



Genetic, Environmental and Clinical Factors Related to Treatment Failure of Cervical Precancerous Lesions in a Cohort of Brazilian Women

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Authors' contributions

This work was carried out in collaboration between all authors. Author IFS designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors RJK and SK managed the final approval of the manuscript, guaranteed the data security and evaluated the statistical analysis. Authors VAGP and AMAS managed the data collection and saw the patients. All authors read and approved the final manuscript.

Research Article

Received 1st April 2013
Accepted 11th June 2013
Published 28th June 2013

ABSTRACT

Aims: To ascertain the risk of pre-cancerous treatment failure considering selected genetic, environmental, and clinical characteristics among Brazilian women.

Study Design: A prospective study developed in a cohort of women treated for Cervical Intraepithelial Neoplasia (CIN) lesion.

Place and Duration of Study: Gynecology Oncology day clinic of Brazilian National Cancer Institute, between October 2004 and May 2006.

Methodology: We included 285 women (age range 18-75 years) with CIN submitted to lesion excision. All patients were interviewed at admission to identify epidemiological and

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clinical characteristics, having blood samples collected, and a colposcopic examination performed. *TP53* polymorphism was ascertained using PCR-RFLP. After treatment, the study population was followed up with Pap-tests during two years. Treatment failure was evaluated using histological confirmation of any altered tissue. Kaplan-Meyer curves and Cox Proportional Risk Model were used for data analysis.

Results: Frequencies of *TP53* polymorphisms were: Arg72Pro genotype(Arg/Pro)-177(62.1%); Arg72 genotype(Arg/Arg)-55(19.3%); Pro genotype (Pro/Pro)-53(18.6%). Women with endocervical margins involvement showed an HR 7.01(1.73-28.44). Current smoking was statistically related to CIN treatment failure (HR:3.90,95%CI:1.28-11.91). Comparatively to Arg/Arg, the risks for treatment failure were: HR 1.51(95%CI:0.23-9.80) for Arg/Pro women; and HR:1.41 (95%CI:0.31-6.52) for Pro/Pro.

Conclusion: Surgical margins involvement and current tobacco smoking presented independent risks for CIN treatment failure among the studied population. Pro/Pro genotype seems to be associated with CIN treatment failure.

Keywords: Cervical cancer; epidemiology; TP53 polymorphism; pre-cancer prognosis; tobacco smoking.

1. INTRODUCTION

Cervical cancer prevention is preventable through the detection and treatment of precancerous lesions, known as cervical intraepithelial neoplasia (CIN) [1]. Nevertheless, this neoplasia still accounts for the second most common cancer in women worldwide and the main cause of cancer-related mortality among women in developing countries [2]. An essential element in the prevention of cervical cancer is a safe and effective CIN treatment. However, a meta-analysis reported that the rate of post-treatment disease following excision methods is around 30% in developing countries [3]. This is particularly worrying as a large amount of these women will be screened just once in their lives, and treatment failure will charge them a very high risk of invasive disease [4]. Therefore, CIN treatment failure may substantially reduce the benefits of screening.

Several risk factors have been strongly associated to CIN treatment failure and most of them are also related to CIN development, such as age, tobacco smoking, persistent HPV infection [5], and genetic host factors such as *TP53* polymorphism [1]. The *TP53* gene encodes the p53 protein, which is involved in a series of pathways, such as apoptosis, transcription, DNA repair, maintenance of genomic stability, and cell cycle control [6]. Polymorphisms in the *TP53* gene have been hypothesized to modify the conformations of the p53 protein, affecting the ability of HPV to bind and to degrade p53, thus potentially acting in early cervical carcinogenesis [7].

Polymorphism rs1042522 at codon 72 of the *TP53* has been investigated as a genetic co-factor since 1998, after HPV16E6 protein being reported to better degrade p53 protein containing arginine[G] at codon 72 than p53 containing proline[C] at that position[8]. Hence, homozygous women to Arginine allele would have a seven times higher risk for cervical cancer than those being heterozygous or homozygous to Proline allele [8].

More than 100 studies have been conducted aiming to investigate the role of polymorphism at codon 72 at *TP53* gene on the risk of cervical cancer development, but showing inconsistent results [9]. Despite the fact that some researchers have studied p53 polymorphism as a prognostic marker in cervical cancer, these studies were developed

among women with advanced cervical cancer [10], and none of them evaluated the effect of this polymorphism among women with precancerous lesion.

