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Detection of cervical intraepithelial neoplasia using impedance spectroscopy: a prospective study

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Objective To compare cervical impedance spectrometry in the cervical epithelium of women with cervical intraepithelial neoplasia (CIN) and normal epithelium.

Design Prospective observational study.

Setting Colposcopy clinic, Jessop Wing, Royal Hallamshire Hospital, Sheffield, UK.

Population Eighty-seven women referred to colposcopy with a moderate or severely dyskaryotic smear.

Methods A pencil probe incorporating four gold electrodes was used to measure an electrical impedance spectrum from cervical epithelium. Colposcopy examinations, including probe positioning, were recorded by video to allow for correlation between results obtained from colposcopic impression, histopathological examination of colposcopically directed punch biopsies and the impedance measurements.

Main outcome measures Cervical impedance derived parameters *R*, *S* and *C* were assessed to see if there was a significant difference in values obtained in CIN and normal squamous epithelium. Analysis was based upon matching the electrical components measured to those identified by cellular modelling as being most sensitive for premalignancy.

Results From normal epithelium through CIN 1 to CIN 2/3, *R* decreased by a factor of 4.5, *S* increased by a factor of 2.5 but *C* remained unchanged.

Conclusions Cervical impedance spectrometry provides a potentially promising screening tool with similar sensitivity and specificity to currently used screening tests, but with the potential advantage of providing instant results. Further work is currently being undertaken to improve the probe in its clinical use.

INTRODUCTION

Cervical cancer is the second most common cancer worldwide, affecting 471,000 women per year with an associated mortality of 233,000. In England and Wales, there are 2700 new cases annually.[1] However, it is potentially preventable by screening and treating the precancerous phase, cervical intraepithelial neoplasia (CIN).

Screening programmes that have been developed are based on the use of exfoliative cervical cytology. However, this test has poor sensitivity, approaching only 50% in some studies when used as a single test. Many of the programmes are effective because of the long pre-invasive phase of CIN, which may last many years. This gives multiple opportunities to detect underlying CIN, which permits an efficient screening programme, once properly organised. Nonetheless, the operation of such a programme is expensive, with an estimated cost of £150 million per year in the UK alone.

In developing countries (which account for about 80% of all cases worldwide), efficient cervical screening programmes are difficult to develop, mainly due to logistical and economic reasons, although the tests' sensitivity and ability to obtain a rapid result also contribute to the problem. There is therefore a need to develop other tests for the detection of CIN, which may make a major impact not only in the developing countries but also in improving the efficiency and the reliability of screening programmes in the developed world.

A number of other screening methods have been investigated including visual inspection of the cervix following the application of acetic acid, colposcopy, cervicography, speculoscopy, molecular (HPV DNA) techniques, fluorescence spectroscopy and electrical measurement techniques.[2] Biological tissues have an electrical impedance, which is a function of frequency. The reason for this dependence is that tissues contain components, which have both resistive and capacitive (charge storage) properties. Both the size of the impedance, as well as the dependence of impedance on frequency, are related to tissue composition. Different tissue structures are associated with different frequency bands within an impedance spectrum. At high frequencies (>1 GHz), molecular structure is the determining factor; whereas at low frequencies (<100 Hz), charge accumulation at large membrane interfaces will dominate. At frequencies of a few kHz to 1 MHz, sometimes referred to as the β dispersion region, cell structures are the main determinant of

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tissue impedance. Within the β dispersion region, low frequency current can be considered as passing through the extracellular space, the current has to pass around the cells and the resistance to flow will depend upon the cell spacing and arrangement. However, at higher frequencies, current can penetrate the cell membranes, hence, pass through both intra- and extracellular spaces. Current will pass through the cells and will thus be determined by intracellular volume and possibly by the size of the nucleus.

Cervical epithelium is a highly structured, stratified tissue that exhibits changes associated with CIN, including an increase in the nuclear cytoplasmic ratio, loss of the layer of flattened cells close to the surface and an increase in the volume of extracellular space. This formed the basis for predicting the associated tissue impedance spectra likely with CIN. Our previous research work[3] has shown that a pencil probe incorporating four gold electrodes can be designed and used to measure an electrical impedance spectrum from cervical epithelium. The objective of the pilot study was to use impedance spectral measurements from the female cervix to assess the agreement between practical measurements and the predictions made by taking into account the known cell arrangements.

