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The detection of cervical intraepithelial neoplasia by electrical impedance spectroscopy: The effects of acetic acid and tissue homogeneity

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ABSTRACT

Objective. To evaluate the efficacy of an electrical impedance probe (Epitheliometer) in the diagnosis of high grade cervical intraepithelial neoplasia (CIN) in women referred with cervical smear abnormalities and to assess the effect of acetic acid (AA) and tissue boundaries on the measurements.

Methods. A prospective observational study was undertaken in the colposcopy clinic. One hundred and sixty-five women, either with a clinical indication or abnormal cervical cytology, were recruited into the study. A pencil type probe was used to record impedance spectra from 12 points on the cervix before and after the application of 5% AA. Spectra were also recorded from tissue boundaries. Colposcopic examinations, including probe positioning, were video recorded to allow for correlations between histopathological diagnosis of colposcopically directed biopsies, colposcopic impression and the diagnosis based on impedance measurements.

Results. Receiver operating characteristic (ROC) curves were derived. The areas under the curves (AUCs) to discriminate original squamous from high grade CIN were 0.80 (pre AA) and 0.79 (post AA). Comparison of these curves showed no significant difference, indicating that application of AA does not produce a large change in spectra. The probe could distinguish tissue boundaries from homogeneous tissue points.

Conclusion. The Epitheliometer has the potential to be used as an adjunct to colposcopy in the diagnosis of high grade CIN. It has the advantage of real time results, decreasing the need for diagnostic cervical biopsies, and facilitates a wider use of the 'see and treat' policy without the risk of overtreatment.

Introduction

Cancer of the cervix remains the second most common cancer among women worldwide. Cervical cancer is potentially preventable by screening and treatment of high grade cervical intraepithelial neoplasia (CIN) [1]. Conventional cervical cytology when used as a single test has a reported specificity of 95%–98% but a sensitivity of only 50% [2]. The effectiveness of the cervical programme therefore relies on multiple opportunities at identifying and treating CIN. The use of liquid based cytology (LBC) has shown a reduction in the number of inadequate smears, but a recent systematic review and meta-analysis by Arbyn et al. [3] found that LBC did not improve the detection of high grade CIN and that LBC was neither more sensitive nor specific than conventional cytology.

Cervical screening programmes using either conventional or LBC are highly expensive and poor countries find it financially and logistically impossible to organise nationwide screening programmes, leading to a high incidence and mortality from cervical cancer. Cytological screening also has a 'test to result delay,' leading to anxiety in women. There is, therefore, the need to develop other tests that not only improve the detection of high grade CIN but also provide real time results, so that women with abnormal smears can be appropriately managed.

Colposcopic examination including directed biopsies has been proposed as the gold standard in the evaluation of abnormal cervical cytology. However there is increasing evidence demonstrating its poor performance with only 54.8% of women with CIN3 being diagnosed in the colposcopy arm of the ALTS study [4]. Various new technologies, usually employing optical spectroscopy, have been investigated to improve the detection of CIN and have reported sensitivity to detect high grade CIN in the range of 70%–95% and specificity of 50%–83% [5].

The current study was aimed at detecting high grade CIN with a probe that measures tissue electrical impedance. Biological tissue has electrical impedance, which is a measure of the total opposition a circuit presents to electric current. Electrical impedance depends on the frequency of current applied. At frequencies of a few kilohertz to 1 MHz, known as the β dispersion region, cell structure and

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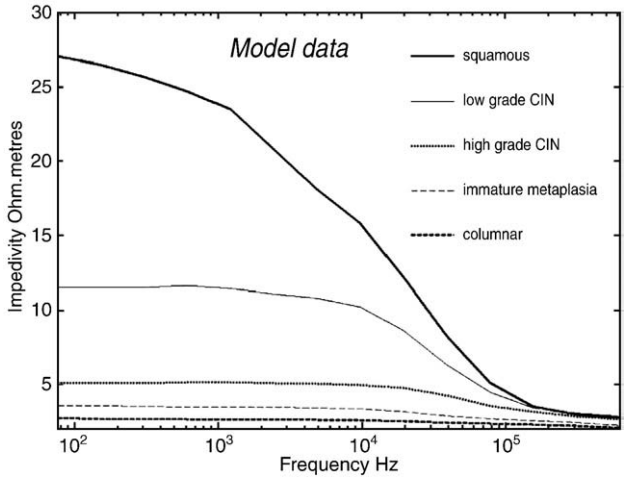

Fig. 1. Impedance curves derived from finite element modelling.

arrangement are the main determinant of tissue impedance. At higher frequencies, electric current can penetrate cell membranes and impedance will be determined by intracellular structures and possibly the size of the nucleus. At lower frequencies, electric current is unable to penetrate cell membranes and current flows mainly through the extra cellular space. Impedance at lower frequencies thus depends on cell spacing and arrangement [6].

