EJGO European Journal of Gynaecological Oncology

The influence of biopsy in cervical high-grade squamous intraepithelial lesion, evaluated by HPV E6/E7 mRNA, Pap test, and conization results

J.E. Cordeiro Valença[1], A.K. Gonçalves[2], I.D. Cotrim Guerreiro da Silva[3], J. Eleutério Junior[4], C. Buarque Valença[5], T. Buarque Valença[5], M.L. Bezerra Menezes[6], R. Arraes de Alencar Ximenes[1]

[1] Federal University of Pernambuco – UFPE, Recife – PE; [2] Federal University of Rio Grande do Norte-UFRN, Natal – RN
[3] Federal University of São Paulo– UFSP, São Paulo – SP; [4] Federal University of Ceará – UFC, Fortaleza – Ceará
[5] Pernambucana College of Health – FPS, Recife – PE; [6] University of Pernambuco– UPE, Recife – PE (Brazil)

Summary

Objective: To evaluate the influence of biopsy on cervical intraepithelial neoplasia (CIN). *Materials and Methods:* A study was conducted involving 124 women underwent colposcopy-guided biopsy. At the first appointment, the women answered the survey questionnaire, cervical samples were collected for Papanicolaou (Pap) testing and the HPV E6/E7 mRNA test. At the second appointment at three to four months after the first, samples were collected from 81 patients with indications for conization, Pap test, and HPV E6/E7 mRNA testing before they underwent the procedure. PCR was used to detect HPV mRNA. The percentage of negative results before and after the biopsy was evaluated. The agreement between the tests results was evaluated using Cohen's kappa. *Results:* Sixty-two patients (76.4%) were between 21 and 40 years of age, 35 (43.2%) had four or more pregnancies, 41 (50.5%) had their sexual debut at 16 years of age or more, and 52 patients (64.2%) had undergone five or more Pap tests. The initial biopsy was negative for CIN2/3 in 14 (12.3%) patients; however, all patients were submitted to conization. Among those women with biopsy showing CIN2/3 (66 [81.5%]), 7.41% showed CIN1 and 14.81% were negative in the conization (kappa = 0.2052). The E6/E7 test performed before and after biopsy showed the best level of agreement by the kappa coefficient (0.7491) *Conclusions:* A higher percentage of negative results were observed in the histopathology, cytopathology, and E6/E7 after biopsy, suggesting that biopsy could affect the regression of CIN.

Key words: HPV; Cervix; mRNA E6/E7; CIN.

Introduction

Cervical cancer is the fourth most common cancer among women worldwide. In 2012, 528,000 new cases of cervical cancer and 266,000 deaths were estimated [1]. In Brazil, 15,590 new cases (15.33/100,000) have been estimated for 2014, and 5,160 deaths were recorded in 2011 [2]. It remains a major public health problem. New methods of prevention should become available and accessible for women of all countries through well-organized programs [3].

The Papanicolaou (Pap) test remains the most used diagnostic and screening method. However, this test has limitations, particularly in terms of sensitivity, which varies among laboratories, from 30% to 87%, requiring its repetition at regular intervals [4]. In recent decades, the importance of the human papillomavirus (HPV) in the development of cervical cancer and precursor lesions has led to the development of new techniques for detecting HPV [5, 6]. More recently, the test for detecting HPV E6/E7 mRNA has been shown to be valid because whereas the HPV DNA tests only identify the viral presence, the mRNA test monitors the oncogenic activity. Detection of active HPV transcription increases the prognostic value of the test, which appears to be more appropriate for risk assessment than the DNA tests are [7-10].

Despite these advances, the definitive diagnosis of precancerous lesions and cervical cancer should be performed through guided incisional biopsy [11, 12]; however, histopathological confirmation through cervical conization is necessary [13, 14]. Even if neoplasias are confirmed by a diagnostic incisional biopsy, sometimes the lesion removed by surgical procedure often presents negative histopathological results, [15], and few studies have addressed the results of negative conizations [16].

In 1966, Richart suggested that the biopsy procedure could completely eradicate cervical intraepithelial neoplasia (CIN), either directly by complete removal or indirectly by altering the balance between the host and the neoplasia, leading to regression of the residual areas, thus making the biopsy both a diagnostic and therapeutic tool [17, 18]. In another study evaluating the evolution of low-grade squamous intraepithelial lesions (LSIL), it was observed that the lesions submitted to biopsy exhibited a higher percent-

Revised manuscript accepted for publication October 6, 2015

Eur. J. Gynaecol. Oncol. - ISSN: 0392-2936
XXXVIII, n. 1, 2017
doi: 10.12892/ejgo3280.2017

7847050 Canada Inc.
www.irog.net

age of regression compared with the lesions examined only by cytology [19].

