FUNDAÇÃO OSWALDO CRUZ
ESCOLA NACIONAL DE SAÚDE PÚBLICA
DOUTORADO EM SAÚDE PÚBLICA
DEPARTAMENTO DE EPIDEMIOLOGIA E MÉTODOS QUANTITATIVOS EM SAÚDE

Contribuição dos fatores clínicos, epidemiológicos e genéticos na evolução das lesões precursoras do câncer de colo de útero.

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Trabalho apresentado na Escola Nacional de Saúde Pública / Fundação Oswaldo Cruz, vinculado ao Departamento de Epidemiologia e Métodos Quantitativos em Saúde do Programa de Doutorado em Saúde Pública, visando a obtenção do grau de Doutora em Saúde Pública, sob a orientação do Professor Doutor. Sergio Koifman.

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"Os homens tornaram-se cientistas porque esperavam encontrar lei na natureza, e esperavam encontrar lei na natureza porque criam em um Legislador."

C.S. Lewis.

"O que me agrada na experiência é a sinceridade que nela percebo. Você pode tomar quantos desvios quiser; mas basta manter os olhos bem abertos, que logo verá a placa de alerta. Talvez você se tenha enganado, mas a experiência não tenta enganar ninguém. **O universo se mostra fiel sempre que você o testa com justiça."**

C.S. Lewis

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Sumário

Lista de Abreviaturas

AGUS	Atypical Glandular Cell of Undetermined Significance (Atipia de Células Escamosas de Significado Indeterminado)
Arg	Arginine
ASCUS	Atypical Squamous Cell of Undetermined Significance (Atipia de Células Escamosas de Significado Indeterminado)
BMI	Body Mass Index
BSF-2	B-cell stimulatory factor
CAF	Cirurgia de Alta Freqüência
CI	Confidential Interval
CIN – I	Cervical Intraepithelial Neoplasia grade I
CIN – II	Cervical Intraepithelial Neoplasia grade II
CIN – III	Cervical Intraepithelial Neoplasia grade III
CIN	Cervical Intraepithelial Neoplasia
CK	Cytokines
CKR	Cytokines-receptors
CNPq	Brazilian National Research Council
CYP	Cytochrome Protein
DST	Doença Sexualmente transmissível
ECC	Endocervical Curettage
FAPERJ	Fundação de Apoio a Pesquisa do Estado do Rio de Janeiro
GST	Gluthathione S-Transferases
HE	Hematoxylin and Eosin
HIV	Human Immunodeficiency Virus
HPV	Human Pappilomavirus (Papilomavirus Humano)
HR	Hazard Ratio
Hr-HPV	High-risk Human Pappilomavirus
HSIL	High-grade Squamous Intraepithelial Lesion
IARC	International Agency for Research on Cancer
IBGE	Instituto Brasileiro de Geografia e Estatística / Brazilian Institute of Statistics and Geography
IBSCC	International Biological Study of Cervical Cancer

IFN-β2	Interferon - β2
IL-6	Interleukin – 6
INCA	Instituto Nacional do Câncer
LEEP	Loop Electrical Excision Procedure
LEETZ	Large Loop Electrosurgical Excision Procedure
LSIL	Low-grade Squamous Intraepithelial Lesion
NIC – I	Neoplasia Intraepitelial Cervical grau I
NIC – II	Neoplasia Intraepitelial Cervical grau II
NIC – III	Neoplasia Intraepitelial Cervical grau III
NIC	Neoplasia Intraepitelial Cervical
OR	Odds Ratio
p53	Proteína do gene *TP*53
PAH	Polycyclic Aromatic Hydrocarbons
PCR	Protein Chain-Reaction (Reação da Cadeia de Polimerase)
PR	Prevalence Ratio
pRb	Proteína do gene retinoblastoma
Pro	Proline
RFLP	Restriction Fragment-Length Polymorphism
SCC	Squamous Cervical Cancer
SIL	Squamous Intraepithelial Lesion
SNP	Single Nucleotide Polymorphism
STD	Sexually Transmitted Disease

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Resumo

Introdução: Apesar dos esforços para controlar a incidência e mortalidade por câncer de colo de útero, esta neoplasia é um problema de saúde pública no Brasil e no mundo. Tem sido internacionalmente observada uma mudança no padrão de incidência do câncer cervical, sobretudo em mulheres jovens, sugerindo uma distribuição heterogênea dos fatores de risco nos diferentes grupos etários. A detecção precoce das lesões precursoras e seu tratamento adequado constituem as ferramentas principais para o controle da incidência e mortalidade por esta neoplasia. No entanto, há um percentual considerável de mulheres cuja evolução caracteriza-se pelo insucesso no tratamento dessas lesões ou apresentam um padrão diferenciado, devido a fatores ainda não muitos bem estabelecidos.
Objetivo: Determinar a contribuição de fatores clínicos, epidemiológicos e genéticos na evolução das lesões precursoras do câncer cervical bem como na falha de tratamento das lesões pré-neoplasicas.
Metodologia: Trata-se de um estudo observacional transversal em mulheres com lesões cervicais com indicação de colposcopia, atendidas num hospital de referência para câncer ginecológico entre outubro de 2004 a Maio de 2006; e um estudo observacional analítico prospectivo de uma coorte de mulheres com lesões precursoras do câncer de colo de útero submetido ao tratamento conservador, seguidas por 2 anos após o tratamento. As pacientes foram submetidas à uma entrevista padronizada buscando identificar os cofatores de risco de interesse no estudo e foi coletado uma amostra de sangue periférico para determinação dos polimorfismos do códon 72 do gene *TP53*, utilizando as técnicas de PCR-RFLP. Foram utilizados os testes de Poisson e de Regressão Logística Múltipla para cálculos das razões de prevalência e OR, respectivamente, com intervalos de confiança de 95% no estudo transversal. Os testes de Kaplan Meier e regressão proporcional de Cox foram utilizados para avaliação da probabilidade condicional de falha no tratamento e estimação das HR, respectivamente.
Resultados: Um total de 318 pacientes assinou o TCLE e cumpriram os critérios de inclusão. Dessas, 136 (42.8%) tinham entre 18-30 anos, 138 (43.4%) entre 31-49 anos, e 44 (13.8%) tinham 50-68 anos. Das 304 coletaram amostras de sangue periférico, 55 (18.1%) eram homozigotos para o alelo Pro, 185 (60.9%) heterozigotos, 64 (21.1) eram homozigotos para o alelo Arg. A distribuição dos polimorfismos está em equilíbrio de Hardy-Weimberg. No grupo mais jovem, paridade e sexarca precoce apresentaram razões de prevalência bruta e ajustada de 1.16 e 1.95, respectivamente. No grupo etário de 31 – 49 anos, a idade na menopausa e tabagismo atual foram fatores de risco independentes para HSIL/Câncer (RP:1.21 e 1.37, respectivamente). A forma heterozigota foi um fator de risco independente para o desenvolvimento de HSIL/Câncer (ORaj=1.92, 95%CI:1.03-3.59). Polimorfismos da p53 interagiram significativamente com uso de contraceptivo oral (OR interação= 3.59,95%CI:1.09-11.84). Envolvimento das margens (HR=7.01), tabagismo atual (HR=3.9) estão estatisticamente associados ao risco de falha no tratamento de lesões precursoras quando o critério de falha utilizado é confirmado histopatologicamente.
Conclusão: Os fatores de risco envolvidos no desenvolvimento do câncer cervical podem variar de acordo com o grupo etário estudado. Fatores genéticos ligados ao hospedeiro (ex. Polimorfismos do gene *TP53*) podem interagir com esses fatores ambientais modulando o risco de HSIL/Câncer. As margens cirúrgicas e os fatores ambientais (tabagismo, sexarca precoce, multiplicidade de parceiros e uso de contraceptivos orais) atuam conjuntamente no risco de falha no tratamento conservador das lesões precursoras do câncer cervical

I – Introdução:

O câncer de colo de útero ainda representa um sério problema de saúde pública, uma vez que é o terceiro tipo de câncer mais comum entre todas as neoplasias, a segunda causa de morte por neoplasia entre as mulheres e ainda é a principal causa de morte por câncer em mulheres em alguns países em desenvolvimento (Masood, 1997; Laczano-Ponce *et al*, 1999; Ferlay *et al*, 2000).

Os estudos epidemiológicos têm revelado que a duração média para que uma célula normal evolua para o estágio de câncer invasor, começando por estágios precoces que são detectáveis e curáveis, é de cerca de dez anos (Holoway *et* al, 1999), possibilitando que sejam feitos esforços preventivos através de programas de rastreamento do câncer cervical (Anderson *et al*, 1998). Embora muitos países desenvolvidos tenham experimentado um acentuado declínio nas taxas de mortalidade e incidência nas últimas décadas, por implementarem efetivos programas de rastreamento (Kjellgren *et al*, 1986; Magnus *et al*, 1987; VanDerGraaf *et al*, 1988), alguns deles vêm observando, recentemente, uma estagnação nesta tendência de declínio, e até um ligeiro aumento na incidência de câncer cervical em mulheres jovens (Llorca *et al*, 1999; Liu *et al*, 2001).

No Brasil, embora já seja possível verificar uma tendência de declínio nas taxas de mortalidade por câncer de colo uterino no conjunto das capitais brasileiras (Koifman *et al*, 2002), possivelmente devido a uma melhor implementação dos programas de rastreamento em massa (Torres *et al*, 2003), as taxas de mortalidade ainda são consideradas elevadas (Ferlay *et al*, 2000).

O câncer de colo de útero pode ser detectado ainda em estágios precoces, que se caracterizam por células displásicas, localizadas na superfície do epitélio cervical. As lesões pré-invasivas são chamadas de Neoplasias Intraepiteliais Cervicais (NIC), que são

classificadas em graus I, II e III os quais refletem o seu comportamento biológico (Kurman & Solomon, 1994). Essa nomenclatura sofreu uma revisão em 2001, classificando as NIC I como lesões de baixo grau e colocando as NIC II e III num mesmo patamar biológico, classificando-as como lesões de alto grau. As alterações celulares que não podem ser classificadas como neoplasia intraepitelial cervical, mas merecem uma investigação melhor são classificadas como atipias de células escamosas de significado indeterminado (ASCUS) ou atipias de células glandulares de significado indeterminado (AGUS) (Kurman & Solomon, 1994).

As lesões pré – neoplásicas, se não tratadas, apresentam um potencial diferenciado de regressão, persistência e progressão, segundo a sua classificação histológica. De acordo com a literatura, lesões do tipo NIC I teriam um potencial de regressão maior (62% a 70%) quando comparadas às NIC II e III (45% a 55%) num período de 11 a 43 meses (Nasiell *et al,*1986; Silva *et al*, 2003). Por outro lado, a literatura mostra que as NIC I apresentam um menor potencial de progressão (da ordem de 4,9% a 16%) que as NICs II e III (30% a 42%), mas o potencial de persistência dessas lesões ainda permanece controverso (Nasiell *et al,*1986; Murthy *et al*, 1990Silva *et al*, 2003).

Existem vários fatores que podem estar envolvidos na evolução do câncer de colo de útero, mas é consenso que a infecção pelo Papilomavirus Humano (HPV), sua persistência e carga viral são os principais fatores envolvidos na evolução das lesões pré-neoplásicas (Muñoz *et al*, 1997; Franco *et al*, 1999a; Schlecht *et al*, 2003). No entanto, para que a doença ocorra, é necessária a associação com outros fatores de risco que atuariam como co-fatores (Parazzini *et al*, 1997; Kjaer *et al*, 1998; Szarewski *et al*, 2001; Lacey Jr. *et al*, 2001). Destacam-se entre estes o tabagismo (Szarewski *et al*, 2001), a susceptibilidade genética (Storey *et al*, 1998), a imunossupressão (Dal Maso *et al*, 2001), o comportamento

sexual de risco (Schiffman *et al*, 1996; Wideroff *et al*, 1999) e o uso de contraceptivos orais (Makoto *et al*, 1998), sendo que estes dois últimos estão intimamente relacionados ao risco de infecção pelo HPV.

Num estudo onde avaliamos a evolução de uma coorte de mulheres com diagnóstico de NIC I, II e III referidas a um polo de patologia cervical no Rio de Janeiro (Silva *et al*, 2003, dados não publicados), verificou-se que as lesões precursoras do câncer têm um potencial de negativação espontânea de 70% para as lesões de baixo grau, e de 50% para as lesões de alto grau. Além disso, entre as mulheres submetidas a exérese da lesão inicial, observou-se que aproximadamente 36% apresentaram falha no tratamento, e que o resultado histológico não esteve associado com o risco de insucesso no tratamento, sendo a idade o único fator apresentando significância estatística tanto para o risco de persistência de alterações cervicais quanto para o risco de insucesso no tratamento (Silva *et al*, 2003, dados não publicados). Esses resultados apontam para a necessidade de verificar o papel da suscetibilidade genética, dos fatores clínicos e epidemiológicos no processo de evolução das lesões precursoras do câncer, considerando a variação no tempo, do *status* de HPV e dos co-fatores de risco.

Em continuidade a esta linha de investigação, o presente conjunto de trabalhos que compõem esta tese procurou explorar as seguintes hipóteses:

1. O padrão de aumento na incidência do carcinoma de células escamosas colo de útero e adenocarcinoma em mulheres jovens no Brasil, e também observado em diferentes regiões do mundo a partir das décadas de 1970 e 1980, poderia ser decorrente das mudanças comportamentais a partir da revolução sexual iniciada na década de 1960, potencializando a geração no aumento da incidência das doenças sexualmente transmissíveis, incluindo a infecção por HPV.

2. A introdução do uso da pílula anti-concepcional entre mulheres em idade fértil na década de 1960 e o aumento da prevalência de tabagismo entre mulheres a partir deste período, poderiam explicar parcialmente o aumento da incidência do carcinoma *in situ* de colo uterino, além de sua possível interação com fatores genéticos do hospedeiro.

1.1. O efeito da idade na incidência de câncer cervical.

A incidência de câncer associada à idade tem sido fundamental para os diversos estágios do conceito de carcinogênese (Moolgavkar *et al*, 1992). A inclinação (variação) da curva de incidência associada a idade é informativa do número de estágios discretos ou eventos mutacionais requeridos para a manifestação do tumor. O pico da taxa de incidência em certa idade e a tendência de queda em idades mais avançadas pode significar a existência de uma sub-população de risco, cessação de uma exposição ou o desaparecimento de células alvo.

Em 1999, o *Office for National Statistics* da Inglaterra publicou um estudo de tendência temporal entre 1971 a 1992 naquele país, demonstrado a existência de um aumento na incidência de adenocarcinoma cervical em mulheres nascidas entre os anos de 1953-1957 (de 30-34 anos, no final da década de 70 e início 80), quando comparadas àquelas nascidas entre 1937 a 1941. Posteriormente, Sasieni & Adams (2001) publicaram outro estudo de tendência temporal também realizado na Inglaterra entre os anos de 1971 e 1994. Além de reproduzir os mesmos resultados, os autores demonstraram um efeito coorte de idade nesta população, no que se refere ao aumento da incidência de adenocarcinoma cervical em mulheres jovens, tendo sido esses achados também encontrados em outras populações (Liu *et al*, 2001).

A razão para o crescimento exponencial de adenocarcinoma em mulheres jovens nas décadas de 70 e 80 ainda é desconhecida. Este padrão poderia ser parcialmente explicado pelas mudanças nas normas sexuais desde a revolução sexual na década de 60, que resultou no aumento generalizado de doenças sexualmente transmissíveis e início da atividade sexual em idade precoce (Hofferth *et al*, 1987). Uma outra possível explicação seria a introdução do uso de pílulas anticoncepcionais entre as mulheres desde a década de 60, a qual estaria associada à evolução do adenocarcinoma *in situ* de colo uterino, mesmo controlando-se o efeito decorrente da infecção pelo HPV 18 (Lancey *et al,* 1999). Se ambas hipóteses etiológicas forem verdadeiras, as coortes de mulheres jovens provavelmente continuarão sob maior risco de desenvolvimento de adenocarcinoma e carcinoma adenoescamoso cervical. No entanto, o conhecimento atual sobre esses fatores ainda não pode explicar completamente o recente aumento específico de adenocarcinoma invasor de cérvice uterina (Liu *et al*, 2001).

Os dados de câncer cervical obtidos em estudos seccionais de muitas populações revelam um pico nas idades de 40-60 anos, que são excepcionalmente precoces para qualquer carcinoma (Gustafsson *et al*, 1997). Dois tipos de dados de incidência idade-relacionada foram observados no período anterior ao rastreamento em massa no mundo: um incluindo países como Suécia, onde a incidência máxima notada foi entre 40-50 anos de idade, seguida por um rápido declínio; e outro, onde se observou um pico mais amplo, entre 50 e 70 anos de idade, seguido por um lento declínio (Gustafsson *et al*, 1997).

No Brasil, o rastreamento cervical foi introduzido na década de 50, mas somente uma minoria de mulheres era rastreada e o programa era pouco organizado. O rastreamento durante as décadas de 70 e 80 era mais comum em mulheres mais jovens do que nas mais idosas, fenômeno observado também em outros lugares do mundo (Sasieni & Adams,

2001). Até o início da década de 90, era consenso que o rastreamento em mulheres com menos de 25 anos oferecia apenas um benefício extra, marginal, a nível populacional, por causa da baixa freqüência de câncer cervical nesta faixa etária e a alta prevalência de lesões pré-clínicas de baixo grau, cuja maioria regredirá (Miller *et al*, 1990). Os dados de países escandinavos confirmam que na idade de 20-24 anos a taxa de lesões de baixo grau era cerca de 3 vezes maior do que a taxa de lesões de alto grau entre os anos de 1979 e 1995. Esta taxa foi duas vezes maior na faixa etária de 25 a 49 anos, mas declinava consideravelmente nas faixas etárias seguintes, desaparecendo na idade de 65 anos (Sigurdsson, 1999). No entanto, mesmo em países nórdicos, onde a cobertura populacional e a qualidade dos programas de rastreamento cervical são elevadas, vem se observando um aumento na detecção de lesões de alto grau no grupo etário de 20-24 anos e um aumento da incidência de doença invasora para as idades de 20-29 anos (Sigurdsson, 1999b). Este aumento da incidência de lesões precursoras e câncer cervical na coorte de mulheres com idades mais jovens confirmam a importância de se iniciar o rastreamento em mulheres com idade inferior a 25 anos.

Na realidade, para se obter um benefício máximo, qualquer programa de rastreamento precisa identificar aqueles indivíduos que estão sob significante risco de desenvolver a condição. A infecção pelo HPV é o evento precoce mais importante na carcinogênese cervical e tem seu pico de prevalência em mulheres que estão entre a adolescência e início da segunda década de vida. Este parece ser o período onde ocorre a máxima exposição sexual, seguido pelo período de maior prevalência de Neoplasias Intra-epiteliais Cervicais (NICs) ao final da segunda e início da terceira década de idade, posteriormente a qual quando o risco de infecção parece cair (Bauer *et al,* 1993). Portanto, mulheres nas quais a NIC ainda não desenvolveu e em quem o risco de adquirir a infecção

pelo HPV é pequeno, estão provavelmente sob muito baixo risco de virem a desenvolver uma NIC. Isto foi confirmado em estudos retrospectivos sobre a incidência de NICs em mulheres com mais de 50 anos, que mostraram que as lesões precursoras são raramente identificadas em mulheres que foram, de modo adequado, rastreadas previamente, em idades superiores a 50 anos (Sigurdsson, 1999, Cruicksshank *et al*, 2002).

1.2 - O papel do HPV na carcinogênese cervical

O papillomavirus humano (HPV) é um vírus que contém DNA de dupla-fita circular com aproximadamente 7.500 - 8.000 pares de bases (Pfister, 1994). São conhecidos mais de 90 tipos diferentes de HPV e cerca de 20 destes possuem tropismo pelo trato genital inferior (Villa *et* al, 1996). Os tipos mais freqüentemente associados ao câncer cervical são os 16, 18, 31 e 45, sendo conjuntamente responsáveis por cerca de 75% dos casos de câncer de colo do útero (Coker *et al*, 2001; Muñoz *et al*, 2003).

Uma vez em contato com o tecido epitelial cervical normal, o HPV introduz seu material genético no DNA da célula hospedeira (processo de transdução gênica), ocasionando mutações que se acumulam e podem progredir para a malignidade. Embora o processo de transdução de material genético viral para células normais seja comum em mulheres com amostras cervicais positivas para HPV, esse processo não resulta necessariamente em malignidade (Louro *et al*, 2000).

A replicação do DNA dos HPVs inicia-se com uma fase de modesta amplificação, através da entrada da camada basal da célula de um epitélio ferido. Após a cura da ferida, a maioria das células retorna ao estado de descanso. O epitélio estratificado é reparado pelas divisões das células basais, sendo similar a um epitélio não infectado, exceto que as células em divisão são mais freqüentes. A atividade transcricional é baixa e o DNA viral replica

somente quando as células basais e parabasais entram na fase S do ciclo celular, resultando num estado de baixo número de cópias do DNA viral. Depois as células ascendem, deixam o ciclo celular e submetem-se à diferenciação progressiva (Chow & Broker, 1994).

Os tipos de HPV genital de médio e alto risco expressam proteínas com potencial oncogênico (E6 e E7), que aumentam a probabilidade de reiniciação da transcrição, tornando-se capazes de imortalizar as células infectadas (Sherman *et al*, 1992; Villa *et al*, 1996). O processo pelo qual isso ocorre pode ser explicado pelo fato de que a proteína E7 liga-se ao produto do gene retinoblastoma (RB), que é uma fosfoproteína cuja forma fosforilada regula negativamente a entrada para a fase S do ciclo celular (Dyson *et al*, 1989). Por sua vez, a proteína E6 liga-se à proteína supressora de tumor p53 degradando-a (Sedman *et al,* 1991).

Vale ressaltar que embora a expressão dos genes E6/E7 possa cooperativamente induzir a imortalização celular, eles não podem induzir o fenótipo tumoral diretamente. Portanto, são necessárias alterações adicionais na expressão gênica celular para que as células adquiram um fenótipo maligno (Pei *et al*, 1993; Stöpler *et al*, 1994). Dado que os HPVs induzem proliferações epiteliais que mostram um crescimento limitado e que, freqüentemente, regridem espontaneamente (Franco *et al*, 1999b), a progressão tumoral está também sujeita a fatores ambientais e/ou restritos ao hospedeiro (Coker *et al*, 2001).

Diversos métodos biomoleculares para a detecção do HPV têm sido descritos nesta última década. Os métodos baseados na amplificação de DNA são os mais sensíveis, principalmente o da Reação em Cadeia da Polimerase (PCR real time), pois permitem a detecção de baixa carga viral e minimizam o erro de classificação do *status* de infecção do HPV (Trofatter *et al*, 1997).

