ORIGINAL RESEARCH

Biopotential Assessment—An Alternative in Oral Squamous Cell Carcinoma Diagnostics: A Study

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ABSTRACT

Aim: The aim of this study was to ascertain the relation between surface electrical potential and the presence of cancer in the buccal mucosa and their correlation.

Materials and methods: A device was designed to measure the skin potentials on the face, and various head and neck carcinomas can be detected in a noninvasive way. The present study is a case control study in the ratio 1:1, comprising two groups of 10 individuals each. The two distinct groups of the study are as follows:
1. Normal subjects group consisting of individuals with no habit of tobacco, with the absence of any lesion active or passive on the skin or buccal mucosa.
2. Cancer patients group consisting of individuals attending the head and neck services at the Tata Memorial Hospital, Mumbai, with the habit of tobacco consumption and having a lesion on the buccal mucosa with biopsy confirming diagnosis of squamous cell carcinoma (SCC).

Results: Sites of SCC were significantly electropositive compared with control sites in normal tissue. But noncancerous lesions yielded no potential difference between the lesion and control sites.

Conclusion: The skin surface potential values are maintained in an individual with no cancerogenesis, whereas in oral squamous cell carcinoma (OSCC) the lesion values are more electropositive than the surrounding areas. This can be used to detect OSCC.

Significance: The device designed is patient-compliant and can be used in cancers of breast, colon, etc. More research work is recommended on skin surface potentials.

Keywords: Electric, Electrodes, Oral squamous cell carcinoma, Skin surface potentials.

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INTRODUCTION

The relationship between bioelectricity and electrical technology has been a long and a two-way street. Its history[1] may be traced back through many centuries, but the starting point that could be considered is the work of Galvani in 1760s, who discovered the electric battery formed by dissimilar metals in an electrolytic solution, and the stimulation of nerves by the current so generated. Through the 19th century, the work of Helmholtz, who was both an eminent physicist and physiologist, put forward the concept of electrical double layer, which is fundamental to the understanding of bioelectric potentials existing at membrane interfaces in all biological systems.

The biological systems[2] have electrical activity associated with them. This activity can be a constant DC electric field, a constant flux of charge-carrying particles or current, or a time-varying electric field or current associated with some time-dependent biological or biochemical phenomenon. All healthy living cells have a membrane potential of about –60 to –100 mV. The negative sign of the membrane potential indicates that the inside surface of the cell membrane is relatively more negative than the immediate exterior surface of the cell membrane.[3] In a healthy cell, the inside surface of the cell membrane is slightly negative relative to its external cell membrane surface.[4] When one considers the transmembrane potential of a healthy cell the electric field across the cell membrane is enormous, being up to 10,000,000–20,000,000 V/m.[5]

The healthy cells intracellularly maintain a high concentration of potassium and a low concentration of sodium. But when cells are injured or cancerous, sodium and water flow into the cells and potassium, magnesium, calcium, and zinc are lost from the cell interior and the cell membrane potential falls.[6-8] Many authors have reported that cancer cells have: (a) Higher intracellular sodium, (b) higher content of unstructured water, (c) lower intracellular potassium, magnesium, and calcium concentrations, and (d) more negative charges on their cell surface.[7-10] The available evidence suggests that cancer develops

within a background of dysregulated proliferation encompassing a field. Hence, preliminary studies which use invasive needle electrode indicate that the region of electrical depolarization extends to the skin surface in quadrants of the breast which harbors malignancy, Davies.[11]

The cancer cells have lower transmembrane potentials than normal cells and altered membrane permeability. These cell membrane changes interfere with the flow of oxygen and nutrients into the cells and impair aerobic metabolism causing cancer cells to rely more on anaerobic metabolism for energy production. Anaerobic metabolism, excessive sodium concentrations, low transmembrane potential, and pH alterations in turn create intracellular conditions more conducive to cellular mitosis.

ELECTRICAL DETECTION OF NEAR SURFACE CARCINOMA

Experimental results have shown that during oncogenesis there are changes in the ionic composition of the cell. There are changes in the cell membrane composition as well. During oncogenesis the intracellular Na+ concentration increases and K+ concentration decreases, and the permeability for Na+ ions also increases. The extracellular fluid would then reflect as changes in the electrical potential of the cell. These changes in the potential could affect the surrounding electric field and can be measured on the surface if these changes are close to the skin surface.

In case of breast cancer, majority of tumors are superficial and palpable. In such cases skin surface potential measurements can have diagnostic value, especially in reducing unnecessary diagnostic tests with inconclusive findings. Proliferative changes in the breast epithelium are an intrinsic aspect in the development of breast cancer and results in regions of epithelial electrical depolarization within the breast parenchyma, which extend to the skin surface. Diagnostic information can be obtained from noninvasive and nonimaging test based on skin surface electropotentials.

