Relationship between colposcopy and bioelectric parameters in cervical squamous epithelium in women in Caldas, Colombia in premenopausal, menopausal and postmenopausal stages

P Gallego-Sanchez, G Olarte-Echeverri, W Aristizabal-Botero, J Rojas-Diaz and C Ruiz-Villa

1 Biophysics Instrumentation Laboratory, Physics Department, Natural and Exact Science Faculty, Caldas University, Manizales, Colombia
2 CI2DT2 – Innovation and Development Technology Center, Engineering Faculty, Caldas University, Manizales, Colombia
3 Informatics and Systems Department, National University of Colombia

E-mail: paula.gallego@ucaldas.edu.co

Abstract. The aim of this research was to measure the structural changes in the squamous epithelium of women in the perimenopausal stages through colposcopic and electrical impedance spectroscopy (EIS). 167 women between 44 and 69 years of age were classified into three groups: premenopausal, menopausal and postmenopausal. Each of them underwent colposcopic examinations and were evaluated by electrical impedance spectroscopy to measure the resistivity of those tissues in two sets of measurements: firstly, after the application of normal saline solution (NSS) at 0.9% and secondly, after being impregnated with 4% acetic acid (AA). It was found that the resistivity of the extracellular matrix (R) of the cervical squamous tissue, measured with NSS and with AA, decreases progressively from the premenopausal stage (NSS 16.7 +/- 15.0 Ω·m; AA 22.3 +/- 14.2 Ω·m) until the postmenopausal stage (NSS 7.0 +/- 8.1 Ω·m; AA 9.6 +/- 10.6 Ω·m). The characteristic frequency (Fc) of the impedance spectra increases significantly for the mentioned stages from 38.6 kHz to 102.3 kHz with NSS and from 29.5 kHz to 86.4 kHz with AA. It was evidenced that, as the years of amenorrhea increase, the electrical resistivity of the tissues decreases progressively.

Introduction

The end of women’s reproductive life, the menopausal stage, is marked by a decrease in the number of oocytes in the ovaries and a change in the secretion of hormones such as gonadotropins and sex steroids [1]. The menopausal phenomenon is a physiological phenomenon in women that can also be produced by iatrogenic methods, such as: drugs, surgery or radiotherapy [2]. According to the Stages of Reproductive Aging Workshop + 10 (STRAW + 10), the menopausal transition is a stage comprising several phases. The menopause or early postmenopause, is a phase that begins after one year of amenorrhea and it is classified in stages +1a, +1b, +1c [3-5]. The menopausal phase is
preceded by two previous phases called early and late menopausal transition, classified in stages -2, -1 and 0. In the menopausal transition, the menstrual cycles are anovulatory and symptoms such as hot flushes, sweating and abnormal uterine bleeding can occur [6, 7]. The late postmenopause phase, stage +2, goes from five or six years after the last menses for the remaining lifespan [3, 4].

The hypoestrogenic state related to the decrease in estriol in the menopausal transition is responsible for symptoms in the genitourinary tract [8]. In the perimenopausal process, changes in the squamous epithelium of the cervix, such as maturation decreasing and the appearance of atrophy are presented [9].

The atrophic cervix shows changes in the squamous and columnar epithelia and the disappearance of the intermediate and superficial cells, while the basal and parabasal cells remain. Clinically, a smaller cervix can be observed, which can bleed easily. Colposcopy shows a thin epithelium enabling the blood vessels of the stroma and sub-epithelial bleeding to be seen, while the squamous-columnar junction goes into the endocervical canal. In the Pap smear, the atrophic epithelium of postmenopause can be confused with intraepithelial lesions (CIN 1, CIN2), leading to unnecessary procedures and follow-ups [9].

Electrical Impedance Spectroscopy (EIS) is a technique used in the characterization of different materials and biological tissues. In EIS, a small alternating current is usually applied to the tissue at a range of frequencies, from a few kHz to a few MHz. The current is supplied through a pair of electrodes through a tetrapolar probe. The potential difference between points of the tissue is measured by the other pair of electrodes. The tissue transfer impedance to each of the signal frequencies is obtained from the ratio between the measured voltage and the applied current. With the calculated impedance values and the different frequencies, curves or spectra can be constructed to associate each curve with the different types of tissues and their pathological states [10-15].