O *International Biological Study of Cervical Cancer* (IBSCC) avaliou material de câncer de colo de útero de vinte e dois países, detectando HPV em 93% dos tumores, confirmados pela histopatologia, usando o teste de Reação em Cadeia da Polimerase (PCR) (Bosh *et al*, 1995). Nas reavaliações dessas biópsias realizada por Walboomers e colaboradores (1999), a prevalência foi de 99,7%. No Brasil, os estudos que avaliam a prevalência da infecção pelo HPV, por subtipo viral, ainda são escassos.

1.3 - A susceptibilidade genética na carcinogênese cervical

Considerando-se os conhecimentos atuais sobre a história natural da infecção por HPV, sabe-se que embora um grande número de mulheres estejam infectadas por este vírus, apenas uma pequena proporção das mesmas desenvolverão câncer cervical. Este fator aponta para o papel de fatores ambientais e genéticos que poderiam estar interagindo, e assim modulando o desenvolvimento do câncer cervical. O gene supressor de tumor *TP53* está localizado no cromossomo 17p13.1 e consiste de 11 exons, cuja proteína está no centro da regulação do ciclo celular, influenciado a transcrição e atividades de vários fatores de replicação e transcrição, reparo do DNA e apoptose, e é conhecido como o guardião do genoma (Lombard *et al*, 2005). Tem sido observado que em cerca de 50-55% de todas as neoplasias humanas, a proteína da p53 está alterada por mutações somáticas em células tumorais (Rudin et al, 2001; Lombard *et al*, 2005).

Na população geral existe um polimorfismo comum no gene supressor tumoral *Tp53*, no éxon 4, códon 72 codificando dois alelos distintos que são funcionalmente equivalentes com respeito ao HPV; um codifica a A*rginina* no aminoácido residual 72 (*Arg* alelo), e o outro codifica a *Prolina* no aminoácido residual 72 (*Pro* alelo) (Franco *et al*, 1999b). Em 1998 Storey e colaboradores demonstraram que, ao nível molecular, a

oncoproteína E6 do HPV poderia mediar a degradação da proteína p53*Arg* mais eficientemente do que a da proteína p53*Pro,* sugerindo que indivíduos *Arg*-alelo teriam um risco maior para o desenvolvimento do câncer cervical na presença da infecção pelo HPV 16 ou 18, do que indivíduos *Pro*-alelo.

Desde então vários estudos foram realizados em vários grupos populacionais na tentativa de reproduzir estes resultados, mas os achados têm sido variados nas diversas regiões do mundo (Cenci *et al*, 2003; Cho *et al*, 2003). Alguns desses estudos conseguiram encontrar os mesmos resultados que Storey e colaboradores (1998) (Zehbe *et al*, 1999; Agorastos *et al,* 2000; Nagpal *et al*, 2002), enquanto outros não conseguiram mostrar que houvesse relação entre o polimorfismo no códon 72 do gene *Tp53* e o risco de desenvolvimento de câncer cervical (Wang *et al*, 1999; Madeleine *et al*, 2000; Tenti *et al*, 2000; Nishikawa *et al*, 2000). Pegoraro *et al* (2002) mostraram que a homozigozidade para o alelo *Arg* no códon 72 do *Tp53* pode constituir um fator de risco para a carcinogênese do câncer cervical, mas na ausência de infecção para o HPV 16/18.

A maioria desses estudos, entretanto, foram limitados pelo pequeno número de pacientes com alterações cervicais (Giannoudis *et al*, 1999; Zehbe *et al*, 1999; Madeleine *et al*, 2000; Tenti *et al*, 2000), poucos levaram em conta o *status* (presença, subtipo e carga viral) do HPV (Wang *et al*, 1999; Madeleine *et al*, 2000; Tenti *et al*, 2000) e de outros co-fatores de risco (Madeleine *et al*, 2000). Adicionalmente, nenhum destes estudos avaliou prospectivamente o efeito do polimorfismo no códon 72 do gene *Tp53* na evolução para o câncer cervical, levando em consideração a variação do *status* de infecção pelo HPV e de outros fatores de risco. Além disso, os estudos têm mostrado que a prevalência deste polimorfismo apresenta uma variabilidade étnica em sua distribuição, sendo que as diferenças mais marcantes são observadas entre as populações caucasianas e japonesas

comparadas às africanas, nas quais as razões de freqüências dos alelos *Arg/Pro* são invertidas (Beckman *et al*, 1994; Weston *et al*, 1997)

Num estudo realizado no Brasil por Makni e colaboradores (2000), o polimorfismo do códon 72 foi determinado cegamente por três laboratórios distintos para avaliar o impacto da variação inter-laboratorial na determinação do polimorfismo no códon 72 do *Tp53,* e na validade da associação entre este polimorfismo e o risco de câncer cervical HPV-induzido. Os autores enfatizaram que há um efeito da variabilidade inter-laboratorial na detecção da associação entre o polimorfismo do *Tp53* e o câncer cervical, mostrando que quando foram retiradas as discordâncias, a OR foi de 8,3 a favor da associação entre o polimorfismo do *Tp53* e o câncer cervical. Esta variabilidade poderia ser contornada através da validação inter-observador no processamento e análise dos marcadores de susceptibilidade do hospedeiro.

1.4. Tratamento

O tratamento das lesões precursoras depende, principalmente, da sua severidade e extensão, da visibilidade da junção escamocolunar e do desejo da paciente de preservar a fertilidade (Loizzi *et al*, 1992; Nagai *et al*, 2000; Soutter *et al*, 2001). Os tipos de tratamento para essas lesões podem variar desde técnicas radicais, como a histerectomia, até aos mais conservadores como a biópsia por cone (conização), ablação a lazer, curetagem ou cirurgia de alta freqüência (CAF) (Huang *et al*, 1999; Nuovo *et al*, 2000).

A eficácia do tratamento através da cirurgia de alta freqüência (CAF) está relacionada a algumas condições, como a presença ou ausência de doença residual, status das margens, condições da peça cirúrgica, condições da paciente e experiência do cirurgião (Soutter *et al*, 1997; Huang *et al*, 1999; ASCP, 2001).

A identificação da presença ou ausência de invasão, pelo patologista, é dependente da qualidade do material obtido. Considerando que o espécime obtido pela CAF pode variar em termos de número, tamanho e quantidade de artefatos térmicos presentes no tecido, torna-se necessário o cumprimento de certos pré-requisitos visando evitar dificuldades na interpretação. Caso estejam presentes em grande quantidade, esses artefatos térmicos podem impossibilitar a avaliação das margens (Konno *et al*, 1999; Livasy *et al*, 1999).

Vários investigadores vêm tentando identificar os fatores relacionados à evolução do epitélio escamoso cervical, após o tratamento da lesão pré–neoplásica, mas ainda não existe consenso em relação àqueles que poderiam ser considerados preditores de recorrência da doença (Huang *et al*, 1999; Livasy *et al*, 1999; Costa *et al*, 2002; Acladious *et al*, 2002; Cruickshank *et al*, 2002).

Tem sido relatado que a recorrência de displasia ocorre em 5–35% após o tratamento conservador como conização e ablação, o que pode ser devido às falhas no tratamento primário ou ao desenvolvimento de nova lesão precursora (Soutter *et al*, 1997; Bollen *et al*, 1999). Em outras palavras, a maioria das recorrências ocorre provavelmente devido à persistência de doença ou à infecção sub-clínica de HPV que não foi completamente erradicada (Bollen *et al*, 1999; Cruickshank *et al*, 2002).

Muito embora o status das margens dos espécimes possa ser determinado, ainda não está muito bem definido se este seria preditor confiável da evolução pós-CAF (Hanau *et al*, 1997). Sabe-se, atualmente, que a melhor forma de seguimento de pacientes após CAF é através da citologia, associada ou não à colposcopia, como indicador prognóstico da probabilidade de recorrência (Hanau *et al*, 1997; Livasy *et al*, 1999; Acladious *et al*, 2002)

Existem várias razões para a presença de doença residual após uma aparente CAF completa (margens negativas para neoplasia), que incluem dificuldades da retirada total do

estroma cervical, lesões multifocais na zona de transformação e a possibilidade de desenvolvimento de novas lesões durante o período de seguimento (Hanau *et al*, 1997), além da ação dos co-fatores envolvidos na carcinogênese cervical (Kjellberg *et al*, 2000).

2 – Justificativa :

Apesar dos esforços para controlar a incidência e mortalidade por câncer de colo de útero, esta neoplasia é um problema de saúde pública no Brasil e no mundo. Além disso, tem sido internacionalmente observada uma mudança no padrão de incidência do câncer cervical. Este vem aumentando, sobretudo em mulheres jovens, sugerindo uma distribuição heterogênea dos fatores de risco nos diferentes grupos de mulheres que atingiam a maturidade sexual em períodos de transformações comportamentais relacionadas ao sexo feminino, influenciando seus hábitos de vida e práticas sexuais.

A detecção precoce das lesões precursoras e seu tratamento adequado constituem as ferramentas principais para o controle da incidência e mortalidade por esta neoplasia. No entanto, há um percentual considerável de mulheres cuja evolução caracteriza-se pelo insucesso no tratamento dessas lesões ou apresentam um padrão diferenciado, devido a fatores ainda não muitos bem estabelecidos. Embora os fatores etiológicos clássicos associados ao desenvolvimento do câncer cervical (infecção pelo HPV, tabagismo, numero de parceiros sexuais, início de atividade sexual em idade precoce, paridade e uso de contraceptivos orais), possam explicar em parte o motivo das falhas ao tratamento, ainda se desconhece se estes estariam homogeneamente distribuídos ao longo do tempo nas diferentes coortes de nascimento. Além disso, fatores genéticos do hospedeiro relacionados à suscetibilidade para o desenvolvimento do câncer cervical, parecem também ter uma influência no padrão de desenvolvimento desta neoplasia.

São poucos os estudos no Brasil que avaliam o efeito das variáveis clínicas, e epidemiológicas e genéticas na evolução do câncer cervical, segundo a coorte de nascimento. Portanto, a realização de estudos que avaliem o efeito dos fatores clínicos, epidemiológicos e genéticos na carcinogênese cervical, além seus efeitos após o tratamento

das lesões precursoras, são importantes e necessários, pois seus resultados podem contribuir para a avaliação prognóstica das mulheres com lesões pré-neoplasicas. Desta maneira, permitiriam a identificação de subgrupos mais suscetíveis para o desenvolvimento das lesões e para o insucesso do tratamento conservador, possibilitando o direcionamento da definição de normas da vigilância citopatológica e acompanhamento destes sub-grupos de mulheres com riscos mais elevados de virem a apresentar insucesso no tratamento.

3 – Objetivos :

3.1. Geral:

Determinar a contribuição de fatores clínicos, epidemiológicos e genéticos na evolução das lesões precursoras do câncer cervical bem como na falha de tratamento das lesões pré-neoplasicas.

3.2. Específicos:

1. Determinar a distribuição dos fatores de risco selecionados para câncer de colo uterino (menarca, idade ao primeiro intercurso sexual, paridade, número de parceiros sexuais ao longo da vida, história de DSTs, uso de contraceptivos orais e tempo de uso de contraceptivos orais, tabagismo, tempo de tabagismo e resultado da colposcopia) nas coortes de nascimento de 1937-55, 1956-75, e 1976-1988.

2. Determinar o efeito do polimorfismo no códon 72 do exon 4 do gene *TP53* e sua interação com fatores de risco ambientais selecionados no desenvolvimento do câncer cervical.

3. Determinar a contribuição dos diferentes fatores genéticos, clínicos e epidemiológicos no prognóstico pós-tratamento das lesões precursoras do câncer de colo de útero, considerando diferentes definições de falha no tratamento.

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5. Artigo – 1

Epidemiological and clinical characteristics of women with pre-cancer and cervical cancer in Rio de Janeiro, Brazil.

Abstract

Objective: to determine the prevalence of epidemiological and clinical aspects associated to the development of cervical cancer in three different birth cohorts of Brazilian women, from the state of Rio de Janeiro. *Methods:* From October 2004 to May 2006, a cross sectional study was carried out among 318 Brazilian women with histological diagnosis of negative, pre-cancer and cancer lesion, including 136 women in the 1976-88 birth cohort, 138 in the 1956-1975 cohort, and 44 in the 1937-1955 cohort. Antecedents of exposures to possible risk factors and clinical characteristics were ascertained through an interview-administered questionnaire, and colposcopy test and lesion excisions were carried out, when indicated. Crude and adjusted prevalence ratio (PR) and the respective 95% confidence intervals of different risk factors were ascertained using Poisson regression analysis in three different birth cohorts: 1937-1955; 1956-1975; 1976-1988. *Results:* When comparing the negative results group vs. HSIL/cancer group, number of pregnancy (adjusted PR: 1.16, 95% CI: 1.01-1.34), early menarche (12 years or younger (adjusted PR: 1.95, 95% CI: 1.17-3.25)), and parity higher than the unity (crude PR: 1.45, 95% CI: 1.09-1.91), were statistically related to cervical cancer in the 1976-1988 cohort. Menopause age (adjusted PR: 1.21, 95% CI: 1.04-1.21) and current tobacco smoking (adjusted PR: 1.37, 95% CI: 1.10-1.70) were independent risk factors for cervical cancer in the cohort 1956-1975; and menopause age (adjusted PR: 1.13, 95% CI: 0.98-1.18), abortions (adjusted PR: 1.44, 95% CI: 0.58-3.59), sexual partners (adjusted PR: 1.41, 95% CI: 0.57-3.49) and tobacco smoking (adjusted PR: 1.19, 95% CI: 0.55-2.60) were associated to cervical cancer in the 1937-1955 birth cohort (statistically not significant results). *Conclusion:* The younger studied cohorts (1976-1988 and 1956-1975) are at higher risk of HSIL and cancer development when compared to the oldest cohort (1937-1955), even controlling for age at menarche, precocity of sexual intercourse, number of sexual partners, oral contraceptive use, tobacco smoking and parity.

Key words: Epidemiology, pre-cancer lesion, cancer, cross-sectional study.

Introduction

Cervical cancer is the second major cause of cancer deaths among women worldwide, and is still the main cause of cancer deaths in some developing countries [1, 2, 3]. Studies of historical trends show that cervical cancer incidence in Brazil has changed since 1980, but still is a public health problem in this country [4, 5]. In Brazil, cervical cancer incidence rates have ranged between 34.71/100,000 women in Belém to 41.40/100,000 women in Goiânia and 22.60/100,000 women in São Paulo [6]**.** In the state of Rio de Janeiro, the expected incidence rate for 2005 was 28.7/100,000 women and the mortality rate between 1995 and 1999, adjusted to the world population, was 5.27/100,000 [7]. The specific mortality rate from cervical cancer in this state varies from 0.03 in women 15-19 years old to 17.60 in 60-69 years old, with an increasing rate trend (0.84/100,000 at age 20-29 yrs; 3.97/100.000 at age 30-39 yrs, 9.44/100.000 at age 40-49 yrs and 14.21/100.000at age 50-59 yrs). [7]

Age-specific trends in cervical cancer mortality have been studied in developed countries. According to a trend study developed in Canada [8], a marked mortality decline from cervical cancer in younger women has occurred between 1953 and 1972, whereas larger reductions in mortality occurred in older age groups from 1973 to 1997. On the other hand, the author observed an increase in the incidence of adenocarcinoma and adenosquamous carcinoma among young women since the early 1970's, suggesting a birth cohort phenomenon.

Although data evidence of a change in the screened population over time from younger to older women, provides a partial explanation for these age-specific trends in cervical cancer mortality [9], the observed increase in adenocarcinoma incidence may be related to the onset of sexual intercourse at an earlier age, changes in sexual habits, and increased transmission of

HPV among younger women [10,11,12]. A well documented report on premarital sexual activity among teenage women in the United States concluded that young teenagers continued to engage in intercourse at earlier ages and in increasing partner number since the sexual revolution of the late 1960s [13].

The known risk factors for cervical cancer include HPV infection, age, early sexual exposure, multiple sexual partners, and sexual contact with high-risk males [14]. However, even people sharing the same environment, varied susceptibility to a specific disease due to physiological, environmental, and genetic characteristics - the latter known as one's genetic susceptibility to a disease -, is usually observed. The relationship between certain gene polymorphisms and cervical carcinogenesis has been studied by many researchers. However, the results from these studies produced no clear conclusions because many tended to over-focus the bio-molecular bases without taking the involvement of environmental factors into consideration [15]. Besides sexual behavior, cigarette smoking has also been reported as an environmental risk factor for cervical cancer, which interacts with HPV as a carcinogenic cofactor [16, 17].

An epidemiological study based on the severity of lesions, carried out in Brasilia, Brazil, reported that among Brazilian women with high-grade lesions or cervical cancer, 66% showed HPV positive results [18]. The study also showed that 50% of women with low-grade lesion showed HPV positive results, with 62% being the overall prevalence. This study showed a high prevalence of HPV 16 (49.2%), followed by HPV-58 (13.4%) and HPV-31 (11.2%) among all levels of lesions. HPV-16 and -18 were responsible for 53.7% of HPV positive results among Brazilian women from central Brazil [18]. Moreover, according to the Brazilian National Cancer Institute [19], the prevalence of tobacco smoking among women under 18 years old varies from 12% in São Luiz to 35% in

Goiânia. In Rio de Janeiro (from 2002 to 2003), current tobacco smoking prevalence among women under 18 years attending school was 19.3%, and 42.6% of the interviewed women reported they had already smoked at least once [19].

This information shows that behavioral and environmental risk factors and clinical characteristics in different birth cohorts of Brazilian women need to be studied in order to propose new strategies to prevent and control cervical cancer according to the age groups under higher risk of cancer development. Thus, the purpose of this study is to determine the prevalence of epidemiological and clinical aspects related to cervical cancer development in three different birth cohorts of Brazilian women from the state of Rio de Janeiro, seeking possible birth cohort specific cofactors associated with the progression to malignancy, elucidating their role in cervical cancer pathogenesis.

Material and Methods

Study Population

The patients were selected from a group for a histopathological result following an altered cytology result (Pap smear). They were referred to the Hospital of Cancer – II at the National Cancer Institute (Rio de Janeiro, Brazil), a public center offering free health care, and recruited for the study, from October 2004 to May 2006.

Study Design

The magnitude of association between HSIL/CC and selected risk factors at the diagnosis was firstly ascertained using a cross-sectional approach. Further, such association of the clinical evolution of the observed lesions over a period of time was analyzed using Poisson regression.

Inclusion criteria: Patients were eligible for this study if they were older than 18 years old, had been not submitted to any cervical treatment in the past 6 months, were free of any psychiatric disease, were either literate or illiterate and bringing a literate relative to witness the informed consent explanation, and if they accepted to sign the informed consent.

Exclusion criteria: Patients were excluded from this study if they refused to sign the informed consent, were HIV positive, and were left untreated because they had no lesion at the time of the colposcopy test, and presented no changes at the Pap smear test taken during the colposcopy exam.

Included patients were then interviewed to ascertain antecedents of exposures to possible risk factors and clinical characteristics, and then they were submitted to colposcopy. Biopsy, partial ablation with diathermy and electrocauterization were used for LSILs, and conization was the treatment of choice for HSILs.

Clinical Tests

After providing informed consent and answering the questionnaire, the patient underwent washing of the cervix and lower genital tract with 4-6% acetic acid solution, and visual inspection under colposcopy. The colposcopy impression, lesion size and suspected grade were reported. Biopsies were made of suspicious lesions; and a Pap smear test was carried out if the colposcopic evaluation was unsatisfactory. When the result of the Pap exam at the time of colposcopy was altered, endocervical curettage (ECC) was performed.

The histological diagnosis was made through slides stained with hematoxylin and eosin (HE) for grading of the cervical lesion according to the Bethesda System [20] in

LSIL, including condylomata, ASCUS/AGUS and cervical intraepithelial neoplasia grade I (CIN I); in HSIL, including CIN II and CIN III/ *in situ* carcinoma (SCC). Histological normal tissues presenting only reactive/reparative changes were classified as negative (disease-free). Biopsy specimens were colposcopically directed, obtained with a punch biopsy device, placed in 10% formalin, and embedded and stained in a routine manner.

Two oncology gynecology surgeons performed the colposcopic exams and Pap smear tests. Histological and cytological specimens were evaluated at the Department of Pathology, Brazilian National Cancer Institute.

Data collection protocols

Age, ethnicity (skin color), education level, age at menarche, age at first sexual intercourse, parity, number of abortions, menopausal status, number of sexual partners, history of sexually transmitted diseases (STDs), contraceptive method use, co-morbidity diseases history (diabetes mellitus, hypertension and obesity), smoking status (current, past and passive smoking) and previous Pap smear tests were obtained from an interview-administered questionnaire. Peripheral blood samples were taken for HIV, Hepatitis B and C infection tests and genetic Single Nucleotide Polymorphism (SNPs) analysis. All patients were interviewed by two trained nurses applying standardized procedures.

Tobacco consumption was classified as never smoked, no passive smoking; passive smoking only; former smoker, and current smoker. Smoking habit variables were defined as the cumulative exposure of daily cigarette packs smoked, expressed as pack years. For this analysis we considered 1g of tobacco for 1 cigarette, 4 cigarettes for each cigar, and 3 cigarettes for each pipe [21].

Variables Description

In Brazil, race is classified by skin color, and according to the Brazilian Institute of Statistic and Geography, skin color must be self reported to avoid race discrimination [22]. Since Brazil is a multiethnic country, we considered ethnic classification as a self reported skin color. Smoking *status* was only assigned for those women who had smoked 100 cigarettes or more during their whole life, and former smokers were classified only as those who quit smoking at least 6 months before the interview date [21]. The Body Mass Index (BMI) strata used were taken from World Health Organization reference [23]. A Poisson regression analysis was used in which HSIL and cancer were define as outcomes of interest and compared to the occurrence of negative and LSIL histological results.

Cohort Classification

Three birth cohorts were analyzed in this study: women had born between the years 1976-1988; women born between the years 1956-75; and women born between the years 1937-1955. Therefore, considering 1970 as the year of the main turning point of the sexual behavior revolution in Brazil, the studied population comprised of women who were currently 18-30 yrs old, 31-49 yrs old, and 50-70 yrs old when the interviews were carried out.

Sample Size

The sampling procedures were carried out on 9,409 women population from Rio de Janeiro State who presented an altered Pap smear exam and were covered by the Brazilian National Public Health System in 2002. In this year, 5,314 (56.5%) women presented CIN

I, 2,014 (21.4%) presented CIN II, 1,423 (15.12%) presented CIN III, 264 (2.8%) presented squamous cancer, 31 (0.33%) adenocarcinoma *in situ,* and 77 (0.82%) presented invasive adenocarcinoma in the State of Rio de Janeiro (Conprev/INCA, 2004 non-published data). Considering such prevalence as the expected values and considering a difference of 5-15% of each lesion degree prevalence as the worse expected value, samples of 42 women with CIN 1, 61 women with CIN 2, 49 with CIN 3, and 113 with cervical cancer were required to obtain statistically significant estimates in the studied population.