REVIEW OF LITERATURE

As seen in Table 1, the concept budded in the 1990s and various studies were attempted. It may be concluded that this technique will be useful for the near surface carcinoma. Notable among these is HFN cancers – particularly squamous cell carcinoma (SCC). In India the prevalence of SCC is relatively high due to tobacco usage—both as cigarettes and analogues as well as chewing tobacco. As per the recent Global Tobacco Survey (GATS) India Report (2010), the current use of smokeless tobacco among adult males in India is as high as 32.9% and among females it is 18.4%. Overall 26 of the adult population consume smokeless types of tobacco. Owing to the widespread nature of the disease, an inexpensive modality for detection and amount of intervention will be particularly useful. This study set out to explain the electrical potential measurements as an inexpensive and noninvasive alternate method to traditional methods.

Most of the clinical research has been focused on evaluating the effectiveness of the test for the differential diagnosis of localized suspicious cancerous lesions. The next step would be for further development and clinical assessment of the technology as a new modality for cancer screening. For this application, additional sensors would be utilized to allow measurement of electropotentials independent of lesion location and for asymptomatic individuals. The objective would be identification of high-risk patients by detecting abnormal levels of relative depolarization, as reflected by higher electropotential differentials. These patients could then be referred for imaging or other tests. Initial pilot studies

Table 1: Early researchers of skin surface potentials in cancer

Study	Author	Notes
Measurements of cell membrane potential in breast tissue and in breast epithelial cells to explore the relation between cell membrane potentials, oncogenesis and electrical potentials previously measured on the breast surface.	Marino et al 1994[12]	The potentials were in the range of −16.2±2.8 mV for benign cases. And −13.3±2.2 mV for malignant breast lesions.
A multi-centric study was carried out in 661 women to see whether measurement of the breast electrical activity with surface sensors could distinguish benign and malignant breast disease [Biofield test]	Cuzik et al 1998[13]	They found a highly significant trend of progressive electrical changes according to the characteristics of biopsied tissue. Discriminatory information was obtained in both pre-menopausal and post-menopausal women. The test procedure was similar to an ECG where in non-invasive sensors were used to measure the skin surface potentials. The results revealed specificity of 55% and sensitivity at 90% for palpable lesions.
A biofield diagnostic test was done on 182 women scheduled either for mammography or ultrasound or both	Subbhuraam VS, 2012[14]	This test demonstrated high values of sensitivity 96.23, specificity 93.80% and accuracy 94.51%. The clinical study results showed that this modality can help physicians to differentiate benign and malignant breast lesions.

using a nondirected or screening-type array indicate that cancers produce higher differentials than benign lesions or normal tissue.

Another application of this technology would be for the diagnosis of recurrent cancer. In the irradiated breast, e.g., mammography has been shown to have a sensitivity of only 64% for recurrent carcinoma in patients who previously had undergone conservative surgery. The Biofield diagnostic array[12] could be utilized for these cases because the region of suspicion, i.e., the site of the previous cancer, is identifiable. Pilot studies are currently under way in Europe to determine the potential effectiveness of the Biofield test for this application. Monitoring the effectiveness of therapy may be another potentially useful application of the technology.

Currently, it is difficult to assess the effectiveness of therapy prior to mortality reduction endpoints in randomized trials. An alternative approach might be to evaluate tissue proliferation before, during, and after cycles of therapy. There is evidence[11] to support the use of the skin surface potential measurements for the assessment of tissue proliferation. The evidence comes from the multicenter trials, in which it was found that proliferative and atypical benign lesions produced greater electropotential differentials than nonproliferative benign lesions.

The key aspect of this technology is its ability to sample electrical potentials concurrently from an array of many sensors placed on the skin. This allows multiple comparisons between sensor sites for the detection of abnormal proliferation, which reveal regions or pockets of relative depolarization on the surface of the skin, analogous to pockets of low- and high-pressure systems seen on weather maps. The technology in its present form confers a number of inherent advantages. These include:

- The test is completely noninvasive and there is no pain associated with the procedure.
- There is no exposure to ionizing radiation or other energy.
- The test is simple to implement and can be performed by a technician.
- Conducting the test takes about 15 minutes and the result is available immediately.
- The test can be repeated as often as needed.
- The test result is objective and does not require an expert for interpretation.
- The test is cost-effective, hence can be widely used.
- Since the test is noninvasive, simple to use, and is cost-effective, it could be eventually integrated into various health care settings where primary or

basic evaluation occurs and may provide adjunctive information for assisting in the resolution of screen detected abnormalities or suspicious palpable lesions.