To our knowledge, no studies to date have evaluated *TP53* polymorphism on precancerous lesion treatment failure, taking into account involvement of the margins, environmental and methodological factors, such as reliable exposure assessment, that have been also related to CIN treatment failure [11]. The present study was carried out aiming to address this gap towards ascertaining the risk of CIN treatment failure exploring the existing genetic (*TP53* polymorphism at codon 72), environmental and clinical risk factors among Brazilian women.

2. MATERIALS AND METHODS

2.1 Study Population and Design

The characteristics of the study design have been previously described[12]. In short, the population study included 285 women enrolled among those attending a reference center of public health service providing free oncological care (INCA) in the city of Rio de Janeiro, Brazil. All gynecologic oncological outpatients showing altered Pap test results (CIN1, CIN2/3, but not invasive cervical cancer) from October 2004 to May 2006, were invited to participate if fulfilling the following criteria: age over 17 years old, no cervical treatment in the past 6 months before the study enrollment, no psychiatric diseases, no current pregnancy, having an intact uterus, and those agreeing to participate by signing an informed consent. The present study was approved by the Ethical Committee from Brazilian National Cancer Institute and from National School of Public Health, Brazil.

2.1.1 Sample size estimation

Considering that treatment failure incidence may vary between 5 to 35%, in order to be conservative, we consider an incidence of treatment failure of 15% in the exposed group, and an expected difference between exposed and non-exposed group of 2.0 (OR=2.0), a sample of 279 women would be necessary to obtain statistically significant estimates ($error\ I=0,05$; power=80%).

2.2 Clinical Procedures and Follow-Up

After agreeing on signing the informed consent, all women were invited to answer a questionnaire with epidemiologic, environmental and clinical information applied by two trained registered nurses, and the patient was then submitted to a Colposcopy test. At colposcopy test, suspicious lesions were eligible to be totally extracted by LEEP. Those women with no visible lesion at colposcopic examination had another Pap test taken. If that cytology was altered those women were submitted to another colposcopic examination. In this second Colposcopy if there was a visible lesion, it was treated by LEEP; If there was no visible lesion, an endocervical curettage (ECC) was performed, in order to identify (and treat) the lesions that were in the endocervix, which is not completely visible at colposcopy. The date of a complete treatment (which means a complete lesion excision) was chosen as the startpoint of the study. After clinical examination and cervical biopsy, peripheral blood samples were obtained for single nucleotide polymorphism (SNP) tests.

According to the official Brazilian protocol for monitoring cytological abnormalities, all women receiving CIN treatment were monitored during the following two years, and a Pap smear

exam should be carried out every 3 months on the first year, and every 6 months on the second year [13]. In order to guarantee patients compliance and the cytological alteration assessment, all women enrolled were followed-up during a two years period in pre-scheduled return visits. Pap smears were obtained and epidemiological variables (such as smoking status, parity, sexual behavior) were updated upon each visit. Referrals for colposcopy and colposcopic-directed punch biopsies were indicated for patients with persistent abnormal cytological findings. The patients with colposcopic-directed punch biopsies that revealed CIN1 or CIN2/3 lesions were either referred for repeated cone biopsy if fertility was to be preserved or for hysterectomy for women who had completed their family planning or had another gynecological indication (eg. menometrorrhagia). Follow-up length was either defined as the period of time that lapsed from the treatment (complete excision of the lesion) date until a confirmed diagnosis of treatment failure, or the last follow-up date in which participants showed no evidence of disease. Failure was defined as and altered Pap test with histological confirmation in the follow-up period, among those women with at least one normal Pap test between the treatment and the altered Pap test.

Those who missed appointments were contacted by phone and/or letter. These attempts at contacting subjects were repeated once a month until an appointment could be rescheduled or the woman explicitly stated that she wished to drop out of the study.

2.3 Data Collection

A longitudinal study involving repeated measurements was carried out recording the age at enrollment, skin color, sexual behavior, and reproductive history, history of sexually transmitted diseases, contraceptive use, smoking, and cervical outcome.

2.3.1 Variables categorization

Histological diagnosis was graded according to the Bethesda System [14] including condylomata and cervical intraepithelial neoplasia grades I(CIN-1), and CIN2-3/*in situ*. 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 as Low grade Squamous Intraepithelial Lesion(LSIL) and as High grade Squamous Intraepithelial Lesion(HSIL).

