HUMAN PAPILLOMAVIRUS AND ENVIRONMENTAL FACTORS PREDICT TREATMENT FAILURE OF CERVICAL PRECANCEROUS LESIONS AMONG BRAZILIAN WOMEN: A HOSPITAL BASED COHORT

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Abstract—Cervical cancer is preventable through the detection and treatment of precancerous lesions. However treatment failure occurs around 5-35%, and there is no consensus about the risk factors involved. Although it has been indicated that HR-HPV in conjunction with cytology should be useful for monitoring women treated for CIN, few HPV infected women will develop persistent HPV infections and progress to cancer, suggesting that additional factors are necessary for treatment failure, such as tobacco smoking and oral contraceptive use. Aim: to evaluate the effect of HPV and selected environmental factors on the risk of CIN treatment failure. A prospective study was conducted on a cohort of 205 women histologically confirmed CIN1–3 who were treated at Brazilian National Cancer Institute from October/2004 to May/2006. A questionnaire with epidemiological and clinical information was administered by two trained registered nurses. Women were followed for 24 months with cytological exams and cervical smears were taken for HPV tests. Mean age was 35(±11) years old; mean age at sexual onset was 17(±3) years old. Current oral contraceptive use(HR=2.05;95%CI:1.08-3.88), current tobacco use(HR=1.87;95%CI:1.08-3.24), and a positive HPV result up to 6 months after treatment

(HR=2.35;95%CI:1.39-3.97) were statistically associated with CIN treatment failure in 24 months. Women who are current smokers, oral contraceptive users, and who are HPV positive in the first 3-6 months after treatment, are at higher risk of treatment failure.

Keywords—Cervical cancer, CIN-treatment failure, HPV, tobacco smoking, oral contraceptive, epidemiology, environmental

1. Introduction

Developed countries have seen a dramatic decrease in the incidence of, and mortality from, invasive cervical cancer in the last 50 years because of mass routine screening with the Pap smear (Broto et al, 2008). In contrast to this striking result, cervical cancer is the second most common cancer among women and the leading cause of cancer death in developing countries probably due to inadequate use of the screening services (Ferlay et al, 2012). In Brazil, the incidence rates of cervical cancer vary widely according to the geographical region investigated. On one hand North and Northeast regions have the highest mortality rates, varying from 11/100,000 women in Rio Branco (state of Acre) to 12/100,000 women in São Luiz (state of Maranhão) respectively; on the other hand, Southeast presents the lowest mortality rate reaching 5/100,000 women in Rio de Janeiro (state of Rio de Janeiro) (MS, 2013). This variation is due to different reasons, including the organization and efficiency of screening and early detection programs, which include Pap-test covertures, treatment and follow-up of pre-cancerous lesions detected (WHO, 2005). However, these reasons alone cannot fully explain the high cervical cancer incidence and mortality.

Cervical cancer is preventable through the detection and treatment of precancerous lesions, known as cervical intraepithelial neoplasia (CIN), which are classified as High grade Squamous Intraepithelial lesion (HSIL) and Low grade Squamous Intraepithelial Lesion (LSIL). Loop electrosurgical excision procedure (LEEP) is the recommended procedure for the treatment of cervical intraepithelial neoplasia (CIN) grade 2⁄3, as this procedure can easily be used on an outpatient basis. However, treatment failure occurs around 5-35%, and there is no consensus about the risk factors involved (Silva, Koifman & Mattos, 2009; Ghaem-Maghami et al, 2007; Acladious et al, 2002). Known risk factors for recurrence of cervical dysplasia are size of the lesion and incomplete excision at the endocervical margin (Ghaem-Maghami et al, 2007; Biggrig et al 1994). However, the histopathologic evaluation of resection margins status is far from perfect, since cervical lesions may recur in 3.1-15% of patients who had conization specimens with clear margins (Ghaem-Maghami et al, 2007; Biggrig et al 1994).