Hence, a study to compare the skin surface potentials in normals and patients suffering from oral squamous cell carcinoma (OSCC) was undertaken.

MATERIALS AND METHODS

The block diagram shown in Figure 1 provided reading in each channel to measure the skin surface potentials with reference point and shows the data using alphanumeric display.

The above-mentioned surface potential measurement circuit was made using MSP430F1611 with all necessary peripherals, analog circuit, and power management unit. The block diagram shows different parts of the board with specific tasks. The modules are described as follows:

- *Processing unit:* The printed circuit board (PCB) was built around MSP430F1611 microcontroller, typically designed for acquiring data from external peripheral devices. The analog data was converted into digital value by using analog to digital converter (ADC) and after processing, the data was displayed on a 20×4 alphanumeric LCD panel. The inbuilt digital port and digital to analog converter (DAC) control the analog signal for processing by using a level shifter to keep the ADC input value positive. (Successive approximation register type of ADC can convert only positive analog input voltage.)
- *Multiplexer unit:* The circuit consisted of four 4:1 multiplexers that receive analog signal from the electrodes and feed to the analog section according to the channel selection provided by the controller. The multiplexing is achieved by using two integrated circuits (ICs) 74HC4052 (dual 4:1 multiplexers).
- *Analog unit:* The circuit was designed to receive ± 60 mV at input side, which converts to the value of 0–2.5 V and fed to the ADC of the microcontroller. The analog consisted of three parts built using IC OP07.
- *Amplifier section:* This section was a noninverting amplifier with a gain of 3, and the amplifier takes input from the multiplexer and feeds it to the filter.
  - *Filter section:* This section was second-order Chebyshev low pass filters with a cutoff frequency of 1.6 Hz. These filters only pass voltages near to DC and block all unwanted signals (like electrocardiogram, electromyography, respiration interference, power line noise). Then these voltages are fed to the controller through level shifter.
  - *Level shifter section:* Since the controller ADC would not be able to handle any negative voltage, so to

measure negative voltage the input was shifted to a positive value using a level shifter.

- *Power supply unit:* The circuit was designed to work on the 9 V battery. The regulator IC LM7806 provided 6 V to the analog section. A supply inverter IC ICL7660 provided a negative voltage to the analog section. In order to provide power to the controller, a low dropout (LDO) regulator IC LP2981 was used, which provided 3.3 V to the mixed signal processor (MSP).
- *Display unit:* This section consisted of a 20 × 4 alpha-numeric display with digital level shifters. The MSP fed the information to the level shifter IC 74HC8T245 which shifted the data to 5 V logic from 3.3 V and drive the LCD display directly.
- *Programming:* The microcontroller was programmed to check every input skin potential and show it on the display.

The present study was a case control study in the ratio 1:1, comprising two groups of 10 individuals each. The two distinct groups of the study are:

1. *Normal subjects group:* This consisted of individuals with no habit of tobacco, with the absence of any lesion active or passive on the skin or buccal mucosa. This group has medically fit subjects.
2. *Cancer patients group:* This consisted of individuals attending the head and neck services at the Tata Memorial Hospital, Mumbai, with the habit of tobacco consumption. Each individual has a lesion on the buccal mucosa, with biopsy confirming diagnosis of SCC.

As shown in Figure 1, a device designed for measuring skin surface potentials was utilized. It comprised a single PCB provided with various other components, fabricated as shown in Figure 2.

After acquiring ethical clearance and patient consent, each individual from the above group was selected for the study. Eight channels were placed on the skin surface of the face bilaterally. First four on the right side followed by the other four on left. Special care was taken to choose subjects who were freshly shaven [skin prepared] to avoid readings with any artifacts or noise in the recordings. The electrodes were stuck separately, such that there was no overlap of even the adhesive material. All care was taken to prevent any leakage of the electro-gel beneath the electrodes, thereby avoiding any shorting in the circuits. Channels 1 to 4 were placed on the affected side of the face and channels 5 to 8 were placed on the contralateral side of the face. Channel 1 paired with channel 5; channel 2 paired with channel 6; channel 3 paired with channel 7; channel 4 paired with channel 8; i.e., these channels were placed bilaterally symmetrical on either side of the face. The reference electrode was put in the sternum.

In the cancer group, channel 4 was kept on the skin overlying the suspicious cancer area. All the other channels were placed on normal overlying skin of the face. Both the groups were subjected to following comparisons and assessment of various parameters was done (Table 2):

Parameter 1: Channel 4 has to be specifically compared to its anatomically similar contralateral side which is nondiseased. The difference of this anatomical site was denoted by D1.