Tobacco smoking was classified according to IARC definition [15] 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 [16] and classified as white and non-white. All analyses were additionally stratified by age at first intercourse (≤ 16 , and >16 years old), number of full-term pregnancies/parity (≤ 2 and >2), number of sexual partners (1-2, 3-4, ≥ 5), a new partner during follow up (0 and ≥ 1), number of abortions in a lifetime (0, 1-2, and >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.

2.3.1.1 Outcome definitions

The main outcome was histologically confirmed precancerous lesion or cancer diagnosed during the follow up visits.

2.4 TP53 Polymorphism Genotyping

After DNA was extracted from leukocytes of peripheral blood through the salting procedure[17], genotyping for p53 polymorphism at codon 72 was carried out using a PCR-RFLP based method[18]. The forward and reverse primers used for amplification of a 296bp fragment containing the polymorphic region were 5'- ATC TAC AGT CCC CCT TGC CG -3' and 5'- GCA ACT GAC CGT GCA AGT CA -3', respectively. Digestion with the restriction enzyme BstU1(New England Biolabs, Beverly, MA) was used to digest 5uL PCR product with overnight incubation at 60°C. Bands of cut and uncut products were visualized on 3% agarose gels. The presence of the Arg allele was indicated by bands of 169bp and 127 bp, wherein no digestion of the Pro allele could be observed (Fig. 1).

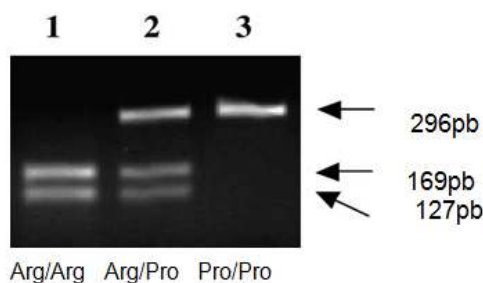


Fig. 1. Gel presenting the bands of TP53 polymorphism genotypes (1-Arg/Arg, 2-Arg/Pro, 3-Pro/Pro) in three different DNA samples, using PCR-RFLP methods[12]

2.5 Statistical Analysis

Chi-square test was carried out to evaluate differences between the frequencies, considering a significant level of 5%. Kaplan-Meier method was used to estimate cumulative risks of treatment failure, being compared using the Log-Rank test (CI:95%). A Cox proportional hazard regression analysis was performed to estimate the crude and adjusted hazard ratio (HR) of treatment failure according to the different associated risk factors, considering a significance level of 5%. All analysis was carried out using SPSS software package version 17.0 for windows (SPSS, IBM, Armonk, NY, USA).

3. RESULTS

According to Table 1, TP53 polymorphism was distributed as follows: 177(62.1%) women showing an Arg72Pro genotype (Arg/Pro), 55(19.3%) homozygous for an Arg72 genotype (Arg/Arg), and 53(18.6%) homozygous for a Pro72 genotype(Pro/Pro), which was in Hardy-Weinberg equilibrium. Around 47% women were under 30 years old, 69.1% were non-white, 75.4% had HSIL cytology at the beginning of the study, and 35.7% had margin involvement after treatment. The prevalence of current tobacco use was 28,1%, and most of treatment failure were CIN 2/3(62,5%).

Table 1. Environmental, clinical and genetic host factors distribution in the studied hospital cohort, Brazil

Variable	Overall^a	%	(CI: 95%)
TP53 Polymorphism	N		
Pro/Pro	53	18.6	(14.1 – 23.1)
Arg/Pro	177	62.1	(56.5 – 67.7)
Arg/Arg	55	19.3	(14.7 – 23.9)
Age (years)			
18 – 30	134	47.0	(41.2 – 52.8)
31 – 45	95	33.3	(27.9 – 38.8)
≥ 45	56	19.6	(15.0 – 24.3)
Ethnicity (skin color)			
White	88	30.9	(25.5 – 36.2)
Non-white	197	69.7	(63.4 – 74.4)
Sexual onset (years)			
≤ 16	145	49.1	(45.1 – 56.7)
> 16	140	40.9	(43.3 – 54.9)
Abortion			
0	156	54.7	(49.0 – 60.5)
1 to 2	110	38.6	(32.9 – 44.2)
> 2	19	6.7	(3.8 – 9.6)
Margin involvement			
Clear	153	64.3	(59.3 – 71.5)
Ectocervix	43	18.7	(13.4 – 23.3)
Endocervix ^c	38	15.9	(11.5 – 21.0)
Tobacco use			
Never	144	50.5	(44.7 – 56.3)
Former	61	21.4	(15.6 – 26.2)
Current	80	28.1	(22.9 – 33.3)
Sexual partners			
1 – 2	96	33.7	(28.2 – 39.2)
3 – 4	88	30.9	(25.5 – 36.2)
≥ 5	101	35.4	(29.9 – 41.0)
Oral contraceptive use			
Never	44	15.4	(11.2 – 19.6)
Former	66	23.2	(18.3 – 28.1)
Current	175	61.4	(55.8 – 67.1)
Duration of oral contraceptive use^b			
≤ 77 months	127	53.1	(46.8 – 59.5)
> 77 months	112	46.9	(40.5 – 53.2)
Duration of tobacco smoking^b			
≤ 10 years	44	31.2	(23.6 – 38.9)
> 10 years	97	68.8	(61.1 – 76.4)
Cytology at entrance			
LSIL	66	25.6	(18.3 – 28.1)
HSIL	215	75.4	(70.4 – 80.4.4)
Cancer	04	1.4	(-)