Cervical epithelium is a highly structured stratified tissue that exhibits changes as it progresses from normal epithelium to high grade CIN. Changes include loss of stratification, increase in the nuclear cytoplasmic ratio and an increase in the extra cellular space. This formed the basis of our finite element modelling (FEM), a computational tool that predicts the electrical properties of biological tissues.

In our previously reported work, FEM in three dimensions was used to predict the impedance spectra from squamous, columnar, metaplastic, low and high grade CIN tissues (Fig. 1) [7].

The aim of the current study was to evaluate the performance of electrical impedance spectroscopy, as measured by the 'Epitheliometer,' in discriminating between tissue types and to assess its potential for use as an adjunct to colposcopy. Impedance measurements were taken over a range of frequencies (76.3 Hz to 625 kHz) where tissue impedance depends mainly on cell spacing and cellular arrangement. Secondary outcomes were to determine if application of acetic acid to the cervix altered tissue impedance and to assess if the probe was able to identify tissue boundaries in order to remove ambiguous measurements.

Methods

One hundred and sixty-five women attending the colposcopy clinic, with any cervical smear abnormality or a clinical indication for colposcopy, were recruited into the study. Of the 165 women, 124 formed the main part of the study and 41 women entered the boundary detection investigation. Impedance measurements taken irrespective of the phase of the menstrual cycle in view of previous published data [8]. Women pregnant at the time of colposcopy and post menopausal women were excluded. Post menopausal women were excluded as lack of estrogen may lead to atrophy of the cervical epithelium and this was not factored into our model. Pregnant women were excluded as the cervix tends to become more vascular during pregnancy and this will change impedance spectra. Ethics approval for the study and informed consent from participants was obtained.

Women recruited to the study were in the age group of 20–55 years. The majority of the study population, about 73% were non-smokers. Impedance measurements were made using a 5.5 mm diameter pencil probe with four 0.6 mm diameter gold electrodes mounted flush with the face of the probe. A current of <20 μA p-p was passed between an adjacent pair of electrodes and the resulting potential measured between the two remaining electrodes. Measurements were made at 16 frequencies, doubling the frequency in steps from 76.3 Hz to 625 kHz.

The probe was used to take 12 measurements from the cervix, four from the outer ectocervix and eight at the cervical os covering the region of the transformation zone. Improved technology enabled us to take more points from the transformation zone in this study than in our earlier studies [8–10]. This should increase our detection of CIN without unduly prolonging the procedure. 5% acetic acid was then applied to the cervix and 12 further measurements taken. Impedance measurements were transferred to a laptop. Colposcopy was performed and biopsies taken if clinically indicated. The entire procedure was video recorded for subsequent correlation of data. Fig. 2 summarises our study protocol.

The probe assesses tissues lying beneath the area covered by the four electrodes. In the transformation zone, this area may have more than one histological type as a result of either physiological metaplasia or CIN. A probe with the capability of injecting current through adjacent and diagonal drives was used to evaluate if the probe could differentiate tissue boundaries. Boundary data collection was collected from 41 women after the application of acetic acid to facilitate tissue differentiation. Measurements were made at 258 homogenous tissues and 209 tissue boundaries; squamous–columnar ($n=112$), squamous–CIN ($n=78$) and columnar–CIN ($n=19$). Data analysis was used to determine if the probe could differentiate between homogeneous and tissue boundary sites.