The present study aimed to evaluate changes in the cytology, histology, and HPV mRNA expression results after biopsy in a group of women referred with Pap test results compatible with high-grade squamous intraepithelial lesions (HSIL) or atypical squamous cells cannot exclude high-grade squamous intraepithelial lesions (ASC-H).

Materials and Methods

A cohort study was conducted from July 2010 to November 2013 assessing the changes in cytology, histology, and HPV mRNA after a colposcopy-guided cervical biopsy in women treated at the hospital of the Federal University of Pernambuco (Universidade Federal de Pernambuco - UFPE), Brazil.

One hundred forty-three (143) women were enrolled in the study, 143 with HSIL. Those who agreed to participate signed an informed consent form, and a standardized questionnaire was used to obtain information on the demographic characteristics of these women. At the first appointment, a gynecological examination was performed to collect cervicovaginal samples for Pap testing and for the detection of HPV mRNA. One hundred twenty-four patients underwent colposcopy-guided biopsy. After three to four months, samples were collected from 81 patients with indication for conization. Before this procedure, samples were collected for Pap and HPV E6/E7 mRNA testing. Then, the results before and after the biopsy were evaluated.

The amplification and detection of E6/E7 mRNA were performed by a polymerase chain reaction (PCR) in cervicovaginal material preserved and placed in a microfuge tube containing 1.5-ml buffered methanol and stored at -20°C until examination for the qualitative determination of E6/E7 mRNA of HPV types 16, 18, 31, 33, and 45. All reactions were processed with an internal control that detects the human gene U1A mRNA to ensure the integrity of the RNA and of the reagents. The manufacturer's protocol was strictly followed.

The questionnaires were entered into a software (double entry), comparing the database through the validate function. The McNemar chi-square test was used to compare categorical variables. Agreement between the test results was assessed using Cohen's kappa, as described by Fleiss [20]. The study was approved by the Research Ethics Committee of the UFPE.

Results

The characteristics of the 81 women submitted to biopsy and conization were as follows: 62 (76.4%) were between 21 and 40 years of age, and none was under 21 years of age; 53 (65.4%) have a sexual partner, 59 (72.8%) had never smoked, and 80 (98.77) did not use drugs; 35 (43.2%) had four or more pregnancies; 41 (50.5%) had their sexual debut at 16 years of age or older; 51 (62.9%) had three or less sexual partners; 53 (61.4%) did not use contraception method; eight (9.88%) referred regular use of condoms, and 52 patients (64.2%) had undergone five or more Pap tests (Table 1).

The initial biopsy was negative for CIN2/3 in 14 (12.3%) patients; however, all patients were submitted to conization. Among those women with biopsy showing CIN2/3 (66 [81.5%]), 7.41% showed CIN1 and 14.81% were negative in the conization, while the cytology collected before these negative conizations also was negative ($p = 0.069$). When evaluating the agreement between the incisional biopsy and the conization by the kappa coefficient, a value of 0.2052 was obtained, which was considered reasonable (95% CI: 0.20-0.39) (Table 2). Hence, the biopsy, compared with the conization, showed 84.12% sensitivity for the high-grade, 27.77% specificity, 80.30% positive predictive value (PPV), and 33.33% negative predictive value (NPV) for CIN2/3.

Assessing only the cytological results before and after the biopsy, there were 11 cases (20.75%) that became negative three to four months after the biopsy and two cases (33.33%) that were negative and became positive for HSIL. In the LSIL cases before biopsy, two (40%) became HSIL. On the other hand, three (5.66%) with diagnostic before biopsy of HSIL became LSIL with the following cytology. The level of agreement between the pre- and post-biopsy cytology, as determined by the kappa coefficient, was considered fair (0.2830) (Table 3).