In previous studies by this group of researchers [15-17], it was observed that the resistivity of the squamous epithelium in healthy women of fertile age was different from that of healthy women in the perimenopause stage, in which the tissues presented extracellular resistivity values (R) in a range between 5 and 15 Ω-m, similar to those that occur in low-grade intraepithelial lesions. In the normal transition from the fertile age to the menopausal stage, different phenomena occur that affect the electrical resistivity of the cervical squamous tissue, produced by the decrease in the thickness and loss of cellular layers in this epithelium.

From these observations, it was proposed to systematically evaluate the different phases of menopause through colposcopic changes and tissular resistivity values. This approach could help to reduce the positive-false rate reported by Pap smears in perimenopausal stages.

1. Materials and methods

Women using the public health service were recruited for the project “Implementation of the program for the diagnosis and control of chronic non-transmissible diseases and cervical and breast cancer with the support of ICT in the department of Caldas”. After the study had been approved by the ethics committee of Caldas University and informed consent obtained, they underwent colposcopic, cytological and electrical impedance spectroscopy (EIS) examinations.

The clinical data of the patients were collected between 2015 and 2017 in an electronic medical record designed for that purpose. From the general database of the program, 167 women over the age of 44 with a normal cytology result were included in this study. From this group, 92 women were classified as early menopausal transition, stages -2, -1 (women who have not stopped menstruating but who have the characteristic symptoms of this cycle) and 75 in the early postmenopausal stage, level +1a; the latter were divided into women who have not menstruated for between one and five years and women who have not menstruated for more than five years. The latter are named late postmenopausal, stage +2, according to STRAW +10 menstrual cycle criteria. Patients who presented acetowhite lesions, leukoplasias, previous conization, prolapse of the uterus and antecedents of pelvic radiotherapy were not considered for this study. The sample was obtained from Caldas University health service and at hospitals in 14 other municipalities in the department of Caldas, Colombia.

The colposcopic examination was performed using a video-coloscope designed and patented by a gynecologist-oncologist participating in this project. The electrical impedance spectroscopy measurements were taken with a tissue impedance-meter that applies a current of 20 μA peak-to-peak through a tetrapolar probe (two current and two voltage electrodes) and by a frequency sweep between 2 kHz and 1.6 MHz, enabling a spectrum to be obtained that shows the real part or the resistivity magnitude as a frequency function [10-17], which can be mathematically adjusted to a Cole-Cole model [18, 19] given by the equation (1):
\[ Z = R_{\text{inf}} + \frac{R_0 - R_{\text{inf}}}{1 + (\frac{\sigma}{F_c})} \]  

This equation (1) enables \( R_0 \) (resistivity at very low frequency), \( R_{\text{inf}} \) (resistivity at very high frequency) and the characteristic frequency \( F_c \) to be calculated. The \( \alpha \) parameter is a constant that increases with tissue heterogeneity. In this study we take \( \alpha = 0 \) when considering the portion of tissue measured is relatively homogeneous. With these variables, it is possible to calculate the electrical parameters that allow the characterization of the tissue: resistivity of the extracellular matrix \( R \), resistivity of the intracellular matrix or cytoplasm \( S \), capacitance of the cellular membrane \( C_m \) and the characteristic frequency \( F_c \) related to the capacitance of the cell membrane or the storage properties of electrical charge at the interfaces of the membrane. The calculations are made with equations (2) and (3) [15].

\[ R = R_0 ; \quad S = \frac{R_0 R_{\text{inf}}}{R_0 - R_{\text{inf}}} \]  

\[ F_c = \frac{R_0 - R_{\text{inf}}}{2 \pi C_m R_0^2} \]

This set of parameters can be associated with a parallel, equivalent electrical circuit formed by two branches: the first is a resistor \( R \) that represents the extracellular space and the other branch is a series formed by a resistor \( S \) that represents the intracellular space and a capacitor \( C_m \) that represents the membrane capacitance [10, 15].