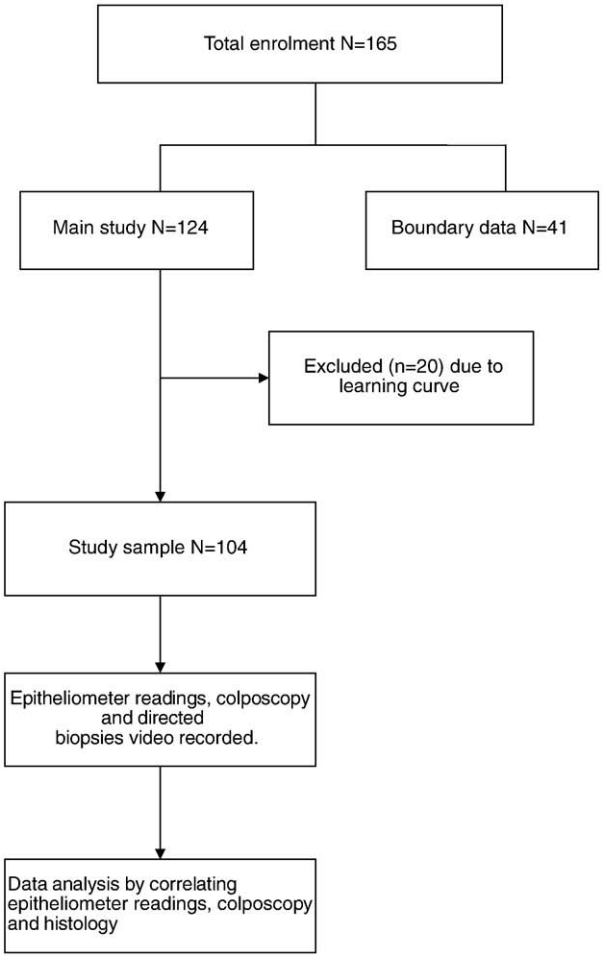

Fig. 2. Figure of protocol.

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Results

One hundred and twenty-four women were recruited into the main study. A 'learning curve' was noticed for impedance measurements taken from the first 20 women of the study and these women were excluded from further analysis. Table 1 lists the indication for referral for colposcopy. Colposcopic examination was satisfactory in all cases.

Impedance spectra were recorded from 1093 points prior to the application of acetic acid. Of these, 204 points were rejected. Twenty-five points were rejected as the probe was unable to take an impedance measurement because of poor contact. One hundred and seventy-nine points were rejected as a definite colposcopic impression could not be assigned either due to poor quality of the video recording or if the probe was placed on a tissue boundary. Eight hundred and eighty-nine points with a clear colposcopy result and good impedance data were analysed; 636 points were squamous, 113 were immature metaplasia, 85 were high grade CIN and 45 were columnar. Ten points were classified as human papillomavirus (HPV) infection, inflammation, mature metaplasia or low grade CIN.

A total of 1068 post acetic acid impedance measurements were collected from the 104 women. After rejecting unsuitable points as with the pre acetic acid data, 839 points were analysed with 597 squamous, 114 immature metaplasia, 76 high grade, 37 columnar and 15 points classified as mature metaplasia, HPV, inflammation or low grade CIN.

Data analysis

Impedance spectra were compared with a template for CIN3 determined from the finite element model of cervical epithelium. Mean deviations of the measured spectra from the template were used to categorise the measurements as normal squamous epithelium, columnar, high grade CIN [CIN2+] or immature metaplasia. The deviations were used point by point and compared with the colposcopic impression obtained from the videos and histological diagnoses. To evaluate if the probe could differentiate high grade CIN from all other tissue types, receiver operating characteristic (ROC) curves were drawn using the Medcalc statistical package. A per woman analysis was performed using the impedance probe results classifying women either as normal or having high grade CIN.

Pre acetic acid data

ROC curves derived for normal squamous epithelium and high grade CIN showed an AUC of 0.80 indicating a good separation. Sensitivity was 78.8% with a specificity of 73%. ROC curves were derived for CIN *vs* all other tissue types. The AUC was 0.77 with a sensitivity of 85.9% and a specificity of 62.6%. For the per woman analysis, women were divided into either the normal group or the high grade CIN group depending on colposcopic diagnosis. The AUC was 0.75 with a sensitivity of 88.4% and a specificity of 63.9%. The sensitivity and specificity values are those where the sum of the sensitivity and specificity values was at a maximum. To separate the women into normal and CIN groups the value of the deviation from the high grade CIN template, derived from the above sensitivity and specificity values, was used.

Table 1
Types of smears in women recruited into the study.

Mild dyskaryosis	34
Moderate dyskaryosis	16
Severe dyskaryosis	27
Borderline	20
Post coital bleeding	5
Inflammatory	1
Glandular neoplasia	1

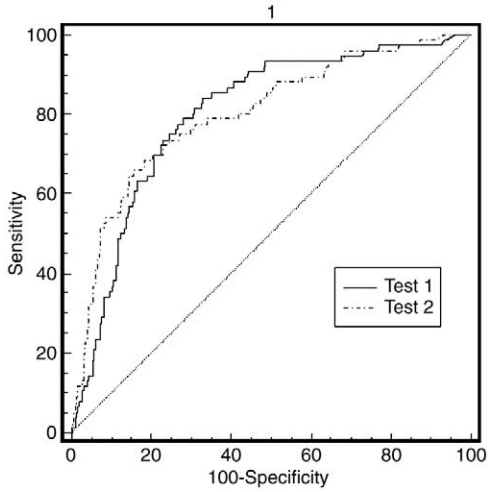

Fig. 3. Comparison of pre- and post-AA ROC curves – squamous *vs* high grade CIN.