In the pilot study on 124 women,[4] changes in the spectra were observed in association with premalignant changes. The sensitivity and specificity obtained in the detection of premalignant changes were at least as good as those obtained from cervical smear testing. The observed changes in impedance spectra can be explained by modelling the known structural changes in the top 400 μm of cervical epithelium associated with CIN 1, 2 and 3.[5,6]

Our current research objective is to improve the performance of the probe and to address practical issues in order to facilitate routine clinical use of the technique.

METHODS

Impedance measurements were made using a 5.5-mm diameter pencil probe, with four 1-mm diameter gold electrodes mounted flush with the face of the probe and spaced equally on a circle. A current of 20 μA peak-to-peak was passed between an adjacent pair of electrodes and the resulting potential measured between the two remaining electrodes. The ratio of the measured potential to the amplitude of the imposed current determines a transfer impedance. Measurements were made at eight frequencies by doubling the frequency in steps between 4.8 and 614 kHz.

Impedance measurements were made before acetic acid was applied for the purposes of colposcopy. The probe was placed in eight positions on the cervix. These were as for the cardinal points of the compass with four positions close to the border with the endocervical canal and the remaining four well into the normal squamous epithelial surface of the cervix. Impedance measurements were made serially at 67 frames per second and input to a computer. Colposcopy examinations, including probe positioning, were recorded by video to allow for correlation between results obtained from colposcopic impression, histopathological examination of colposcopically directed punch biopsies and the impedance measurements. To qualify as 'normal', squamous epithelium had to lie outside the transformation zone, show no evidence of change with acetic acid and have a positive staining with Lugol's iodine. The whole of the transformation zone was visible in all cases. In nearly all cases, two separate sets of data (each of 100 measurements recorded over 1.5 seconds) were recorded in succession in order to check reproducibility of the measurements. Only the first set of measurements was used for the results presented in this paper.

Calibration was performed by placing the probe in saline of known electrical conductivity. A four-electrode measurement of the transfer impedance spectrum is essentially independent of the contact impedance between electrode and tissue (which is of the order of 1 kΩ compared with the transfer impedance of 100 Ω).

Patient measurements were made in the colposcopy clinic after the study had been approved by an ethics committee and informed consent was obtained from the patients. Women with high grade smears (i.e. moderate or severe dyskaryosis) were recruited. However, three women with mild dyskaryosis were also studied. Postmenopausal women were not included in this study.

The 100 measurements forming the first data set recorded at each measurement position were averaged to give mean values of impedance at each of the eight frequencies. These data, forming an impedance spectrum, were then fitted by a least square deviation method to a Cole equation[7] of the form

$$Z = R_\infty + \frac{R_0 - R_\infty}{[1 + (jF / F_c)]^{(1-\alpha)}}$$

to give estimates of R_0, R_∞ and F_c. R_0 and R_∞ are the impedances (real part) at very low and very high frequencies, respectively; F_c is a frequency and α is a constant. The α increases with the inhomogeneity of tissue but we assumed a value of zero. In this case, an equivalent electrical circuit consisting of a resistor R placed in parallel with a resistor S and capacitor C in series will have an impedance Z, given by the above equation, where

$$R_0 = R,\ R_\infty = \frac{RS}{R+S},\ F_c = \frac{1}{2\pi C(R+S)}$$

Parameters R, S and C can thus be determined from the fitted Cole equation. Because the probe was calibrated in saline of known conductivity, R and S are inversely proportional to conductivity and have the units of Ω m. They can be related to the extra- and intracellular spaces, respectively, as described earlier. C is related to the cell membrane capacitance and is given in units of μF m^{-1}.

RESULTS

A clear colposcopy result and good impedance data were available for 655 measurements made on 87 women. The maximum possible number of measurements was 8 × 87 (i.e. 696). In 41 cases either, the tissue, at the point where the probe had been placed, was not clearly identified either by biopsy or colposcopy or data were rejected on technical grounds.

After comparing colposcopic and histology results, there were found to be 292 measurements from normal squamous epithelium, 123 from CIN 2/3 (high grade) and 34 from CIN 1 (low grade). In addition, 47 points were classified as mature metaplasia, 100 as immature metaplasia and 59 as columnar tissue.

