MICHELLE MOTA SENA

ENVOLVIMENTO DOS POLIMORFISMOS rs28362491 DE *NFKB1* E rs696 DE *NFKBIA* NA INFECÇÃO PELO PAPILOMAVÍRUS HUMANO

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Orientadora: Profa. Dra. Karen Brajão de Oliveira

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"Por vezes sentimos que aquilo que fazemos não é senão uma gota de água no mar. Mas o mar seria menor se lhe faltasse uma gota."

(Madre Teresa de Calcutá)

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RESUMO

O Papilomavírus Humano (HPV) consiste no agente etiológico da infecção sexualmente transmissível mais frequente do trato anogenital. Embora, na maioria das vezes, sua presença não leve à manifestações clínicas, sendo uma infecção facilmente resolvida em grande parte dos casos, quando há a persistência por HPVs de alto risco em conjunto com características intrínsecas ao hospedeiro, tais como estado imunológico, padrões comportamentais e fatores genéticos, é possível que haja o desenvolvimento de lesões intraepiteliais escamosas, as quais podem evoluir para o câncer cervical (CC) caso não exista identificação e tratamento adequados. No contexto da resposta imunológica, proliferação, diferenciação, apoptose, dentre outros, a via do NF-kB atua na regulação de inúmeros genes e alterações estruturais e/ou funcionais em componentes dessa via podem propiciar o desenvolvimento de diversos tumores. Tendo em vista que a presença de alterações nesta via sob as circunstâncias da infecção pelo HPV, bem como as suas possíveis relações com as lesões induzidas pelo vírus e com o CC ainda não estão bem estabelecidas, o objetivo deste estudo foi avaliar a influência dos polimorfismos rs28362491 de *NFKB1* e rs696 de *NFKBIA* na infecção por HPV e no desenvolvimento de lesões pré-neoplásicas e neoplásicas em 334 pacientes do sexo feminino. Para tal, as amostras foram submetidas à Reação em Cadeia da Polimerase para detecção viral e posterior restrição enzimática para genotipagem dos polimorfismos, cujos resultados foram observados em gel de poliacrilamida 10% corado com nitrato de prata. Por meio da aplicação de questionário, foram obtidas informações socio-demográficas e de comportamento sexual das pacientes. O vírus esteve presente em 163 mulheres, sendo mais frequente entre aquelas que desconheciam o vírus, com idade ≤24 anos, fumantes, solteiras e que tiveram ≥4 parceiros sexuais durante a vida. As pacientes com lesões precursoras declararam ter tido mais parceiros durante a vida do que aquelas com resultado citológico normal, enquanto que, quando comparadas a estas últimas, as pacientes com CC relataram desconhecer as formas de transmissão do vírus, apresentaram idade ≥55 anos, em sua maioria viúvas, com baixa escolaridade, renda mensal <1 salário mínimo, que tiveram ≥5 gestações, cujos partos foram, em geral, do tipo normal. Quanto aos polimorfismos, os genótipos de *NFKB1* homozigotos para inserção (II) e AA para *NFKBIA*, quando combinados por meio de regressão logística multinominal com valores ajustados para fatores confundidores, demonstraram exercer papel protetor contra a infecção pelo HPV, não sendo observada influência sobre o desenvolvimento de lesões e progressão para o CC. Assim, o presente trabalho demonstrou pela primeira vez que os polimorfismos rs28362491 e rs696 estão envolvidos com a proteção contra a infecção pelo HPV. Tendo em vista o resultado promissor, estudos adicionais permitirão compreender de que forma essas alterações genéticas atuam na situação de infecção e, por fim, uma maior população de estudo será necessária para a validação dos resultados encontrados.

Palavras-chave: HPV. Via do NF-kB. Lesões cervicais. Colo de útero. Diagnóstico molecular.

SENA, Michelle Mota. **Involvement of rs28362491 polymorphism of *NFKB1* and rs696 of *NFKBIA* in Human Papillomavirus infection.** 2019. 87 p. Dissertation (Postgraduate Program in Experimental Pathology) – Universidade Estadual de Londrina, Londrina, 2019.