Table 1. — *Sociodemographic characteristics of women with diagnosis of HSIL and submitted to biopsy and conization.*

Sociodemographic aspects		N	%
Age (years)	21-30	28	34.57
	31-40	34	41.98
	41-50	10	12.35
	> 50	9	11.11
Marital status	Single	28	34.57
	Has a partner	53	65.43
Smoking habit	Never	59	72.84
	Smoker	15	18.52
	Former smoker	7	8.64
Drug use	Never	80	98.77
	User	1	1.23
Pregnancies	0	5	6.17
	1	11	13.58
	2-4	30	37.04
	≥ 4	35	43.21
Sexual debut	< 16 years old	40	49.38
	≥ 16 years old	41	50.52
Partners	1	10	12.35
	2-3	41	50.62
	4-9	22	27.16
	≥ 10	8	9.88
Contraception	None	53	65.43
	Hormonal	23	28.40
	Condom	5	6.17
Condom use	Never	20	24.69
	Sometimes	25	30.86
	Always	8	9.88
	Have used	28	34.57
Number of Pap tests	< 5	29	35.8
	≥ 5	52	64.2
Total		81	100.00

Table 2. — *Evaluation of the diagnostic agreement index between the initial biopsy and the histology result of conization.*

Directed Biopsy	Conization			
	Negative N (%)	CIN 1 N (%)	CIN 2/3 N (%)	*p*
Negative	1 (20)	0	4 (80)	0.069
CIN 1	1 (10)	3 (30)	6 (60)	
CIN 2/3	10 (15.5)	3 (4.55)	53 (80.3)	

Pearson chi^2 (4) = 8.7068, Pr = 0.069.

Expected

Agreement	Agreement	Kappa	Std. Err.	Z	Prob > Z
70.37%	65.20%	0.1485	0.0809	1.84	0.0332

*Kappa coefficient = 0.2052 (0.20−0.39) = fair agreement.
**Kappa coefficient of ≥ 0.20 = minimal correlation between the incisional biopsy and CAF.

Values of Kappa Interpretation [21]

< 0	No agreement
0−0.19	Poor agreement
0.20−0.39	Fair agreement
0.40−0.59	Moderate agreement
0.60−0.79	Substantial agreement
0.80−1.00	Almost perfect agreement

Table 3. — *Evaluation of the diagnostic agreement index between the cytology at the time of biopsy and three to four months after biopsy.*

Cytology on the same day of the biopsy	Cytology 3/4 months after the biopsy			
	Negative N (%)	LSIL N (%)	HSIL N (%)	*p*
Negative	4 (66.67)	0	2 (33.33)	0.008
LSIL	1 (20)	2 (40)	2 (40)	
HSIL	11 (20.75)	3 (5.66)	39 (73.58)	

Pearson chi^2 (4) =13.9087, Pr = 0.008.

Expected

Agreement	Agreement	Kappa	Std. Err.	Z	Prob > Z
70.31%	58.59%	0.2830	0.0884	3.20	0.0007

*Kappa coefficient = 0.2830 (0.20−0.39) = fair agreement.
**Kappa coefficient of ≥ 0.20 = minimal correlation between cytology before and after the biopsy.

Values of kappa interpretation [21]

< 0	No agreement
0−0.19	Poor agreement
0.20−0.39	Fair agreement
0.40−0.59	Moderate agreement
0.60−0.79	Substantial agreement
0.80−1.00	Almost perfect agreement

The E6/E7 test performed before and after biopsy showed the best level of agreement by the kappa coefficient (0.7491). The percentage of negative results was higher after than before the biopsy, 25.35% and 16.90, respectively. The tests that were negative before the biopsy were also negative after, while among the tests that were positive before the biopsy, 10.17% became negative after biopsy (Table 4).

Table 4. — *Evaluation of the diagnostic agreement index between the E6/E7 tests performed before and after biopsy.*

Test E6/E7 on the same day of the biopsy	Test E6/E7 3/4 months after the biopsy		
	Negative N (%)	Positive N (%)	Total N (%)
Negative	12 (100)	0	12 (100)
Positive	6 (10.17)	53 (89.83)	59 (100)

Pearson chi^2(1) = 42.5198, Pr = 0.0.

Expected

Agreement	Agreement	Kappa	Std. Err.	Z	Prob > Z
91.55%	66.32%	0.7491	0.1149	6.52	0.0000

*Kappa coefficient = 0.7491 (0.60−0.79) = substantial agreement.
**Kappa coefficient of ≥ 0.20 = minimal correlation between the E6E7 tests before and after biopsy.