During the colposcopic examination, a cervical-uterine cytology was taken for each of the patients, followed by cleaning of the cervix with normal saline solution 0.9% (NSS) and then, under colposcopy, the spectroscopy probe was positioned in each of the four points of the cervix (clockwise: 12, 3, 6, 9) to perform two sets of EIS measurements. This procedure was repeated after observing and assessing cervical stratified squamous epithelia impregnated with acetic acid 4% (AA). In total, 16 EIS measurements were taken from each patient, 8 with NSS and 8 with AA. The cervical-uterine cytology reports were given based on the Bethesda 2001 system. The data collected by EIS were processed in the Matlab® platform, in which, using a nonlinear, least squares adjustment algorithm designed for this study, the parameters corresponding to the Cole-Cole model were calculated. The atypical data were removed from this set of parameters, eliminating those that exceeded the 90th percentile. The values removed due to poor contact of some of the electrodes with the tissue, or also due to inadequate positioning of the probe at the moment when the EIS measurements were taken, were far from those obtained for normal cervical squamous epithelia in previous studies.

A statistical analysis was performed with the remaining data using the statistical software StatGraphics Centurion® in order to determine whether these data adjust to a normal or Gaussian distribution by Shapiro-Wilks tests and normal probability curves. Mann-Whitney nonparametric tests were also performed to evaluate if the differences between the medians of the groups into which the information was separated, are statistically significant. There were six measurement groups: three for each premenopausal stage multiplied by two types of liquids (NSS and AA) used in the colposcopic examination. The procedures were recorded in photographs and videos to be used in the correlation sessions between the gynecologist-oncologists and the bioengineers participating in the project. With the average values of \( R_{\text{inf}}, R_0 \) and \( F_c \), the representative spectra of the different types of tissues used in this research were constructed.

2. Results
A total of 2,672 electrical impedance spectra were obtained, 1,336 with NSS and 1,336 with AA in the three stages of perimenopause in which the patients were classified. From these, 499 were removed because they presented atypical values that exceeded the 90th percentile taken as reference. The electrical parameters \( (R, S, C_m \) and \( F_c \) were calculated for the remaining spectra using adjustment to the Cole-Cole equation (1). From these parameters, only \( R \) and \( F_c \) were used in this study because they best characterize these epithelia.

The Shapiro-Wilks normality tests and the normal probability graphs showed that the data of \( R \) and \( F_c \) do not adjust to a normal or Gaussian distribution (P-value <0.01) in each of the subgroups in
which the population was divided. The variation coefficients showed high variability with values above 60% (Table 1).

When measuring the squamous epithelium of premenopausal women, over the age of 44 and who continue to menstruate, first using the NSS, an average and standard deviation for the $R$ value of 16.7 +/- 15 $\Omega \cdot m$ was obtained, which increases to 22.3 +/- 14.2 $\Omega \cdot m$ when the measurements are made after impregnating the tissue with AA; the medians were respectively 11.4 $\Omega \cdot m$ with NSS and 21.9 $\Omega \cdot m$ with AA. When evaluating patients in the menopausal group who had not menstruated between one and five years, $R$ was found to have an average value of 7.7 +/- 7.9 $\Omega \cdot m$ with NSS and 18.4 +/- 15.4 $\Omega \cdot m$ with AA; the medians were respectively 4.6 $\Omega \cdot m$ with NSS and 16.6 $\Omega \cdot m$ with AA. For women who had not menstruated for more than five years, the average $R$ values of measurements with NSS were 7 +/- 8.1 $\Omega \cdot m$ and median of 3.3 $\Omega \cdot m$; after applying AA the average $R$ was 9.6 +/- 10.6 $\Omega \cdot m$ and the median 3.9 $\Omega \cdot m$ (Table 1, Figure 1). In Figure 1 we observed that the electrical resistivity of the tissues decreases when perimenopause progresses from the premenopausal stage to the postmenopausal stage.