ABSTRACT

Human Papillomavirus (HPV) is the etiologic agent of the most frequent sexually transmitted infection of anogenital tract. Although not always its presence leads to clinical manifestations, being an infection easily resolved in most cases, when there is persistence by high-risk HPVs in conjunction with intrinsic host characteristics such as immune status, behavioral patterns and genetic factors, it is possible that squamous intraepithelial lesions develop, which may progress to cervical cancer (CC) in the absence of adequate identification and treatment. In the context of immune response, proliferation, differentiation, apoptosis, among others, the NF-kB pathway regulates innumerable genes, and structural and/or functional alterations in components of this pathway may favor the development of several tumors. Considering that the presence of alterations in this pathway under circunstances of HPV infection, as well as its possible relations with the virus-induced lesions and CC have not yet been well established, the purpose of this study was to evaluate the influence of the rs28362491 polymorphism of *NFKB1* and rs696 of *NFKBIA* in HPV infection and the development of pre-neoplastic and neoplastic lesions in 334 female patients. For this, the samples were submitted to Polymerase Chain Reaction for viral detection and subsequent enzymatic restriction for genotyping, whose results were observed in 10% polyacrilamide gel stained with silver nitrate. Through the application of questionaire were obtained socio-demographic and sexual behavior information about the patients. The virus was present in 163 women, being more frequent among those who did not knowledge about the virus, aged ≤24 years, smokers, single and who had ≥4 sexual partners during their lifetime. Patients with precursor lesions reported having had more partners during lifetime than those with a normal cytological results, whereas when compared to the latter, CC patients reported not aware the viral transmission forms, were aged ≥55 years, widows, with a low education level, monthly income <1 minimum wage, who had ≥5 full-term pregnancies, whose deliveries were normal. Considering the polymorphisms, the combination of *NFKB1* homozygous insertion (II) and *NFKBIA* AA genotypes, through multinomial logistic regression with adjusted values for confounding factors, demonstrated to play a protective role against HPV infection, and no influence on lesion development and progression to CC was observed. Thus, the present study demonstrated for the first time that rs28362491 and rs696 polymorphisms are involved in protection against HPV. In view of the promising result, additional studies will allow us to understand the way in which these genetic changes act in the infection situation and, finally, a large study population will be necessary in order to validate the results found.

Key words: HPV. NF-kB pathway. Cervical lesions. Cervix. Molecular diagnosis.

LISTA DE ILUSTRAÇÕES

LISTA DE ABREVIATURAS E SIGLAS

AS-PCR	*Allele specific-polymerase chain reaction*
BAFF	*B-cell activating factor*
BaP	*Benzo[a]pyrene*
BC	*Bladder cancer*
BCL-2	*B-cell lymphoma 2*
BMI	*Body mass index*
BRC	*Breast cancer*
CC	*Cervical cancer*
CIN	*Cervical intraepithelial neoplasia*
CLL	*Chronic lymphocytic leukemia*
COPD	*Chronic obstructive pulmonary disease*
COX	*Cyclooxygenase*
CRC	*Colorectal cancer*
CSCC	*Cervical squamous cell carcinoma*
DNA	*Deoxyribonucleic acid*
EDTA	*Ethylenediamine tetracetic acid*
ESCC	*Esophageal squamous cell carcinoma*
EGFR	*Epidermal growth factor receptor*
E6AP/UBE3A	*Ubiquitin ligase E6-associated protein*
GC	*Gastric cancer*
GEPC	*Gastroenteropancreatic cancer*
HCC	*Hepatocellular cancer*
HL	*Hodgkin's lymphoma*
HNSCC	*Head and neck squamous cell carcinoma*
HPV	*Human Papillomavirus*
HR-HPV	*High-risk HPV*
HSIL	*High squamous intraepithelial lesion*
HWE	*Hardy-Weinberg equilibrium*
IBD	*Inflammatory bowel disease*
IkB	*Inhibitory NF-kB protein*
IKK	*IkB kinase*