Current post-treatment follow-up protocols are mainly based on serial cytology. Since colposcopy adds little to the detection rate of residual/recurrent CIN (Biggrig et al 1994), neither the British nor the American Societies for Colposcopy and Cervical Pathology nor the Brazilian Ministry of Health recommend mandatory colposcopy follow-up (Gardeil et al, 1994; Benchimol, Mergui & Uzan, 2005; MS, 2011). High-risk human papillomavirus (HR-HPV) detection techniques have been proposed in the follow-up of patients treated for CIN2–3 because of their high sensitivity and negative predictive value (NPV) for detecting residual/recurrent disease as a good index of disease clearance. Thus, growing evidence indicates that HR-HPV in conjunction with cytology should be useful for monitoring women treated for CIN (Bar-Am et al, 2003; Alonso et al, 2006; Arbyn et al, 2012). However, it has been suggested that, since HPV is detected in many asymptomatic low grade CIN lesions and only a minority of persistent HPV infections progress to cancer, additional factors are necessary if malignant change is to occur, such as tobacco smoking, oral contraceptive use, and clinical factors (Acladious et al, 2002; Aerssens et al, 2009).

Tobacco smoking may be responsible for the exposure to chemical carcinogens present in cigarette smoke. Smoking appears

to be the most important factor affecting the progress of CIN after HPV infection (Acladious et al, 2002). Long-term oral contraceptive use has been thoroughly studied epidemiologically, and correlates to cervical cancer in most studies (Marks et al, 2010). In vitro studies on cervical cell lines transfected with HPV and animal studies indicate that sex steroid hormones are capable of inducing cancer. Human studies have identified that oral contraceptive use is more strongly associated with reduced risk of HPV clearance, rather than new HPV infection, in young women who are long term hormonal contraceptive users (Marks et al, 2010; Marks et al, 2011).

Consequently, gynecologists face diagnostic problems for adequate follow-up of patients after primary treatment for cervical dysplasia. A better indicator for imminent recurrent dysplasia might be helpful in addition to the existing cytological screening. Thus, the aim of the present study is to evaluate the effect of HPV and selected environmental factors on the risk of CIN treatment failure.

2. Methods

2.1 Study population and design

A prospective study was conducted on a cohort of outpatients histologically confirmed CIN1–3 who were treated by LEEP at the Hospital of Cancer-II from Brazilian National Cancer Institute, Rio de Janeiro, between October 2004 and May 2006. Inclusion criteria were CIN 1 to 3 confirmed in the conization specimen, patients attending at least two follow up visits and age 17+ years old. Exclusion criterion was not signing the informed consent. Eighteen women (3,9%) refused to sign the informed consent. Thus, 205 women were included in the study. The present study was approved by the Ethical Committee from Brazilian National Cancer Institute and from National School of Public Health, Brazil, and all included women signed the Informed Consent.

2.2 Data collection and variable classification

After signing the informed consent, all women were invited to answer a questionnaire with epidemiological and clinical information administered by two trained registered nurses. The interview was carried out recording the age at enrollment, skin color, sexual behavior, and reproductive history, history of sexually transmitted diseases, contraceptive use, and smoking history. Tobacco smoking was classified according to IARC definition (IARC, 2004) as current (over 100 cigarettes smoked in a lifetime), former smoker (quit smoking in the past 6 months at least) and non-smoker. Oral contraceptive use was graded as current (regular use for at least a month by the time of enrollment), former (those who quit using for at least one month before the interview) and non-user. Ethnicity was classified as the self-reported skin color (IBGE, 2009) and classified as white and non-white. All analyses were additionally stratified by age at first intercourse ($\leq$15, and >15 years old), number of full-term pregnancies/parity (<2 and $\geq$2), number of sexual partners (< 5, $\geq$5), a new partner during follow up (0 and $\geq$1), number of abortions in a lifetime (< 2, and $\geq$2), and presence of sexually transmitted diseases at follow up (yes/no). Categorizations of these last variables were based on the mean and median split.

