2.611				

**Table 4:** Comparison of bioimpedance of different clinical stages at different frequencies

Frequency	TNM stage	Mean $\pm$ SD	f-value	p-value	R-square	Statistical significance
20 Hz	Stage I	4881 $\pm$ 262.5	9.996	0.0006	0.4347	Yes
	Stage III	4376 $\pm$ 121.3				
	Stage IV	4500 $\pm$ 181.6				
50 KHz	Stage I	369.8 $\pm$ 18.09	0.007753	0.9923	0.0005961	No
	Stage III	369.5 $\pm$ 27.80				
	Stage IV	368.3 $\pm$ 27.85				
1.3 MHz	Stage I	80.67 $\pm$ 11.58	0.4051	0.6711	0.03022	No
	Stage III	85.57 $\pm$ 2.645				
	Stage IV	137.3 $\pm$ 207.4				
2.5 MHz	Stage I	45.30 $\pm$ 1.353	0.04325	0.9577	0.003316	No
	Stage III	44.75 $\pm$ 3.605				
	Stage IV	45.03 $\pm$ 3.061				
3.7 MHz	Stage I	40.80 $\pm$ 1.100	1.353	0.2762	0.09423	No
	Stage III	39.65 $\pm$ 2.249				
	Stage IV	40.83 $\pm$ 1.601				
5 MHz	Stage I	26.87 $\pm$ 2.802	0.8159	0.4532	0.05906	No
	Stage III	29.73 $\pm$ 3.567				
	Stage IV	29.01 $\pm$ 3.382				

## DISCUSSION

In this study, pre- and post-experimental oral temperatures of all the patients as well as controls were recorded as bioimpedance of the tissue varies with the temperature. Results showed that only 3 patients of the OSCC group and 1 subject of the control group had a change of 0.1°C in oral temperature after the experiment. Such temperature changes might be due to the patient's real oral temperature change or due to instrumentation error during measurement as the body temperature thermometer has an accuracy of  $\pm 0.1^\circ\text{C}$ . For the other patients, their pre- and post-experimental oral temperatures were constant. Among the patients, the lowest and the highest oral temperatures were 35.8 and 39.6°C respectively, with a temperature difference of only 3.8°C. Among the healthy subjects, the lowest and the highest oral temperatures were also 36.6 and 36.8°C, respectively, with the temperature difference of only 0.2°C. No statistically significant difference was found on patients' pre- and post-experimental oral temperatures as well as on control subjects' pre- and post-experimental oral temperatures. Also, no statistically significant difference was found between the pre-experimental oral temperatures of patients and that of healthy subjects as well as between the post-experimental oral temperatures of patients and that of healthy subjects. Because the oral temperature difference among the patients was not large, the effect of temperature on patients' bioimpedance value was assumed to be minimal.

Measurements of the electrical properties of tissues, impedance, phase angle, real part and imaginary part of impedance can be done in two or four-electrode configuration. The instrument assembly is critical, and hence

in the present study, a four-electrode impedance measurement was used so that the measured bioimpedance could be essentially independent of the contact impedance between electrode and tissue. Since a four-electrode configuration was employed in the instrumentation and size of the lesion could be small, the disposable probe and the sensing area were needed to be as small as possible. Therefore, we kept the sensing area of about 9 mm<sup>2</sup> with the probe size of 5  $\times$  3 mm thick  $\times$  100 mm long. This ensured the reduction of inter-site and inter-individual variation. Four electrical parameters (Z,  $\theta$ , R and X) were assessed in the  $\alpha$ - and  $\beta$ -dispersion regions to see if significant difference in values obtained in patient's and healthy subject's oral mucosa existed. Our findings showed that only specific frequencies within the  $\alpha$ - and  $\beta$ -dispersion regions were useful in distinguishing the OSCC tissue and normal tissue.

Only the measurement at 20 Hz and 50 kHz could significantly distinguish the OSCC tissue from normal tissue. At low frequencies, the capacitive cell membranes have a high bioimpedance, and the sent current is confined to the narrow extracellular pathways of the tissue. This results in a high bioimpedance. However, in OSCC tissue, these pathways are wider, and thus offer less bioimpedance. Moreover, reduced cell volume allows electric current to take a relatively straight pathway. This results in a very low levels of bioimpedance of OSCC tissue.<sup>23</sup>

In the present study, impedance values decreased from 4493  $\pm$  216.9 to 28.85  $\pm$  3.481  $\Omega$  for OSCC patients and from 15490  $\pm$  287.2 to 30.13  $\pm$  2.601  $\Omega$  for controls as the measurement frequency increased from 20 Hz to 5 MHz. At 20 Hz the impedance for OSCC patients ranges from 4236.5 to 5159.1  $\Omega$  with a mean of 4493  $\pm$  216.9  $\Omega$ ;

whereas for controls the impedance values ranged from 14939.1 to 15926.9  $\Omega$  with a mean of  $15490 \pm 287.2 \Omega$ . At 50 KHz the impedance for OSCC patients ranges from 309.1 to 414.1  $\Omega$  with a mean of  $370.0 \pm 26.45 \Omega$ ; whereas for controls the impedance values ranged from 830.3 to 800.4  $\Omega$  with a mean of  $817.1 \pm 7.227 \Omega$ . Statistically significant difference was noted between the study and control group at 20 Hz and 50 KHz with a  $p < 0.0001$ . Similar results were obtained by Ching et al<sup>24</sup> and Sun et al<sup>25</sup> in their studies on tongue tissue. Results of the study conducted by Ching et al<sup>24</sup> showed that impedance (Z) of cancerous tongue tissue (CTT) ( $Z = 4318 \Omega$  at 20 Hz and  $372 \Omega$  at 50 kHz) was significantly smaller than that of the surrounding normal tongue tissue (NTT) ( $Z = 12772 \Omega$  at 20 Hz and  $783 \Omega$  at 50 kHz).