Values of kappa interpretation [21]

< 0	No agreement
0−0.19	Poor agreement
0.20−0.39	Fair agreement
0.40−0.59	Moderate agreement
0.60−0.79	Substantial agreement
0.80−1.00	Almost perfect agreement

Discussion

The possibility of using any test that could predict negative results on conization is really attractive. Specifically in this study, the assessment of the biopsy and cytology, as well as the results of the E6/E7 mRNA test performed before the biopsy and repeated three to four months after the biopsy revealed that E6/E7 test showed a strong agreement, while the other two tests showed a weaker agreement.

Cones with diagnosis of CIN2/3 that had biopsy diagnosing negative or CIN 1 had cytology performed just before negative. Previous studies have shown that a negative biopsy or one with CIN1 and the conization exhibiting CIN2/3 may occur because the conization can detect lesions in the cervical canal, which are detectable by cytology but not by incisional biopsy. Such a result can also be obtained if the biopsy is not performed on the area with higher atypia or even in cases of small lesions that are missed by the biopsy but detected in the conization [22, 23]. Therefore, in cases of cytology with HSIL in which colposcopic lesions are not detectable or with a negative or CIN1 biopsy, conization should be indicated, except in special situations, adolescent, and pregnant women [24].

In some cases, the initial cytological examination performed at the same time as the directed biopsy showed negative results or LSIL, while a second cytological examination performed at the day of conization exhibited HSIL. Pap smear performed on the day of the biopsy were false-negative, which can occur depending on the conditions and techniques used for collection of the material [25].

Most of the conization results confirmed the high-grade lesions diagnosed by biopsy, in agreement with previous

studies [26], and there was a higher agreement between the CIN2/3 of the biopsy and of the conization, compared with the CIN1, as previously shown [27].

Others studies observed failure to confirm the biopsy result by conization in approximately 15% of cases [26, 28], similar to the percentage found in the present study. According to Ostör, even high-grade atypia can regress. The author observed that the lesions regressed in even greater percentage than in the present study; however, in the other studies, the follow-up period was always longer than one year [29].

Considering that the biopsy and/or cytology revealed HSIL (CIN2/3) followed by a negative conization has several possible explanations, such as an incorrect diagnosis by biopsy, neoplasia regression after biopsy, removal of the lesion by the biopsy, insufficient cone sampling, small neoplastic foci removed at conization but not shown in the histological sections, presence of histopathological characteristics, failure to remove the neoplasia and technical difficulties related to the conization [26, 28, 30], and a change in the balance between the host and the neoplasia, with regression of residual areas of CIN [17, 18]. In a review following-up cases of low-grade lesions, the group monitored by biopsy displayed a higher percentage of lesion regression compared with the group monitored only using cytology [19].

Similar to the results obtained by histopathology, in the Pap test, a lower percentage of positive results and higher percentages of negative results were observed after the biopsy. However, half of the negative cytological samples collected at the time of conization were false-negative which might have the same explanations as those for the false-negative cytology at the time of biopsy [25].

The HPV E6/E7 mRNA test has been considered a marker of disease progression [7]. Women who had negative results before the biopsy maintained the same results afterward, while there were cases of positive results in the sample collected before the biopsy that became negative afterward. Therefore, a higher percentage of negative results were observed after the biopsy. Because E6/E7 mRNA is a marker and detects viral activity of HPV [31], the results obtained indicate the lesion severity [8]; however, the E6/E7 test only detects HPV types 16, 18, 31, 33, and 45, which are responsible for 86% of high-grade atypia and cervical cancer [32]. Positive cytological and/or histopathological results with a negative E6/E7 test results may be attributed to other HPV types. [8].

Despite the possibility of false-positive biopsy results or false-negative conization results, the present study found a higher percentage of negative results after biopsy in the histological examination and in two additional tests, thereby allowing attribution of these negative results to the biopsy, as suggested previously and in the present study[29]. Kraus *et al.* suggested that these negative conizations may eventually be reduced through the introduction of HPV tests for preoperative evaluation [33].

Multicenter and/or population-based studies or investigations with a larger number of subjects may confirm the effect of incisional biopsy on the regression of HPV-induced lesions, and further studies may find a diagnostic method that can prevent surgery in cervices negative for CIN.

Acknowledgements

The study was supported by the National Council for Scientific and Technological Development - Brazil (Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq) - Proc. 477348/2010-6. The authors were partially supported by CNPq (scholarship 308818/2013-0 to R.A.A.X.).