2.3 Clinical procedures

Following the interview, patients were then submitted to a Colposcopy test. Colposcopy was performed after preparing the cervix with 3% acetic acid. Colposcopic findings were described following the Rome criteria

(Stafl & Wilbanks, 1991). All women with abnormal cytology (ASC-US or higher) and an abnormal transformation zone underwent a colposcopy-directed Loop Electrical Excision Procedure (LEEP). When the transformation zone was not visible, only partially visible, or no colposcopy abnormality was identified, endocervical curettage was also performed.

The cervix was exposed using an adapted speculum allowing smoke evacuation. After delineating the area of abnormality with Lugol's iodine, 1 ml of local anesthetic was injected in each quadrant. The loop was selected according to the size of the area to be excised. When an endocervical extension was suspected, a second selective endocervical sweep was performed. Exceptionally, when the exocervical lesion was too large to be accommodated by a single sweep, excision was achieved with two or more systematic sweeps. The base of the resulting crater was then coagulated by ball diathermy.

2.4 Histological and cytological specimen processing

LEEP specimens were anatomically oriented and pinned to cork support and fixed in 10% formalin. After processing the whole specimen in 3-12 paraffin blocks (median 6), the excision samples were thoroughly examined. If there was more than one sweep, each one of them was independently included. Surgical margins were identified with ink and were carefully examined. Surgical specimens were examined histologically and were analyzed for classification of the lesions and the status of the resection margins. These pathologists had no prior knowledge of the patient`s clinical data and HPV status. The biopsies were assessed according to the Bethesda System (Solomon et al, 2002) including condylomata and CIN-1, and CIN2-3.Histological normal tissue presenting only reactive/reparative changes was categorized as negative(disease-free). Involvement of surgical margins was classified as clear (no margin involvement), ectocervix involvement only, or endocervix involvement. Cytological results were classified according to the Bethesda System for Cytological results, as Low grade Squamous Intraepithelial Lesion(LSIL) and as High grade Squamous Intraepithelial Lesion(HSIL).

All histological and cytological slides were analyzed (and revised) by two pathologists from the Integrated Cytopathology Technology Section (SITEC) of the Brazilian National Cancer Institute, which diagnoses less than 5% of undetermined squamous cell carcinoma and/or undetermined adenosquamous cell carcinoma (ASCUS/AGUS) (Zardo et al, 2009).

2.5 Follow-up routine

Post-treatment control was scheduled at 3, 6, 9, 12, 16, 20 and 24 months. In every visit after LEEP, a Pap smear was performed. Exocervical smear was obtained with an Ayre spatula and the endocervical sample using a cytobrush; both were put onto a glass slide. All specimens were stained using the Papanicolaou method. The mean follow-up period was 22.16 ± 3.56 months (range, 20–40). Mean number of visits after treatment was 6.0 (median 5; range 2 to 9). Two swabs from the cervix were taken for HPV DNA at 3 or 6 months after treatment (118 and 87 cases respectively), placed at a modified Carnoi medium (1 part of acetic acid and 2 parts of ethanol 100%) and stored at 4^{o}C.

2.6 Cervical DNA extraction and HPV testing

DNA extraction from cervical follow-up

swabs was performed by vortex of the modified Carnoi solution medium, incubated containing the swabs. Cervical samples were submitted to pH neutralization step with addition of5ml de *Phosphate-Buffered Saline* 1x(PBS) and added20ug/uL of proteinase K. DNA isolation was performed with Wizard®Genomic DNA Purification kit (Promega, Madison, WI, USA) following the manufacturer's instructions. The quality and validity of the extracted DNA specimen was assessed by inclusion of *Beta*-globin gene-specific primers in the polymerase chain reaction (PCR). Only specimens with detectable *Beta*-globin were used in this analysis.

HPV DNA amplification was performed by nested PCR, using PGMY primers (Gravitt et al, 2000) in the first round, andGP05/06+ primers (da Roda Husman et al, 1995) in the second round. First round was performed using 100-200 ng of genomic DNA, and the forward and reverse PGMY primers used for amplification of a 450bp fragment containing the L1 HPV capsid. Second round was carried out using 5uL of first round PCR product, and forward and reverse GP5/6 primers, for amplification of a 140bp fragment. DNA isolated from CaSKi (HPV 16) lineage was used as positive control, while Milli-Q water was used as negative control.