IL-1β	*Interleukin-1 beta*
IL-6	*Interleukin-6*
IL-8	*Interleukin-8*
LC	Lung cancer
LCR	*Long control region*
LR-HPV	*Low-risk HPV*
LSIL	*Low squamous intraepithelial lesion*
MAF	*Minor allele frequency*
MHC	*Major histocompatibility complex*
MM	*Multiple myeloma*
MMP	*Metalloproteinase*
NEMO	*NF-kB essential modulator*
NF-kB	*Nuclear factor-kappa B*
NIK	*NF-kB inducing kinase*
NPC	*Nasopharyngeal carcinoma*
NSLC	*Non-small cell lung cancer*
OC	*Oral contraceptive*
ORF	*Open region frame*
OSCC	*Oral squamous cell carcinoma*
PAH	*Polynuclear aromatic hydrocarbons*
PC	*Prostate cancer*
PCR	*Polymerase chain reaction*
PCR-CGE	*Polymerase chain reaction with capillary gel electrophoresis*
PCR-KASP	*Polymerase chain reaction kompetitive allele specific*
PCR-PAGE	*Polymerase chain reaction-polyacrilamide gel electrophoresis*
PCR-RFLP	*Polymerase chain reaction-restriction fragment length*
PCR-SSCP	*Polymerase chain reaction-single-strand conformation polymorphism*
pRb	*Retinoblastoma protein*
RANKL	*Receptor activator of NFkB ligand*
RHD	*REL-homology domain*
SIL	*Squamous intraepithelial lesion*
SNP	*Single nucleotide polymorphism*
TE	*Tris-EDTA*
TNF-α	*Tumor necrosis factor-α*

TLR	*Toll-like receptor*
TWEAK	*TNF-related weak inducer of apoptosis*
UICC	*International Union Against Cancer*
URR	*Upstream regulatory region*
VEGF	*Vascular endothelial growth factor*

SUMÁRIO

1 INTRODUÇÃO

O câncer cervical (CC) consiste no quarto tipo de câncer mais prevalente em mulheres mundialmente, com estimativas de aproximadamente 570.000 novos casos e mais de 311.000 mortes anualmente (WHO, 2018). No Brasil, este tipo de câncer é o terceiro mais comum a acometer mulheres, ficando atrás somente do câncer de mama e de cólon e reto (ao se excluir os casos de câncer de pele não melanoma) e são esperados 16.370 novos casos para o biênio 2018/2019, representando 8,1% do total de tumores esperados para este período no país (INCA, 2018).

Dentre os diversos fatores relacionados com o desenvolvimento do CC, destaca-se a persistência da infecção pelo Papilomavírus Humano (HPV) (SAKAMOTO et al., 2018), a qual é considerada como sendo a infecção transmitida por relação sexual diagnosticada mais frequentemente (WANG et al., 2018). Mundialmente, as infecções virais contribuem para cerca de 15 a 20% de todos os cânceres humanos, sendo que 5% de todos estes tumores são causados pelos HPVs (GUPTA; KUMAR; DAS, 2018). O envolvimento deste vírus é reconhecido em virtualmente 100% dos casos de CC (STEINBACH; RIEMER, 2018), além de já ter sido constatada a presença de certos tipos de HPV em cerca de 50% dos cânceres vulvares, de 30 a 50% dos cânceres de pênis, de 60 a 90% dos cânceres de vagina e de 25 a 30% dos cânceres de orofaringe (SASAGAWA; TAKAGI; MAKINODA, 2012).

Embora já seja bem descrito o envolvimento do HPV como fator etiológico do CC, a infecção por si só não é suficiente para o desenvolvimento de tal tumor, sendo necessária a combinação de outros fatores, tais como tabagismo, consumo de álcool, estado imunológico, número de parceiros sexuais, paridade, idade da primeira gravidez a termo, bem como perfil genético do hospedeiro (PALLAVI; ANOOP; SHOWKET, 2015).

1.1 PAPILOMAVÍRUS HUMANO (HPV)

1.1.1 O vírus

Pertencentes à família *Papillomaviridae*, já são descritos mais de 200 tipos de HPV capazes de infectar seres humanos, os quais, de acordo com a sequência nucleotídica da fase de leitura aberta (ORF) codificadora da proteína de capsídeo L1, são divididos filogeneticamente em cinco gêneros distintos: Mu-papilomavírus, que inclui somente três membros; Nu-papilomavírus, com um único membro; Beta e Gama-papilomavírus, responsáveis por infecções cutâneas inaparentes; e finalmente, Alfa-papilomavírus, onde estão incluídos os principais tipos virais associados com o desenvolvimento de cânceres anogenitais e de orofaringe (BZHALAVA; EKLUND; DILLNER, 2015; DOORBAR, 2018).