Statistical Analysis

Prevalence ratios (PR) were obtained to evaluate the magnitude of association between HSIL/CC development and risk factors, using a univariate analysis (CI: 95%). Later, multivariate analysis was accomplished to obtain the prevalence ratio adjusted by those variables that were shown to be statistically significant on univariate analysis, using Poisson regression analysis (CI: 95%). In order to determine the association between each variable, and disease susceptibility, the dichotomous outcome of the subject's status (negative/LSIL vs. HSIL/Cancer) was regressed against each explanatory variable, adjusted by age at first sexual intercourse, pack-year smoked (when indicated), number of sexual partners and contraceptive use. In order to build the final predictive model the inclusion criteria used were the level of significance under 20% ($p \leq 0.20$) and the biological plausibility of the cervical cancer development.

Variables expressed as means were evaluated using independent Student's t-test to assess the mean differences between the lesion groups. The distribution of variables between the groups was evaluated using *Chi-square* test and Mantel-Haentszel procedures

for trend analysis. Probability values less than 0.05 were considered significant. All analyses were done using STATA program (5.0 version, Stata Press, College Station, TX). This project was approved by the ethical committee of the Brazilian National Cancer Institute.

Results

From October 2004 to May 2006, 463 women met the inclusion criteria and 18 patients (3,9%) refused to participate. From 445 patients who agreed to sign the informed consent, 318 patients (71.5%) were submitted to the lesion excision or biopsy (Table 1), and 127 (28.5%) were left untreated because they had no lesion at the colposcopy test and presented no changes at the Pap smear test taken at the time of colposcopy exam. The demographic and clinical characteristics of enrolled women according to the studied birth cohort are presented at table 2.

Comparing the 1976-1988 cohort (women currently 18-30 years old) to the older cohorts (1956-1975 and 1937-1955) we identified that there are more women who were born in Rio de Janeiro state and had completed elementary/middle school among the younger cohort. On the other hand, there are more women who are migrants from North/Northeastern Regions of Brazil, who work as domestic maids and who had not even completed elementary/middle school in the older cohorts. Comparing the younger cohort (18-30 years old) to the intermediate age cohort (31-49 years old), either histological results of cervical cancer or negative results were more prevalent among older women ($p<0.05$). On the other hand, the histological result of LSIL is significantly more prevalent among younger women.

Comparing the younger cohorts (18-30 and 31-49) to the oldest (50-68 years old), ASCUS/AGUS and cancer cytological results were statistically significantly more prevalent among the oldest cohort, while HSIL is more prevalent among the younger cohorts. Hypertension was significantly more prevalent among the oldest cohort as compared to the intermediate age cohort (31-49 years old), and showing a linear trend among the cohorts prevalence (table 2).

The distribution of frequencies of epidemiological and clinical characteristics according to the lesion degree in each birth cohort is presented in table 3. Parity–and contraceptive use for more than 60 months were statistically more prevalent in the HSIL/cancer group in the youngest cohort. In the 1956 – 1975 cohort (women currently 31-49 years old), menopause age, pack-year smoked, first sexual intercourse before 16 years old, 3 or more lifelong sexual partners, and current smoking habits were statistically related to the highest degree of lesion. However, in the oldest cohort, only menopause age, current oral contraceptive use and body mass index were significantly related to the level of lesion.

Crude and adjusted prevalence ratio for High SIL and cancer development, according to birth cohort are shown in table 4. In the youngest cohort, parity and early menarche presented an adjusted prevalence ratio statistically significant (PR adjusted: 1.16 and 1.95, respectively), and the number of delivery events showed a significant crude prevalence ratio (PR crude: 1.45). In the 1956 – 1975 cohort, menopause age showed a significant adjusted risk of 1.21 for HSIL/cancer development, while current tobacco smoking was shown to be a risk factor for HSIL/cancer development in this group (PR crude and adjusted: 1.59 and 1.37, respectively). Despite the fact that menopause age (adjusted PR = 1.13, 95% CI: 0.98-1.32), number of abortions (PR=1.44, 95% CI: 0.58-3.59), life number of sexual partners (adjusted PR = 1.41, 95% CI: 0.57-3.49), current oral contraceptive use

(adjusted PR=1.15, 95% CI: 0.44-2.99), former and current smoking habits (adjusted PR= 1.20, 95% CI: 0.54-2.65) have shown to be risk factors for HSIL/cancer development in the oldest cohort (1937-1955), none of them were statistically significant.

To be either born in the 1956 – 1975 or in the 1976 – 1988 cohorts showed an excess risk for developing a HSIL/cancer, as compared to the oldest cohort (1937-1955), even controlling by menarche, age at sexual onset, number of sexual partners, oral contraceptive use, tobacco smoking habits and number of deliveries. It was also observed that women, who had 3 to 4 sexual partners during their life, had a statistically significant increased risk for developing HSIL/cancer (RP: 1.342, 95% CI: 1.05-1.71). Current tobacco smoking was also an independent risk factor for HSIL/cancer development (PR=1.373,*p=0.007*).

Discussion

The development of cervical carcinoma correlates closely with the presence of certain human Papillomavirus types, such as HPV-16 and HPV-18. The HPV infection was reported as being a high risk factor to cervical cancer (prevalence higher than 95%), its contribution to the risk of disease development being greater than any other recognized determinant [24]. However, a large number of sexually active women are infected with HPV, but only a fraction of them will develop cervical cancer after a long latency period. This fact suggests behavioral, environmental and genetic cofactors, interacting into the carcinogenic transformation of HPV-infected cervical epithelium [18, 25, 26]. In this sense, the several characteristics of the sexual behavior identified as risk factors for cervical cancer represent different surrogate markers of HPV infection, which is the single most important etiologic factor of cervical cancer. The risk factors predisposing women to HPV

infections have been classified into five categories: (1) sexual behavior, (2) smoking habits, (3) reproductive history, (4) nutritional factors, and (5) immunosuppression [27, 28]. The observed increase in the incidence of adenocarcinoma and adenosquamous carcinoma since the early 1970s has been attributed to the sexual revolution in the late 1960s [10, 17, 29]. Nevertheless, current knowledge regarding the specific risk factors related to cervical cancer development among women from different birth cohorts is still unclear.

We believe that our data can be considered as a representative sample of women with cervical diseases in Rio de Janeiro, taking into account that the Brazilian National Cancer Institute is a local and national benchmark service to provide health care to such conditions. In this sense, it was interesting to observe the smaller inclusion of affected women from the 1937–1955 cohort (13.8%) as compared to the 1956-75 and 1976-88 birth cohorts (43.4% and 42.8%, respectively). Since screening for cervical cancer in Brazil has been mainly strengthened as a systematical program after 1998, this phenomenon could be either explained by the low Pap test coverage of this age group (especially if we consider that older women in Brazil still show embarrassment when they undergo the Pap exam), or as a consequence of their poorer survival after diagnosis by the screening program. The third hypothesis is that, according to the natural history of cervical cancer, precursor lesions are actually less incident in this age group.

The results from this study reveal that some risk factors associated to the development of cervical cancer may vary according to the birth cohort as observed in Rio de Janeiro, and this can result from behavioral, cultural, social and educational differences among these groups. The finding that 40.9% of women from the oldest cohort (compared to 14.7% among the youngest cohort) are migrants from North/Northeastern Region of Brazil and have a lower educational level could indicate to us a clue to those differences. There

are many cultural and educational differences between the regions of Brazil. Women from some areas, like the North and Northeastern Regions, usually have a lower educational level and a more conservative behavior as compared to women from the southeastern side of the country. Thus, although 1938-1955 cohort women were 15-32 years old in the 1970s, the sexual revolution does not seem to have had much influence on their behavior. On the other hand, in the 1956-1975 and 1976-1988 cohorts, 63% and 76.5% (respectively) of the studied women were born in Rio de Janeiro state and probably have inherited all the cultural and behavior changes that occurred among women after oral contraceptives diffusion observed in the 1960's and 1970's in Western industrialized countries. Such influence might be reflected in histological results, where 38.6% were negative for any kind of lesion among the oldest cohort, while only 14.7% and 26.8% of women had such a result among the 1976-1988 and 1956-1975 cohort, respectively. As expected, there were significantly more LSIL and less cancer results (LSIL: 25.7% and 03.7%) among the youngest cohort, when compared to the 1956-1975 and 1937-1955 cohorts (LSIL1956-75: 13.8%; cancer 1956-75: 10.9% and LSIL1937-55: 06.8%; cancer 1937-55: 13.6%). Considering the latency needed to develop a cervical HSIL, a higher prevalence of these lesions would be expected among older women. Unexpectedly, however, a higher HSIL prevalence (55.9%) was only seen in the youngest cohort as compared to the older (HSIL1956-75: 48.6%; HSIL1937-55: 40.9%), with a significant linear trend between them.

We have shown that epidemiological and clinical characteristics have a significant different distribution among the studied cohorts, when comparing the degree of lesion among them (table 3). Mean menopause ages were higher in the HSIL/cancer group in the older cohorts, but behavioral risk factors such as early sexual onset,

number of sexual partners and smoking were significantly more prevalent in the HSIL/cancer group in the 1956-1975 cohort as compared to the oldest and youngest cohorts. This might possibly be explained by the fact that this group acquired their life style habits and sexual and reproductive behavior immediately following the strong change of patterns related to the female role in industrialized societies. Thus, when compared to the oldest women, they had an earlier sexual onset, had an increased number of sexual partners, and started smoking earlier and smoked for longer periods of time (the latest, when compared to the youngest women too). Those changes in the behavior patterns must be true for the youngest age group too, but because the health effects of smoking only become fully evident 20-40 years after smoking onset, the effect of smoking habits among the youngest cohort could not be fully appraised in this study. The number of sexual partners was not statistically different between normal/LSIL vs. HSIL/cancer groups in the youngest cohort, probably because it has been increasing in this whole cohort. The effect of this risk factor will probably be further depicted when this cohort reaches the next decades. Despite the fact that the Brazilian government has invested in tobacco control programs for school children, health centers and work environments, it could partially explain the tobacco smoking habit pattern in the youngest cohort, since those programs only became fully implemented in late 1999 to 2000. Respecting the latency period, a longer period of time would be needed to detect any change in the behavioral habit related to those programs, and also a longer period of time to find any difference in the incidence of HSIL and cervical cancer.

Known risk factors such as smoking, parity, menarche and the number of sexual partners were not statistically significant in the oldest age group. On the other hand, current

oral contraceptive use was significantly higher in the HSIL/cancer group in this cohort, suggesting that older women who are sexually active might be at higher risk of cancer development. Cruickshank *et al* [30] developed a case-control study in the United Kingdom, seeking to identify those risk factors associated with the development of an abnormal Pap smear over the age of 50, in particular, the HPV status. The authors found that HPV positivity was significantly associated with the change of sexual partner after age 40, which probably results from new infection, but could be a surrogate marker for previous sexual behavior.

According to our findings, women currently 18-30 years old showed an increment for cervical cancer development of 16% for each pregnancy and a 26% higher risk among women who had had more than one child. This observation could be possibly explained by the fact that women who had had an early age at menarche (adjusted PR= 1.95) would have experienced higher opportunities to have a higher parity comparativelly to those women who had older age at menarche. These results corroborate the findings of Hofferth *et al* [13], who reported an increasing number of sexual partners and an earlier age at sexual onset among teenage women in US. If the above female sexual behavior scenario had also occurred in Brazil, younger birth cohorts would be likely to continue to be at higher risk for developing cervical cancer.

Oral contraceptive use and the length of oral contraceptive use were important risk factors among the youngest cohort, but not among the older, showing 1.11-fold risk and 1.08-fold risk to former and current users, respectively. A 1.22-fold risk was also observed for women who were users over 60 months compared to women who used oral contraceptive for less than one year. This suggests that oral contraceptive use can be a low magnitude risk factor for cervical cancer development and not only a sexual behavior

marker. Parazzini *et al* [31] reported that estrogen – progesterone stimulation favors or accelerate cervical carcinogenesis, possibly through selective carcinogenic transformation, glucocorticoid-dependent, made by HPV (especially HPV16). On the other hand, Kanai et al [32] reported that cervical squamous cells express estrogen-receptors (ER) and progesterone-receptors (PR) that regulate their growth and differentiation through sexual steroids hormones. The authors suggest that cervical malignant transformations are related to abnormal ER and PR expression and to cyclins, kinase cyclin-dependent and p53 over-expressions (that are cellular cycle related factors), suggesting that cellular tumors escape from normal growth control, through sexual hormones, and take potential active growth by aberrant expression of those cellular cycle related factors.

In the 1956-1975 cohort, menopause age and current tobacco smoking habit showed to be independent risk factors for cancer development. There was a significant increment of 21% for each year of menopause age and a risk 1.37-fold higher for current smokers as compared to those who never smoked. Though it was not statistically significant, the present study showed 1.43- and 1.13-fold increased risk among women who smoked > 15 years in the 1976-1988 and 1956-1975 cohorts, compared to women who smoked for less than 15 years. On the other hand, the risks among former smokers were 1.44-fold (1976-1988 cohort), 1.20-fold (1956-1975 cohort) and 1.20-fold (1938-1955 cohort). Another finding is that the risk increases with the number of pack-year smoked in the youngest cohort (PR: 1.02 for each pack-year smoked). These observations suggest a cumulative or late-promoting effect of cigarette smoke on cervical neoplastic transformation. Our data is supported by epidemiological studies showing a relationship with cervical cancer to current or former smokers, with the highest risks generally observed on long-term basis or with high-intensity smokers [17, 33]. Biological explanations for this association is given by biochemical studies

showing that cigarette smoking derivatives such as nicotine and cotinine [16], including some procarcinogens such as N-nitrosamines [34], are present in the cervix.

The predictive model shows that belonging to any of the younger cohorts is an independent risk factor for HSIL/cancer development, compared to the oldest cohort. The number of sexual partners (ranging from 3-4) was also an independent risk factor for cancer development. Of particular interest is the role of cigarette smoking as a contributing risk factor for cervical cancer. Our findings show that women who are current smokers have a 1.37-fold risk for HSIL/cancer, as compared to those who never a smoked; and it was statistically significant even when controlled by cohort, menarche, and age at sexual onset, oral contraceptive use, number of sexual partners and number of deliveries. Former smokers have an independent risk of 1.19-fold, but it was not statistically significant.

There are two main possible mechanisms that explain an association between cigarette smoking and cervical cancer. First, absorbed carcinogenic components of cigarette smoking reach the cervix (transported in the blood stream) inducing DNA damage to promote cervical carcinogenesis [16, 34]. In addition, tobacco smoke appears to lead to suppression of the immune system and of serum immunoglobulin and pronounced depression of antibody levels [35]. Cytokines (CK) act by binding to specific CK-receptors (CKR) expressed by the CK-producing cells themselves (autocrine signaling) or by non-producer cells (paracrine signaling) which are of particular interest to oncologists. Although the action of many CK is confined to the site where they are produced, some CK bind (e.g. IL-6) on distal CKR + target cells. IL-6 (also known as B-cell stimulatory factor (BSF-2) and interferon β2 (IFN β2), an inflammatory cytokine produced by T-cells, B-cells, keratinocytes and monocytes) plays a role in the development of cervical neoplasia and contributes to the immune response, which may be affected by smoking [16].

Recently the genetic polymorphisms among the human population are actively being studied to correlate differential susceptibility to cervical carcinoma [36]. Major classes of carcinogens present in tobacco and tobacco smoke are converted into DNA reactive metabolites by cytochrome P450 (CYP)-related enzymes, several of which display their action favored by specific genetic polymorphism. Individual susceptibility to cancer is likely to be modified by the genotype for enzymes involved in the activation or detoxification of carcinogens in tobacco and repair of DNA damage. CYP1A1 is a member of cytochrome P450 family, a phase I enzyme that is important to the metabolic activation of polycyclic aromatic hydrocarbons (PAHs), carcinogens found in tobacco smoke, and it is expressed in human cervical cells [36, 37].

Other metabolic steps including conjugation of reactive metabolites by phase II enzymes could play important roles in ultimately determining whether initial bioactivation of the xenobiotic has positive or negative consequences for carcinogenesis. Several carcinogens present in tobacco smoke are inactivated by gluthathione S-transferases (GSTs). The GSTs are multifunctional phase II enzymes that detoxify activated forms of chemical carcinogens, such as polycyclic aromatic hydrocarbons (PAHs). The GSTs conjugate glutathione to various potentially carcinogenic compounds, which facilitate the excretion of a wide range of carcinogens, reactive oxygen species, and chemotherapeutic agents, with a variety of substrate specificities [38]. There is some evidence that polymorphisms in activating and detoxifying enzymes may affect the modulation of reactive species that form DNA adducts, and cause somatic mutation [39].

The present study suggests that risk factors for HSIL/cervical cancer may play different roles among the three birth cohorts of Brazilian women (1938-1955; 1956-1975-1976-1988). In this study, women who currently are 50-68 yrs old seem to experience a

lower risk for cancer development than women from 18-30 and 31-49 yrs old. Parity, early age at menarche and parity over 1 are independent risk factors for women of 18-30 years old, while menopause age and current tobacco smoking habits were statistically related to HSIL/cancer development among women from 31-49 years old. Though menopause age, current and former tobacco smoking habits, current contraceptive use, number of sexual partners and number of abortions (over 1) were independently related to HSIL/cancer development among women from 50-68 yrs old, they showed no significance in the multivariate analysis. Our data suggest that, among women from Rio de Janeiro, Brazil, cervical cancer prevention and early detection programs must have different approaches among specific cohorts to be effective.

Current smoking habits (PR: 1.37) were independently associated to the risk of developing HSIL and cancer, showing that special care must be taken about this issue. Because the health effects of smoking only become fully evident many years after the widespread uptake of smoking, the full global impact of smoking on women's health will not be seen for some decades. Many women, even in developed countries, are unaware of the extension of the risk of cervical cancer. This study can provide evidence to support tobacco control programs focused on Brazilian women who are at higher risk of developing cervical cancer all over the country.

Conclusions

This study suggests that risk factors for HSIL/cervical cancer may have played different roles along generations of Brazilian women in Rio de Janeiro. In this sense, our data suggest that cervical cancer control programs in the studied population should have

different approaches among specific cohorts to be effective and to achieve cervical cancer prevention and early detection.

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Table 1. Characteristics of Women included in the study, Rio de Janeiro, Brazil

Variables	N	%	IC (95%)
Total	**318**	**100.00**	
Age (Years)			
18-30	136	42.76	(37.3–48.2)
31-49	138	43.40	(37.9–48.8)
50-68	44	13.84	(10.0–17.6)
Birth Region			
- State of Rio de Janeiro	207	65.09	(59.9–70.3)
- Others southeast/South states	36	11.32	(7.8 - 14.8)
- North/Northeast side	75	23.58	(18.9 – 28.3)
Occupation			
- House wife	98	30.82	(25.7 – 35.9)
- House keeper	71	22.33	(-)
- Unskilled worker	15	4.72	(2.4 – 7.0)
- Student	15	4.72	(2.4 – 7.0)
- Others	119	37.42	(32.1 – 42.7)
Ethnicity			
- White	95	29.87	(24.8–34.9)
- Black	64	20.13	(15.7–24.5)
- Multiethnic	159	50.00	(44.5–55.5)
Education Level			
- < Complete Elementary/Middle	167	52.52	(47.0–58.0)
- Complete Elementary/Middle school	85	26.73	(21.9–31.6)
- Complete High School	53	16.67	(12.6–20.8)
- Complete/incomplete College Degree	13	4.09	(1.9–6.3)
Marital Status			
- with no partner	104	32.70	(27.5–37.9)
- with a partner	224	70.44	(65.4–75.5)
Reference Cytology			
- CIN 1	16	5.03	(2.6–7.4)
- CIN 2	132	41.51	(36.1–46.9)
- CIN 3	112	35.22	(30.0–40.5)
- ASCUS /AGUS	50	15.72	(11.7–19.7)
- Invasive cancer/Adenocarcinoma	8	2.52	(0.8–4.2)
HIV			
- Yes	6	1.89	(0.4 – 3.4)

- No	312	98.11	(96.6 – 99.6)
HCV			
- Yes	10	3.14	(1.2 – 5.1)
- No	304	95.60	(93.3 – 97.9)
HBS- Ag			
- Yes	23	7.33	(4.4 – 10.1)
- No	291	91.51	(88.4 – 94.6)
Diabetes Mellitus			
- Yes	12	3.77	(1.7 – 5.9)
- No	306	96.23	(91.4 – 98.3)
Hypertension			
- Yes	47	14.78	(10.9 – 18.7)
- No	271	85.22	(81.3 – 89.1)

Table 2. Deographic and Clinical Characteristics of Enrolled Women, according to birth cohort, Rio de Janeiro, Brazil