2.7 Criteria for treatment failure

Criteria for treatment failure were based on positive cytology exams, in such a way that women presenting one positive Pap smear for HSIL/cancer and those presenting two consecutive cytological LSIL results were classified as failure. In the last cases we considered the date of the second Pap smear test as the date of failure. Women who did not present any altered Pap test at the end of 24 months were censored in the cohort. Those women who did not complete 24 months of follow up were censored in the date of the last Pap test. All positive Pap smear test were revised by two specialized pathologists from the SITEC/INCA, who were blinded for the Pap smear result at entrance and HPV status of the women.

2.8 Statistical Analysis

Descriptive analysis was carried out to evaluate the distribution of the variable, and their respective 95% Confidential Interval (95% CI). Survival analysis was performed at the 24-month follow-up after LEEP treatment, aiming to evaluate the risk of treatment failure, according to each exposure variable, using the pairwise Kaplan-Meier method (Log-Rank test: 95%). Crude and adjusted *Hazard Ratios* for treatment failure according to the different associated risk factors were estimated using Proportional Cox Regression method, considering a significant level of 5%. A multiple proportional Cox regression model was constructed to evaluate predicting factors for CIN treatment failure. Independent variables shown to be statistically significant at uni-variate analysis were included in the proportional Cox regression model through the *Enter* method, using the default values as criteria to enter($p<0.010$) and remove ($p>0.05$). The criteria to keep the variable in the final model were either presenting biological meaning or statistical significance in the bivariate analysis.

A t-trend test was carried out aiming to explore the occurrence of possible trends with categorical variables risk estimates. The joint effect of HPV vs tobacco smoking, and HPV vs Oral contraceptive use on the risk of treatment failure was explored, but no statistical significance was found. Epi Info software (version 6.04d,2001); Center for Disease Control and Prevention (CDC),Atlanta, Georgia, USA) and SPSS software (version 17.0) were used

to carry out the analysis.

3. Results

In the studied population, mean age was 35 (±11) years old, mean age at sexual onset was 17 (±3) years old. The mean time of follow up was 22.16 months and median were 24 months. Less than 10% of follow-up loss was present. Most of the study's population were under 45 years old (79.0%), were current oral contraceptive users (63.9%), and presented a Histology result at entrance of CIN-2/3 (56.1%). Margins were involved in 33.7% of women and 38.0% of all participants were HPV positive up to 6 months after treatment (Table 1).

At the end of 24 months 148 (72.2%) women were censored in the cohort and 57 (27.8%) cases of treatment failures were determined. From these cases 40 (19.5% of the participants) were classified as LSIL while 17 (8.3%) were classified as HSIL. Risk of treatment failure was significantly different among those women who were former and current users of oral contraceptive, in the 12 and 24 months after treatment (16.3% and 30.6% among former users; 19.4 and 33.3% for current smokers). Comparing never/former smokers to current smokers showed higher risk of treatment failure in 12 and 24 months (11.4% and 19.7% vs. 24.4% and 33.3%, respectively). Risks of treatment failure were statistically higher among those women presenting margin involvement in 12 and 24 months (24.4% and 35.2%, respectively) compared to those presenting clear margins. HPV positive status also significantly increased the risk of treatment failure at 12 months (24.4%) and at 24 months (33.9%) after treatment (Table 2).

Table 3 presents crude and adjusted HR for CIN treatment failure. During 24 months of follow up, current oral contraceptive use was statistically associated with CIN treatment failure (HR=2.05; 95%CI:1.08-3.88), even after controlling by age, ethnicity and HPV status. Having a positive HPV result up to 6 months after treatment also was statistically associated to CIN-treatment failure, independently of age and ethnicity (HR=2.35; 95%CI:1.39-3.97). Current tobacco use (HR=1.87; 95%CI:1.08-3.24) and margin involvement (HR=1.91; 95%CI:1.06-3.42) were also significantly associated with CIN treatment failure. However, only HPV status, oral contraceptive use and tobacco smoking remained in the predictive model (table 4). According to this model, having an HPV positive test at 3 or 6 months after treatment increased the risk of treatment failure 2.38 (95%CI:1.41-4.04) fold when compared to women with an HPV negative test, independently of age, oral contraceptive use, and tobacco smoking.