O HPV consiste em um vírus epiteliotrópico, não-envelopado composto por um capsídeo proteico icosaédrico e por ácido desoxirribonucleico (DNA) dupla fita circular, o qual contém cerca de 8000 pares de bases (pb) e é responsável por codificar oito ou nove ORFs (DOORBAR, 2006). As proteínas virais codificadas são classificadas de acordo com o momento de síntese, sendo denominadas proteínas precoces (E, do inglês *early*) aquelas produzidas logo ao início da infecção, enquanto que as proteínas sintetizadas tardiamente são conhecidas como proteínas tardias (L, do inglês *late*) (JOHANSSON; SCHWARTZ, 2013). Além das regiões responsáveis por codificar as proteínas precoces E1, E2, E4, E5, E6 e E7 e as proteínas tardias L1 e L2, existe uma terceira região do genoma, que é não codificante e denominada *long control region* (LCR) ou *upstream regulatory region* (URR), a qual contém a sequência regulatória transcricional de um ou mais promotores e atua na regulação da expressão das oncoproteínas E6 e E7 (GUPTA; KUMAR; DAS, 2018).

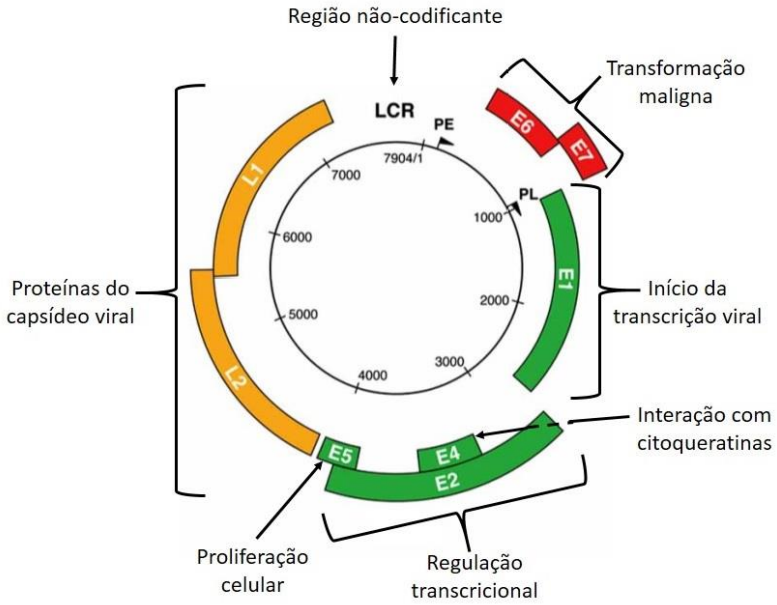
Envolvidas com o início da replicação e regulação da transcrição viral, E1 e E2, atuam respectivamente, como uma DNA helicase, catalisando o desenrolamento do DNA e promovendo o recrutamento da maquinaria de replicação; e como um fator de transcrição viral e proteína acessória para replicação, sendo capaz de ativar ou reprimir a transcrição conforme evidenciado em diferentes sistemas experimentais (HEBNER; LAIMINS, 2006; DOORBAR, 2016; GRAHAM, 2016).

A proteína E4 induz alterações citomorfológicas específicas do HPV em células epiteliais infectadas por meio da sua interação com citoqueratinas e do colapso dos filamentos intermediários (SZENTIRMAY et al., 2005); enquanto que E5 atua estimulando o crescimento celular por meio da sua ligação ao receptor do fator de crescimento epidérmico (EGFR), promovendo a transmissão de sinais mitogênicos para o núcleo (JOHANSSON; SCHWARTZ, 2013); além de, sob condições experimentais, atuar como oncogene impedindo a degradação de receptores de superfície para fatores de crescimento e sendo capaz de transformar algumas linhagens murinas *in vitro* (SZENTIRMAY et al., 2005). Além disso, estes dois produtos virais atuam na regulação das funções virais tardias, exercendo papeis na liberação do vírion e na evasão imunológica, como é o caso de E5 que inibe a apresentação do peptídeo viral pelo Complexo principal de histocompatibilidade (MHC) classe 1, sendo necessários também para a replicação e produção viral (DOORBAR, 2016; BORDIGNON et al., 2017).