Variables	1976 – 1988 (18-30 years old) N = 136		1956 – 1975 (31-49 years old) N = 138		1937 – 1955 (50-68 years old) N = 44		
	N (%)	**95% CI**	**N (%)**	**95% CI**	**N (%)**	**95% CI**	*p-trend*
Birth region							
- State of Rio de Janeiro	104 (76.5)*φ	(69.3 – 83.6)	87 (63.0)*π	(55.0 – 71.1)	16(36.4)φ π	(2.21 – 50.6)	***P = 0.0000***
- Southeast/South states	12 (8.8) φ	(4.1 – 13.6)	13 (9.4)	(5.1 – 15.2)	10 (22.7) φ	(10.3 – 35.1)	***P = 0.0360***
- North/Northeast side	20 (14.7) *φ	(8.8 – 20.7)	37 (26.8) π	(19.4 – 34.2)	18 (40.9) φ	(26.4 – 55.4)	***P = 0.0002***
Occupation							
- House wife	48 (35.3)	(27.3 – 43.3)	38 (27.5)	(20.1 – 35.0)	12 (27.3)	(14.1 – 40.4)	*P = 0.2062*
- House keeper	19 (14.0) *φ	(8.1 – 19.8)	39 (28.3) *	(20.7 – 35.8)	13 (29.5) φ	(16.1 – 43.0)	*P = 0.0048*
- Unskilled worker	06 (4.4)	(1.0 – 7.9)	08 (5.8)	(1.9 – 9.7)	01 (2.3)	-	*P = 0.8014*
- Student	14 (10.3)	(5.2 – 15.4)	0 (0.0)	-	01 (2.3)	-	*P = 0.0010*
- Others	49 (36.0)	(28.0 – 44.1)	53 (38.4)	(30.3 – 46.5)	17 (38.6)	(24.2 – 53.0)	*P = 0.6854*
Ethnicity							
- White	46 (33.8)	(28.0 – 44.1)	33 (23.91)	(16.8 – 31.0)	16 (36.4)	(22.1 – 50.6)	*P= 0.6572*
- Black	30 (22.1)	(15.1 – 29.0)	26 (18.84)	(12.3 – 25.4)	08 (18.2)	(6.8 – 29.6)	*P = 0.4829*
- Multiethnic	60 (44.1)φ	(35.8 – 52.5)	79 (57.25) *	(49.0 – 65.5)	20 (45.4)	(30.7 – 60.2)	*P = 0.3329*
Education Level							
- < Complete Elementary/Middle	53 (39.0) *φ	(30.8 – 47.2)	79 (57.1) *π	(49.0 – 65.5)	35(79.5)φ π	(67.6 – 91.5)	*P = 0.0000*
- Complete Elementary/Middle school	50 (36.8) φ	(28.7 – 44.9)	31 (22.4) *	(15.5 – 29.4)	04 (9.1)	-	*P = 0.0001*
- Complete High School	27 (19.8)	(13.1 – 26.6)	22 (15.9)	(9.8 – 22.0)	04 (9.1)	-	*P = 0.0967*
- Complete/incomplete College Degree	06 (4.4)	(1.0 – 7.9)	06 (4.3)	(0.9 – 7.8)	01 (2.3)	-	*P = 0.6134*
Marital Status							
- with no partner	48 (35.3)	(27.3 – 43.3)	44 (31.9)	(24.1 – 39.7)	12 (27.3)	(14.1 – 40.4)	
- with a partner	88 (64.7)	(56.7 – 72.7)	94 (68.1)	(60.3 – 75.9)	32 (72.7)	(62.2 – 87.8)	*P = 0.3089*
Reference Cytology							
- LSIL	12 (8.8)	(4.1 – 13.6)	4 (2.9)	-	0 (0.0)	-	*P = 0.0065*
- HSIL	107 (78.7) φ	(71.8 – 85.6)	106 (76.8)π	(69.8 – 83.9)	08(18.2)φ π	(6.8 – 29.6)	*P = 0.0000*
- Ascus/Agus	17 (12.5) φ	(6.9 – 18.1)	23 (16.7)π	(10.4 – 22.9)	33(75.0)φ π	(62.2 – 87.8)	*P = 0.0000*
- Câncer	0 (0.0)	-	03 (2.2)	-	03 (6.8)	-	*P = 0.0120*
Histological results							
- Negative	20 (14.7) *φ	(8.8 – 20.7)	37 (26.8) *	(19.4 – 34.2)	17(38.6) φ	(24.2 – 53.0)	*P = 0.0004*
- LSIL	35 (25.7) *	(18.4 – 33.1)	19 (13.8) *	(8.0 – 19.5)	03 (6.8)	-	*P = 0.0011*
- HSIL	76 (55.9)	(46.8 – 63.5)	67 (48.5)	(40.2 – 56.9)	18 (40.9)	(26.4 – 55.4)	*P = 0.0024*
- Cancer	05 (03.7) *	(1.0 – 7.9)	15 (10.9) *	(5.7 – 16.1)	06 (13.6)	(3.5 – 23.8)	*P = 0.0120*
Diabetes Mellitus							
- Yes	0 (0.0)	-	05 (3.6)		07 (15.9)	(5.1 – 26.7)	*P = 0.0000*
- No	136 (100)		133 (96.4)		37 (84.1)	(73.3 – 94.9)	
Hypertension							
Yes	3 (2.2)	-	23 (16.7)π	(10.4 – 22.9)	21 (47.7) π	(33.0 – 62.5)	*P = 0.0000*
No	133 (97.8)		115 (83.3)π	(77.1 – 89.6)	23 (52.3) π	(37.5 – 67.0)	

* *p ≤ 0.05 (Between 1976 – 1988 cohort vs* 1956 – 1975 *cohort);*

φ *p ≤ 0.05 (Between 1976 – 1988 cohort vs.* 1937 – 1955 *cohort)*

π p≤ 0.05 (Between 1956 – 1975 cohort vs. 1937 – 1955 cohort

Table3. Distribution of epidemiological and clinical characteristics according to the lesion degree in each birth cohort, Rio de Janeiro

Variables	1976 – 1988 (18-30 years old) N = 136 (100%)		1956 – 1975 (31-49 years old) N = 138 (100%)		1937 – 1955 (50-68 years old) N = 44 (100%)	
	Negative/LSIL (n=55)	HSIL/Cancer (n=81)	Negative/LSIL (n=56)	HSIL/Cancer (n=82)	Negative/LSIL (n=20)	HSIL/Cancer (n=24)
Menopause (Mean ± SD)	-	-	46.7 (0.6)*	42.5 (0.7)*	46.5 (7.5)*	50.0 (2.8)*
Number Pregnancies (Mean ± SD)	1.6 (1.5)*	2.3 (1.5)*	3.2 (2.0)	3.7 (2.2)	4.1 (2.1)	4.2 (3.1)
Pack-year smoked (Mean ± SD)	3.4 (4.5)	5.9 (6.7)	12.0 (10.6)*	19.2 (15.9)*	33.0 (41.5)	22.0 (26.0)
Menarche age						
≤ 12 years	28 (50.9)	50 (61.7)	39 (69.6)	52 (63.4)	12 (60.0)	14 (58.3)
> 12 years	27 (49.1)	31 (38.3)	17 (30.4)	30 (36.6)	08 (40.0)	10 (41.7)
Age at first intercourse						
≤ 16 years	35 (63.6)	61 (75.3)	15 (26.8)*	39 (47.6)*	05 (25.0)	07(29.2)
> 16 years	20 (36.4)	20 (24.7)	41 (73.2)	43 (52.4)	15(75.0)	17 (70.8)
Number of Deliveries						
0-1	39 (70.9)	39 (48.1)	16 (28.6)	16 (19.5)	02 (10.0)	06 (25.0)
> 1	16 (29.1)*	42 (51.9)*	40 (71.4)	66 (80.5)	18 (90.0)	18(75.0)
Number of Abortions						
0-1	49 (89.1)	72 (88.9)	47 (83.9)	64 (78.0)	14 (70.0)	14 (58.3)
> 1	06 (10.9)	09 (11.1)	9 (16.1)	18 (22.0)	06 (30.0)	10 (41.7)
Number of Sexual Partners						
1 – 2	21 (38.2)	25 (30.9)	21 (37.5)	15 (18.3)	11 (55.0)	09 (37.5)
3 – 4	16 (29.1)	29 (35.8)	13 (23.2) *	33 (40.2) *	03 (15.0)	10 (41.7)
≥ 5	18 (32.7)	27 (33.3)	22 (39.3)*	34 (60.7)*	06 (30.0)	05 (20.8)
Number of STD episodes						
0	46 (83.6)	69 (85.2)	49 (87.5)	68 (82.9)	13 (65.0)	21 (87.5)
≥ 1	09 (16.4)	12 (14.8)	07 (12.5)	14 (17.1)	07 (35.0)	03 (12.5)
Oral Contraceptive status						
- Current use	38 (69.1)	57 (70.4)	29 (51.8)	42 (51.2)	6 (30.0)*	13 (54.2)*
- Former use	07(12.7)	17 (21.0)	17 (30.3)	27 (32.9)	9 (45.0)	4 (16.7)
- Never use	10 (18.2)	07 (8.6)	10 (17.9)	13 (15.9)	5 (25.0)	7 (29.2)
Contraceptive use length (months)						
0	10 (18.2)	07 (8.6)	11 (19.6)	14 (17.1)	05 (25.0)	07 (29.2)
1 – 60	28 (50.9)	38 (47.0)	16 (28.6)	31 (37.8)	06 (30.0)	08 (33.3)
≥ 60	17 (30.9)*	36 (44.4)*	29 (51.8)	37 (45.1)	09 (45.0)	09 (37.5)
Tobacco Smoking status						
- Current smoker	10 (18.2)	20 (24.7)	14 (25.0)*	39 (47.6)*	4 (20.0)	8 (34.8)
- Former smoker	04 (7.3)	14 (17.3)	13 (31.2)	18 (22.0)	9 (45.0)	8 (34.8)
- Never smoke	41 (74.5)	47 (58.0)	29 (51.8)	25 (30.5)	7 (35.0)	7 (30.4)
Tobacco smoking length (years)						
≤ 15	14 (100)	30 (88.2)	10 (37.0)	11 (19.3)	04 (30.8)	06 (37.5)
> 15	0 (0.0)	04 (11.8)	17 (63.0)	46 (80.7)	09 (69.2)	10 (62.5)
Total	14(100)	34(100)	27(100)	57(100)	13(100)	16(100)

p ≤ 0.05 (between groups: Negative/LSIL vs. HSIL/Cancer

Table 4. Crude and adjusted prevalence ratio for High SIL and cancer development, according to birth cohort, among Brazilian Women.

Variables	1976 - 1988 (18-30 years old)		1956 - 1975 (31-49 years old)		1937 – 1955 (50-68 years old)	
	Crude PR (CI:95%)	* adj. PR (CI:95%)	crude PR (CI:95%)	* adj. PR (CI:95%)	crude PR (CI:95%)	* adj. PR (CI:95%)
Menopause age	-	-	0.44 (0.25-0.76)	1.21 (1.04-1.41)	1.08(0.99-1.18)	1.13 (0.98- 1.32)
Number Pregnancy	1.10 (1.02- 1.20)	1.16 (1.01-1.34)	1.04 (0.98-1.11)	1.03 (0.98-1.09)	1.01(0.91- 1.11)	1.03 (0.89- 1.20)
Pack-year smoked	1.02 (0.99-1.04)	1.02 (0.99-1.05)	1.01 (1.00-1.019)	1.00 (0.99-1.01)	0.99 (1.00- 1.01)	0.99 (0.98- 1.01)
Menarche						
> 12 years	1.00	1.00	1.00	1.00	1.00	1.00
≤ 12 years	1.03 (0.85-1.25)	1.95 (1.17-3.25)	1.12 (0.84-1.49)	1.22 (0.94-1.57)	1.03 (0.59-1.81)	0.89 (0.38- 2.09)
Age at sexual onset						
> 16 years	1.00	1	1	1	1	1
≤ 16 years	1.27 (0.92-1.76)	1.06 (0.65-1.73)	1.41 (1.07-1.86)	1.20 (0.95-1.51)	1.10 (0.60-2.01)	0.81 (0.35-1.87)
Parity						
0-1	1	1	1	1	1	1
> 1	1.45 (1.09-1.91)	1.26 (0.82-1.91)	1.24 (0.88-1.77)	1.02 (0.78-1.32)	0.67 (0.35-1.26)	0.65 (0.23- 1.80)
Number of Abortions						
0-1	1	1	1	1	1	1
> 1	1.01 (0.65-1.57)	1.43 (0.80-2.59)	1.16 (0.83-1.62)	1.26 (0.97-1.65)	1.25 (0.71-2.19)	1.44 (0.58- 3.59)
Number of Sexual Partners						
1 – 2	1	1	1	1	1	1
3 – 4	1.18 (0.84-1.67)	0.67 (0.39-1.17)	1.72 (1.16-2.55)	1.09 (0.81-1.48)	1.71 (0.91-3.21)	1.41 (0.57-3.49)
≥ 5	1.10 (0.78-1.57)	0.91 (0.57-1.44)	1.46 (0.98-2.15)	1.05 (0.79-1.41)	1.01 (0.47-2.17)	0.89(0.31 - 2.49)
Oral Contraceptive						
- Never use	1	1	1	1	1	1
- Former use	1.72 (0.98-3.03)	1.11 (0.64-1.83)	1.08 (0.71-1.66)	0.89 (0.64-1.24)	0.53 (0.22-1.24)	0.42 (0.13- 1.36)
- Current use	1.46 (0.88-2.41)	1.08 (0.57-0.44)	1.05 (0.70-1.56)	0.93 (0.70-1.24)	1.17 (0.62-2.23)	1.15 (0.44- 2.99)
Contraceptive use length						
0	1	1	1	1	1	1
1 – 60 months	1.40 (0.83-2.35)	0.99 (0.57-1.73)	1.18 (0.78-1.77)	0.90 (0.67-1.22)	0.98 (0.48-1.99)	0.77 (0.27- 2.21)
≥ 60 months	1.65 (0.98-2.78)	1.22 (0.69-2.18)	1.00 (0.67-1.49)	0.93 (0.69-1.27)	0.86 (0.43-1.71)	0.82 (0.29- 2.32)
Tobacco Smoking						
- Never smoke	1	1	1	1	1	1
- Former smoker	1.46 (0.99-2.14)	1.44 (0.97- 2.13)	1.25 (0.85-1.85)	1.20 (0.94-1.54)	0.94 (0.46-1.93)	1.20 (0.54- 2.65)
- Current smoker	1.25 (0.89-1.75)	1.31 (0.93-1.87)	1.59 (1.15-2.20)	1.37 (1.10-1.70)	1.33 (0.65-2.73)	1.19 (0.55- 2.60)
Tobacco smoking length						
≤ 15 years	1	1	1	1	1	1
> 15 years	1.47 (0.82-2.61)	1.43 (0.76-2.72)	1.39 (0.96-2.03)	1.13 (0.90-1.43)	0.88 (0.43-1.77)	0.74 (0.35- 1.60)

**RP adjusted by Number of Sexual Partners, Contraceptive use, Age at sexual onset and Pack-year smoked*

Table 5. Predictive model for the HSIL/cervical cancer development among enrolled population, Rio de Janeiro, Brazil.

Variable		RP	CI (95%)	*p-value*
Cohort :	1937 – 1955	1.00		
	1956 – 1975	1.05	(0.77 - 1.44)	*0.739*
	1976 – 1988	1.15	(0.82 - 1.63)	*0.419*
	p-trend = 0.2262			
Menarche:	> 12 years old	1.00		
	≤ 12 Years old	1.02	(0.84 - 1.25)	*0.813*
Age at sexual onset:	> 16 years old	1.00		
	≤ 16 years old	1.18	(0.95 - 1.47)	*0.136*
Number of Sexual Partners:	1 – 2	1.00		
	3 – 4	**1.34**	**(1.05 - 1.71)**	***0.018***
	≥ 5	1.10	(0.86 - 1.42)	*0.442*
	p-trend = 0.1122			
Oral Contraceptive:	- Never use	1.00		
	- Former use	1.12	(0.81 - 1.54)	0.491
	- Current use	1.20	(0.90 - 1.59)	*0.210*
	p-trend = 0.0010			
Tobacco Smoking:	- Never smoke	1.00		
	- Former smoker	1.19	(0.91 - 1.54)	*0.205*
	- Current smoker	**1.37**	**(1.09 - 1.73)**	***0.007***
	p-trend = 0.0018			
Number of Deliveries:	0 -1 child	1.00		
	> 1 child	1.24	(0.99 - 1.56)	*0.065*

6. Artigo – 2

***TP53* genetic polymorphisms and environmental risk factors associated with cervical carcinogenesis in a cohort of women with cervical lesions in Rio de Janeiro, Brazil.**

Abstract

Objective: This study evaluated the prevalence of p53 polymorphisms at codon 72 and their association with environmental risk factors in a sample of women requiring health care in an oncological reference center in Rio de Janeiro, Brazil. **Methods:** A cross-sectional study was conducted with 304 women with histological diagnoses of negative, pre-cancerous and cancerous lesions between October 2004 and May 2006. Antecedents of exposure to possible environmental risk factors were ascertained through an interview-administered questionnaire, and whenever indicated, colposcopy tests and lesion excisions were performed. Genomic DNA was extracted from leukocytes of peripheral blood, and genotyping of p53 polymorphism was conducted using polymerase chain reaction and restriction fragment-length polymorphism methods. Crude and adjusted odds ratio (OR), and their 95% confidence intervals, were ascertained for selected risk factors and allelic groups among Normal, Low-SIL, and High-SIL/Cancer strata using logistic regression analysis. **Results:** The observed p53 polymorphisms distribution in this population was 64(21.1%) Arg/Arg, 55(18.1%) Pro/Pro, and 185(60.9%) Arg/Pro. Women who were heterozygous (Arg/Pro) showed an independent risk for cervical HSIL/Cancer (adjusted OR:1.92,95%CI:1.03-3.59, controlled by age, ethnicity and age at menarche) when compared to Pro allelic homozygous cases. Age at sexual onset up to 16 years old (adjusted OR:1.97,95%CI:1.18-3.30), lifelong 3-4 sexual partners (adjusted OR:2.38,95%CI:1.32-4.28), current smoking (adjusted OR:2.32,95%CI:1.31-4.13), and smoking over 10 years (adjusted OR:2.52, 95%CI:1.042-6.09), were found as independent risk factors to cervical HSIL/Cancer. **Conclusion:** Women showing p53arg/pro72 profile have shown a higher risk of HSIL/cancer development when compared to p53/pro72 in the studied sample after control for selected confounders. Early sexual onset, multiple sexual

partners, and current and past tobacco smoking were independent risk factors to pre-cancer and cancer development in this study.

Key words: pre-cancerous lesion, cervical cancer, HSIL, LSIL, p53 polymorphism

Introduction:

Cervical cancer is a major public health problem in Brazil, with its incidence and mortality rates as high as in Africa and Asia [1]. The main risk factor for pre-cancerous lesions and invasive cancer of the cervix is Human Papillomavirus (HPV) infection. However, despite the large amount of sexually active women who are infected with HPV, only a small fraction of them will develop cervical cancer subsequently to a long latency[2]. This fact points to the role of genetic and environmental cofactors triggering the carcinogenic transformation of the cervical epithelium infected by HPV infection.

Infections of the genital tract by HPV are initiated when the virus invades the epithelium by micro abrasion and persists along certain period of time. It can progress to a pre-cancer stage, and eventually, to invasive cervical cancer lesions. However, infection by HPV can also be cleared by the innate immune system or by other mechanisms, and regression into a pre-cancer status may occur [3]. Experimental data provides evidence that the E6 and E7 oncoproteins of High Risk-HPV (HR-HPV) are primarily expressed in human keratonocytes, and only the intact expression of these oncogenes efficiently maintain the immortalization of the cells infected with HR-HPV[4, 5]. Furthermore, it has been shown that E6 and E7 oncoproteins are able to bind, respectively, with the tumor suppressor gene p53 and retinoblastoma proteins (pRb). Both oncoproteins enable the deregulation of the cell cycle control mechanism, in which p53 and pRb play a fundamental role because they show tumor-suppressive and cell-cycle growth inhibitory properties [6].

Recently, several studies have been published highlighting the role of p53 polymorphisms as a risk factor for cervical cancer [7-10]. It has been reported that codon 72 in exon 4 of p53 protein is polymorphic, either encoding an arginine (Arg), or a proline (Pro) amino acid residue. It has also been suggested that the homozygous Arg variant of

p53 is more susceptible to HR-HPV E6 mediated proteolysis than the homozygous Pro variant of p53. This could possibly be related to a higher susceptibility to HPV-induced malignant disease in individuals carrying the homozygous Arg allele [11]. Although many studies have tried to reproduce these findings, the results have been controversial [7, 8, 12, 13]. Such apparent discrepancy may arise from the fact that those studies have been carried out in different ethnic population groups, thus suggesting that p53 polymorphism distribution may be influenced by the genetic variations of diverse population groups. Moreover, some studies were either short sampled, or did not consider the effect of environmental risk-factors on the natural history of disease.

Therefore, considering the absence of consensus on the debatable role of p53 polymorphism at codon 72 according to the individual genetic susceptibility relatively to environmental risk factors, such as HPV, smoking and others in cervical carcinogenesis, and the inexistence of similar studies carried out among Brazilian women, this investigation was planned and developed aiming to evaluate the role of p53 polymorphism at codon 72 on cervical HSIL/cancer in a sample of women from Rio de Janeiro, Brazil.

Material and Methods

Study population and study design

Patients were selected from a group of women referred to *Hospital do Cancer (INCA -II)*, Brazilian National Cancer Institute, located in the city of Rio de Janeiro, a public health center offering universal oncological care. They were recruited for the study from October 2004 to May 2006. A cross-sectional approach was used to ascertain the magnitude of association between p53 codon 72 polymorphisms and selected risk factors at the diagnosis

of HSIL/cervical cancer. This research was performed after approval by the Human Investigations Ethical Committee from the Brazilian National Cancer Institute.

Inclusion criteria

Patients were eligible for this study if older than 18 yr. old, had not been submitted to a conservative cervical treatment in the 6 months previous to the current diagnosis, were free of any psychiatric disease, were literate (or illiterate but bringing a literate relative to witness the informed consent explanation) and have accepted to sign an informed consent.

Exclusion criteria

Patients were excluded from this study if they refused to sign the informed consent, had no lesion at the time of colposcopy, or presented no changes at the Pap smear test taken during the colposcopy exam.

Data collection and variable description

All patients were interviewed by two trained nurses using standardized procedures. Age, ethnicity(skin color) , education, age at menarche, age at first sexual intercourse, parity, number of abortions, menopausal status, number of sexual partners, oral contraceptive use, diabetes mellitus antecedents, and smoking *status* (current, former and never smoked) were obtained from an interview-administered questionnaire.

In Brazil, race is usually stated by skin color, and according to the Brazilian Institute of Statistics and Geography, data collection of skin color must be self-reported in the country to avoid race discrimination[14]. Since Brazil is a multiethnic country, ethnicity is classified by self-reported skin color. Smoking *status* was only assigned for those women

who reported to have smoked over 100 cigarettes during their life. Former smokers were classified only as those who have quit smoking at least 6 months before the interview [15].

After the interview, participants underwent colposcopy. Biopsy or partial ablation with diathermy and electrocauterization was used for LSILs. Loop Electrical Excision Procedure (LEEP) was the treatment of choice for HSILs. Peripheral blood samples were taken to test for HIV, Hepatitis B and C infections and ascertainment of genetic single nucleotide polymorphism (SNPs) analysis.

The histological diagnosis was obtained through hematoxylin and eosin (HE) stained slides for grading of the cervical lesion according to the Bethesda System [16]: in LSIL, including condylomata, ASCUS/AGUS and cervical intraepithelial neoplasia grade I (CIN I); in HSIL, including CIN II and CIN III/ *in situ* carcinoma (SCC). Histological normal tissue presenting only reactive/reparative changes were classified as negative (disease-free).

DNA Extraction and P53 genotyping

Genomic DNA was extracted from leukocytes of peripheral blood, using a salting procedure protocol described by Miller et al (1988) [17], and then diluted in water Milli Q. The DNA content of each sample was analyzed by spectrophotometry and about 0.1-10μg of DNA was taken from each sample for polymerase chain reaction (PCR) amplification.