4. Discussion

Women treated for CIN must be followed regularly to monitor the eventual altered cytology, since treated women are still at increased risk for subsequent invasive cervical cancer compared to the general population during at least 10 years and maybe up to 20 years after treatment (Soutter, Sasieni & Panoskaltsis, 2006). In the present study the treatment failure rate, evaluated over 24 months, varied from 8.3% for HSIL to 19.5% for LSIL. The risk of recurrent CIN is higher in women who have ≥ 5 sexual partners, who are former/current smokers; who are former/current oral contraceptive users, presented involved margins, and had a positive HPV in the first follow up visit. These results are consistent with the natural history of cervical cancer, whose development depends on the HPV infection and the effects of other environmental and clinical co-factors. However, the timing for such development is shorter among women treated for CIN stressing the need of a follow up in the first

24 months after treatment.

Positive HPV test from 3-6 months after treatment was independently associated to CIN treatment failure among Brazilian women, in up to 24 months of follow up (Adjusted HR=2.38; 95%CI: 1.41-4.04). Similar results were found by Aerssens et al (2009) among 138 Belgian and Nicaraguan women diagnosed with CIN 2/3 and treated by LEEP. According to their findings, combining abnormal cytology and the presence of HR HPV within the first 6 months after treatment gave the best correlation with residual/recurrent disease during follow-up (OR 10.2; 95% CI: 2.2–48.3). The sensitivity of this combination was 84.6% and specificity 65.0% (Aerssens et al, 2009). Also, in a meta-analysis developed by Zielinski et al (2004), it was proposed that combined cytology and HPV testing at 6 and 24 months after treatment and referral back for a 5-yearly routine cytological screening would be needed if all examinations are negative (Zielinski et al, 2004).

Although the etiopathogenesis of cervical cancer is mainly related to HPV infection, the persistent infection is not a sufficient factor in the neoplastic transformation. Epidemiological studies have demonstrated that cigarette smoking increases the risk of developing cervical cancer as well as the risk of treatment failure in women infected with HPV (Acladious et al, 2002; Marks et al, 2011; Woodman, Collins & Young, 2007). We have found that comparing never/former smoker women to current smokers showed a statistically higher risk of CIN treatment failure at 12 and 24 months after treatment (24.4% and 33.3%, respectively). Among the 60 carcinogens compounds identified in tobacco, polycyclic aromatic hydrocarbons (HPA), including the ubiquitous environmental carcinogen benzo[a]pyrene (B[a]P) are among the most toxic and carcinogenic (Hoffman, Hoffman & El-Bayoumy, 2001). Studies *in vitro* and *in vivo* have found that cells infected with HPV are capable of generating high levels of both detoxification metabolites and increased the levels of B[a]P metabolites that are known to damage DNA when compared with controls (Trushin et al, 2012; Alam et al, 2008).

Additionally, we have found that current tobacco smoking was independently associated with CIN treatment (adjusted HR=1.85; 95%CI:1.07-3.19), but duration of tobacco use did not seem to be associated with it. Similar results were found by Acladiouset al (2002) in a prospective multi-center cohort study that was conducted on 958 women in the North West of England, who attended the colposcopy clinics at 3 hospitals for treatment of CIN. According to the results of this study, women who were currently smokers presented a risk 19.34 fold for CIN treatment failure, when compared to never smokers, even controlling by post-treatment HPV infection. Also, the authors showed that the risk increased for every ten cigarettes per day currently smoked. Those results suggest that, concerning to the risk of CIN treatment failure, current women smokers who are diagnosed with CIN may have some benefit if they decide to quit smoking.