The derived Cole equation parameters for the three tissue groups are shown in Fig. 1. Non-parametric Mann–Whitney two-tailed tests were performed and showed that there are several significant separations of the groups. The values for *R* and *S* separate normal squamous epithelium tissue from the CIN 2/3 tissues ($P < 0.0001$ in both cases). *R* and *S* also separate normal squamous epithelium from CIN 1 tissues ($P < 0.0001$ in both cases). *S* separates CIN 1 from CIN 2/3 tissues ($P = 0.03$). The values for *C* do not show any significant changes.

To assess the statistical independence of the estimated values for *R* and *S*, a Pearson correlation was performed using the pooled data for normal squamous epithelium, CIN 1 and CIN 2/3 tissues. The r^2 was 0.259, which can be interpreted as showing that 25.9% of the variation in *R* can be attributed to variations in *S* and *vice versa*.

From normal squamous epithelium through CIN 1 to CIN 2/3 (Fig. 1), *R* decreases by a factor of about 4.5, *S* increases by a factor of about 2.5 but *C* does not change.

To assess the usefulness of the technique as a screening test, we derived receiver operating characteristic (ROC) curves[8] for the normal squamous epithelium and CIN 2/3 tissue groups (Figs 2 and 3). ROC curves show the sensitivities (1 − the fraction of false negatives) and specificities (1 − the fraction of false positives) with these variables used as discriminants between the normal squamous epithelium and CIN 2/3 tissue groups. If there is discrimination between the two groups, the curve is displaced upwards and to the left. The area under the curve indicates the degree of separation: an area of 0.5 corresponds to no discrimination and an area of 1.0 to perfect separation.

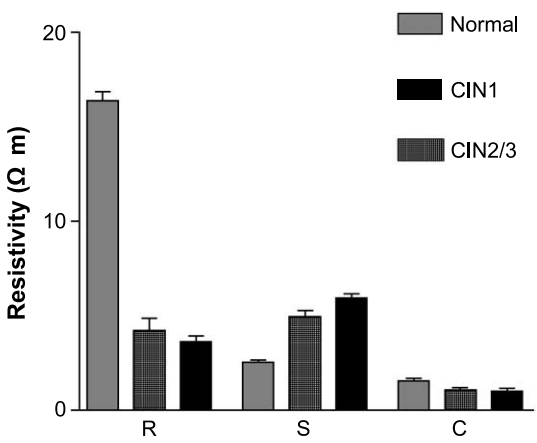

Fig. 1. Mean values of *R*, *S* and *C* in normal squamous epithelium, CIN 1 tissue and CIN 2/3 tissue. *R* and *S* are in Ω m. *C* is a capacitance with the units of $\mu F\ m^{-1}$ (number of points for normal squamous epithelium = 292, CIN 1 = 34 and CIN 2/3 = 123; bars indicate standard error).

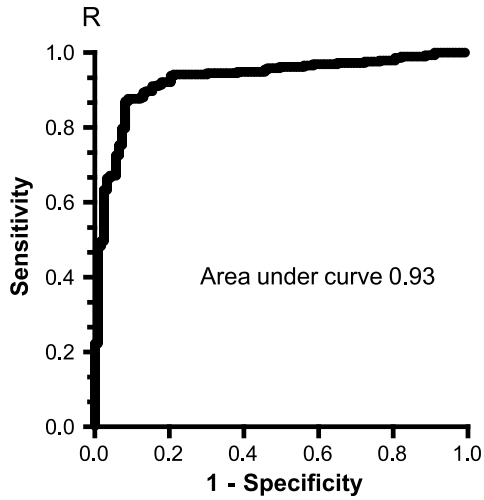

Fig. 2. ROC curves from the data (*R*) for normal squamous epithelium and the CIN 2/3 tissues.

As in the pilot study, the data were also grouped for each woman so that we could make comparisons between the electrical impedance measurements and the results of both the referral smear test and the outcome of the colposcopy examination.

To provide a single indicator for each woman, *R*/*S* was first calculated for each of the eight measurement sites. This was an attempt to take into account the fact that *R* decreases and *S* increases as we progress from normal squamous epithelium through CIN 1 to CIN 2/3. The lowest value of *R*/*S* (*R*/*S* minimum) was then taken as the outcome for each woman on the basis that this should identify the greatest abnormality. However, we found that this method of identification included a number of tissue sites, which were identified by colposcopy as columnar or immature metaplasia tissues. To limit this confusion, we excluded sites where *R* was less than or equal to 2.12 Ω m (the 25% centile for the CIN 2/3 group) when taking the minimum value of *R*/*S* in each woman. In this study, but not in the pilot study, we also analysed the data by defining CIN by centile limits on *R*, *S* and *R*/*S*. If any of the measurements on a particular woman were within these limits, then a positive impedance result was returned.