Genotyping of p53 in codon 72 was performed by PCR and restriction fragment-length polymorphism (RFLP). 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. The PCR mixture contained approximately 0.1-10μg of genomic DNA, 2U of Platinum Taq

polymerase (Invitrogen, São Paulo, Brazil), 10pmol of a pair of primers and 5mM of dNTPs in 50µl of final volume. PCR was performed at 94ºC for 4min to initial denaturation, followed by 35 cycles of denaturation at 94ºC for 40sec, annealing at 58ºC for 40sec and extension at 72ºC for 40sec. The final step of elongation was performed at 72ºC for 5min. Following PCR, 5µl aliquots were removed and subjected to digestion with the restriction enzyme BstU1 (New England Biolabs, Beverly, MA). Digested DNA was electrophoresed on 3% agarose gels for 2hs, following ethidium bromide staining. Gels were photographed by UV light transluminator. The presence of the wild-type Arg allele was indicated by bands of 169 and 127 bp, whereas no digestion of the mutant Pro allele could be observed.

Sample Size

Sampling was carried out based on the available population data covered by the Brazilian National Public Health System in Rio de Janeiro State in 2002 presenting an altered Pap smear exam (9,409 women). In this year, 5,314 (56.5%) women presented CIN I, 2,014 (21.4%) presented CIN II, 1,423 (15.1%) presented CIN III, 264 (2.8%) presented squamous cancer, 31 (0.3%) adenocarcinoma *in situ,* and 77 (0.8%) presented invasive adenocarcinoma in the State of Rio de Janeiro (Conprev/INCA, 2004 non-published data).

Literature reports that the Arg allele homozygous at *Tp53* gene codon 72 may range around 27-53% among women with cervical pre-cancerous lesions [13, 18] and from 42-56% among women with cervical cancer [19, 20]; such variability ethnically dependent. Considering the large genetic variation in the Brazilian population, our sampling was carried out using an estimated intermediary homozygous Arg allele prevalence of 35% for pre-cancer, and of 50% for cancer lesions, assuming the worst acceptable values of,

respectively, 25% and 42%. According to these parameters, a 5% type I error and study power of 80%, a sample of 235 women would be required.

Statistical analysis

Distribution of frequency for socio-demographic characteristics and environmental factors, according to p53 Polymorphism in the study population, were compared using a chi-square test. Modelisation using non-conditional logistic regression was used to evaluate the association between p53 genotype and HSIL/Cancer. After adjusting for other potential confounders, the respective odds ratios (OR) and the respective 95% confidence intervals (CI) were obtained. Environmental and genetic interactions between p53 polymorphism and selected environmental risk factors were explored towards interaction odds ratios, and the respective 95% confidence intervals were ascertained using a case-only approach [21]. Statistical analyses were performed using STATA version 8.

Results

From October 2004 to May 2006, 304 women met the inclusion criteria, and according to histological results, more than 50% were HSIL/cancer. The observed prevalence of the different p53 polymorphisms were 64 (21.1%) of Arg/Arg, 55(18.1%) of Pro/Pro, and 185(60.9%) of Arg/Pro (table 1).

The distributions of the environmental characteristics according to each of *TP53*codon 72 genotype are presented in table 2. Heterozygous Arg/Pro genotype was the most prevalent, being observed among 69.1% of white participants, 67.2% of black, and 53.0% of multiethnic cases. Pro allele homozygous cases were the lowest prevalent type among white women, while homozygous for the Arg allele type presented the lowest

prevalence among black women. Concerning the histological results, there is a decreasing prevalence of Pro allele homozygosis according to lesion degree (27.1% for normal tissue, 17.2 for LSIL, 15.3% for HSIL, and 11.5% for cancer).

When evaluating the variable according to the lesion degree (HSIL/Cancer vs. Normal/LSIL), HSIL/cancer was more prevalent among the heterozygous form (63.2%) when compared to Normal/LSIL (36.8%, $p < 0.001$), while among homozygous Pro allele form the prevalence of HSIL/cancer was lower than Normal/LSIL (47.3% and 52.7%, respectively, $p < 0.001$, table 3). Early age at sexual onset ($\leq$ 16 years old), parity higher than 1, number of sexual partners, tobacco smoking and duration of tobacco use, were statistically related to the cervical lesion degree (table 3).

Crude and adjusted *odds ratios* between genetic/environmental variables and HSIL/cancer development are presented in table 4. Heterozygous Arg/Pro was independently associated with HSIL/cancer development, comparatively to Pro/Pro genotype ($_{adj}$OR=1.92,95%CI:1.03-3.59). The observed environmental risk factors independently associated with HSIL/cancer development were early sexual onset ($_{adj}$OR=1.97,95%CI:1.18-3.30), 3-4 sexual partners in a lifetime ($_{adj}$OR=2.38,95%CI:1.32-4.28), current tobacco use (adj. OR=2.32, CI 95%: 1.31-4.13), and duration of tobacco use of over 10 years ($_{adj}$OR=2.52,95%CI:1.04-6.09). A statistically significant trend in tobacco use status was also observed.

Associations between p53 polymorphism and HSIL/Cancer stratified by environmental variables are presented in table 5. According to this table, p53 polymorphism seems to be interacting with environmental variables, where among black women heterozygous Arg/Pro presented a risk 5.20 fold higher than Arg/Arg form women (*p=0.016*). Among women with a partner the risk for HSIL/Cancer for Arg/Pro form was

2.44 fold higher when compared to Arg/Arg form (*p=0.035*). Heterozygous Arg/Pro presented an increased risk for HSIL/Cancer among women who had her first sexual intercouse up to 16 years old when compared to Arg/Arg women (OR=2.58 vs OR=1.06, *p=0.026*).

Estimated genetic-environmental interaction between p53 polymorphisms and selected environmental risk factors are presented in table 6. A statistically significant interaction odds ratio between the Arg/Pro polymorphism and current contraceptive use (OR=3.59,95%CI:1.09-11.84) was observed. Current contraceptive use also showed an interaction odds ratio with Arg/Arg genotype (OR=3.82,95%CI:0.78-19.85), though it was not statistically significant. Former oral contraceptives use also seems to present an interaction with p53 polymorphism, but also not statistically significant ($OR_{A/P}$=2.13,95%CI:0.56-8.30; $OR_{A/A}$=2.29, 95%CI:0.37-14.99). Early sexual onset (up to 16 years old) presented an increased interaction odds ratio with Arg/Pro genotype (OR=1.29,95%CI:0.51-3.29), and with Arg/Arg genotype (OR=2.67,95%CI:0.79-9.16). Increased but not statistically significant interaction odds ratios were also found between smoking and homozygous Arg allele, showing an $OR_{A/A}$=1.37,95%CI:0.35-5.53, among current smokers, and an $OR_{A/A}$=1.18,95%CI:0.27-5.17, among former smokers.

Discussion

The *Tp53* tumor suppressor gene is located on chromosome 17, and encodes a 393-amino-acid nuclear phosphoprotein, p53 [22]. *Tp53* mutations have been related to 50-55% of cancer cases and therefore, play an important role in carcinogenesis. The p53 gene has a critical function in several cellular processes, such as cell cycle arrest, gene transcription, DNA repair, and apoptosis. In case of DNA damage, p53 arrest cell cycle division in G1

phase, leading to the initiation of the DNA repair process or, in more severe cases, to apoptosis [22]. If *Tp53* is mutated, the normal functions of the protein may not be activated, leading to the development of cancer as a consequence of uncontrolled cell proliferation. Storey et al (1998) [11] observed that Caucasian women homozygous for arginine (Arg72/p53) were about seven fold more susceptible than heterozygous to present HPV-mediated p53 degradation leading to cervical cancer, suggesting that p53 represents an important risk factor for cervical carcinogenesis. Nevertheless, those results have not been yet confirmed by other studies developed in ethnically different populations [12, 23, 24].

In the present study, we have tested the hypothesis that the polymorphism at codon 72 of *TP53* gene may be associated with the degree of cervical lesions, considering their possible interaction with environmental risk factors. Interestingly, when all participants in our study were analyzed simultaneously, we detected that the frequencies of p53-Arg/Pro alleles fit the Hardy-Weimberg Equilibrium. These results seem to be supported by a quite similar p53-Arg/Pro distribution reported by a breast cancer case-control study developed in India, in which the prevalence of such alleles among controls was 19.5% for Pro/Pro genotype, 22% for Arg/Arg, and 58,5% for Arg/Pro [25].

It is worth highlighting that some studies developed in Europe, South Africa, and Korea reported a higher arginine allele prevalence [11, 12, 23, 25, 26], despite inconsistency of such findings among other populations [24, 25]. The reasons for such heterogeneity could either result from ethnic variability, or from the inverse reported frequency distribution of Arginine allele in different populations according to the geographical distance from the Equator[27].

The findings of our study are probably explained by the fact that Brazilians are one of the most ethnically mixed populations worldwide, which resulted from five centuries of

interethnic mixture within the autochthonous Amerindians, European colonizers (mainly Portuguese, but also, French, Dutch and Spanish), the African slaves, and in the last century, by other communities such as Italians, Germans, Spanish, Polish, Arabs, Japanese, among several others [28]. Thus, genetic variability is common and would be expected in such scenario. In addition, exposure to environmental risk-factors such as tobacco smoking, HPV infection and oral contraceptive use have increased in Brazil [29, 30]. Thus, all the aforementioned conditions may explain the observed higher frequency of Arg/Pro form (table 2) in all lesion degree categories in the studied population when analyzed their Arg allele distribution of frequencies (60.3% LSIL, 68.7% HSIL, and 53.8% cancer).

Our study revealed that heterozygous Arg/Pro allele women in our sample were at higher risk of HSIL/cancer development when compared to homozygous ones, even after adjustment for age, age at menarche and ethnicity. Our data support the results reported by Kim et al (2000) [26] among Korean women who presented a 9.5 folds higher risk for cervical cancer among Arg allele heterozygous women, compared to homozygous ones.

Tobacco use was statistically related to cervical cancer development in this study (OR_{adj} = 2.32, 95% *CI* 1.31-4.13 among current smokers, and OR_{adj} = 1.5, 95% 0.81-2.77 among former smokers), presenting a statistically significant dose-response effect among the categories either for crude or adjusted ORs (table 4). Tobacco use over 10 years or longer was also an independent risk factor for cervical cancer in our sample (OR_{adj} = 2.52, 95% CI 1.04-6.09). Biological evidence supporting these findings was reported by studies showing that nicotine levels, and levels of its major metabolite cotinine, were increased, respectively, forty-fold and four-fold, in the cervical mucus of healthy female smokers [31] and in women with CIN [32] as compared to serum levels.

Moreover, either DNA repair defects related to carcinogenesis or DNA damage have been found in cervical tissue of smokers with high DNA adduct levels [33, 34]. Benzo(a)pyrene has been detected in cervical tissue, and DNA adducts in smokers were detected twice as often than non-current smokers. Additionally, cell growth and DNA damage induced by benzo(a)pyrene was higher in HPV-16 immortalized cervical cells than in normal tissue [35].

Though we were not able to accomplish HPV detection in this study, some surrogate variables of such exposure, such as an early sexual onset (before 16 years old) and the amount of sexual partners (3-4 in a lifetime), were statistically associated with cervical cancer development, respectively, OR_{adj}=1.97(95%CI:1.18-3.30), and OR_{adj}=2.38(95%CI: 1.32-4.28).

Interaction of viral DNA with the host genome thereby enabling expression of viral oncogenes E6 and E7 seems to be a necessary step in immortalization; this probably does not occur without the presence of co-factors [2-6]. Risky sexual behavior, oral contraceptive use, and smoking have been reported among the proposed risk factors for cervical neoplasia. Risky sexual behavior, such as number of sexual partners and early age at sexual onset, is now recognized as a surrogate for cervical HPV infection. In the case-only analysis carried out with our data (table 6), a suggested interaction between p53 polymorphism at codon 72 and selected environmental risk factors was observed.

According to this analysis, increased interaction odds ratios between p53 SNP and oral contraceptive (OC) use were observed: for OC users, $OR_{A/P}$=3.59,95%CI:1.09-11.84; $OR_{A/A}$=3.82,95%CI:0.78-19.85; and among former OC users, risk estimates were $OR_{A/P}$=2.13,95%CI:0.86-8.30; $OR_{A/A}$=2.29, 95% CI 0.37-14.99.

In relation to p53 SNPs and smoking interaction, the following interaction odds ratios were observed: $OR_{A/A}$ =1.37,95%CI:0.35-5.53 among current smokers, and $OR_{A/A}$=1.18, 95%CI:0.27-5.17 among former users.

Reviewing the acknowledged mechanisms related to the natural history of cervical carcinogenesis, possible explanations for the findings observed in our study involving a possible interaction between *TP53* genetic polymorphisms (Arg/Pro allele) and oral contraceptive use or smoking, were explored.

Tumor suppressor gene *TP53* encodes the p53 protein, which is activated in response to DNA damage, it causes cell cycle arrest by blocking the cell at the G1 and G2 phase prior to DNA replication and mitosis, thereby aiding the DNA repair process and preventing mutations [36]. The HPV *E6* oncogenes bind to p53 and direct its rapid degradation in a step thought to be important in viral DNA replication [37]. Additionally, mutations of the *Tp53*, with a subsequent decreased capacity of DNA repair, is one of the mechanisms that contributes to cancer growth [38]. It was found also reported that tobacco nitrosamines and polycyclic aromatic hydrocarbons seem to increase mutations of *p53* in lung cancer [39]. Also, a mitogenic effect of nicotine on normal and HPV-16 DNA-transformed squamous cervical epithelial cell lines has been observed [40]. Smoking has been significantly associated with cervical cancer cell proliferation, measured as the DNA S-phase fraction [41].

In addition, serum progesterone levels have been significantly associated with cervical cancer proliferation. When the serum-estradiol/progesterone ratio was estimated among premenopausal women, a strong positive association was found not only with survival length, but also with S-phase fraction [42].

Both estrogen and progesterone receptors are present in cervical Neoplasia; the expression of both receptors is higher in immature squamous metaplasia of the transformation zone than in the ectocervix [43]. HPV has a tendency to transfect cells with progesterone receptors. Both HPV 16 and HPV 18 contain progesterone and glucocorticoid response elements that increase expression of the *HPV E6* and *E7* oncogenes, considered crucial in cell transformation [44]. Moreover, in the epithelium of the transformation zone, where cervical neoplasia is initiated, 16- α -hydroxylation of estradiol occurs resulting in 16-α-hydroxyestrone, which is linked to malignant transformation of estrogen-sensitive cells infected by HPV [45].

Conclusion

The balance between proliferation, mutations and apoptosis is important for maintenance of tissue homeostasis. A combined effect of *TP53* mutations, increased proliferation of HPV-infected cell and decreased apoptosis (that may result from higher degradation of homozygous Arg allele) during estrogen/progesterone or tobacco exposures, could be one possible mechanism to explain the increased interaction found between those factors in this study.

At this point, nevertheless, these results may simply generate hypothetical evidence involving the possible interaction between the Arg/Pro allele polymorphisms of *TP53* gene with some environmental exposures, namely smoking and exogenous hormonal exposure in the natural history of cervical carcinogenesis. However, further corroboration of the presented results by other studies is needed.

Conflict of Interest Statement

The authors declare that there are no conflict of interest between the research supporters and the authors, since this project is supported by governmental institutions which also have academicals purpose.

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Article Précis

Women showing p53Arg/Pro72 polymorphism are at higher risk of developing cervical cancer, and a genetic-environmental interaction may occur involving p53Arg polymorphism, with either exogenous hormone or tobacco use.

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Table1. Distribution of frequencies of genetic, socio-demographic, and environmental variable in a women cohort with altered cytology (n=304), Rio de Janeiro, Brazil.

Variable	*N (%)	CI:95%
P53 polymorphism		
P/P	55 (18.1)	(13.8 - 22.4)
A/P	185 (60.9)	(55.4 – 66.3)
A/A	64 (21.1)	(16.5 – 25.6)
Age		
18-30 years old	136 (44.7)	(39.1 – 50.3)
31-50 years old	129 (42.4)	(36.9 – 48.0)
>50 years old	39 (13.8)	(9.1 – 16.6)
Ethnicity		
White	94 (30.9)	(25.7 – 36.1)
Black	61 (20.1)	(15.6 – 24.6)
Multiethnic	149 (49.0)	(43.4 – 54.6)
Education		
< High school	113 (37.2)	(31.7 – 42.6)
≥ High School	191 (62.8)	(57.4 – 68.3)
Marital *Status*		
With no partner	100 (32.9)	(27.6 – 38.2)
With a partner	204 (67.1)	(61.8 – 72.4)
HIV *status*		
Positive	6 (2.0)	(0.4 – 3.5)
Negative	297 (97.7)	(96.5 – 99.6)
HBS-Ag *status*		
Positive	22 (7.3)	(4.4 – 10.2)
Negative	279 (92.7)	(89.8 – 95.6)
Diabetes Mellitus *status*		
Positive	10 (3.3)	(1.3 – 5.3)
Negative	291 (95.7)	(94.7 – 98.7)
Age at Menarche		
≤ 12 years old	119 (39.1)	(33.7 – 44.6)
> 12 years old	185 (60.9)	(55.4 – 66.3)
Age at sexual onset		
≤ 16 years old	146 (48.0)	(42.4 – 53.6)
> 16 years old	158 (52.0)	(46.4 – 57.6)
Parity		
0-1	115 (37.8)	(32.4 – 43.3)
>1	189 (62.2)	(56.7 – 67.6)
Abortion		
0-1	249 (81.9)	(77.6 – 86.2)
>1	55 (18.1)	(13.8 – 22.4)
Sexual Partners		
1-2	95 (31.3)	(26.0 – 36.5)
3-4	98 (32.2)	(27.0 – 37.5)
≥ 5	111 (36.5)	(31.1 – 41.9)
contraceptive use		
Never	52 (17.1)	(12.9 – 21.3)
Former	74 (24.3)	(19.5 – 29.2)
Current	178 (58.6)	(53.0 – 64.1)
** Duration of contraceptive use		
1-60 months	122 (48.8)	(42.7 – 55.0)
≥ 60 months	128 (51.2)	(45.0 – 57.4)
Tobacco use		
Never	150 (49.3)	(43.7 – 55.0)
Former	65 (21.4)	(16.8 – 26.0)
Current	89 (29.3)	(24.2 – 34.4)
*** Duration of tobacco use		
≤ 10 years	40 (31.8)	(20.3 – 34.9)
> 10 years	105 (68.2)	(65.1 – 79.7)
Reference cytology		
LSIL	63 (21.0)	(16.2 – 25.3)
HSIL	235 (76.8)	(72.6 – 82.0)
Cancer	6 (2.0)	(0.4 – 3.5)
Histological result		
Normal	70 (23.0)	(18.3 – 27.8)
LSIL	58 (19.1)	(14.7 – 23.5)
HSIL	150 (49.3)	(43.7 – 55.0)
Cancer	26 (8.6)	(5.4 – 12.7)

** Total may vary because of missing values; ** among former and current users only; *** among former and current smokers only.*

Table 2. Distribution of frequencies of p53 polymorphism, according to socio-demographic and environmental variable in a women cohort with altered cytology, Rio de Janeiro, Brazil.

Variable	P/P	A/P	A/A	*p-value* (X^2)
	*N (%)	*N (%)	*N (%)	
Age				
18-30 years old	22 (16.2)	87 (64.0)	27 (19.9)	
31-50 years old	26 (20.2)	75 (58.1)	28 (21.7)	*0.883*
>50 years old	07 (17.9)	23 (59.0)	09 (23.1)	
Ethnicity				
White	09 (9.6)	65 (69.1)	20 (21.3)	
Black	16 (26.2)	41 (67.2)	04 (6.6)	*0.001*
Multiethnic	30 (20.1)	79 (53.0)	40 (26.8)	
Education				
< High school	29 (15.2)	115 (60.2)	47 (24.6)	*0.063*
≥ High School	26 (23.0)	70 (61.9)	17 (15.0)	
Marital *Status*				
With no partner	34 (16.7)	123 (60.3)	47 (24.6)	*0.388*
With a partner	21 (21.0)	62 (62.0)	17 (17.0)	
Age at Menarche				
≤ 12 years old	16 (13.4)	79 (66.4)	24 (20.2)	*0.184*
> 12 years old	39 (21.1)	106 (57.3)	40 (21.6)	
Age at sexual onset				
≤ 16 years old	36 (24.7)	86 (58.9)	24 (16.4)	*0.008*
> 16 years old	19 (12.0)	99 (62.7)	40 (25.3)	
Parity				
0-1	23 (20.0)	67 (58.3)	25 (21.7)	*0.734*
>1	32 (16.9)	118 (62.4)	39 (20.6)	
Abortion				
0-1	46 (18.5)	148 (59.4)	55 (22.1)	*0.531*
>1	09 (16.4)	37 (67.3)	09 (16.4)	
Sexual Partners				
1-2	21 (22.1)	57 (60.0)	17 (17.9)	
3-4	15 (15.3)	63 (64.3)	20 (20.4)	*0.617*
≥ 5	19 (17.1)	65 (58.6)	27 (24.3)	
Oral Contraceptive use				
Never	13 (25.0)	26 (50.0)	13 (25.0)	
Former	17 (23.0)	44 (59.5)	13 (17.6)	*0.183*
Current	25 (14.0)	115 (64.6)	38 (21.3)	
** Duration of contraceptive use	20 (16.4)	78 (63.9)	24 (19.7)	*0.972*
1-60 months	22 (17.2)	80 (62.5)	26 (20.3)	
≥ 60 months				
Tobacco use				
Never	27 (18.0)	92 (61.3)	31 (20.7)	
Former	13 (20.0)	38 (58.5)	14 (21.5)	*0.989*
Current	15 (16.9)	55 (61.8)	19 (21.3)	
*** Duration of tobacco use				
≤ 10 years	09 (18.4)	31 (63.3)	09 (18.4)	*0.813*
> 10 years	19 (18.1)	62 (59.0)	24 (22.9)	
Histological result				
Normal	19 (27.1)	33 (47.1)	18 (25.7)	
LSIL	10 (17.2)	35 (60.3)	13 (22.4)	*0.040*
HSIL	23 (15.3)	103 (68.7)	24 (16.0)	
Cancer	03 (11.5)	14 (53.8)	09 (34.6)	

** Total may vary because of missing values; ** among former and current users only; *** Among former and current smokers only.*

Table 3. Distribution of frequencies of histological results, according to genetics, socio-demographic and environmental variable in a women cohort with altered cytology, Rio de Janeiro, Brazil.