Post acetic acid data

ROC curves derived to discriminate original squamous from high grade CIN showed an AUC of 0.79 with a sensitivity of 78.9% and a specificity of 66%. The AUC for high grade CIN *vs* all other tissue was 0.74 with a sensitivity of 73.6% and a specificity of 63%. The per woman analysis gave an AUC of 0.74 with a sensitivity of 89.7% and a specificity of 50% showing that the probe may be used as an adjunct to colposcopy to clinically diagnose women with high grade CIN.

Comparison of pre and post acetic acid data

Fig. 3 shows pre and post acetic acid ROC curves derived to differentiate original squamous from high grade CIN. The difference between the two curves was not statistically significant ($P=0.98$). There was no significant difference between pre and post acetic acid ROC curves derived to differentiate between high grade CIN and all other tissue ($P=0.68$), showing that acetic acid does not cause a large change in impedance spectra.

Comparison of Epitheliometer diagnosis with histology

The Epitheliometer diagnosis of tissue type and histological diagnosis at the site of biopsy were compared by studying data from points where the Epitheliometer reading was almost coincident with the biopsy site. In all cases the colposcopic impression was of high grade disease. At all sites the Epitheliometer and histological diagnosis were in agreement, with 18 sites identified by both as high grade CIN and 4 sites identified as non-CIN, suggesting a 100% sensitivity and specificity, albeit on a small sample set.

Boundary data

Boundary data were collected from a further 41 women giving spectra from 209 tissue boundaries and from 258 sites of homogeneous tissue. Data sets were analysed by using a quantity (n) where n is the ratio between null and the normal measurements.

$$n = \left| \frac{null}{norm} \right|$$

Where:

null is the measurement taken from diagonally opposing electrodes at a frequency of 78.1 kHz.

norm is the measurement taken from adjacent electrodes.

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n was calculated for all the data, boundary and homogeneous subsets. Best separation of the two sets was found using a value of $n = 0.18$. The homogeneous data set was analysed initially by deriving ROC curves to discriminate between squamous and high grade CIN (AUC = 0.86). ROC curves were derived using the same dataset after excluding impedance measurements where n was greater than 0.18. Exclusion of inhomogeneous data appeared to improve the sensitivity to detect high grade CIN but was not statistically significant. Using a more severe limit by decreasing the threshold for $n = 0.1$ increased the AUC significantly (0.98), but this involved rejecting 85% of the measurements and might prolong the measurement time.

Discussion

Histologically, CIN is associated with loss of stratification and differentiation, increased nuclear cytoplasmic ratio and increase in the extra-cellular space. Previous studies suggested a six-fold increase in the extra-cellular space when compared to normal squamous tissue [9]. This increase in extra-cellular space decreases impedance at low frequencies in CIN. Thus, original squamous epithelium has high impedance while high grade CIN has lower impedance. Finite element modelling provided templates for impedance spectra for the various cervical tissue types and the templates were similar to impedance spectra measured from the cervix. The best fit with the measured data was obtained for models with an epithelium of 300–400 μm in depth with a surface layer of mucus of 10 μm [10].

Analysis was initially performed on a per point basis and the AUC for the pre AA ROC was 0.80 and for the post AA ROC 0.79, showing a good discrimination between the two tissue types. In a clinical setting it is important to diagnose if a woman referred with smear abnormality has high grade CIN as this will inform further management. To assess if the probe was able to identify women with high grade CIN, a per woman analysis was performed. The AUC was 0.75 with a sensitivity of 88.4% and a specificity of 63.9% showing that the probe may be used as an adjunct in the colposcopy clinic to diagnose women with high grade CIN.