Tanto E6 como E7 são as principais proteínas responsáveis pelo processo de transformação maligna das células infectadas, sendo que a primeira é capaz de se ligar à proteína ubiquitina ligase associada a E6 (E6AP/UBE3A), promovendo a degradação do produto gênico do supressor tumoral p53, fator de transcrição o qual está envolvido no reparo do DNA, parada do ciclo celular e apoptose; e E7 ao se ligar à proteína Retinoblastoma (pRb), desloca o fator de controle da transcrição E2F e leva à expressão constitutiva dos genes responsivos ao mesmo, promovendo ativação do ciclo celular (BASHAW et al., 2017), que tem por consequência o aumento da proliferação celular, favorecendo o aumento da replicação viral.

Finalmente, L1 e L2 são proteínas estruturais sintetizadas apenas em células epiteliais escamosas totalmente diferenciadas, coincidindo com a montagem do capsídeo viral maduro e são importantes para que a infectividade viral seja eficiente (SZENTIRMAY et al., 2005; DOORBAR, 2006; BORDIGNON et al., 2017) (Figura 1).

Figura 1 – Organização genômica do HPV



Estrutura genômica do HPV contendo a região não codificante (LCR), a região expressa durante o início da infecção (E1-E7) e a que é expressa mais tardiamente (L1-L2). PE: promotor dos genes precoces; PL: promotor dos genes tardios. Fonte: modificado de CID-ARREGUI, 2009.

O vírus HPV infecta preferencialmente queratinócitos da camada basal de epitélios da superfície cutânea e membranas mucosas, sendo que os que infectam estas últimas são classificados baseando-se em seu potencial oncogênico em tipos de baixo risco (*low-risk*: LR-HPV) e de alto risco (*high risk*: HR-HPV) (BASHAW et al., 2017). Enquanto que as manifestações clínicas decorrentes da infecção pelos tipos de baixo risco variam de completamente assintomática até o desenvolvimento de papilomas benignos ou verrugas, causadas pelos tipos HPV6 e HPV11 em aproximadamente 90% dos casos, os HPVs de alto risco são considerados agentes etiológicos de malignidades anogenitais, bem como de diferentes formas de cânceres orofaríngeos (BORDIGNON et al., 2017; LIU et al., 2018; STEINBACH; RIEMER, 2018). São conhecidos pelo menos 40 tipos capazes de infectar a mucosa do trato genital (WANG et al., 2018), sendo que destes, 13 são reconhecidos como oncogênicos, sendo eles os HPV16, HPV18, HPV31, HPV33, HPV35, HPV39, HPV45, HPV51, HPV52, HPV56, HPV58, HPV59 e HPV68 (SAKAMOTO et al., 2018).

1.2.2 A infecção

A principal forma de infecção pelo HPV ocorre horizontalmente por meio do contato sexual pele-a-pele ou mucosa-mucosa (SCHIFFMAN et al., 2007; SAKAMOTO et al., 2018), embora já estejam descritas algumas outras formas menos comuns, como por exemplo por autoinoculação ou durante a passagem pelo canal de parto infectado ao nascer (SABEENA et al., 2017). Apesar de existirem evidências de que o uso consciente do preservativo poderia reduzir o risco de CC (SHEPHERD; PEERSMAN; NAPULI, 2009), este oferece apenas uma proteção parcial, visto que algumas áreas possivelmente contaminadas não são totalmente encobertas, como vulva e bolsa escrotal (WEAVER, 2006).

A infecção é extremamente comum em mulheres jovens durante a primeira década de atividade sexual (SCHIFFMAN et al., 2007), sendo que alguns estudos demonstraram que após o primeiro contato sexual mais de 50% das mulheres estarão infectadas pelo HPV e, mais de 80% das mulheres entrarão em contato pelo menos uma vez com o vírus no decorrer de sua vida (SASAGAWA; TAKAGI; MAKINODA, 2012; SAKAMOTO et al., 2018). Em geral, o sistema imunológico é capaz de conter a infecção, sendo responsável pela eliminação de cerca de 90% das infecções espontaneamente dentro de 3 anos, entretanto, alguns tipos de HPV de alto risco são capazes de levar à persistência da infecção em 10 a 12% dos casos, os quais podem evoluir para câncer invasivo, o que ocorre em menos de 1% dos casos (SASAGAWA; TAKAGI; MAKINODA, 2012; GUPTA; KUMAR; DAS, 2018).