Long-term use of combined oral contraceptives (COCs) has been shown in epidemiological studies to be associated with cervical cancer diagnosis among HPV-positive women (Moreno et al, 2002; Appleby et al, 2007). The present study shows that oral contraceptive use was statistically associated with CIN-treatment failure indicating a dose-response effect in the three categories of risks evaluated (never/former/current) in 12 months as well as 24 months after treatment. Multiple theories have been advanced to

explain the association between long-term oral contraceptive use and cervical cancer risk including: increased risk of high-risk (HR) HPV infection among oral contraceptive users, a direct carcinogenic effect of estrogen and progesterone by up regulation of viral oncogene expression (Ruutu et al, 2006), and the ability for estrogens to promote tumor growth and persistence (Shai et al, 2007; Chung et al, 2008). Present study shows statistically higher risk of treatment failure among current OC users compared to never users (HR=2.05; 95%CI:1.08-3.88), even controlling for age, ethnicity and HPV status. Although prevalence of HPV infection after treatment and current tobacco smoking were higher among current OC users (22.9% and 18.5%, respectively), compared to former (8.8% and 6.8%, respectively) and never users (6.6% and 5.9%, respectively), it was possible to observe that the current use of oral contraceptive statistically increased the risk of CIN treatment failure in 2.01 folds, when compared to never use women, even controlling for HPV status and tobacco smoking. Despite of the fact that several studies have reported higher prevalence of HPV infection among current OC users (Marks et al, 2010; Marks et al, 2011), a cohort study developed by Marks et al (2011) has shown that current OC use may increase the risk of HPV persistence but not new HPV detection.

Although inferring causal associations between OC use and HPV infection are difficult, due to residual confounding related to sexual behavior and unmeasured behaviors (eg, underreporting or unknown risk from male sex partners), hormonal contraception has been hypothesized to facilitate HPV-mediated cervical carcinogenesis through a variety of pathways such as increased expression of viral oncogenes (Ruutu et al, 2006) and tumor promotion and persistence (Shai et al, 2007; Chung et al, 2008). Recently Marks et al (2010) showed that human peripheral blood mononuclear cells stimulated with HPV 16 virus–like particles and treated with estradiol and progesterone have lower concentrations of interferon-c, interleukin-12p70, and tumor necrosis factor–a production and increased concentrations of interleukin-10, transforming growth factor–b, and forkhead box p3 gene expression compared with non-hormone-treated cells (Marks et al, 2010). These results might be suggesting that estrogen/progesterone may also increase the risk of invasive cervical cancer by suppressing the host immunological response to infection.

Our study has limitations and strengths that need to be considered in drawing inferences from our findings. First, this study has a small sample size which may reduce the statistical power to detect possible interactions between the studied variables. Second, considering that we have studied a hospital based cohort, a population based study is needed to confirm the present results. However, the present study has strengths that must be pointed out. First, we relied on accurate data collection and clinical procedures since the cytological and histological slides were all revised by two pathologists specialized on cervical cancer analysis, all the colposcopic examinations and LEEP were performed by two gynecologist oncologist surgeons, and all the environmental information was obtained in a very accurate manner by two trained nurses. Second, a short cervical sampling interval of 3 months in the first year and of 4 months in the second year, allowed us to evaluate accurately HPV infection and recent altered Cytological tests. Third, we used a nested PCR using PGMY primers in the first, and GP 5/6 in the second round (both are degenerated primers), which are very sensitive protocol to detect HPV L1 capsid gene.

5. Conclusion

No consensus regarding the necessary duration of the post-treatment surveillance exists. Thus, our results suggest that age, margin status, current tobacco smoking, oral contraceptive use, and HPV status from 3 to 6 months after treatment, could be indicators that predict CIN treatment failure, allowing for the shortening of the follow-up period. It would means that such women require longer and more intensive follow-up than those who are never/former smokers, never used OC, and were HPV negative. Therefore, women should be more aware of the hazards smoking presents in relation to cervical cancer, and smokers should be encouraged to stop smoking after treatment for CIN. On the other hand, women treated for CIN that are current OC users should be informed about other contraceptive method options.