Histology at treatment			
CIN 1	129	45.3	(39.5 – 51.0)
CIN 2-3	156	54.8	(49.0 – 60.5)
Treatment Failure			
CIN 1	4	25.0	(-)
CIN 2/3	10	62.5	(-)
Cancer	2	12.5	(-)

^a Total may vary according to missing values; ^aAnalysis carried out among former and current users, only. ^c This category includes endocervical margin involvement and both margins involvement.

In Table 2 we observed that women more likely to present treatment failure in 24 months were those who were current tobacco users (12.9%,*p*=.0176); CIN 2/3 histological result at the follow-up startpoint (7.8%,*p*=.0247); and having ectocervix (9.8%) or endocervix (14.7%) margin involvement (*p*=.0017).

Table 2. Probability of CIN treatment failure, studied hospital cohort (n=285), Rio de Janeiro, Brazil, 2004-2008

Variable	% Treatment failure	
	24 months	^b Log-Rank test: 95%
TP53 Polymorphism		
Arg/Arg	6.0	
Arg/Pro	6.8	0.7433
Pro/Pro	6.2	
Age		
18 – 30	11.9	
31 – 45	6.7	0.6855
> 45	7.5	
Skin color		
White	21	
Non-white	4.6	0.037
Oral contraceptive use		
Never	3.1	
Former	13.5	0.1006
Current	11.5	
Duration of contraceptive use^a		
≤ 77 months	4.2	0.3375
> 77 months	7.5	
Tobacco use		
Never	4.7	
Former	3.7	0.0176
Current	12.9	
Duration of tobacco use^a		
≤ 10 years	4.6	0.2438
> 10 years	10.9	
Histology		
CIN-1	3.6	0.0247
CIN 2-3	7.8	
Margin involvement		
Clear	3.1	
Ectocervix	9.8	0.0017
Endocervix ^c	14.7	

Menarche		
> 13 years old	5.2	0.9077
≤ 13 years old	6.1	
Sexual onset		
> 16 years old	7.1	0.2380
≤ 16 years old	4.6	
Years from menarche to sexual onset		
> 3	6.3	0.5355
≤ 3	5.4	
Sexual partners		
1 – 2	4.5	
3 – 4	4.9	0.1688
≥ 5	10,4	
Abortion		
0	13.0	
1 to 2	8,4	0.1549
> 2	19,8	
Parity		
≤ 2	7,4	0.3356
> 2	5,5	

^aAnalysis carried out among former and current users, only. ^bLog-Rank test obtained from Kaplan-Meier analysis. ^cThis category includes endocervical margin involvement and both margins involvement.

Margin involvement was an independent risk factor for CIN treatment failure, (HR=7.01;95%CI:1.73-28.4). Current tobacco use was also an independent risk factor for CIN treatment failure (HR=3.90;CI:95%=1.28-11.91). Comparatively to CIN1 women at the follow-up startpoint, women with CIN2/3 histological result showed an HR=4.67(95%CI:1.27-17.05)(Table 3). The joint effect of tobacco smoking and *TP53* polymorphism in the risk of treatment failure was explored, but no statistical significance was found.