A infecção pelo HPV é favorecida pela presença de microlesões no epitélio, as quais permitem o acesso do vírus às células da camada basal e, assim, a formação do reservatório viral que se mantém sob baixos níveis de expressão gênica até o início da diferenciação epitelial, momento no qual ocorre a ativação do ciclo replicativo do HPV com consequente geração de milhares de cópias virais por célula infectada e a expressão de genes tardios e montagem do vírion subsequente (BORDIGNON et al., 2017). Até então, além de serem mantidos em um baixo número de cópias, os genomas virais se mantêm sob a forma episssomal, isto é, desagregado do material genômico, diferentemente dos tumores associados ao HPV, nos quais em geral as infecções são não produtivas e nenhuma progênie viral infecciosa é produzida, sendo isso justificado pela integração das sequências do

DNA viral ao genoma do hospedeiro em certos locais cromossômicos fragilizados, podendo levar ao aumento da expressão de proto-oncogenes celulares (GUPTA; KUMAR; DAS, 2018).

Quando há a persistência da infecção, o vírus pode permanecer sob o estado latente, ou ainda levar ao desenvolvimento de lesões, sendo que a presença destas é correspondente à fase pré-maligna. A classificação das lesões é realizada com base no grau de severidade das anormalidades histológicas ou citológicas presentes. No Brasil, têm-se como regras a classificação de Richart (1967) para o exame histopatológico e a classificação baseada no Sistema Bethesda (2001) para o exame citológico. No exame histopatológico, as lesões são classificadas baseando-se na proporção de espessura do epitélio escamoso constituído por queratinócitos maduros e diferenciados em: neoplasias intraepiteliais cervicais grau 1 (displasia leve), 2 (displasia moderada) ou 3 (displasia severa/carcinoma *in situ*) (*cervical intraepithelial neoplasia:* CIN1, 2 ou 3), enquanto que no exame citológico, as lesões são divididas em lesões intraepiteliais escamosas de baixo ou alto grau (low-grade squamous intraepithelial lesion: LSIL ou *high-grade squamous intraepithelial lesion:* HSIL) e adenocarcinoma *in situ*, sendo que LSIL é equivalente a CIN1 e HSIL, a CIN2 E CIN3 (BRASIL, 2012, 2016).

Na fase maligna, os dois principais subtipos tumorais encontrados consistem no carcinoma de células escamosas e no adenocarcinoma, sendo que enquanto o primeiro tem tido uma redução importante na sua incidência desde a implementação da triagem citológica, o segundo tem crescido significativamente quando comparado ao primeiro, representando cerca de 20% de todos os cânceres cervicais, valor bem superior aos observados na década de 1970, onde era observada uma incidência entre 5 e 10% (MABUCHI et al., 2012).

Durante a infecção por HPV, o fator de transcrição-kappa B (NF-kB) tem importante papel na resposta imune do hospedeiro, por meio da ativação de moléculas de adesão e proteínas quimioatraentes essenciais para o recrutamento de células inflamatórias que atuarão no combate do vírus. Durante a progressão das lesões para CC, o NF-kB se torna constitutivamente ativado, favorecendo o acúmulo de citocinas pró-inflamatórias que atuam diretamente para iniciação e progressão tumoral (TILBORGHS et al., 2017).

1.2 VIA DO NF-KB

1.2.1 Proteínas e vias de sinalização

O NF-kB foi primeiramente descoberto por Sen e Baltimore em 1986 como um fator de transcrição capaz de se ligar ao promotor da cadeia leve kappa de imunoglobulina em células B (YANG et al., 2014) e é composto por cinco membros: c-Rel, RelB, RelA (p65), p50/105 e p52/100, os quais são codificados pelos genes *REL*, *RELB*, *RELA*, *NFKB1* e *NFKB2*, respectivamente (FU et al., 2017) (Tabela 1). Tanto p105 como p100 consistem em moléculas citoplasmáticas, as quais ao passarem por processamento proteassomal, têm seus domínios de anquirina degradados, levando à maturação, respectivamente, de p50 e p52, moléculas capazes de chegarem ao núcleo e se ligarem ao DNA (SKAUG; JIANG; CHEN, 2009).