D1 = channel 4 – channel 8

Parameter 2: Readings of channel 4 have to be compared with the channels on the same side, i.e., the diseased side. Hence the average of channels 1 to 3 has to be taken, presuming that channels 1 to3 clinically do not not overly on the cancerogenic site. The difference is denoted by D2.

Thus, D2 = channel 4 – average of channels 1 to 3

Parameter 3: The skin surface potential on both half of the face has to be evaluated to judge the difference in the clinically affected side as compared to sound normal side of the same individual. This difference is denoted by D3.

D3 = average of channels 1,2,3,4 – average of channels 5 to 8

The numerical values obtained thus were subjected to Student's t-test.

Table 2: Diagrammatic representation of parameters evaluated after obtaining the readings

	Normal group	Cancer patients
D1 (Comparing Ch 4 with 8)	√ [Interpretation 1]	√ [Interpretation 4]
D2 (Comparing Ch 4 with mean of Ch 1 to 3)	√ [Interpretation 2]	√ [Interpretation 5]
D3 (Bilateral comparison)	√ [Interpretation 3] Comparing right half with the left half	√ [Interpretation 6] Comparing cancerous side from the normal side

The numerical values obtained thus were subjected to Student's t-test

- Data given for two groups were:

	Normal	*Cancer*
Sample size	$n_1 = 10$	$n_2 = 10$
Mean	X_1	X_2
Standard deviation	SD_1	SD_2

STATISTICAL EVALUATION

Applying the Fundamentals of Null Hypothesis

Null Hypothesis: There is no significant change in the surface potential measurements

(Normal Group):

D1: Channel 4 – channel 8 = 0 (Not significant)

D2: Channel 4 – (Average of channels 1 to 3) = 0

(Not significant)

D3: That is, Left-hand side of the face and right-hand side of the face have no difference

LHS – RHS = 0 (Not significant)

Thus, in the normal group subjects obey Null Hypothesis.

Similarly, when cancerogenesis sets in the area affected it is unable to maintain the surface potential.

{Cancer Group}:

D1: Channel 4 – channel 8 ≠ 0 (Significant)

D2: Channel 4 – (average of channels 1 to 3) ≠ 0

(Significant)

D3: That is, Comparing averages of all channels of cancerous side *vs* normal side has no difference

LHS – RHS ≠ 0 (Not significant)

RESULTS

In normal subjects (Table 3), the skin surface potential values should be equal bilaterally, i.e., not significant as there was no carcinogenesis. Statistical data reveals that there was no significant difference between Channel 4 and 8 for 90% confidence interval. These findings are similar to earlier results that sites of SCC were significantly electropositive compared with control sites in normal tissue, but that noncancerous lesions yielded no potential difference between the lesion and control sites.

DISCUSSION

Oral cancer is a problem of great concern globally. Nowadays, with the introduction of smokeless tobacco in the Eastern Asia, the abuse has percolated among the teenagers and male to female ratio of the habit is comparable. Cancer is curable if detected early. It is the dream of every clinician to curb the menace caused by this life-threatening disease. Extensive survey and screening protocols are designed to diagnose the disease at a very early stage.

Wilson and Jungner[13] have mentioned about the criteria for a disease to be categorized to undergo a screening protocol. Since oral cancer meets at least three of those criteria, screening measures seem to be seriously warranted. Owing to the cost implications and the possibility for over-diagnosis (false positive result), strict criteria are needed to evaluate the screening program and to determine its appropriateness. Similar to pap smear, which has stood the test of time for diagnosing cervical cancers, there is no test available to diagnose oral cancer.

Vascular-related streaming potentials and neural activity may contribute to the genesis of the electrical potentials, but they arise primarily from Nernst potentials across various tissue membranes. The altered electrical potentials reflect the presence of transformed cells during oncogenesis. This effect is due to change in interstitial K+ concentration that arises from alterations in the activity of K+ channels. Surface electrical potentials shows a change in individuals with cancer, if measured on the skin. It is found that decreased intracellular K+ concentration occurs during oncogenesis, and this account for the observed association between elevated electrical potentials and cancer. Decreased intracellular K+ suggests a higher extracellular K+ level, and the cancer site would therefore tend to be electropositive compared with a control site in normal tissue because addition of relatively few K+ could produce electrical potentials comparable in magnitude.