References

[1] Ferlay J., Soerjomataram I., Ervik M., Dikshit R., Eser S., Mathers C., *et al.*: "GLOBOCAN 2012 v1.0, Cancer incidence and mortality worldwide: IARC Cancer Base No. 11". Lyon, France: International Agency for Research on Cancer, 2013. Available at: http://globocan.iarc.fr.

[2] Instituto Nacional do Câncer (Brazilian Cancer Institute). Rio de Janeiro: Brazilian Ministry of Health; c1996-2014. Available at: http://www2.inca.gov.br/wps/wcm/connect/tiposdecancer/site/home/colo_utero/definicao

[3] Arby M., Castellsagué X., de Sanjosé S., Bruni L., Saraiya M., Bray F., *et al.*: "Worldwide burden of cervical cancer in 2008". *Ann. Oncol.*, 2011, *22*, 2675.

[4] Nanda K., McCrory D.C., Myers E.R., Bastian L.A., Hasselblad V., Hickey J.D., Matchar D.B.: "Accuracy of the Papanicolaou test in screening for and follow-up of cervical cytologic abnormalities: a systematic review". *Ann. Intern. Med.*, 2000, *132*, 810.

[5] Castellsagué X.: "Natural history and epidemiology of HPV infection and cervical cancer". *Gynecol. Oncol.*, 2008, *110*, S4.

[6] Cox J.T.: "History of the use of HPV testing in cervical screening and in the management of abnormal cervical screening results". *J. Clin.Virol.*, 2009, *45*, S3.

[7] Cattani P., Zannoni G.F., Ricci C., D'Onghia S., Trivellizzi I.N., Di Franco A., *et al.*: "Clinical performance of HPV E6 and E7 mRNA testing for high-grade lesions of the cervix". *J. Clin. Microbiol.*, 2009, *47*, 3895.

[8] Lie A.K., Kristensen G.: "Human Papillomavirus E6/E7 mRNA testing as a predictive marker for cervical carcinoma". *Expert Rev. Mol. Diagn.*, 2008, *8*, 405.

[9] Liverani C.A., Ciavattini A.: "High risk HPV DNA subtypes and E6/E7 mRNA expression in a cohort of colposcopy patients from Northern Italy with high-grade histologically verified cervical lesions". *Am. J. Transl. Res.*, 2012, *4*, 452.

[10] Lie A.K., Risberg B., Borge B. Sandstad B., Delabie J., Rimala R., *et al.*: "DNA- versus RNA-based methods for human papillomavirus detection in cervical neoplasia". *Gynecol. Oncol.*, 2005, *97*, 908.

[11] Eftekhar Z., Izadi-Mood N., Yarandi F., Khodamoradi M., Rahimi-Moghaddam P.: "Can we substitute brush cytology for biopsy in the evaluation of cervical lesions under the guidance of colposcopy?" *Int. J. Gynecol. Cancer*, 2005, *15*, 489.

[12] Starzewski J., Gózdz S., Chil A., Piasek G., Plutecki J., Smorag L., *et al.*: "Postoperative verification of cervical intraepithelial neoplasia grade". *Wiad. Lek*, 2003, *56*, 162. [Article in Polish]

[13] Szurkus D.C., Harrison T.A.: "Loop excision for high-grade squamous intraepithelial lesion on cytology: correlation with colposcopic and histologic findings". *Am. J. Obstet. Gynecol.*, 2003, *188*, 1180.

[14] Matthews K.S., Rocconi R.P., Case A.S., Estes J.M., Straughn J.M. Jr., Huh W.K.: "Diagnostic loop electrosurgical excisional procedure

for discrepancy: preoperative factors predict presence of significant cervical intraepithelial neoplasia". *J. Low. Genit. Tract. Dis.*, 2007, *11*, 69.

[15] Murta E.F., Silva A.O., Silva E.A.: "Clinical significance of a negative loop electrosurgical excision procedure, conization and hysterectomy for cervical intraepithelial neoplasia". *Eur. J. Gynaecol. Oncol.*, 2006, *27*, 50.

[16] Diakomanolis E., Haidopoulos D., Chatzipapas I., Rodolakis A., Stefanidis K., Markaki S.: "Negative cone biopsies. A reappraisal". *J. Reprod. Med.*, 2003, *48*, 617.