The colposcopy and biopsy results were used to place women into either a CIN group or a 'normal' group. All of the 'normal' group had at least two colposcopic examinations,

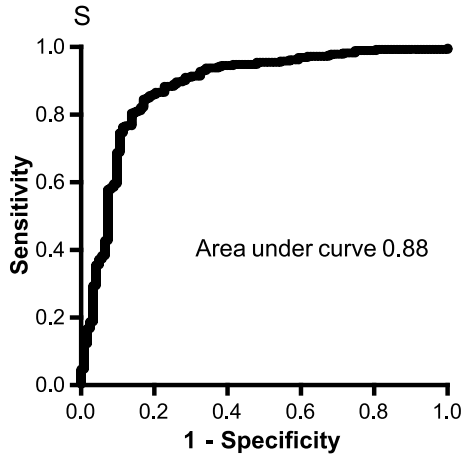

Fig. 3. ROC curves from the data (*S*) for normal squamous epithelium and the CIN 2/3 tissues.

six months apart, with repeat cervical cytology and biopsies. If any tissue of CIN 1 or CIN 2/3 was identified, then the woman was placed in the CIN group. There were 61 in the CIN group and 21 in the 'normal' group. Five were excluded from the total of 87 women. Women were excluded if we obtained fewer than six out of the possible eight measurements or the outcome of the colposcopy investigation was ambiguous.

The *R*/*S* minimum results when compared with the CIN, 'normal' classification using the ROC curve revealed an area under the curve at 0.707. A sensitivity of 67% can be obtained with a specificity of 67%.

If we categorise the 82 women on the basis of the impedance results and use the 75% centile point (0.70) for *R*/*S* minimum as the borderline, then impedance produced the following performance measures: sensitivity 75% (46/61), specificity 43% (9/21), positive predictive value 79% (46/58) and negative predictive value 38% (9/24). If we define CIN using the 25 and 75 centiles on *R*, *S* and *R*/*S* (i.e. $2.0 < R < 3.4$, $4.2 < S < 7.5$ and $0.31 < R/S < 0.55$), then we obtain the following: sensitivity 75% (46/61), specificity 71% (15/21), positive predictive value 88% (46/52) and negative predictive value 50% (15/30). In this study, cervical cytology had a positive predictive value of 74% (61/82).

DISCUSSION

The major changes in cervical tissue in the pre-cancerous stages are the breaking down of superficial cell layering and increases in the size of cell nuclei. The Cole equation, which has been fitted to the measured impedance spectra, provides the parameters *R*, *S* and *C*.

R is determined by the conduction pathways through the extracellular space, and is thus sensitive to the packing of cells into layers. In normal squamous epithelium, we would expect to see a high value for *R* as current has to track around the cell layers, hence, take a long resistive path. In CIN 1 and CIN 2/3, the superficial cell layering of normal squamous epithelium is absent, hence, *R* is greatly reduced. The observed changes in *R* in this prospective study fit well with this model. *S* is determined by the conduction paths through the intracellular space. If the conductance of the intracellular space is reduced in CIN, then *S* will increase in value. There is a well-documented large increase in mean nuclear size in CIN 2/3 tissue[9,10] and this can be expected to cause some increase in the value of *S*. The observed changes show a large increase in *S*. This change is actually larger than can be explained solely by an increase in nuclear size.[5] *C* is determined by the structure of the cell membrane. We found no change in the value of *C*.

Our results show a very good separation of the measurements made on normal squamous epithelium and on CIN 1 and CIN 2/3 graded tissues. The ROC curves (Figs 2 and 3) show that sensitivities and specificities of 80–90% can be obtained in detecting the changes associated with CIN 2/3 in individual biopsy proven sites. The analysis on a 'per woman' basis gives lower sensitivities and specificities of about 70%. These figures are slightly worse than found in the pilot study. The fact that there were more sites of immature metaplasia and of columnar tissue in the prospective group than in the pilot study group would be expected to give a small increase in false-positive results.

The value of *R* is slightly reduced in the prospective study when compared with the pilot study.[4] The most likely reason for this difference is that a different probe was used in the prospective study. While the construction specification was unchanged, small differences in electrode spacing and the flatness of the face of the probe can produce changes in *R* as the thickness of mucous trapped between the tissue and the probe may vary.