Variable	HSIL/cancer	Normal/LSIL	*p-value*
P53 polymorphism			
P/P	26 (47.3)	29 (52.7)	
A/P	117 (63.2)	68 (36.8)	0.056
A/A	33 (51.6)	31 (48.4)	
Age			
18-30 years old	78 (57.4)	58 (42.6)	
31-50 years old	76 (63.2)	53 (41.1)	0.948
>50 years old	22 (56.4)	17 (43.6)	
Ethnicity			
White	54 (57.4)	40 (42.6)	
Black	31 (50.8)	30 (49.2)	0.391
Multiethnic	91 (61.1)	58 (38.9)	
Education			
< High school	115 (60.2)	76 (46.0)	0.173
≥ High School	61 (54.0)	52 (39.8)	
Age at sexual onset			
> 16 years old	73 (50.0)	73 (50.0)	0.005
≤ 16 years old	103 (65.2)	55 (34.8)	
Parity			
0-1	59 (51.3)	56 (48.7)	0.045
>1	117 (61.9)	72 (38.1)	
Abortion			
0-1	142 (57.0)	107 (43.0)	0.310
>1	34 (61.8)	21 (38.2)	
Sexual Partners			
1-2	45 (47.4)	50 (52.6)	
3-4	67 (68.4)	31 (31.6)	0.013
≥ 5	64 (57.7)	47 (42.3)	
Oral Contraceptive use			
Never	27 (51.9)	25 (48.1)	
Former	43 (58.1)	31 (41.9)	0.618
Current	106 (59.6)	72 (40.4)	
** Duration of contraceptive use			
1-60 months	72 (59.0)	50 (41.0)	0.528
≥ 60 months	76 (59.4)	52 (40.6)	
Tobacco use			
Never	76 (50.7)	74 (49.3)	
Former	39 (60.0)	26 (40.0)	0.024
Current	61 (68.5)	28 (31.5)	
*** Duration of tobacco use			
≤ 10 years	105 (52.8)	94 (47.2)	0.009
> 10 years	71 (67.6)	34 (32.4)	

** Among former and current users only; *** among former and current smokers only.

Table 4. Crude and Adjusted OR related to HSIL/cancer development, among Brazilian women.

Variable	Crude OR	CI: 95%	*Adj. OR	CI: 95%
P53 polymorphism				
P/P	1		1	
A/P	1.92	(1.04 – 3.52)	1.92	(1.03 – 3.59)
A/A	1.19	(0.58 – 2.44)	1.08	(0.52 – 2.27)
Education				
< High school	1		1	
≥ High School	1.29	(0.81 – 2.06)	1.28	(0.78 – 2.08)
Age at sexual onset				
> 16 years old	1		1	
≤ 16 years old	1.87	(1.18 – 2.97)	1.97	(1.18 – 3.30)
Parity				
0-1	1		1	
>1	1.54	(0.96 – 2.46)	1.67	(0.99 – 2.79)
Abortion				
0-1	1		1	
>1	1.22	(0.67 – 2.22)	1.36	(0.72 – 2.57)
Sexual Partners				
1-2	1		1	
3-4	2.40	(1.34 – 4.31)	2.38	(1.32 – 4.28)
≥ 5	1.51	(0.87 – 2.63)	1.54	(0.89 – 2.69)
Oral Contraceptive use				
Never	1		1	
Former	1.28	(0.63 – 2.62)	1.34	(0.65 – 2.76)
Current	1.36	(0.73 – 2.54)	1.42	(0.75 – 2.67)
** Duration of contraceptive use				
1-60 months	1		1	(0.62 – 1.74)
≥ 60 months	1.02	(0.61 – 1.68)	1.04	
Tobacco use				
Never	1		1	
Former	1.46	(0.81 – 2.64)	1.50	(0.81 – 2.77)
Current	2.12†	(1.22 – 3.68)	2.32†	(1.31 – 4.13)
*** Duration of tobacco use				
≤ 10 years	1		1	
> 10 years	1.44	(0.71 – 2.90)	2.52	(1.04 – 6.09)

** OR adjusted by age, age at menarche, and ethnicity; ** among former and current users only; *** among former and current smokers only. † p-trend*<0.05

Table 5. Association between p53 polymorphism and HSIL/Cancer, stratified by environmental variables.

Variable	OR stratified (CI:95%)			*p-value*
	Pro/Pro	Arg/Pro	Arg/Arg	(X^2 test)
Age				
18-30	1	1.96 (0.76 - 5.04)	1.29 (0.42 - 4.00)	*0.303*
31-49	1	2.33 (0.94 - 5.79)	1.17 (0.40 - 3.40)	*0.104*
50-68	1	0.97 (0.18 - 5.38)	0.94 (0.13 - 6.87)	*0.998*
Ethnicity (skin Collor)				
White	1	0.40 (0.08 - 2.09)	0.23 (0.4 – 1.42)	*0.232*
Multiethnic	1	2.04 (0.87 – 4.80)	1.35 (0.52 – 3.50)	*0.229*
Black	1	5.20 (1.42 – 19.04)	1.00 (0.80 – 12.86)	*0.016*
Education				
< High School	1	2.40 (1.05 - 5.49)	1.52 (0.60 – 3.86)	*0.084*
≥ High School	1	1.41 (0.57 – 3.49)	0.70 (020 – 2.41)	*0.390*
Marital *Status*				
With no partner	1	1.26 (0.46 – 3.40)	1.02 (0.28 – 3.68)	*0.868*
With a partner	1	2.44 (2.13 – 5.29)	1.32 (0.54 – 3.21)	*0.035*
Menarche				
> 12 years old	1	2.52 (1.19 – 5.32)	1.58 (0.65 – 3.84)	*0.044*
≤ 12 years old	1	1.14 (0.38 – 3.38)	0.66 (0.18 – 3.35)	*0.499*
Age at sexual onset				
> 16 years old	1	0.92 (0.32 – 2.65)	0.69 (0.22 – 2.20)	*0.723*
≤ 16 years old	1	2.58 (1.15 – 5.76)	1.06 (0.36 – 3.09)	*0.026*
Sexual partners				
1 - 2	1	3.20 (1.08 - 9.43)	1.75 (0.45 – 6.77)	*0.077*
3 - 4	1	1.25 (0.37 – 4.17)	0.75 (0.18 – 3.03)	*0.631*
≥ 5	1	1.44 (0.51 – 4.03)	0.97 (0.30 – 3.13)	*0.616*
Abortion				
≤ 1	1	1.79 (0.92 – 3.49)	1.13 (0.58 – 2.48)	*0.134*
> 1	1	2.60 (0.59 – 11.49)	1.56 (0.24 – 10.03)	*0.409*
Tobacco use				
Never	1	1.98 (0.83 – 4.73)	0.92 (0.32 – 2.64)	*0.098*
Former	1	1.31 (0.37 – 4.68)	1.54 (0.33 – 7.33)	*0.854*
Current	1	2.56 (0.78 – 8.36)	1.50 (0.38 – 5.95)	*0.258*
Duration of tobacco use				
≤ 10 years	1	2.29 (1.07 – 4.91)	1.28 (0.51 – 3.21)	*0.052*
> 10 years	1	1.43 (0.48. – 4.20)	0.97 (0.28 – 3.38)	*0.678*
Oral Contraceptive use				
Never	1	0.85 (0.22 – 3.33)	0.28 (0.05 – 1.41)	*0.201*
Former	1	2.50 (0.79 – 7.85)	2.29 (0.52 – 10.01)	*0.274*
Current	1	2.30 (0.95 – 5.52)	1.57 (0.57 -4.43)	*0.146*
Duration of contraceptive use				
1 – 60 months	1	2.06 (0.76 – 5.58)	1.71 (0.52 – 5.67)	*0.356*
≥ 60 months	1	2.68 (1.02 – 7.05)	1.97 (0.62 – 6.23)	*0.127*
Parity				
0 – 1	1	2.31 (0.86 – 6.18)	2.39 (0.74 – 7.66)	*0.204*
> 1	1	1.64 (0.74 – 3.64)	0.74 (0.29 – 1.89)	*0.082*

Table 6. Interaction between p53 polymorphism and selected environmental risk factors (Case-only study).

TP53 Polymorphism	Smoking				
	Never	Former		Current	
	N	N	$OR_{interaction}$ (95% CI)	N	$OR_{interaction}$ (95% CI)
P/P	11	07	1	08	1
A/P	53	23	0.68 (0.21 - 2.25)	41	1.06 (0.35 - 3.23)
A/A	12	09	1.18 (0.27 - 5.17)	12	1.37 (0.35 - 5.53)
	Oral contraceptive use				
	Never	Former		Current	
	N	N	$OR_{interaction}$ (95% CI)	N	$OR_{interaction}$ (95% CI)
P/P	08	07	1	11	1
A/P	15	28	2.13 (0.56 - 8.30)	74	3.59 (1.09 - 11.84)
A/A	04	08	2.29 (0.37 - 14.99)	21	3.82 (0.78 - 19.85)
	Number of sexual partners				
	1 - 2	3 - 4		≥ 5	
	N	N	$OR_{interaction}$ (95% CI)	N	$OR_{interaction}$ (95% CI)
P/P	06	10	1	10	1
A/P	32	45	0.84 (0.56 - 8.30)	40	0.75 (0.21 – 2.57)
A/A	07	12	1.03 (0.21 – 5.05)	14	1.20 (0.25 - 5.77)
	Sexual onset				
	> 16 years old			≤ 16 years old	
	N			N	$OR_{interaction}$ (95% CI)
P/P	13			13	1
A/P	51			66	1.29 (0.51 – 3.29)
A/A	09			24	2.67 (0.79 - 9.16)
	Number of abortions				
	> 1			≤ 1	
	N			N	$OR_{interaction}$ (95% CI)
P/P	04			22	1
A/P	25			92	1.49 (0.43 – 5.66)
A/A	05			28	0.98 (0.19 - 5.06)

7. Artigo – 3

Genetic, environmental and clinical factors related to treatment failure of cervical precancerous lesions among Brazilian women

Abstract:

Objective: To ascertain the risk of SIL treatment failure considering selected *TP53* polymorphism at codon 72 and environmental and clinical risk factors among Brazilian women. **Methods:** A prospective study was carried out among 285 outpatients treated for cervical precancerous lesion at the Brazilian National Cancer in Rio de Janeiro between 2004-2008. All patients were interviewed at admission to ascertain epidemiological and clinical characteristics, had blood samples collected, had a colposcopic examination, and signed an informed consent. P53 polymorphism was ascertained using PCR-RFLP procedures. After treatment, the study population was followed up with Pap-test exams along 2 years. Treatment failure was evaluated using three different outcomes definitions: any altered Pap test during follow up (failure1); a first HSIL or two subsequent LSIL cytological results (failure2); and histological confirmation of an altered tissue (failure3). Statistical analysis to evaluate failure univariate risks was performed using Kaplan Meier method, and the Proportional Cox Regression to ascertain hazard ratios and their 95% CI. **Results:** P53 polymorphisms frequencies in the studied sample were 177 (62.1%) of Arg72Pro allele (A/P), 55 (19.3%) of Arg72 allele (A/A), and 53 (18.6%) of Pro allele (P/P). The risks for SIL treatment failure related to the involvement of endocervix margins were: $_{adj}HR_{failure1:}$ 1.88 (95%CI:1.19-2.99); $_{adj}HR_{failure2}$: 2.00 (1.02-3.91); $_{adj}HR_{failure3}$: 7.01 (1.73-28.44). Current smoking was also statistically related to an increased risk for SIL treatment failure ($_{adj}HR_{failure2:}$ 1.78 (95%CI:1.03-3.08); $_{adj}HR_{failure3:}$ 3.90 (95%CI:1.28-11.91)). Compared to Arg/Arg form, the risks for treatment failure were: Arg/Pro: $_{adj}HR_{failure1}$=1.22 (95%CI:0.70-2.12), $_{adj}HR_{failure2:}$ 1.28 (95%CI:0.55-2.98), $_{adj}HR_{failure3:}$ 1.51 (95%CI:0.23-9.80); Pro/Pro: $_{adj}HR_{failure1}$=1.43 (0.91-2.22), $_{adj}HR_{failure2:}$ 1.28 (95%CI:0.64-2.35), $_{adj}HR_{failure3:}$ 1.41 (95%CI:0.31-6.52). **Conclusion:** Our results suggest that P53/Pro72 polymorphim seems to be the associated to and increased risk of SIL treatment failure, despite statistically not significant. Margins involvement and current tobacco smoking presented independent risk for SIL treatment failure among the studied Brazilian women.

Introduction:

Cervical cancer is the second commonest cancer in women worldwide and the main cause of cancer-related mortality in women in developing countries[1]. The causal role of HPV on cervical cancer has been biologically and epidemiologically established[2] and it is now well recognized that persistent infection with high-risk human papillomavirus (HPV) is the primary cause of cervical cancer, present in over 99% of all cases[3, 4]. Organized cytological screening programs have been shown to reduce the incidence of cervical cancer by 80%, but the success of such programs is dependent on the coverage, effective treatment of precancerous lesions and follow-up subsequently to treatment, as women treated for pre-invasive cancer are at risk for recurrence disease.

Several techniques have been used to treat Squamous Intraepithelial Lesion (SIL), including cone biopsy, cryotherapy, laser vaporization and loop electrosurgical excision procedure (LEEP), also known as large loop excision of the transformation zone (LLETZ)[5, 6]. Cone biopsy (or conization) and LEEP are surgical procedures, in which the entire transformation zone is removed by cutting or by use of a loop electrode [6].

The LEEP treatment efficiency is well known and has been widely reported [7, 8]. However, it has also been reported that the recurrence of disease after treatment is relatively high, ranging from 5 to 36%[5, 7] and that HPV infection is one of the main risk factors related to such failure. Fortunately, clearance rate of HPV infection after conservative SIL treatment may vary from 82.3 to 92.2% along 2 years of follow-up[7, 8]. On the other hand, women treated for pre-cancerous lesions have an increased risk for HPV persistence and reappearance [7], which might be related to clinical and behavioral factors such as margins involvement, development of sexually transmitted diseases during follow-up, and amount of sexual partners. Furthermore, the reported variability according to failure

risk of SIL treatment may not be completely explained by HPV infection, as HPV is a necessary, but not sufficient factor for SIL development. Genetic host factors, which are sensitive to their prevalence on the studied population [9, 10], besides epidemiological [11] and methodological factors, such as reliable exposures assessment and outcome definitions, also seem to be involved.

Viral oncogenes, such as E6 and E7, have been shown to be the main contributors to the development of HPV-induced cervical cancer and increased expression, probably due to integration of the viral DNA in the host cell genome, which has been detected in invasive cancers and in a subset of high grade lesion[12]. Inactivation of tumor suppressor p53 and/or retinoblastoma protein (pRb) is a common event for the carcinogenesis of human cell. Both E6 and E7 HPV oncogenes interact with and inhibit the activities of these tumor suppressors [13]. It has been proposed that two wild-type p53 forms, proline or arginine at the amino acid residue 72, might differently contribute to cervical cancer development [14, 15]. The association between p53 polymorphism and cervical cancer development has been supported by cross-sectionals and case-control studies [15-17]. Despite the fact that some researchers have studied p53 protein as a prognostic marker in cervical cancer, they were developed among women with advanced cervical cancer, and contradictory results have been reported [9, 18]. Thus, little is known about the role of p53 polymorphism on SIL treatment failure considering environmental and clinical factors.

Therefore, the aim of this study is to ascertain the risk of SIL treatment failure considering host genetic factors (*TP53* polymorphism at codon 72), environmental, and clinical risk factors according to different failure definitions among Brazilian women.

Material and Methods

Study Population and Design

The study population addressed in this investigation included women enrolled from the coverage of the Hospital of Cancer II, Brazilian National Cancer Institute, an oncologic reference center located in the city of Rio de Janeiro. This institution is part of the Brazilian National Health System, with universal free health care provided for all population, regardless geographic, socio-economic or demographic characteristics.

Women demanding the Gynecologic Oncology outpatient service and showing altered Pap test results (LSIL and HSIL, but not invasive cervical cancer) between October 2004 to May 2006, being further referred to colposcopy, were invited to participate in this investigation if attending the following criteria: being older than 17 yr. having not been submitted to any cervical treatment in the past 6 months, being free of any psychiatric disease, being not currently pregnant, having an intact uterus, no antecedents of hysterectomy after treatment, and agreeing to participate signing an informed consent, further obtained from all study participants. Periodical visits of women who became pregnant during follow-up were temporarily interrupted until delivery, being further invited to participate in the follow-up. All women with a hysterectomy indication at the enrollment or who became HIV+ during the follow-up period were excluded.

Data Collection

This is a longitudinal study involving repeated measurements on the individual participants over time of selected risk factors such as age, ethnicity (skin color) [19], sexual behavior, reproductive history, history of sexually transmitted diseases (STDs), contraceptive methods use, smoking, and cervical outcome (presence of pre-invasive

cervical neoplasia). Serologic tests for HIV infection was carried out on the entered study time and every six months until the end of study follow-up.

Variables Categorization

Histological diagnosis was graded according to the Bethesda System[20] in LSIL, including condylomata and cervical intraepithelial neoplasia grade I (CIN I), and HSIL, including CIN II and CIN III/ *in situ* carcinoma (SCC). Histological normal tissue presenting only reactive/reparative changes was categorized as negative (disease-free). Surgical margins involvement was classified as clear (no margins involvement), ectocervix involvement, only or endocervix involvement. Cytological results were classified as LSIL, including CIN I and undetermined squamous cell carcinoma and/or undetermined adenosquamous cell carcinoma (ASCUS/AGUS), and as HSIL including CIN II and CIN III [20].

Tobacco smoking was classified according to IARC definition [21] as current (over 100 cigarettes smoked in a lifetime), former (those who have quit smoking in the past 6 months at least), and never smokers; information on the age at starting and stopping smoking, the duration of smoking and the average number of cigarettes smoked per day were also collected. The number of cigarettes smoked up to the every follow-up visit was calculated as the average of smoked cigarettes per day multiplied by the number of days up to the visit, at each visit.

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 never user. Duration of contraceptive use was stratified in $\leq$77 months and >77 months. Ethnicity was classified as self-reported skin color [19] and classified as

white, black, and multiethnic. All analyses were additionally stratified by age at first intercourse (≤ 16, and > 16 years), amount of full-term pregnancies/parity (≤ 2 and > 2), lifetime amount of sexual partners (1-2, 3-4, ≥ 5), and a new partner during follow up (0 and ≥ 1), number of abortions in a lifetime (< 2 and ≥ 2), and presence of sexually transmitted diseases at follow up (yes/no).

The studied outcome (cervical lesion treatment failure) was defined according to three different criteria, as follow: the observation of any altered Pap test (LSIL/HSIL) at the first follow-up visit (named failure 1); either a new HSIL/cancer or two subsequent LSIL diagnosis at Pap tests carried out along follow-up (failure 2); and a histologically confirmed altered cervical tissue during follow up (failure 3).

Clinical procedures and Follow-up

After clinical examination, cervical biopsy and peripheral blood samples were obtained for histological diagnosis and single nucleotide polymorphism tests. A questionnaire with required information was also applied by two trained nurses. All patients with LSIL or HSIL lesion underwent washing of the cervix and lower genital tract with 4-6% acetic acid solution, and visual inspection under colposcopy. The colposcopy impression, lesion size and suspected grade were reported. Suspicious lesion were biopsied or totally extracted (by either cone biopsy, partial ablation with diathermy or Loop electrosurgical excision procedure - LEEP), and if the colposcopic evaluation was unsatisfactory Pap smear test was carried out. When the result of the Pap exam done at the time of colposcopy was altered, endocervical curettage (ECG) was performed.

Subjects enrolled into the study were followed during 2-years period in pre-scheduled return visits. The follow-up visits took place at intervals of 3 months in the first year, and at each 4 months in the second year. Pap smears were obtained (whose reports are based on the Bethesda system for cytological diagnosis) and epidemiological variables whose status could change over time (smoking status, parity, STDs history, sexual behavior), were updated in each visit. Referrals for colposcopy and colposcopic-directed punch biopsies were indicated for patients with persistent abnormal cytologic findings, i.e., two consecutive Pap tests with a 3-month interval between them. The patients with colposcopic-directed punch biopsies that reveled CIN 1 or CIN 2-3 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 (e.g. menometrorrhagia). Follow-up length was defined as the period of time lapsed until a confirmed diagnosis of treatment failure by histology since the first treatment, or the last follow-up date in those cases showing no evidence of disease.

Delays in returning for a given appointment cause the following one to be postponed correspondingly, so the timing between the visits may vary. Those who miss appointments were contacted by phone and/or letter. These attempts at contacting subjects were repeated once a month until an appointment could be scheduled or the woman explicitly stated that she wished to drop out of the study.

TP53 polymorphism genotyping

After DNA extracting from leukocytes of peripheral blood through salting procedure protocol [22], genotyping for p53 polymorphism at codon 72 was carried out

using a PCR-RFLP based method. 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 60ºC. Bands of cut and uncut products were visualized on 3% agarose gels. The presence of the wild-type Arg allele was indicated by bands of 169 and 127 bp, whereas no digestion of the mutant Pro allele could be observed.

Statistical analysis

Chi-square test for heterogeneity and further conditional probability of treatment failure according to studied variables and their hazard ratios ascertainment were carried out.

The Kaplan-Meier method was used to estimate the cumulative risk of treatment failure, being the ascertained curves compared using Log-Rank test. The amount of smoked cigarettes was used as a conditional variable. Finally, a Cox proportional hazard regression analysis was performed to examine the hazard ratio (HR) of treatment failure according to the different associated risk factors considering different failure definitions. All analyses were carried out using SPSS software package version 10.0 for windows (SPSS Inc., Chicago, IL).

Results

Environmental, clinical and genetic host factors distributions of the enrolled participants are presented at table 1. According to this table, P53 polymorphisms are distributed as follow 177 (62.1%) women showing an Arg72Pro allele (A/P), 55 (19.3%) just an Arg72 allele (A/A), and 53 (18.6%) just a Pro allele (P/P). The majority of participants (47%) were under 30 years old, multiethnic origin(48.8%), had HSIL cytology at the study entrance (75.4%), and had clear margins at treatment (64.3%).

Table 2 presents the probability of SIL treatment failure in 24 months of follow-up, according to different failure definitions. According to this table the probability of SIL treatment failure increases with the tobacco smoke use status varying from 17.6% among never users to 32.2% among current users, in failure 2 (log rank chi square, p = 0.0237); and from 4.7% among never users to 12.9% among current users, if considered histology results as failure (log rank chi square, p=0.0176). Similar results are seen to oral contraceptive use status, where the probability of failure 2 varied from 17.6% among never users to 33.9% among current users (log rank chi square, p=0.095), and for failure 3, varied from 4.7% among never users to 11.5% among current users (log rank chi square, p =0.1006). Duration of tobacco smoking use over 10 years increased statistically the probability of presenting at least one altered Pap smear in 24months (failure 1), reaching a SIL treatment failure likely of 64.1% (log rank chi square, p =0.0231). Margin involvement was statistically related to SIL treatment failure regardless the used definition, increasing with the depth of margins involvement, and ranging from 15.9% observed in clear margins to 29.0% whenever presenting an endocervix involvement (failure 2). When analyzing the treatment failure defined according to the histological result, it ranged from 3.1% among

cases with clear margins to 14.7% when endocervix margins were involved (log rank chi square, p =0.0017).