[17] Richart R.M.: "Influence of diagnostic and therapeutic procedures on distribution of cervical intraepithelial neoplasia". *Cancer*, 1966, *19*, 1635.

[18] Chenoy R., Billingham L., Irani S., Rollason T., Luesley D., Jordan J.: "The effect of directed biopsy on the atypical cervical transformation zone: assessed by digital imaging colposcopy". *Br. J. Obstet. Gynaecol.*, 1996, *103*, 457.

[19] Saw H.S., Lee, J.K., Lee H.L., Jee H.J., Hyun J;J.: "Natural history of low-grade squamous intraepithelial lesion". *J. Low. Genit. Tract. Dis.*, 2001, *5*, 153.

[20] Fleiss J.L.: "The measurement of interrater agreement, statistical methods for rates and proportions". 2nd ed. New York: John Wiley & Sons, Inc., 1981, 212.

[21] Landis J.R., Koch G.G.: "The measurement of observer agreement for the categorical data". *Biometrics*, 1977, *33*, 159.

[22] Nam K., Chung S., Kwak J., Cha S., Kim J., Seob J., *et al.*: "Random biopsy after colposcopy-directed biopsy improves the diagnosis of cervical intraepithelial neoplasia grade 2 or worse". *J. Low. Genit. Tract. Dis.*, 2010, *14*, 346.

[23] Bulten J., Horvat R., Jordan J., Herbert A., Wiener H., Arbyn M.: "European guidelines for quality assurance in cervical histopathology (review)". *Acta Oncol.*, 2011, *50*, 611.

[24] Massad L.S., Einstein M.H., Huh W.K., Katki H.A., Kinney W.K., Schiffman M., *et al.*: "2012 updated consensus guidelines for the management of abnormal cervical cancer screening tests and cancer precursors". *J. Low. Genit. Tract. Dis.*, 2013, *17*, S1.

[25] Franco R., Amaral R.G., Montemor E.B.L., Montis D.M., Morais S.S., Zeferino L.C.: "Factors associated with false-negative cervical cytological results". *Rev. Bras. Ginecol. Obstet.*, 2006, *28*, 479. [Article in Portuguese]

[26] Witt B.L., Factor R.E., Jarboe E.A., Layfield L.J.: "Negative loop electrosurgical cone biopsy finding following a biopsy diagnosis of high-grade squamous intraepithelial lesion: frequency and clinical significance". *Arch. Pathol. Lab. Med.*, 2012, *136*, 1259.

[27] Duesing N., Schwarz J., Choschzick M., Jaenicke F., Gieseking F., Issa R., *et al.*: "Assessment of cervical intraepithelial neoplasia (CIN) with colposcopic biopsy and efficacy of loop electrosurgical excision procedure (LEEP)". *Arch. Gynecol. Obstet.*, 2012, *286*, 1549.

[28] Livasy C.A., Moore D.T., Van Le L.: "The clinical significance of a negative loop electrosurgical cone biopsy for high-grade dysplasia". *Obstet. Gynecol.*, 2004, *104*, 250.

[29] Ostör A.G.: "Natural history of cervical intraepithelial neoplasia: a critical review". *Int. J. Gynecol. Pathol.*, 1993, *12*, 186.

[30] Koc N., Sahin D., Ayas S.: "Reevaluation of negative cone biopsy results after a positive cervical biopsy finding". *J. Low. Genit. Tract. Dis.*, 2013, *17*, 154.

[31] Jeanteta D., Schwarzmanna F., Trompb J., Melchersc J., Wurffd A., Oosterlakenb T., *et al.*: "NucliSENS® EasyQ® HPV v1 test testing for oncogenic activity ofhuman papillomaviruses". *J. Clin. Virol.*, 2009, *45*, S29.

[32] Muñoz N.: "Human papillomavirus and cancer: the epidemiological evidence". *J. Clin.Virol.*, 2000, *19*, 1.

[33] Kraus I., Molden T., Ernø L.E., Skomedal H., Karlsen F., Hagmar B.: "Human papillomavirus oncogenic expression in the dysplastic portion; an investigation of biopsies from 190 cervical cones". *Br. J. Cancer*, 2004, *90*, 1407.

Corresponding Author:
J.E. CORDEIRO VALENÇA, M.D., PhD
Rua Pereira Simões, 463 / 1201
Bairro Novo – Olinda - PE
53.030-060 (Brazil)
e-mail: jeffersonvalenca25@hotmail.com