Results of the study conducted by Sun et al<sup>25</sup> showed that impedance (Z) of CTT ( $Z = 4356 \Omega$  at 20 Hz and  $381 \Omega$  at 50 kHz) was significantly smaller than that of the surrounding NTT ( $Z = 13295 \Omega$  at 20 Hz and  $764.8 \Omega$  at 50 kHz) as well as that in healthy subjects ( $Z = 14459 \Omega$  at 20 Hz and  $816.9 \Omega$  at 50 kHz).

Variability of bioimpedance values between patients was seen because of number of reasons which include different electrode and tissue contact impedances related to fluid content of the saliva, slight variations in pressure applied between the probe and tissue and inherent patient to-patient tissue variation.

Conductivity is a measure of the mobility of ions in the extracellular fluid in the presence of an electric field. The tissue composition determines travel of current, and thus electrical conductivity is specific for a tissue. Increased cellular water and salt content, altered membrane permeability, changed packing density and different orientation of cells seen in OSCC results in high conductivity. Conductivity is inversely proportional to resistance and thus bioimpedance, and hence OSCC tissue shows lower levels of bioimpedance as compared to normal tissues.

Our results are also in accordance with the *in vivo* study conducted by Wan et al<sup>26</sup> on prostate cancer. They found that at frequencies ranging from 0.4 kHz to 25.6, the conductivity in cancerous tissue was significantly larger than that of normal tissue. It is hypothesized that this relationship may arise from the larger blood volume present in the highly vascularized cancerous regions; blood has a higher conductivity than normal prostatic tissues. This finding is contradictory to that reported previously in multiple *ex vivo* studies. These reports have suggested that cancerous tissue has a significantly lower conductivity than benign tissue ( $p < 0.05$ ) at frequencies ranging from 0.1 to 100 kHz, and that the permittivity of cancerous tissue is significantly greater than that of normal tissues at 100 kHz ( $p < 0.0001$ ).

These contradictory findings may stem from differences between *in vivo* and *ex vivo* tissue properties. Blood-concentrated vascularization may be the predominant factor affecting the bioimpedance of the OSCC in *in vivo* tissues. On the other hand, tissue architecture and cell density become the dominating factors affecting the electrical properties under *ex vivo* conditions. Our findings are also similar to other studies in the literature reporting bioimpedance of breast cancer and cervical cancer.

For clinicopathological correlation, the 50 OSCC patients were divided into four groups according to the TNM staging as stages I to IV and into three groups according to the histopathological grade as well, moderate and poor. Six cases were found to be in stage I, three cases in stage II, 17 cases in stage III, and 24 cases in stage IV. Moreover, 16 of the cases were found to be well differentiated, eight cases were moderately differentiated and six cases were poorly differentiated. Impedance values of the three groups classified according to TNM stage and histopathological grades were compared at frequencies of 20, 50, 1.3, 2.5, 3.7 and 5 MHz. Impedance values were seen to decrease from stages I to IV. Statistically significant differences in values of impedance were observed between stage I ( $4881 \pm 262.5 \Omega$ ) and stage IV ( $4500 \pm 181.6 \Omega$ ) at frequency of 20 Hz ( $p = 0.0060$ ) and also between stage I ( $4881 \pm 262.5 \Omega$ ) and stage III ( $4376 \pm 121.3 \Omega$ ) at frequency of 20 Hz ( $p = 0.0005$ ). However, no statistically significant difference was noted in the other parameters of phase angle, real and imaginary part of impedance between the three stages.

Furthermore, impedance values declined as the histological grade progressed from well to poor. Statistically significant differences in values of impedance were also observed between grades well ( $4557 \pm 260.8 \Omega$ ) and poor ( $4347 \pm 76.12 \Omega$ ) only at 20 Hz ( $p = 0.0004$ ). Moreover, no statistically significant difference was noted in the other parameters of phase angle, real and imaginary part of impedance between the three grades. To the best of our knowledge, reports on impedance values in relation to different stages and grades of OSCC were not found in the available literature, therefore, comparison was not possible.

## CONCLUSION

In conclusion, significant differences in OSCC from control group were observed at 50 kHz electrical properties (Z,  $\theta$ , R and X) measurement. It was also found that Z and R of OSCC were generally smaller than that of control group. The advantage of this method as a potential screening test is that it can provide an immediate result and may be used by those with minimal training in the setting of primary care or in the developing world. To



further explore its role, we recommend future studies on oral potentially malignant disorders and correlation with different grades of epithelial dysplasia. Moreover, studies in community setup are needed to realize effectiveness of this technique.

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