Involvement of endocervix margins were an independent risk factor for SIL treatment failure, ranging from $adjHR_{failure1=}$1.88-fold (CI:95%=1.19-2.99) to $adjHR_{failure3=}$7.01-fold (CI:95%=1.73-28.44) as compared to clear margins (table 3). Current tobacco smoking use was also an independent risk factor for SIL treatment failure as compared to never smokers, presenting an $adjHR_{failure2}$=1.78 (CI:95%=1.03-3.08) and $adjHR_{failure3}$=3.90 (CI:95%=1.28-11.91). Duration of tobacco use (> 10 years), current oral contraceptive use, the amount of sexual partners ($\geq$ 5), HSIL histological result at treatment, and STD at follow-up showed increased association to SIL treatment failure but no significance was found (table 3).

Interestingly, Pro72 and Arg72Pro p53 polymorphisms showed increased risks for SIL treatment failure comparatively to Arg72 allele, presenting adjusted risks of, respectively, 1.44-fold and 1.51-fold for histologically confirmed cervical lesion (failure 3 definition), despite statistically not significant. Similar patterns were observed for the other failure definitions.

Conditional probabilities for SIL treatment failure according to the number of cigarettes smoked are presented (table 4 and Figure 1). The probability of SIL treatment failure showed a statistically significant association with margin involvement among smokers (over 5,000 cigarettes smoked in a lifetime). On the other hand, the probability of treatment failure was inversely associated with the age, being higher among women 18-30 years old than observed among older one.

Current oral contraceptive use compared to never use (failure 2 definition = 28.7% vs. 26.8%; failure3 definition =9.3% vs. 5.9%, respectively) and pregnancy at follow up

seem to be influenced by the number of cigarettes smoked, related to the probability of SIL treatment failure in all three outcome definitions (failure1=48.5% vs. 46.5%; failure2=41.4% vs. 23.3%; failure3=23.8% vs. 6.4%, for pregnancy vs. no pregnancy, respectively).

Discussion

The role of *TP53* polymorphism either on cervical cancer development or on cervical cancer treatment failure has been worldwide explored by many researchers and the results have been frequently controversial [9, 14-16, 23]. In half of human cancers the tumor suppressor p53 is damaged by somatic mutation in tumor cell. This protein is at the center of cell regulatory pathways when reacting to cellular stress [24]. Apoptosis, cell cycle arrest at the G1 checkpoint, and cellular senescence are all mechanism triggered by activated p53, thereby preventing cancer growth. When p53 is damaged, cancer cells continue to multiply. The SNP rs1042522 is located in exon 4 and results in Arginine to Proline amino acid substitution in position 72 of the p53 protein**.** However, there are marked functional differences between both p53-protein forms. In summary, the Arg72 form is more efficient in apoptosis induction, whereas the Pro72 form induces more G1 arrest and enhances activation of p53 dependent DNA repair [25]. The frequency of the Pro72 allele or Arg72Pro ranges from 70% among South Africans to 23% among western Europeans [26]. Pro72 is probably the ancient allele, but the reason for the high frequency of Arg72 among Europeans is unclear.

The p53 genetic polymorphisms distributions were not statistically different in the histological categories among the women recruited in this study. However, women with histological results of invasive cancer at treatment presented a statistically different distribution of such polymorphisms with an increased proportion of Arg/Arg variant

comparing to Pro/Pro variant (data not shown). As women with invasive cancer had had histerectomy, they were therefore excluded from the prospective phase of the study. This fact also may partially explain why Arg/Arg form was not statistically related to the treatment failure. Women with cervical cancer are not elligible to perform this study population since they are not at risk of pre-cancer treatment failure, and therefore they were not included in this analysis. Thus, since the study population is performed by women with pre-cancer lesion who underwent to conservative treatment and, therefore, are at risk of SIL treatment failure, and that the p53 genetic polymorphisms distributions were at Hardy-Waimberg equilibrium among pre-cancer lesions, it is probably possible to consider this study population free of sellection bias.

Although it has been shown that p53Arg polymorphic variant is more susceptible to the effects of HPV E6 than the p53Pro, increasing the risk of cervical cancer development [14], the role of p53 72 polymorphism on SIL treatment failure remains unknown, as a clearance rate of HPV infection after SIL treatment ranges from 82.3% - 92.2% [7, 8]. Despite the no observation of statistical significance, our study results suggest that p53Pro homozygous and 53Arg/Pro heterozygous alleles seems to be risk factors for treatment failure, regardless the use of different outcome definition criteria (table 3). This finding is corroborated by others studies that reported p53Pro as a risk factor for cervical cancer development (OR=4.54, CI:95% 1.00-20.56), endometrial cancer (OR=3.56, CI:95% 2.10-6.64), and ovarian cancer (p=0.002) [10, 27, 28]. According to Bhattacharya et al (2007) [10] this genetic polymorphism 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 transforming potential of the virus. Thus, these authors showed that HLA-B*07 and p53Pro72Pro alleles carriers women were at higher

risk of cervical cancer development when compared to non-carriers of both (OR=14.05, CI:95% 1.11-177.30). Another possible explanation is that, after SIL treatment with LEEP, the role of p53Arg allele (apoptosis) might be more efficient than p53Pro (G1 arrest) and p53Arg72Pro alleles on preventing treatment failure, as after LEEP around 72.9% women clear the HPV infection, only 5.7% have persistent infection and 21.3% are re-infected with the same or different HPV type [7]. This hypothesis is supported by the fact that women with margins involvement (which may suggest residual disease and, therefore, persistent HPV infection) were significantly at higher risk of SIL treatment failure when compared to women with clear margins. On the other hand, there were no statistically significant differences in the conditional probability for treatment failure in 24 moths for all p53 forms (table 3).

Despite the acknowledgement of tobacco smoking as a cause of cervical cancer by the International Agency for Research on Cancer/WHO [29], it remains unclear how different patterns of tobacco use, such as the amount smoked or duration of smoking [11], and if its relationship with others risk factor affects a woman's risk of developing cancer and/or SIL treatment failure. The results of the present study (tables 2 and 3) provide evidence that current smokers, compared to never smokers, are likely to failure on treatment and are at significantly higher risk of treatment failure, even adjusted by age and ethnicity. When analyzing the duration of tobacco use it is also possible to see that long term smokers (>10 years) are more likely to failure on treatmento when compared to smokers under 10 years. These findings may be explained by the fact that defects in DNA repair are related to carcinogenesis, and DNA damage has been found in cervical tissue of smokers with high DNA adduct level [30, 31]. Benzo(a)pyrene has been detected in cervical tissue, and DNA adducts were twice as common in smokers. Also, cell growth and

DNA damage induced by benzo(a)pyrene was higher in HPV-16 immortalized cervical cells than in normal tissue [32].

In studies approaching the association between smoking and cervical cancer, the main emphasis has been addressed on the potential confounding between smoking and cervical HPV infection, which is acknowledged as the main cause of cervical cancer. However, in a study developed by the International Collaboration of Epidemiological Studies of Cervical Cancer [11], which combined cohort and case-control studies from developed and developing countries (9,052 invasive cancer, 4,489 CIN3 cases, and 23,017 controls), evidence of a significantly increased risk of squamous cell cervical carcinoma for current smokers in analyses restricted to women who tested positive for a high-risk HPV was reported.

In our study, HPV infection could not be ascertained. Nevertheless, it was possible to investigate whether smoking increases the risk of SIL treatment failure in women who are equally likely to have been exposed to, rather than infected with, HPV. Thus, this was accomplished through stratification by two of the main proxy variables for HPV exposure, which are the amount of sexual partners and the occurrence of margins involvement, whose occurrence suggests residual disease.

The probabilities of SIL treatment failure according to the number of smoked cigarettes are presented at Figure 1. The analyzed data suggests that women who reported having 3-4 or 5 or more sexual partners in a lifetime were more likely to failure on the treatment when they smoked more than 5,000 cigarettes (according to failure 3 definition, 7.0% and 7.9%, respectively). Despite of the absence of statisticall significance, our results are similar to previous studies which reported that smoking could act increasing the risk of cervical cancer at different stages of natural history of the disease; for example, increasing

the probability of becoming infected given an exposure to HPV, or of developing a persistent cervical HPV infection, both being possibly explained by the tobacco-related immune competence reduction [33, 34]. Another risk factor that was found to be associated to SIL treatment failure was oral contraceptive use with an adjusted risk varying from 1.34-fold (95% CI 0.88-2.01) considering failure1 definition to 3.16-fold (95% CI 0.86-11.57) considering failure3 definition, for current smokers as compared to never smokers. Similar results were also seen among former smokers (table 3). These results are supported by the literature as it has been reported that both estrogens and progesterone receptors are present in cervical neoplasia. The expression of both receptors is higher in immature squamous metaplasia of the transformation zone than in ectocervix [35]. Related to HPV, it is known that HPV has a tendency to transfect cells with progesterone receptors and that both HPV 16 and HPV 18 contain progesterone and glucocorticoid response elements that increase expression of the *HPV E6* and *E7* oncogenes, considered crucial in cell transformation, with gestagenic stimuli [36]. Progesterone and glucocorticoid hormones also enhance HPV mRNA levels rise, and significantly stimulate viral replication [37]. On the other hand, in the epithelium of transformation zone, where cervical neoplasia is initiated, 16-a-hydroxylation of estradiol occurs, yielding to 16-α-hydroxyestrone [38], which is linked to malignant transformation of estrogen-sensitive cells transfected by HPV. Thus, serum estrone was reported to be elevated in patients with CIN who were HPV-positive as compared to HPV-negative women with or without CIN [39].

The results of the present study are consistent with a recent study developed in Sweden (2007) which reported that absence of p53 protein expression was associated with increased serum progesterone and smoking among women with cervical cancer [40]. This interaction is supported by the fact that sex steroid hormones might play a role in the

process of p53 degradation promoted by E6-HPV [41], and by previous studies that show that exposure to smoke increased expression of dysfunctional p53 in gastric cancer [42], and that progesterone down-regulated p53 expression in breast cancer [43]. Another support for these findings are previous studies showing an increased S-phase fraction (mitosis) in smokers, as compared to non-smokers, and in fertile women with serum progesterone $\geq$ 2.6 mol/L as compared to those with lower levels [44]. A decreased survival of premenopausal women with low a serum estradiol/progesterone ratio who eventually died from cervical cancer was also reported [45].

To our knowledge, the present study is the first one which aimed to investigate prospectively the relationship between p53 polymorphism, tobacco smoking, and oral contraceptive use on SIL treatment failure among Brazilian women, considering different outcome definitions. The presented associations of p53/Pro allele with former/current smoking and former/current oral contraceptive use status on SIL treatment failure are biologically plausible, being supported by previous studies. However, as this study could accomplish a relatively small sample size, statistical significance of some of the observed findings with the studied risk factors could have been jeopardized.

If in one hand, considering failure deffinition as any kind of cytologic alteration (failure 1), it would seem that SIL treatment failure is high in our study (table 2); on the other hand, when considering more accurate criterias (failures 2 and 3 definitions), the probability of SIL treatment failure are according to those described in previous studies around the world [5, 7]. It is also possible to verify that as more precise is the failure deffinition of treatmen failure, as higher is the association strength and the statistical significance of our findings as well. This could support the causality hypothesis proposed by our study. Thus, the mentioned results should be confirmed by larger studies to be

developed in different populations. Nevertheless, our findings lend some evidence supporting the hypothesis that women with P53/Pro allele, currently smokers and oral contraceptive users, are at higher risk of SIL treatment failure.

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Table 1. Environmental, clinical and genetic host factors distribution of study population, Brazil.

Variable	Overall*	%	(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)
Multiethnic	139	48.8	(43.0 – 54.6)
Black	58	20.4	(15.7 – 25.0)
Margin involvement			
Clear	153	64.3	(59.3 – 71.5)
Ectocervix	43	18.7	(13.4 – 23.3)
Endocervix	38	15.9	(11.5 – 21.0)
Tobacco smoking 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**			
≤ 77 months	127	53.1	(46.8 – 59.5)
> 77 months	112	46.9	(40.5 – 53.2)
Duration of tobacco smoking**			
≤ 10 years	44	31.2	(23.6 – 38.9)
> 10 years	97	68.8	(61.1 – 76.4)
Cytology at entrance			
CIN I	16	5.7	(02.9 – 08.3)
CIN II	130	45.6	(39.8 – 51.4)
CIN III	85	29.8	(24.5 – 35.1)
ASCUS/AG-US	50	17.5	(13.1 – 22.7)
Cancer	04	1.4	(-)
Histology			
LSIL	129	45.3	(39.5 – 51.0)
HSIL	156	54.8	(49.0 – 60.5)
STD at follow-up			
No	263	92.3	(89.2 – 95.4)
Yes	22	7.7	(04.6 – 10.8)
Pregnancy at follow-up			
No	264	92.6	(89.6 – 95.7)
Yes	21	7.4	(04.3 – 10.4)

* Total may vary because of missing values; ** Analysis carried out among former and current users, only.

Table 2. Probability of SIL treatment failure in 24 months in 285 Brazilian women, according to different failure definitions, Rio de Janeiro (2004-2008).

Variable	% Treatment failure in 24 months, according to failure definition		
	1st altered test (failure1)	1st HSIL/2nd LSIL (failure 2)	Histological result (failure3)
***TP53* Polymorphsm**			
A/A	45.0	21.4	6.0
A/P	59.4	20.4	6.8
P/P	52.7	25.6	6.2
****Log-Rank: 95%**	*0.2033*	*0.6214*	*0.7433*
Age			
18 – 30	50.6	17.9	11.9
31 – 45	56.7	21.3	6.7
> 45	60.7	26.4	7.5
****Log-Rank: 95%**	*0.9583*	*0.4845*	*0.6855*
Ethnicity (skin color)			
White	57.6	19.6	11.1
Multiethnic	50.1	18.3	2.4
Black	63.8	33.3	9.7
****Log-Rank: 95%**	*0.2261*	*0.1877*	*0.0125*
Oral contraceptive use			
Never	51.7	17.6	3.1
Former	56.0	23.8	13.5
Current	67.7	33.9	11.5
****Log-Rank: 95%**	*0.4533*	*0.0945*	*0.1006*
Duration of contraceptive use*			
≤ 77 months	52.7	22.3	4.2
> 77 months	54.0	15.9	7.5
****Log-Rank: 95%**	*0.5943*	*0.0950*	*0.3375*
Tobacco smoking use			
Never	53.1	17.6	4.7
Former	50.1	15.4	3.7
Current	63.2	32.2	12.9
****Log-Rank: 95%**	*0.1985*	*0.0237*	*0.0176*
Duration of tobacco use*			
≤ 10 years	42.5	21.7	4.6
> 10 years	64.1	23.7	10.9
****Log-Rank: 95%**	*0.0231*	*0.7125*	*0.2438*
Histology			
LSIL	46.8	18.1	3.6
HSIL	45.4	22.2	7.8
****Log-Rank: 95%**	*0.7393*	*0.2249*	*0.0247*
Margin involvement			
Clear	42.2	15.9	3.1
Ectocervix	78.0	28.6	9.8
Endocervix	67.6	29.0	14.7
****Log-Rank: 95%**	*0.0000*	*0.0512*	*0.0017*
Menarche			
> 13 years old	54.3	23.8	5.2
≤ 13 years old	54.4	18.4	6.1
****Log-Rank: 95%**	*0.8858*	*0.9547*	*0.9077*
Sexual Onset			
> 16 years old	53.6	23.8	7.1
≤ 16 years old	55.2	16.9	4.6

****Log-Rank: 95%**		*0.8976*		*0.3961*		*0.2380*
Years from Menarche to sexual onset						
> 3	52.7		20.4		6.3	
≤ 3	54.6		20.3		5.4	
****Log-Rank: 95%**		*0.7671*		*0.9428*		*0.5355*
Sexual partners						
1 – 2	55.1		23.5		4.5	
3 – 4	47.4		13.5		4.9	
≥ 5	62.1		23.3		10.4	
****Log-Rank: 95%**		*0.0858*		*0.0318*		*0.1688*
Abortion						
< 2	52.6		19.6		4.2	
≥ 2	58.3		20.8		12.1	
****Log-Rank: 95%**		*0.6190*		*0.5375*		*0.0710*
Parity						
≤ 2	53.6		20.8		7.4	
> 2	55.3		19.4		5.5	
****Log-Rank: 95%**		*0.8030*		*0.5409*		*0.3356*
Pregnancy at follow-up						
No	54.1		19.5		5.4	
Yes	58.3		16.9		5.0	
****Log-Rank: 95%**		*0.8551*		*0.9101*		*0.3981*
STD at follow-up						
No	52.7		18.0		5.1	
Yes	64.2		40.4		9.1	
****Log-Rank: 95%**		*0.5032*		*0.1500*		*0.1037*
Sexual partners at follow-up						
0	51.7		19.8		5.7	
≥ 1	62.6		19.5		6.4	
****Log-Rank: 95%**		*0.2814*		*0.7604*		*0.9991*

Failure 1: First altered Pap test (LSIL/HSIL) at follow-up; **Failure2**: first HSIL/cancer or two LSIL altered Pap test**;** **Failure3**: histologically confirmed altered cervical tissue.

*Analysis carried out among former and current users, only.

** Log-Rank test obtained from Kaplan-Meier analysis.

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

Variables	1st. HSIL/LSIL Pap test (failure1)		1st. HSIL/2nd.LSIL Pap test (failure2)		Histological failure (failure3)	
	Crude HR (CI:95%)	* adj. HR (CI:95%)	crude HR (CI:95%)	*adj. HR(CI:95%)	crude HR (CI:95%)	* adj. HR(CI:95%)
P53 Polymorphism						
Arg/Arg	1	1	1	1	1	1
Arg/Pro	1.27 (0.74 – 2.20)	1.22 (0.70 – 2.12)	1.41 (0.62 – 3.22)	1.28 (0.55 – 2.98)	1.63 (0.27 – 9.77)	1.51 (0.23 – 9.80)
Pro/Pro	1.48 (0.95 – 2.31)	1.43 (0.91 – 2.22)	1.39 (0.70 – 2.76)	1.28 (0.64 – 2.35)	1.79 (0.40 – 8.10)	1.41 (0.31 – 6.52)
Age						
18 – 30	1	1	1	1	1	1
31 – 45	1.03 (0.72 – 1.47)	1.10 (0.75 – 1.54)	1.05 (0.61 – 1.83)	1.06 (0.61 – 1.86)	1.43 (0.46 – 4.45)	1.77 (0.56 – 5.59)
≥ 45	1.06 (0.70 – 1.61)	1.05 (0.69 – 1.59)	1.44 (0.78 – 2.65)	1.43 (0.77 – 2.64)	1.69 (0.45 – 6.02)	1.89 (0.52 – 6.82)
Ethnicity						
White	1	1	1	1	1	1
Multiethnic	0.83 (0.57 – 1.19)	0.85 (0.59 – 1.22)	0.77 (0.44 – 1.34)	0.76 (0.43 – 1.34)	0.14 (0.03 – 0.66)	0.13 (0.03 – 0.62)
Black	1.17 (0.76 – 1.79)	1.15 (0.74 – 1.79)	1.34 (0.71 – 2.51)	1.29 (0.67 – 2.49)	0.95 (0.32 – 2.86)	0.85 (0.27 – 2.71)
Margins Involvement						
Clear	1	1	1	1	1	1
Ectocervix	2.40 (1.58 – 3.64)	2.36 (1.55 – 3.60)	1.74 (0.90 – 3.37)	1.76 (0.90 – 3.41)	3.44 (0.69 – 17.08)	3.81 (0.76 – 19.08)
Endocervix	1.91 (1.21 – 3.00)	1.88 (1.19 – 2.99)	2.08 (1.07 – 4.01)	2.00 (1.02 – 3.91)	8.60 (2.15 – 34.41)	7.01 (1.73 – 28.44)
Tobacco smoking use						
Never	1	1	1	1	1	1
Former	0.88 (0.58 – 1.33)	0.89 (0.58 – 1.36)	0.85 (0.44 – 1.67)	0.85 (0.43 – 1.69)	0.90 (0.18 – 4.67)	0.81 (0.15 – 4.35)
Current	1.29 (0.90 – 1.45)	1.28 (0.89 – 1.86)	1.85 (1.09 – 3.13)	1.78 (1.03 – 3.08)	3.68 (1.23 – 10.97)	3.90 (1.28 – 11.91)
Sexual partners						
1 - 2	1	1	1	1	1	1
3 – 4	0.76 (0.51 – 1.33)	0.77 (0.52 – 1.16)	0.49 (0.25 – 0.96)	0.50 (0.26 – 0.99)	1.44 (0.32 – 6.45)	1.62 (0.36 – 7.38)
≥ 5	1.17 (0.81 – 1.70)	1.16 (0.80 – 1.68)	1.13 (0.67 – 1.93)	1.09 (0.64 – 1.87)	2.98 (0.81 – 11.00)	2.77 (0.75 – 10.24)
Oral contraceptive use						
Never	1	1	1	1	1	1
Former	1.10 (0.75 – 1.61)	1.09 (0.74 – 1.61)	1.25 (0.68 – 2.28)	1.20 (0.65 – 2.22)	3.02 (0.97 – 9.40)	3.03 (0.94 – 9.73)

Current	1.31 (0.86 – 2.00)	1.34 (0.88 – 2.01)	1.90 (1.06 – 3.43)	2.01 (1.10 – 3.65)	2.68 (0.75 – 9.52)	3.16 (0.86 – 11.57)
Duration of OC use**						
≤ 77 months	1	1	1	1	1	1
> 77 months	0.91 (0.64 – 1.29)	0.94 (0.66 – 1.35)	0.62 (0.35 – 1.09)	0.63 (0.35 – 1.12)	1.74 (0.55 – 5.49)	1.96 (0.60 – 6.38)
Duration of tobacco use**						
≤ 10 years	1	1	1	1	1	1
> 10 years	1.79 (1.10 – 2.98)	2.30 (1.26 – 4.19)	1.14 (0.57 – 2.25)	1.28 (0.55 – 2.96)	2.43 (0.52 – 11.31)	2.90 (0.51 – 16.52)
Histology						
LSIL	1	1	1	1	1	1
HSIL	0.95 (0.69 – 1.30)	0.98 (0.71 – 1.35)	1.35 (0.83 – 2.20)	1.49 (0.90 – 2.47)	3.81 (1.10 - 13.37)	4.67 (1.27 – 17.05)
STD at follow-up						
No	1	1	1	1	1	1
Yes	1.20 (0.69 – 2.09)	1.19 (0.69 – 2.07)	1.67 (0.83 – 3.37)	1.65 (0.81 – 3.37)	2.72 (0.77 – 9.57)	2.44 (0.68 – 8.71)
Abortion						
< 2	1	1	1	1	1	1
≥ 2	1.10 (0.74 – 1.64)	1.01 (0.66 – 1.53)	1.19 (0.68 – 2.10)	1.04 (0.57 – 1.88)	2.47 (0.89 – 6.81)	1.72 (0.56 – 5.25)

**HR adjusted by age and ethnicity;* ** Analysis carried out among former and current users, only.
Failure 1: First altered Pap test (LSIL/HSIL) at follow-up; **Failure2**: first HSIL/cancer or two LSIL altered Pap test**; Failure3**: histological confirmation of altered cervical tissue.