![Figure 1. Variation in electrical resistivity of the extracellular space in the perimenopausal tissues with 0.9% saline solution and with 4% acetic acid.](image)

Table 1. Statistical values of bioelectric parameters $R$ and $Fc$ for stratified squamous epithelia in perimenopausal stages. All statistically significant differences with a p-value <0.01 of the Mann-Whitney test except (*) p-value = 0.054.

| Type of tissue | Saline solution 0.9% | Acetic acid 4% |
|---------------|----------------------|----------------|
|               | $R$ (\(\Omega \cdot m\)) | $Fc$ (kHz) | $R$ (\(\Omega \cdot m\)) | $Fc$ (kHz) |
| Premenopausal | Mean | 16.7 | 38.6 | 22.3 | 29.5 |
|               | SD   | 15.0 | 70.3 | 14.2 | 67.4 |
|               | Median | 11.4 | 18.4 | 21.9 | 9.7 |
|               | Variation coefficient | 89.8% | 182.1% | 63.6% | 228.4% |
| Menopausal    | Mean | 7.7 | 69.9 | 18.4 | 52.9 |
|               | SD   | 7.9 | 70.4 | 15.4 | 94.2 |
|               | Median | 4.6 | 44.9 | 16.6 | 18.3 |
|               | Variation coefficient | 102.6% | 100.7% | 83.6% | 178.1% |
| Postmenopausal| Mean | 7.0 | 102.3 | 9.6 | 86.4 |
|               | SD   | 8.1 | 109.5 | 10.6 | 96.2 |
|               | Median | 3.3 | 71.5* | 3.9 | 47.5* |
|               | Variation coefficient | 115.7% | 107% | 110.4% | 111.3% |
Regarding the characteristic frequency $F_c$, it was found that the average value of this parameter increases sequentially from the premenopausal to the menopausal and to the postmenopausal stages, as follows: 38.6, 69.9 and 102.3 kHz respectively in measurements made with NSS; likewise, when measurements were made with AA, this parameter increased as follows: 29.5, 52.9 and 86.4 kHz respectively (table 1; figure 2).

![Figure 2](image1.png)

**Figure 2.** Characteristic variation in frequency of epithelial tissues in perimenopausal stages with 0.9% saline solution and with 4% acetic acid.

The $R_o$, $R_{inf}$ and $F_c$ parameters that were found after adjusting the data to the Cole-Cole equation (1) were used to construct the representative resistivity spectra of the squamous tissues as a function of the signal frequency for the premenopausal, menopausal and postmenopausal stages with NSS and AA. Figure 3 shows that the values of average resistivity in measurements on epithelia cleaned with NSS, at frequencies lower than 20 kHz, were lower in the postmenopausal stage, intermediate in the menopausal stage and higher in the premenopausal stage. In addition, it was observed that resistivity increases when measurements are made on epithelia impregnated with AA. The characteristic frequency $F_c$ is determined by the point where the curve changes concavity and has its maximum gradient [12]. The graph shows that $F_c$ presents a shift to higher frequencies when resistivity decreases in value, that is to say, $F_c$ is lower in the premenopausal stage, increases in the menopausal stage and is higher in the postmenopausal stage.

![Figure 3](image2.png)

**Figure 3.** Resistivity curves of epithelial tissues in perimenopausal stages with 0.9% saline solution and with 4% acetic acid.
3. Discussion

In existing medical literature, they report hormonal changes that women present during the menopause leading to the loss of the superficial and intermediate layers of cervical stratified squamous tissue [20]. With these changes, the structure of the epithelium becomes thinner and atrophic [21]. For this reason, when applying an alternating electrical current on the tissue, a greater percentage of current lines are deviated to the underlying stroma, the latter is a very low resistivity, reticular connective tissue, which is similar to that registered for columnar tissue [22].