It was shown by Coppleson *et al.*[11] that electrical measurements could be used to detect pre-cancerous changes in cervical tissue. Their probe, known as Truscreen,[12] makes bipolar electrical measurements of the response to a 1.25-V pulse applied for 260 μs. The subsequent decay of charge at the tissue/electrode interface is observed. This response will be indirectly related to the low frequency impedance of the electrodes and the underlying tissue. Clinical trials using this probe have not yet been fully reported.

Mitchell *et al.*[13] reviewed several methods for the diagnosis of squamous intraepithelial lesions and derived ROC curves. They give areas under the ROC curves of 0.76 for Papanicolaou smear testing, 0.84 for diagnostic colposcopy and 0.71–0.75 for a fluorescence spectroscopy technique they have developed. In most cases, Mitchell *et al.* grouped CIN 1 and CIN 2/3 tissues together in deriving the ROC curves. Our data in this prospective study are very similar to those we reported previously and again show sensitivities and specificities that are certainly comparable with existing screening methods. The advantage of this method as a potential screening test is that it can provide an immediate result. There are a number of advantages

of a screening test that provides immediate results enabling a positive screen to be investigated further by colposcopy or treated in cases of high grade CIN. This would be of particular benefit in the developing countries where the organisational structure and economical factors limit national screening programmes. In addition, an immediate result from a screening test will reduce the anxiety and wait that many women face after having a smear.

Our current research programme is to use an improved impedance spectrometer to enable measurements to be made more accurately, at 30 instead of eight frequencies and over a wider frequency range. Results from this improved system will be reported later.

CONCLUSIONS

Cervical impedance spectrometry provides a potentially promising screening tool with similar sensitivity and specificity to currently used screening tests, but with the potential advantage of providing instant results. Further work is currently being undertaken to improve the probe in its clinical use.

References

1. National Statistics: Cancer statistics registrations. 2001 MB1, No. 28.
2. Soler ME, Blumenthal P. New technologies in cervical cancer precursor detection. *Curr Opin Oncol* 2000;**12**(5):460–465.
3. Brown BH, Wilson AJ, Bertemes-Filho P. Bipolar and tetrapolar transfer impedance measurements from a volume conductor. *Electron Lett* 2000;**36**(25):2060–2062.
4. Brown BH, Tidy J, Boston K, Blackett AD, Smallwood RH, Sharp F. The relationship between tissue structure and imposed electrical current flow in cervical neoplasia. *Lancet* 2000;**355**:892–895.
5. Walker DC, Brown BH, Hose DR, Smallwood RH. Modelling the electrical impedivity of normal and pre-malignant cervical tissue. *Electron Lett* 2000:1603–1604.
6. Jones DM, Smallwood RH, Hose DR, Brown BH. Constraints on tetrapolar tissue impedance measurements. *Electron Lett* 2001;**37**(25): 1515–1517.
7. Cole KS, Cole RH. Dispersion and absorption in dielectrics. *J Chem Phys* 1941;**9**:341–351.
8. Metz CE. Basic principles of ROC analysis. *Semin Nucl Med* 1978; **8**:283–298.
9. Boon ME, Kok LP. Koilocytotic lesions of the cervix: the interrelation of morphometric features, the presence of papilloma-virus antigens, and the degree of koilocytosis. *Histopathology* 1985;**9**:751–763.
10. Sorensen FB, Bichel P, Jakobsen A. Stereological estimates of nuclear volume in squamous cell carcinoma of the uterine cervix and its precursors. *Virchows Archiv A Pathol Anat* 1991;**418**:225–233.
11. Coppleson M, Reid BL, Skladnev VN, Dalrympl JC. An electronic approach to the detection of pre-cancer and cancer of the uterine cervix: a preliminary evaluation of Polarprobe. *Int J Gynaecol Cancer* 1994;**4**:79–83.
12. Quek SC, Mould T, Canfell K, Singer A, Skladnev V, Coppleson M. The Polarprobe—emerging technology for cervical cancer screening. *Ann Acad Med Singap* 1998;**27**:717–721.
13. Mitchell MF, Cantor SB, Ramanujam N, Tortolero-Luna G, Richards-Kortum R. Fluorescence spectroscopy for diagnosis of squamous intraepithelial lesions of the cervix review. *Obstet Gynecol* 1999; **93**(3):462–470.

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