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List of abbreviation

B[a]P – Benzo[a]pyrene
CIN – Cervical Intraepithelial Lesion
CIN 1 - Cervical Intraepithelial Lesion grade 1
CIN 2 - Cervical Intraepithelial Lesion grade 2
CIN 3 - Cervical Intraepithelial Lesion grade 3
HPV – Human Papillomavirus
HR – Hazard Ratio
HR-HPV – High Risk Human Papillomavirus
HSIL - High grade Squamous Intraepithelial
Lesion
INCA – Instituto Nacional do Câncer (Brazilian National Cancer
Institute) LEEP – Loop Electrosurgical Excision Procedure
LSIL – Low grade Squamous Intraepithelial Lesion
NPV – Negative Predictive Value
PAH – Polycyclic AromaticnHydrocarbons
PBS – Phosphate-Buffered Saline
PCR – Polymerase Chain Reaction.
SIM – Sistema de Informação de Mortalidade (Mortality Data System)
SITEC – Sistema Integrado de Tecnologia Citopatológica (Integrated Cytopathology Technology Section)

Competing interests

All the researchers declare no financial and non-financial competing interests.

Authors` contributions

IFS contributed to the study concept and design, patients` interview, participated in the HPV detection, and carried out the analysis and interpretation of the data. RJK participated in the concept, study design, substantially contributed to the interpretation of the data, and have given the final approval of the version to be published. VWMD proceeded the HPV tests e validations. RCSPF substantially contributed to the histological and cytological analysis, and review of all altered cytological and histological slides. NMSRH substantially contributed to the histological and cytological analysis, and review of all altered cytological and histological slides. SK *(in memoriam)* contributed to the study concept, design, participated in the analysis, interpretation of the data, and have given the final approval of the version to be published.

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Table1. **Epidemiological characteristic of women referred to pre-cancer treatment in Rio de Janeiro, Brazil.**

Variables	[a]N (%)	CI:95%
Age (years)		
18-30	97 (47.3)	40.5 – 54.2
31-45	65 (31.7)	25.3 – 38.1
>45	43 (21.0)	15.4 – 26.5
Skin Color		
White	60 (29.3)	23.0 – 35.5
Non-white	145 (70.7)	64.5 – 77.0
Menarche		
≤13 years old	137 (66.8)	26.7 – 39.6
>13 years old	68 (33.2)	60.4 – 73.3
Sexual Partners		
<5 partners	130 (63.4)	56.8 – 70.0
≥5 partners	75 (36.6)	30.0 – 43.2
Sexual onset		
≤ 15 years old	123 (60.0)	53.3 – 66.7
>15 years old	82 (40.0)	33.3 – 46.7
Menarche-sexual onset timing		
>3 years	98 (47.8)	41.0 – 54.6
≤ 3 years	107 (52.2)	45.4 – 59.0
Parity		
< 2	134 (65.4)	58.9 – 71.9
≥2	71 (34.6)	28.1 – 41.1
Abortions		
< 2	165 (80.5)	75.1 – 85.9
≥2	40 (19.5)	14.1 – 24.9
Oral contraceptive (OC) use		
Never	31 (15.1)	10.2 – 20.0
Former	43 (21.3)	15.4 – 26.5
Current	131(63.9)	57.3 – 70.5
Tobacco use		
Never/ Former	141 (68.8)	62.4 – 75.1
Current	64 (31.2)	24.9 – 37.6
Histology at entrance		
CIN-1	90 (43.9)	37.1 – 50.7
CIN-2/3	115 (56.1)	49.3 – 62.9
Margin status		
Free	116 (66.3)	59.3 – 73.3
Involved[c]	59 (33.7)	26.7 – 40.7
HPV status		
Negative	127 (62.0)	57.1 – 70.5
Positive	78 (38.0)	29.5 – 42.9

[a]Total may change according to missing values; [b] included former and current users, only; [c]This category includes both endocervical and exocervical margin involvement.