Considering the separation between the electrodes that inject current and, under the assumption that the penetration of the current lines is greater up to half the interelectrode distance, in epithelia in perimenopausal stages most of the current flows through the stroma. This is characterized by having low resistivity because it is a tissue formed mainly by networks of collagen and blood vessels that allow a higher flow of current. This can be explained from the electrical model, placing a low resistance in parallel with the resistance of the extracellular fluid. This, in turn, produces a decrease in the total equivalent resistivity that enables the stratified squamous epithelial tissues of the cervix to be characterized. This causes the total extracellular resistivity of stratified squamous tissue in perimenopausal women to be less than that of stratified squamous tissues in healthy women of fertile age [15, 17]. This loss of resistivity becomes more evident as the number of years of amenorrhea increases.

Due to the structure of the healthy squamous epithelium of women of fertile age, the intercellular junctions in the superficial layers are tightly packed and the membranes of the cells are almost fused together, which generates a greater electrical resistivity of the extracellular space (R). When these layers are lost, the extracellular volume increases, due to less packing between the basal cells; consequently, the current flows with less resistance and the R value is reduced.

The increased resistivity of the squamous epithelia, after being impregnated with AA, can be explained by the dehydration that this substance produces on the tissue, causing the narrowing of the current pathways and increasing the extracellular resistivity.

In premenopausal women, resistivity values of epithelia are within the range of normality, similar or slightly lower than those of women of fertile age, which is consistent with the maturation state of the epithelium that women present in this period. In menopausal patients, whose cellular maturation has decreased markedly, there is lower resistivity than in the previous ones, which is lower with NSS but whose resistivity increases with AA at values similar to those obtained in tissues from women in the premenopausal stage; this can be explained if we take into account tissue dehydration and the consequent narrowing of the current pathways.

In the postmenopausal stage, the epithelia present very low resistivity when measured after cleaning with NSS and a little increase occurs with the application of AA on the tissues; this can be explained by the fact that, in this stage, the epithelium is so thin and atrophic that it is constituted solely by layers of basal cells and a few parabasals. AA is deposited on the underlying stroma almost instantaneously after its application. The characteristic frequency increases with years of amenorrhea and with the severity of tissue atrophy; this is due to the fact that this parameter is inversely proportional to the square of the extracellular matrix resistivity, as was shown in the equation (3) and, therefore, when it decreases, the Fc increases, as was found in this research and which had been reported by other authors [12, 17] when they measured this parameter in low resistivity columnar tissues and in tissues with neoplastic lesions.

In this study, only the R and Fc parameters were taken into account, as they are the ones that best explain the electrical behavior and the changes observed in the colposcopy of the stratified squamous tissues in perimenopausal stages. The S parameter was not considered because a significant percentage of the current applied is deviated to the stroma, as mentioned above. Likewise, it has been found in this and other studies [10, 15, 17] that Cm is a parameter that provides little information in the range of frequencies in which this research was conducted, not presenting either a statistically significant difference in any of the groups in which the population under study was divided.

4. Conclusion

Colposcopy, along with the EIS, is a tool that enables bioelectrical parameters such as extracellular space electrical resistivity and characteristic frequency to be determined for cervical squamous tissues. Considering all that has been said above, it is possible to determine the state of these tissues in women in perimenopausal stages. These techniques altogether can improve the diagnostic precision and help to reduce the positive-false rate reported by Pap smear in these stages of women’s life.
Acknowledgement
Caldas University Health Sciences Faculty.
Funded with resources of the Technology and Innovation Science Fund. Royalties General System (RGS-Colombia) BPIN code 2013000100126.