Table 4. Probability of SIL treatment failure among Brazilian women, according to number of smoked cigarettes, Rio de Janeiro (2004-2008).

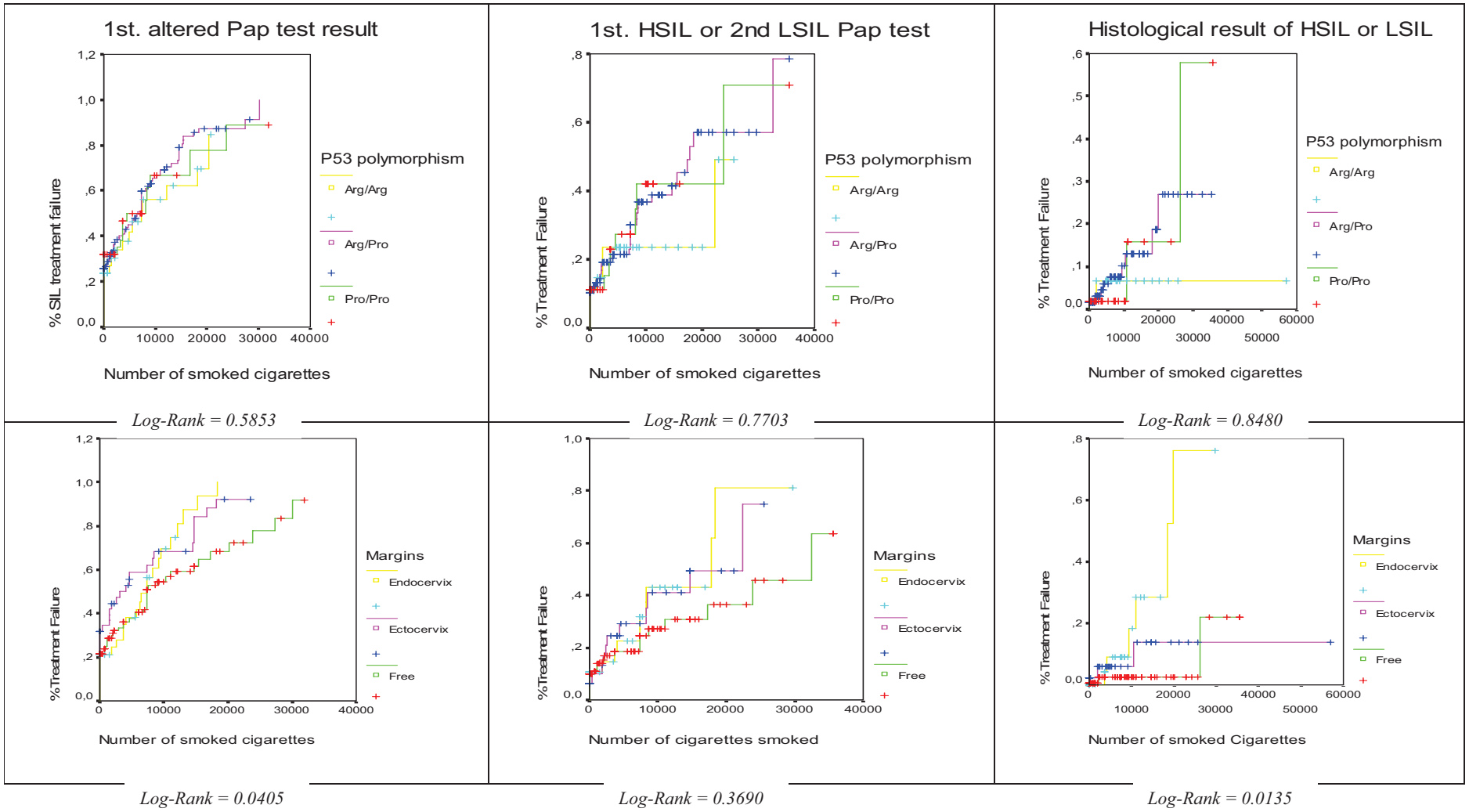
Variables	1st. HSIL/LSIL Pap test (failure1)		1st. HSIL/2nd.LSIL Pap test (failure2)		Histological failure (failure3)	
	% failure more than 5,000 cig	*L-R: 95%*	%failure more than 5,000 cig.	*L-R: 95%*	%failure more than 5,000cig.	*L-R: 95%*
Overall	**45.4**		**23.0**		**6.3**	
P53 Polymorphism						
Arg/Arg	42.0		23.5		6.7	
Arg/Pro	45.9	*0.5853*	23.0	*0.7703*	7.5	*0.8480*
Pro/Pro	50.2		27.4		1.9	
Age						
18 – 30	55.2		25.3		7.0	
31 – 45	40.3	*0.0784*	17.2	*0.5078*	6.4	*0.9757*
> 45	44.8		27.3		5.4	
Ethnicity						
White	52.4		27.8		11.9	
Multiethnic	41.8	*0.1382*	19.7	*0.2080*	1.6	*0.0018*
Black	50.3		28.3		9.3	
Margin involvement						
Clear	37.6		18.5		2.5	
Ectocervix	58.9	*0.0405*	29.2	*0.3690*	5.9	*0.0135*
Endocervix	41.8		22.6		9.1	
Sexual partners						
1 – 2	53.8		29.9		3.1	
3 – 4	43.3	*0.2293*	16.3	*0.0620*	7.0	*0.6469*
> 5	43.6		23.3		7.9	
Oral contraceptive use						
Never	49.5		26.8		5.9	
Former	48.8	*0.9771*	17.5	*0.6587*	4.8	*0.3368*
Current	38.8		28.7		9.3	
Duration of contraceptive use						
< 77 months	54.2	*0.3266*	27.2	*0.0369*	2.4	*0.2988*
> 77 months	42.0		18.4		8.0	
Histology						
LSIL	52.0	*0.1041*	20.9	*0.8518*	3.9	*0.1016*
HSIL	41.1		24.1		7.7	
STD at follow-up						
No	46.9	*0.8513*	23.3	*0.1940*	6.2	*0.2729*
Yes	35.4		28.7		9.1	
Abortion						
< 2	44.1	*0.5117*	21.8	*0.7309*	5.0	*0.1735*
> 2	51.9		30.6		11.0	
Sexual Onset						
> 16 years old	52.5	*0.1821*	32.2	*0.1447*	9.1	*0.0460*

< 16 years old	41.3		16.7		5.3	
Parity						
< 2	47.8	*0.0493*	23.6	*0.4260*	7.3	*0.0628*
> 2	32.7		22.3		4.9	
Pregnancy at follow-up						
No	46.5	*0.3597*	23.3	*0.6616*	6.4	*0.6388*
Yes	48.5		41.4		23.8	

* Analysis carried out among former and current users, only.

Failure 1: First altered Pap test (LSIL/HSIL) at follow-up; **Failure2**: first HSIL/cancer or two LSIL altered Pap test**; Failure3**: histologically confirmed altered cervical tissue.

Figure1. Pre-cancer treatment failure probability according to smoked cigarettes and different failure definitions, Rio de Janeiro, 2004-2008.



8. Considerações Finais

As modificações no padrão de incidência do câncer cervical observadas no Brasil e no mundo desde o final da década de 60, especialmente entre as mulheres jovens, podem ter sido influenciadas pelas transformações nos padrões sexuais das mulheres observadas a partir deste período. Este padrão de aumento parece não sofrer modificações causadas pelos programas de rastreamento e detecção precoce do câncer cervical no Brasil.

Os resultados de nosso estudo sugerem a existência de uma diferença significativa na distribuição das características clínicas e epidemiológicas associadas ao câncer de colo uterino entre os grupos etários estudados (18-30 anos, 31- 49 anos e 50-68 anos) no Rio de Janeiro. Este fato teria contribuído para a observação de que os fatores de risco associados ao câncer cervical podem apresentar diferentes contribuições para este risco dependendo do grupo etário considerado. Esta variabilidade pode resultar de diferenças sociais, como relativas a escolaridade, culturais e comportamentais observadas entre os mesmos.

No entanto, em conjunto, esta heterogeneidade cultural, educacional e comportamental entre os grupos etários da população não seria suficiente para explicar completamente o padrão de distribuição dos riscos de câncer cervical na amostra estudada. O fato de que mulheres de um mesmo grupo etário, e igualmente submetidas a uma dada exposição ambiental, venham, no entanto, a apresentar riscos distintos de desenvolvimento do câncer cervical, aponta para o possível papel dos fatores genéticos ligados ao hospedeiro (como o polimorfismo do gene *TP53*), que estariam interagindo com os fatores ambientais, e modulando desta maneira os diferentes graus de suscetibilidade para o desenvolvimento desta neoplasia.

Assim, nossos achados sugerem que na população brasileira, mulheres que são heterozigoto (p53Arg/Pro72) apresentam um risco 1.92 vezes mais elevado de

desenvolvimento de HSIL/Câncer do que mulheres homozigoto para o alelo Pro (Pro/Pro), independente da idade ao diagnóstico, idade da menarca e cor da pele. Além disso, as formas Arg/Pro e Arg/Arg parecem interagir com fatores ambientais (como tabagismo atual, uso atual de anti-contraceptivos orais, início precoce da atividade sexual e multiplicidade de parceiros) no desenvolvimento do câncer cervical. No entanto, as mulheres homozigoto para o alelo Pro parecem experimentar um maior risco de falha no tratamento das lesões pré-neoplásicas, quando comparadas às mulheres homozigoto para o alelo Arg, sendo que este risco parece aumentar a medida que amplia-se o grau de acurácia do critério de falha. O mesmo padrão de associação entre diferentes critérios de falha é observado para variáveis ambientais (tabagismo, uso de contraceptivos orais, multiplicidade de parceiros) e clínicas (envolvimento das margens cirúrgicas, DST durante o seguimento e número de abortos), mesmo ajustando pela idade e etnia.

Entretanto, nossos resultados apenas são sugestivos de uma possível interação entre os polimorfismos do gene *TP53* no codon 72 do éxon 4 com variáveis clínicas e ambientais, no desenvolvimento do câncer cervical e no risco de falha de tratamento das lesões pré-neoplásicas. Portanto, outros estudos são necessários visando corroborar com os resultados do presente estudo.

Portanto, nossos resultados sugerem que, para que os programas de rastreamento e detecção precoce do câncer cervical sejam efetivos entre as mulheres do Rio de Janeiro, são necessárias diferentes abordagens entre os grupos etários específicos. Além disso, programas voltados para a educação sexual e controle do tabagismo, visando reduzir a exposição a fatores ambientais como o tabaco, uso de contraceptivos orais e exposição precoce ao risco de infecção pelo HPV (início precoce da atividade sexual), que podem interagir com diferentes graus de suscetibilidades do hospedeiro, devem passar a ser

abordagens prioritárias na prevenção primária do câncer de colo uterino se almejarmos um controle eficaz da incidência do câncer cervical no Brasil.

9 - Anexos

Anexo 1

Carta de Aprovação do CEP/INCA

INCA

COORDENAÇÃO DE PESQUISA
Serviço de Pesquisa Clínica-SPC-CPQ
Comitê de Ética em Pesquisa-INCA

Rio de Janeiro, 16 de agosto de 2004

Ilce Ferreira da Silva
Pesquisadora Principal

Ref. Prot. nº 30/04 – A influência do polimorfismo no códon 72 do gene Tp53 na evolução das lesões precursoras do câncer de colo de útero

Prezada Doutora,

Informo que o Comitê de Ética em Pesquisa do Instituto Nacional de Câncer após reanálise decidiu **aprovar** o Protocolo intitulado: A influência do polimorfismo no códon 72 do gene Tp53 na evolução das lesões precursoras do câncer de colo de útero, bem como seu Termo de Consentimento Livre e Esclarecido em 10 de agosto de 2004.

Estamos encaminhando a documentação pertinente para o CONEP, com vistas a registro e arquivamento.

Atenciosamente,

Dr. Luis Otávio Olivatto
Presidente do Comitê de Ética em Pesquisa
CEP-INCA

Anexo 2

Escola Nacional de Saúde Pública / Fundação Oswaldo Cruz

Departamento de Epidemiologia (DEMQS)

FICHA DE COLETA DE DADOS DE PATOLOGIA CERVICAL

Responsável: Ilce Ferreira da Silva Código no estudo: |__|__|__|__||__|

Instituição: _____

Data de Entrada no Estudo: / /

1 – <u>Identificação</u>:

1.1 – Nome: _____ MH:_____

1.2 – Naturalidade: _____ Nacionalidade: _____

1.3 Endereço _____

1.4 Bairro: _____1.5 Cidade:_____

CEP: -

1.6-Ponto de referência: _____

1.7-Tel. Contato: _____

1.8 - Data de Nascimento: / / Idade:_____

2 – <u>Dados Sociodemográficos</u>:

2.1 – Estado Civil: Solteira Casada Viúva Tem companheiro 99

2.2 – Cor: Branca Negra Parda Amarela (oriental) 99

2.3 – Profissão: _____

2.4 – Escolaridade: Não estudou 2º grau completo
1º grau Incompleto 3º grau incompleto
1º grau completo 3º grau completo
2º grau incompleto

3 – <u>História Sexual e Reprodutiva</u>:

3.1.1 – DUM: / / 3.1.2 Menarca: ___ anos **3.1.4 Menopausa:** ____anos

3.1.5 Gesta:____ **3.1.6 Para:**____ **3.1.7 Aborto:** ____ **3.1.8 Início da vida sexual:** |__|__| **anos**

3.1.9 Nº de parceiros: _____

3.2 – DST				**Tratou?**	
	Sim	Não	Quantas vezes	Sim	Não
Gonorréia			_____		
Sífilis			_____		
Herpes			_____		
Condiloma			_____		
HIV			*	*	*

3.2.2 – Uso de contraceptivo? Sim Não |__| Já usou

Qual?	**Tempo de Uso (em anos)**	
Diu	_____	
Diafragma	_____	
Pílula	_____	Qual? _____
Condon	_____	
Outros	_____	**Especificar:**_____

4 – <u>Outras patologias</u>:

4.1 – Patologias	**Sim**	**Não**	**Data diagnóstico**	**Tempo em meses**
Diabetes			/ /	_____
Hipertensão			/ /	_____
Obesidade			/ /	_____
Lupus			/ /	_____

5 – <u>Hábitos:</u>

5.1 – Fumo: Sim Não Ex-fumante

Idade de Início	**Idade que parou**	**Tipo de cigarro (a)**	**Marca**	**Nº/dia**

|__|__| |__|__| |__| _____ |__|__|

|__|__| |__|__| |__| _____ |__|__|

|__|__| |__|__| |__| _____ |__|__|

(a) 1- Manufaturado, com filtro **4- Enroladinho de palha**

2- Manufaturado, sem filtro **(b) Cachimbo.**

3- Enroladinho de papel **(c) Charuto**

(d) Maconha

5.2 – Mora (ou já morou) com alguém que fuma: |__| sim |__| não

Sua idade quando ele(a) iniciou | **Sua idade quando ele(a) parou** | **Nº horas que ele fuma(va) em sua presença**

|__|__| |__|__| |__|__| na semana |__|__| final de semana

|__|__| |__|__| |__|__| na semana |__|__| final de semana

|__|__| |__|__| |__|__| na semana |__|__| final de semana

5.3 – Trabalha (ou trabalhou) em lugar fechado onde as pessoas fumam:

|__| sim |__| não

Sua idade de início | **Sua idade de término** | **Nº horas/dia que estava exposta** | **Nível de Fumaça (1) Muita (2) Pouca / (9) não lembra**

|__|__| |__|__| |__|__| |__|__|

|__|__| |__|__| |__|__| |__|__|

|__|__| |__|__| |__|__| |__|__|

6 – <u>História Ginecológica</u>:

6.1 – ÚltimoPreventivo: / / Resultado: _____

6.2 – Cauterizações anteriore: Sim Não **quantas? _____Há quanto tempo? _____**

6.3 – tratamentos anteriore: Sim Não **quais? _____Há quanto tempo? _____**

7 - Encaminhada como NIC I NIC II NIC III ASCUS/AGUS

7.1 – Condutas:

Apenas colposcopia ( )
Colposcopia e biópsia ( )

Biópsia de ectocérvice ( ) Biópsia de endocérvice( ) EAZT ( )
Fatia anterior e posterior ( ) Fatia anterior ( ) Fatia Posterior ( ) Canal ( )

7.1.A - Citologia 7.1.A 1 – **Data citologia entrata:** / /

7.1.A 2 - **Amostra:** |__| Satisfatória

|__| Insatisfatória

|__| Satisfatória, mas limitada por_____

_____

7.1.B - Colposcopia Data: / /

7.1.B1 – Resultado:

|__| Satisfatória sem lesão

|__| Insatisfatória sem lesão

|__| Insatisfatória com lesão

|__| Satisfatória com lesão

|__| Colo não localizado

7.1.B2: ZONA DE TRANSFORMAÇÃO:

7.1.B2A: Grau 0: ou área iodo clara muda ( )

7.1.B2B: Grau 1: epitélio branco fino ( ) mosaico regular ( ) pontilhado regular( )

7.1.B2D: Grau 2: Sim Não

7.1.B2D1: orifícios glandulares espessados: Sim Não

7.1.B2D2: epitélio branco espessado: Sim Não

7.1.B2D3: mosaico irregular: Sim Não

7.1.B2D4: vasos atípicos: Sim Não

7.1.B2D5: pontilhado irregular: Sim Não

7.1.B2E: Neoplasia Invasiva: Sim Não

7.1.C – Biópsia Sim Não Data: / /

7.1.C1 **– Procedimento:** |__| Retirada total da lesão

|__| Retirada parcial da lesão

|__| Outro:_____

7.1.C2 – **Histopatologia:**

7.1.C3 – **Conclusão:** |__| Negativo para neoplasia

|__| Compatível com HPV

|__| NIC II (Displasia moderada)

|__| NIC III

|__| CA *in situ*

|__| CA invasivo

|__| Adenocarcinoma *In situ*

|__| Adenocarcinoma invasor

|__| Outros_____

7.1.C4 – **Margens Cirúrgicas:**

|__| Livres

|__| Comprometidas

|__| Sem possibilidade de avaliação

|__| Outro_____

7.1.C5 – **Recomendação:**

|__| Citologia

|__| Colposcopia

|__| CAF

|__| Outro_____

7.1.C6 – **Agendamento:** |__|__|__| dias Data: / /

7.1.D – CAF Sim Não Data: / /

7.1.D1 **– Procedimento:** |__| Retirada total da lesão

|__| Retirada parcial da lesão

|__| Outro:_____

7.1.D2 – **Histopatologia:**

7.1.D3 – **Conclusão:** |__| Negativo para neoplasias

|__| Compatível com HPV

|__| NIC II (Displasia moderada)

|__| NIC III

|__| CA *in situ*

|__| CA invasivo

|__| Adenocarcinoma *In situ*

|__| Adenocarcinoma invasor

|__| Outros_____

7.1.D4 – **Margens Cirúrgicas:**

|__| Livres

|__| Comprometidas

|__| Sem possibilidade de avaliação

|__| Outro_____

7.1.D5 – **Recomendação:**

|__| Citologia

|__| Colposcopia

|__| Outro_____

7.1.D6 – **Agendamento:** |__|__|__| dias Data: / /

Entrevistador: _____

Anexo 3 – Ficha de Seguimento

INICIAIS: _____ **MH:** Código no estudo: |__|__|__|__||__||__|

7.2 - |__| Follow-up: / /

Conduta:

7.2.A - Citologia7.2.A 1 - **Amostra:** |__| Satisfatória

|__| Insatisfatória

|__| Satisfatória, mas limitada por_____

_____

7.2.A 2 – **Alterações em Células Epiteliais:**

|__| NIC I |__| CA *in situ*

|__| NIC II |__| CA invasivo

|__| NIC III |__| Efeito citopático compatível com HPV

|__| AGUS |__| Negativo para Neoplasia

|__| ASCUS

7.2.B - Colposcopia **Data: / /**

7.2.B1 – Resultado:

|__| Negativa

|__| Insatisfatória sem lesão

|__| Insatisfatória com lesão

|__| Positiva

|__| Colo não localizado

7.2.B2: ZONA DE TRANSFORMAÇÃO:

7.1.B2A: Grau 0: ou área iodo clara muda ( )

7.1.B2B: Grau 1: epitélio branco fino ( ) mosaico regular ( ) pontilhado regular( )

7.2.B2D: Grau 2: Sim Não

7.2.B2D1: orifícios glandulares espessados: Sim Não

7.2.B2D2: epitélio branco espessado: Sim Não

7.2.B2D3: mosaico irregular: Sim Não

7.2.B2D4: vasos atípicos: Sim Não

7.2.B2D5: pontilhado irregular: Sim Não

7.2.B2E: Neoplasia Invasiva: |__| Sim |__| Não

7.2.C – CAF/Biópsia |__| Sim |__| Não Data: / /

7.2.C1 **– Procedimento:** |__| Retirada total da lesão

|__| Retirada parcial da lesão

|__| Outro:_____

7.2.C2 – **Histopatologia:**

7.2.C3 – **Conclusão:** |__| Compatível com HPV

|__| NIC II (Displasia moderada)

|__| NIC III

|__| CA *in situ*

|__| CA invasivo

|__| Outros_____

7.2.C4 – **Margens Cirúrgicas:**

|__| Livres

|__| Comprometidas

|__| Sem possibilidade de avaliação

|__| Outro_____

7.2.C5 – **Recomendação:**

|__| Citologia

|__| Colposcopia

|__| Outro_____

7.2.D Resultado da PCR:

7.2.D1 – HPV + Sim Não

7.2.D2 – Tipo: _____

7.2.E. Tabagismo:

Iniciou Não (Vá para E3) Parou (vá para E2) Continua (vá para E2b).

7.2.E1 Se sim, Data Início: / / nº cigarros/dia: |__|__|__|

Tempo de tabagismo: _____

7.2.E2 Se parou, Data término: / / Tempo desde o último cigarro: |__|__|__| dias

7.2.E2b – Se Continua: nº cigarros/dia: |__|__|__| Tempo desde a última consulta: |__|__|__| dias.

7.2.E3 Nº de parceiros desde a última consulta: |__|__|__|

7.2.E4 – Gestante: Sim Não

7.2.F DST: Sim Não

	Sim	Não	Data:
Gonorréia			/ /
Sífilis			/ /
Herpes			/ /
Condiloma			/ /
HIV			/ /

7.2.G – Condição da Paciente no estudo:

Câncer Invasor Insucesso no tratamento Em observação Excluída do estudo

7.2.H – **Agendamento:** |__|__|__| dias Data: / /

Entrevistador: _____