Table 2. **Risk of pre-cancer treatment failure among Brazilian women in 24 months of follow up, Rio de Janeiro (N=205).**

Variables	Risk of treatment failure		Log Rank test
	12 months (%)	24 months (%)	*p-value*
Age (years)			
18-30	10.4	21.0	
31-45	14.2	24.5	*0.892*
>45	18.6	32.5	
Skin Color			
White	11.7	21.2	*0.607*
Non-white	16.2	25.1	
Menarche			
>13 years old	16.4	30.3	*0.808*
≤13 years old	14.9	28.2	
Sexual onset			
>15 years old	20.0	20.1	*0.794*
≤ 15 years old	12.3	26.1	
Menarche-sexual onset timing			
>3 years	13.3	22.2	*0.489*
≤ 3 years	17.4	25.5	
Sexual Partners			
< 5	13.9	20.7	*0.062*
≥5	18.1	29.6	
Parity			
< 2	14.4	23.1	*0.158*
≥2	17.2	25.2	
Abortions			
< 2	16.0	24.4	*0.856*
≥2	12.9	21.7	
Oral contraceptive (OC) use			
Never	14.1	19.6	
Former	16.3	30.6	*0.048*
Current	19.4	33.3	
Tobacco use			
Never/ Former	11.4	19.7	*0.027*
Current	24.4	33.3	
Histology at entrance			
CIN-1	12.2	21.2	*0.215*
CIN-2/3	17.9	26.1	
Margin status			
Free	10.5	17.5	*0.026*
Involved[b]	24.4	35.2	
HPV status			
Negative	9.7	17.7	*0.001*
Positive	24.4	33.9	

[a]Included former and current users, only;[b] This category includes both endocervical and exocervical margin involvement.

Table 3.**Crude and adjusted *hazard ratios* for pre-cancer treatment failure among Brazilian women, Rio de Janeiro (n=205).**

Variable	Crude HR (CI:95%)	Adjusted HR[a] (CI:95%)
Age (years)[b]		
18-30	1	1
31-45	1.17 (0.64-2.14)	1.16(0.63-2.13)
>45	1.41 (0.72-2.73)	1.44 (0.74-2.79)
Skin Color		
White	1	1
Non-white	1.16 (0.65-2.08)	1.06 (0.59-1.92)
Menarche		
>13 years old	1	1
≤13 years old	0.93 (0.54-1.61)	1.13 (0.64-1.98)
Sexual onset		
>15 years old	1	1
≤ 15 years old	1.10 (0.63-1.84)	1.02 (0.98-1.03)
Menarche-sexual onset timing		
>3 years	1	1
≤ 3 years	1.20 (0.71-2.03)	1.23 (0.71-2.14)
Sexual Partners		
< 5	1	1
≥5	1.64 (0.97-2.76)	1.62 (0.96-2.73)
Parity		
<2	1	1
≥2	1.46 (0.86-2.46)	1.43 (0.81-2.55)
Abortions		
<2	1	1
≥2	0.94 (0.50-1.79)	0.85 (0.44-1.65)
Oral contraceptive (OC) use		
Never	1	1
Former	1.50 (0.78-2.88)	1.34 (0.69-2.01)
Current	2.16 (1.14-4.10)	2.05 (1.08-3.88)
Tobacco use		
Never/Former	1	1
Current	1.80 (1.06-3.06)	1.87 (1.08-3.24)
Histology at entrance		
CIN-1	1	1
CIN-2/3	1.40 (0.82-2.39)	1.54 (0.90-2.64)
Margin status		
Free	1	1
Involved[c]	1.92 (1.07-3.43)	1.91 (1.06-3.42)
HPV status		
Negative	1	1
Positive	2.36 (1.39-3.98)	2.35 (1.39-3.97)

[a]Adjusted by age, ethnicity and HPV status; *[b] included former and current users, only; [c] This category includes both endocervical and exocervical margin involvement.*

Table 4**. Predictive Model for CIN treatment failure on a cohort of Brazilian Women (N=205)**

Predictor Models	**Adjusted HR[a]**
First follow-up HPV status	
Negative	**1**
Positive	**2.38 (1.41-4.04)**
Oral contraceptives	
Never	**1**
Former	**1.40 (0.72-2.71)**
Current	**2.01 (1.06-3.80)**
Tobacco smoking	
Never/Former	**1**
Current	**1.85 (1.07-3.19)**

[a] *Adjusted by age and all other variables in the model*