Table 3. Crude and adjusted HR for CIN treatment failure among Brazilian Women, Rio de Janeiro (2004-2008)

Variables	Treatment failure in 24 months	
	Crude HR (CI:95%)	^a adjusted
TP53 Polymorphism		
Arg/Arg	1	1
Arg/Pro	1.63 (0.27 – 9.77)	1.51 (0.23 – 9.80)
Pro/Pro	1.79 (0.40 – 8.10)	1.41 (0.31 – 6.52)
Age (years)		
18 – 30	1	1
31 – 45	1.43 (0.46 – 4.45)	1.77 (0.56 – 5.59)
≥ 45	1.69 (0.45 – 6.02)	1.89 (0.52 – 6.82)
Ethnicity		
White	1	1
Non-white	0.15 (0.02 – 1.43)	0.15 (0.02 – 1.14)
Margin Involvement		
Clear	1	1
Ectocervix	3.44 (0.69 – 17.08)	3.81 (0.76 – 19.08)
Endocervix ^c	8.60 (2.15 – 34.41)	7.01 (1.73 – 28.44)

Tobacco use		
Never	1	1
Former	0.90 (0.18 – 4.67)	0.81 (0.15 – 4.35)
Current	3.68 (1.23 – 10.97)	3.90 (1.28 – 11.91)
Sexual partners		
1 – 2	1	1
3 – 4	1.44 (0.32 – 6.45)	1.62 (0.36 – 7.38)
≥ 5	2.98 (0.81 – 11.00)	2.77 (0.75 – 10.24)
Oral contraceptive use		
Never	1	1
Former	3.02 (0.97 – 9.40)	3.03 (0.94 – 9.73)
Current	2.68 (0.75 – 9.52)	3.16 (0.86 – 11.57)
Duration of OC use^b		
≤ 77 months	1	1
> 77 months	1.74 (0.55 – 5.49)	1.96 (0.60 – 6.38)
Duration of tobacco use^b		
≤ 10 years	1	1
> 10 years	2.43 (0.52 – 11.31)	2.90 (0.51 – 16.52)
Histology		
CIN-1	1	1
CIN 2-3	3.81 (1.10 - 13.37)	4.67 (1.27 – 17.05)
Abortion		
0	1	1
1 to 2	1.68 (0.57 – 5.01)	1.31 (0.43 – 4.00)
> 2	3.68 (0.91 – 14.82)	2.85 (0.66 – 12.23)

^aHR adjusted by age and ethnicity; ^bAnalysis carried out among former and current users, only. ^cThis category includes endocervical margin involvement and both margins involvement.

4. DISCUSSION

Recently, two meta-analyses were carried out aiming to investigate the role of *TP53* polymorphism on the risk of cervical cancer. The first one analyzed individual data from 49 different studies worldwide and no association was found when the analysis was restricted to methodologically sound studies [19]. The second reanalyzed 45 studies of the above meta-analysis and found substantial heterogeneity between the estimates from the studies. This was attributable to study populations with genotypic distributions that departed from Hardy-Weimberg equilibrium and may suggest problems with genotyping/laboratory methods or study design [20].

While this inconsistency across studies might reflect a lack of true association, it is also plausible that some of the heterogeneity may be either due to differential haplotypic structure of *TP53* codon 72 polymorphisms in different study population with different backgrounds[21], or due to variations between countries in the association between *TP53* codon 72 polymorphism and cervical cancer, with some countries showing a trend toward increased risk of cervical cancer associated with proline at codon 72[22,23].

Although Arg/Arg genotype is known as being more susceptible to the effects of HPV E6 than the Pro/Pro, increasing the risk of cervical cancer development [8], it doesn't seem to play the same role in the risk of CIN treatment failure, since the present study results suggest that proline at codon 72, but not arginine, was associated with CIN treatment failure, despite lacking statistical significance. Thus, the role of p53 72 polymorphism on CIN treatment failure remains unknown, since HPV infection and its persistence are known as

the main risk factors related to treatment failure [1] and clearance rate of HPV infection after CIN treatment ranges from 82.3%-92.2%[5].

One possible explanation is that after CIN treatment with LEEP, the role of Arg/Arg genotype (apoptosis) might be more efficient in preventing treatment failure than either observed with Pro/Pro (G1 arrest), or Arg/Pro genotypes [23]. Bhattacharya and colleagues [11] reported that proline at codon 72 probably facilitates HPV-16/18-induced transformation of cervical cells by affecting cell cycle regulation. Moreover, the ability of HPV to avoid immune attack might be associated with the transforming potential of the virus. Thus, these authors showed that women who were HLA-B*07 and Pro/Pro carriers were at higher risk of cervical cancer development when compared to non-carriers of both alleles (OR=14.05, CI:95% 1.11-177.30).