Table 3: Normal group

Para meter	Test for	t	p-value	Interpretation
D1	Channel 4 – channel 8	0.505	0.6357	1) No significant difference between Ch 4 and 8 for 90% confidence interval
D2	Channel 4 – (average of channels 1 to 3)	0.9834	0.3706	2) No significant difference between channel 4 and average channels 1, 2, 3 for 90% confidence interval
D3	Comparing left hand side with right hand side of the face [LHS with RHS]	1.4737	0.2	3) No significant difference between left hand side with right hand side of the face for 90% confidence interval

Table 4: Cancer patients group

Parameter	Test for	t	p-value	Interpretation
D1	Channel 4 – Channel 8	2.1965	0.071	4) Significant difference between Channels 4 and 8 for 90% confidence interval
D2	Channel 4 – (average of channels 1 to 3)	2.3129	0.060	5) Significant difference between Ch 4 and average channels 1 to 3 for 90% confidence interval
D3	Comparing averages of all channels of cancerous side *vs* normal side	1.9126	0.105	6) No significant difference between the averages for Cancerous side and the unaffected contralateral side for 90% confidence interval Significant difference for 85% confidence interval

Review of the literature has generated a lot of interest in modalities of screening, which were noninvasive, showed patient compliance, and ease of repeatability in past few years. Skin surface potential measurement has appeared to be lucrative as a screening tool. Hence various experiments were conducted, in which the skin surface potential measurement studies were pursued. It is hypothesized that, if we were able to detect the surface potentials on the surface of the skin using electrodes and quantify them, then that can help in differentiating disease state like cancers from the normal physiologic conditions in man. Thus the skin surface potential measurement (a modality which analyzes the skin surface electrical potentials measured by an array of electrodes) test holds a promise in diagnosing breast cancer. Thus if the device can be used to measure the skin potentials on the face, various head and neck carcinomas can be detected in a noninvasive way, given that the skin surface potentials by and large remain stable and same in a normal individual. The cells are able to maintain the Na–K influx. Hence the difference on the left half of the face as compared to right half of the face should be more or less zero. Statistically evaluating the results should be not significant.

In normal subjects (Table 3), in the right half of the face, the skin surface potential values should be equal, i.e., not significant as there was no carcinogenesis. Statistical data reveals that there was no significant difference between Channel 4 and 8 for 90% confidence interval. This was because the surface potential in normal individual was physiologically maintained equal bilaterally. The value, if compared, was almost zero or less than zero. Hence was not significant.

The present study included all cases of SCC confirmed by gold standard biopsy. Channel 4 was always kept on the area where clinically abnormal multiplication of cell – i.e., cancerogenesis—was suspected. Hence behavior of Channel 4 was compared to various other channels in both the groups. Electrical changes may provide a physical basis for distinguishing between normal and cancerous growth.[14] Our findings are similar to earlier results[15] that sites of SCC were significantly electropositive compared with control sites in normal tissue, but that noncancerous lesions yielded no potential difference between the lesion and control sites. In preliminary studies done by researchers, elevated surface electrical potentials were found to be associated with cancerous lesions beneath the skin in women with palpable breast masses.[16] The aim of the present study was to ascertain the relation between surface electrical potential and the presence of cancer in the buccal mucosa. In cancer patients (Table 4), in the right half of the face, the skin surface potential values would not be the same and hence should be statistically significant. Table 4 also reveals that Channel 4 and the contralateral side, Channel 8, showed statistical significant results. There was a significant difference between Channel 4 and 8 for 90% confidence interval. Also, there is a significant difference between Channel 4 and average channels 1 to 3 for 90% confidence interval.[17,18]

CONCLUSION

The differences in channels of normal subjects are not statistically significant as the normal skin surface potential is always maintained at a particular level. So, the skin surface potential values appear close to each other and their differences are not statistically significant. This implies that they are close to zero. The affected area shows significant difference with the ipsilateral channels as well as the contralateral channels. That means if clinically known cancer lesion is given, prediction of the skin surface potential of the area can be made and then quantified. The affected side may be at the same skin surface potential because of process of cancerogenesis. Hence the difference of the affected side appears "not significant" statistically, according to the findings of interpretation. It is significant only at 85% confidence interval. Larger sample size is hence required to prove a statistical relevance. Trials need to be carried out on cancers occurring in the other parts of the body like breast, colon.

CLINICAL SIGNIFICANCE

Our findings are similar to earlier results that sites of SCC were significantly electropositive compared with control sites in normal tissue, but that noncancerous lesions yielded no potential difference between the lesion and

control sites. In preliminary studies done by researchers, elevated surface electrical potentials were found to be associated with cancerous lesions beneath the skin in women with palpable breast masses. If clinically known cancer lesion is given, prediction of the skin surface potential of the area can be made and then quantified.

ACKNOWLEDGMENT

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