Application of acetic acid removes mucus and debris from the surface of the cervix, causes a coagulation of cell proteins and leads to acetowhite change. Post AA values might be better than pre AA values as we should be able to visualise the acetowhite area denoting CIN. Results showed that the pre AA ROC curves gave slightly better discrimination. It is possible that because of the thinner layer of mucus after the application of AA, the pathways of electrical current are altered. This needs to be studied further with separate templates for post acetic acid analysis. Balas and Potirakis [11] studied the structural and functional alterations in cervical epithelium following application of acetic acid. Their results suggest that application of acetic acid will result in some closing of gap junctions and an increase in proton concentration in both intra-cellular and extra-cellular spaces. Gap junctions have been shown by tissue modeling to have only a small effect [12]. Increased proton concentration will cause a decrease in tissue impedance but hydrogen ions have relatively low mobility and so the change in impedance will be small. We conclude that a small decrease in tissue impedance, leading to small changes in spectral shape, might be expected following the application of acetic acid but these changes are likely to be much less than the changes associated with the large structural differences between normal and CIN epithelium. While the pre AA AUC showed slightly better discrimination of tissue types than the post AA AUC, comparison of the ROC curves found no statistical significance, demonstrating that the application of acetic acid does not markedly change impedance measurements.

Good discrimination of immature metaplasia from high grade CIN remains a problem. This is a difficult area in colposcopy as well, as these tissues become acetowhite and may look similar on visual inspection. In our study, ROC curves to differentiate immature metaplasia from high grade CIN, using any of the study parameters gave an AUC ranging from 0.50 to 0.65. While this is not ideal, in previous studies the AUC for these two tissue types has been 0.51–0.55 showing a definite improvement in the discrimination between these two tissues [8].

Previously published work described earlier versions of the Epitheliometer, used different frequencies, different methods of analysis and different population characteristics [8–10,13]. Results published by Abdul et al. [10] showed good discrimination of original squamous and high grade CIN with an AUC of 0.88. However, the per woman analysis was slightly worse at AUC = 0.65 when compared to the per woman AUC in the current study (AUC = 0.74). It is possible that the use of a wider range of frequencies, in particular lower frequencies, in the current probe has improved tissue differentiation.

The ability of the Epitheliometer to diagnose tissue boundaries was evaluated. In a perfectly homogeneous tissue there is a balance between the two receiving electrodes. When the probe is placed on a boundary between tissue of different resistance the system is unbalanced and this is detected by the probe. It is essential that impedance measurements are not taken from tissue boundaries to reduce the possibility of a false positive diagnosis of CIN and improved AUCs were obtained when impedance measurements taken from tissue boundaries were excluded. This capability will be incorporated into the next version of the Epitheliometer, to reduce the potential for false positive results. Appropriate setting up of recording systems and ensuring that the cervix is not covered with blood or excessive mucus would further bring down the number of points rejected. Quality assurance standards in the probe have also been improved to ensure that only good quality data should be collected.

Evaluation of any colposcopic technique or new screening tool is limited by the availability of a true 'gold standard' as it is not feasible to biopsy every colposcopic abnormality. In an attempt to overcome this problem we analysed 22 high grade colposcopic lesions and compared the histological diagnosis with the Epitheliometer diagnosis. There was 100% concordance between histology and probe diagnosis. The overall results are encouraging and larger numbers of patients are needed to explore this further.

We have demonstrated that the Epitheliometer has the potential to act as an adjunctive test in the colposcopy clinic either as an aid to take better targeted biopsies, replace the taking of biopsies or guide treatment at first visit. In poor countries, where organised call and recall screening programmes are not in place, visual inspection of the cervix with acetic acid (VIA) and visual inspection with Lugol's iodine (VILI) have been used as screening tools. A recent study established a sensitivity of VIA to be 79% and a specificity of 85% with VILI on average 10% more sensitive and equally specific [14].

In resource poor countries the Epitheliometer can be used on VIA positive women over 35 years as a secondary screen to improve the detection of high grade CIN allowing for a single episode of care that includes diagnosis and treatment without histological confirmation. Previous work by Escobar et al. [15] has shown an improvement in specificity in the detection of high grade CIN when optical coherence tomography was used as a diagnostic aid to VIA. In a trial using the Polarprobe which uses both optical and electrical systems, the sensitivity and specificity of the Polarprobe for the diagnosis of invasive cervical cancer was found to be 90% and 91% respectively [16]. However, in the Shanxi province cervical cancer screening study using fluorescence spectroscopy did not improve detection of high grade CIN [17].

The Epitheliometer is a self contained, battery powered, portable device which does not require cervical biopsies for diagnosis of CIN and thus could be used as an inexpensive tool for the diagnosis of CIN. As the probe gives real time results, women diagnosed with high grade CIN can be treated at the same visit without the risk of over treatment. The development of such a device is especially important as the major burden of cervical cancer is borne by poor countries.

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Conflict of interest statement
The authors declare that there are no conflicts of interests.

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