It still remains unclear how different patterns of tobacco use and its relationship with other risk factors affect a woman's risk of developing cancer and/or CIN treatment failure. Our results provide evidence that current smokers, compared to non-smokers, are likely to present CIN treatment failure (Tables 2 and 3). If confirmed by other studies, such observation can be translated into important public health intervention policies towards smoking control among women with CIN lesions.

A large prospective study in UK exploring the role of HPV testing and smoking on CIN treatment failure found that smoking is an independent risk factor for CIN treatment failure. This study results showed that the OR of treatment failure of CIN increases by a factor of 2.58 (95% C.I. 1.7–3.91) for each additional 10 cigarettes a woman smokes daily (ranging 0-30 cigarettes), having this risk indeed persisted after HPV adjustment [24]. The authors suggested that this increased risk estimate may result from a local immunological effect of smoking on the cervical epithelium, or by the formation of carcinogen-DNA adducts in the cervical tissue itself, which may initiate neoplastic transformation [24].

Another important finding was that the histologic lesion CIN 2-3 at treatment significantly increased the risk of treatment failure, comparing to CIN-1 (Table 2 and 3). This might be explained by the fact that CIN 2-3 are related to high-risk HPV (HR-HPV) infection which is more likely to persist and has been found as one of the main risk factor related to CIN treatment failure [24,25]. In the same way, the number of sexual partners presented a dose-response relationship and a strong association with treatment failure (Table 2 and 3), although no statistical significance was found. Number of sexual partner is closely related to HPV infection, since the more sexual partner a woman has the more likely of achieving an HPV infection. Kreimer et al [25] developed a cohort study in USA with a group of women with ASCUS-LSIL. The authors found that HPV infection detected 6 months after treatment may predict CIN development after treatment.

A limitation of the present study was that HPV status could not be ascertained. However, this study tried to deal with that issue through stratification by the number of sexual partners and the involvement of margins, whose occurrence suggests persistence of residual disease.

Our findings provide evidence that women with margins involvement were significantly at higher risk of CIN treatment failure when compared to those with clear margins, regardless of age and ethnicity. Our results are also in agreement with other studies, which reported that the depth of treatment and the completeness of excision are the crucial treatment factors determining a successful outcome [3,26]. As the depth of treatment is dependent on the extent of involvement of the endocervical canal, it is very important to accurately identify

the location of the squamocolumnar junction. The mentioned authors also advocate that when the CIN 2-3 is present at the deep margins, there will be a risk that any residual disease may be an undetected invasive lesion.

It has been suggested that besides environmental and genetic host characteristics, the risk of CIN treatment failure is closely related to clinical conditions, such as the presence of residual disease (surgical margin *status*) and surgeon's expertise [26]. One of the strengths of our study is that surgical procedures (LEEP) were accomplished by only two oncological gynecology surgeons aiming to assure quality of treatment. Since the study population consisted of women with pre-cancer lesion who underwent conservative treatment, and therefore, all of them were at the risk set of presenting the studied outcome (CIN treatment failure), it is improbable that selection bias has occurred, and external validity was provided.

5. CONCLUSION

This study provides evidence that obtaining clear margins particularly clear deep margins (endocervix), when treating CIN is very important to prevent treatment failure, independently of ethnicity and age at the treatment. Moreover, the study pointed out that current tobacco smoking was strongly associated to CIN treatment failure. Thus, women should be more aware of the risks of smoking, and smokers should be encouraged to stop smoking any time before/after CIN treatment.

The present study is the first one which investigated prospectively the relationships between p53 polymorphisms and tobacco smoking on CIN treatment failure among Brazilian women, and the presented results need further confirmation by other investigations.

CONSENT

Authors declare that all study participants signed a written informed consent.

ETHICAL APPROVAL

The present study was approved by the Ethical Committee of both the Brazilian National Cancer Institute and the National School of Public Health. All authors declare that written informed consent was obtained from all patients enrolled in this study.

ACKNOWLEDGEMENTS

This study was partially supported by the Ministry of Health and The Brazilian National Research Council (CNPq), which provided the source of molecular analysis. Authors also thanks to Amy Fairchild, Jeremy Conrad and Anelise Gondar for the English text review. Special thanks to Michelle de Oliveira e Silva for the standardization of molecular analysis procedures.

COMPETING INTERESTS

The authors declare no conflict of interest.

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