References
[1] Honour J 2018 Biochemistry of the menopause Ann. Clin. Biochem. 55 118-33
[2] Davis S R, Lambrinoudaki I, Lumsden M, Mishra G, Pal L, Rees M, Santoro N and Simoncini T 2015 Menopause Nat. Rev. Dis. Primers. 1 1-19
[3] Soules MR, Sherman S, Parrott E, Rebar R, Santoro N, Utian W and Woods N 2001 Executive summary: Stages of Reproductive Aging Workshop (STRAW) Climacteric 4 267–72
[4] Harlow SD, Gass M, Hall JE, Lobo R, Maki P, Rebar R, Sherman S, Sluss P, de Villiers T 2012 Executive summary of the stages of reproductive aging workshop + 10: addressing the unfinished agenda of staging reproductive aging J. Clin. Endocr. Metab. 97 1159–68
[5] Harlow S, and Paramsothy P 2011 Menstruation and the menopausal transition Obstet. Gynecol. Clin. North. Am. 38 3595–607
[6] Monteleone P, Mascagni G, Giannini A, Genazzani A and Simoncini T 2017 Symptoms of menopause - global prevalence, physiology and implications Nat. Rev. Endocrinol. 4 199-215
[7] Grady D 2006 Clinical practice: management of menopausal symptoms N. Engl. J. Med. 355 2338-47
[8] Bulten J, Wilde P, Boonstra H, Gemmink J, Hanselaar, A 2000 Proliferation in “Atypical” Atrophic Pap Smears Gynecol. Oncol. 79 225–29
[9] Sankaranarayanan R, Wesley RS 2005 Manual práctico para la Detección Visual de las Neoplasias Cervicales Centro Internacional de Investigaciones sobre el Cáncer. Organización Mundial de la Salud. Publicación Técnica del CIIC.41 IARC Press Lyon
[10] Brown BH, Tidy JA, Boston K, Blackett AD, Smallwood R H and Sharp F 2000 Relation between tissue structure and imposed electrical current flow in cervical neoplasia The Lancet 355 892-95
[11] Abdul S, Brown BH, Milnes P and Tidy JA 2005 A clinical study of the use of impedance spectroscopy in the detection of cervical intraepithelial neoplasia (CIN) Gynecol. Oncol. 99 S64-S66
[12] Abdul S, Brown B H, Milnes P and Tidy J A 2006 The use of electrical impedance spectroscopy in the detection of cervical intraepithelial neoplasia Int. J. Gynecol. Cancer 16 1823-32.
[13] Balasubramani L, Brown B H, Healy J and Tidy J A 2009 The detection of cervical intraepithelial neoplasia by electrical impedance spectroscopy: The effects of acetic acid and tissue homogeneity Gynecol. Oncol 115 267-71
[14] Brown BH, Milnes P, Abdul S and Tidy JA 2005 Detection of cervical intraepithelial neoplasia using impedance spectroscopy: a prospective study BJOG: an International Journal of Obstetrics and Gynaecology 112 802-6
[15] Olarte G, Aristizabal W, Gallego PA, Rojas-Diaz J, Botero B E and Osorio GF 2007 Detección precoz de lesiones intraepiteliales del cuello uterino en mujeres de Caldas–Colombia mediante la técnica de espectroscopía de impedancia eléctrica Rev. Colomb. Obstet. Ginecol 58 13-20
[16] Olarte G, Aristizabal W, Osorio G F, Rojas-Diaz J 2010 Espectroscopia de impedancia eléctrica en cáncer invasivo del cuello uterino en mujeres de Caldas (Colombia), 2008-2009. Rev. Colomb. Obstet. Ginecol. 61 28-33
[17] Olarte G, Aristizabal W, Gallego PA 2015 Evaluation of electrical impedance spectroscopy for cervical intraepithelial lesions detection Revista Biosalud 14 26-35
[18] Cole K S and Cole R H 1941 Dispersion and absorption in dielectrics I Alternating current characteristics. J. Chem. Phys. 9 341-51
[19] Caicedo M, Rojas-Díaz J, Betancourt S, Aristizábal W and Chaparro J 2009 Algoritmos genéticos difusos para el ajuste del modelo de Cole-Cole. 4° Congreso Colombiano de Computación

[20] Prendiville W and Sankaranarayanan R 2017 Colposcopy and Treatment of Cervical Precancer. Chapter 2 Anatomy of the uterine cervix and the transformation zone IARC

[21] Tabrizi D A2018 Atrophic Pap Smears, Differential Diagnosis and Pitfalls: A Review IJWHRS 6 2-5

[22] Ocampo OH, Ruiz C A, Aristizábal W, Olarte G and Gallego PA 2017 Caracterización del tejido columnar del cérvix mediante espectroscopia de impedancia eléctrica y modelado computacional Revista Biosalud 16 29-31