Tabela 1 – Características dos componentes da família do NF-kB

Proteína	Gene	Cromossomo	Via de sinalização
c-Rel	*REL*	2	canônica
p105/p50	*NFKB1*	4	canônica
RelA	*RELA*	11	canônica
p100/p52	*NFKB2*	10	não canônica
RelB	*RELB*	19	não canônica

Em células em repouso, os dímeros de NF-kB são retidos por proteínas inibitórias, denominadas IkB, as quais os mantêm inativados por meio da interação entre as repetições de anquirinas presentes e os domínios que se ligam ao DNA dos fatores de transcrição (TILBORGHS et al., 2017). Dentre as proteínas que compõem a família do IkB têm-se as clássicas, as quais agem como inibidoras do NF-kB, sendo elas IkBα, IkBβ e IkBε, sendo a primeira a melhor caracterizada; e as proteínas semelhantes ao IkB atípicas IkBζ e Bcl-3, as quais atuam como coativadoras do NF-kB (SKAUG; JIANG; CHEN, 2009).

Uma vez que haja a ativação das vias de sinalização, estas irão diferir a

depender dos fatores que foram ativados, sendo denominadas via canônica ou clássica, na qual há o envolvimento dos fatores c-Rel, RelA e p50; e via não canônica ou alternativa, na qual os dímeros de p52 e RelB são predominantes (WENIGER; KÜPPERS, 2016) (Figura 2).

A ativação da via canônica ocorre na presença de moléculas como fator de necrose tumoral (TNF)-α, interleucina-1β (IL-1β), ligantes de receptores semelhantes ao Toll (TLR) ou ainda radiação e estresse. Todos estes fatores, ao se ligarem aos seus receptores de membrana, induzirão à transdução de sinais que alcançarão o complexo IkB-quinase (IKK). Este complexo ao ser ativado, levará à fosforilação da proteína IkBα, marcando-a para degradação proteassomal (SKAUG; JIANG; CHEN, 2009), e assim, o dímero p50/RelA se torna livre e se acumulará no núcleo, local no qual irá ativar a expressão de genes de proteínas relacionadas à regulação da proliferação, tais como ciclina D1 e C-Myc; de genes envolvidos na metástase, como aqueles que codificam moléculas de adesão e metaloproteinases de matriz; e na angiogênese dependente do fator de crescimento endotelial vascular (VEGF) (KINKER et al., 2016; TILBORGHS et al., 2017). Ativará ainda o gene do inibidor do NF-kB (*NFKBIA*), responsável por codificar IkBα, o qual removerá o fator de transcrição do núcleo, caracterizando assim, uma alça de *feedback* negativo (MOROTTI et al., 2016).

A via não canônica, por sua vez, é desencadeada por alguns membros da família TNF, dentre os quais CD40L, linfotoxina, fator ativador de células B (BAFF) e indutor fraco de apoptose relacionado ao TNF (TWEAK), os quais promoverão a ativação da quinase indutora de NF-kB (NIK), a qual irá fosforilar e ativar os dímeros de IKKα, os quais levarão à poliubiquitinação de p100 e, por consequência, haverá a liberação de p52, o qual se liga à RelB. Com o aumento da disponibilidade do dímero p52/RelB, esses se translocam para o núcleo e estão envolvidos com a transcrição de genes que estão relacionados principalmente com a organogênese linfoide e com o desenvolvimento e sobrevivência de células B (VALLABHAPURAPU; KARIN, 2009; WENIGER; KÜPPERS, 2016).

Enquanto que a via canônica em geral está relacionada com funções anti-apoptótica e promotora tumoral, a via não canônica apresenta diferentes papeis, às vezes suprimindo o tumor e facilitando a apoptose (TILBORGHS et al., 2017). No entanto, ambas as vias estão ativadas em diversos cânceres e essa característica é frequentemente associada com pior prognóstico (HOESEL; SCHMID, 2013).

1.3.2 Polimorfismos rs28362491 e rs696

Tendo em vista a ampla importância que a via do NF-kB exerce por meio da regulação de diversos genes relacionados à resposta imunológica, adesão celular, proliferação, diferenciação, além de apoptose, metástase e angiogênese (ESKANDARI-NASAB et al., 2016; LI et al., 2016), é evidente que alterações funcionais e/ou estruturais nos componentes da via poderão alterar a sua atividade, ocasionando a quebra da homeostase e favorecendo um aumento da susceptibilidade a diversos tumores (LEONE et al., 2018).

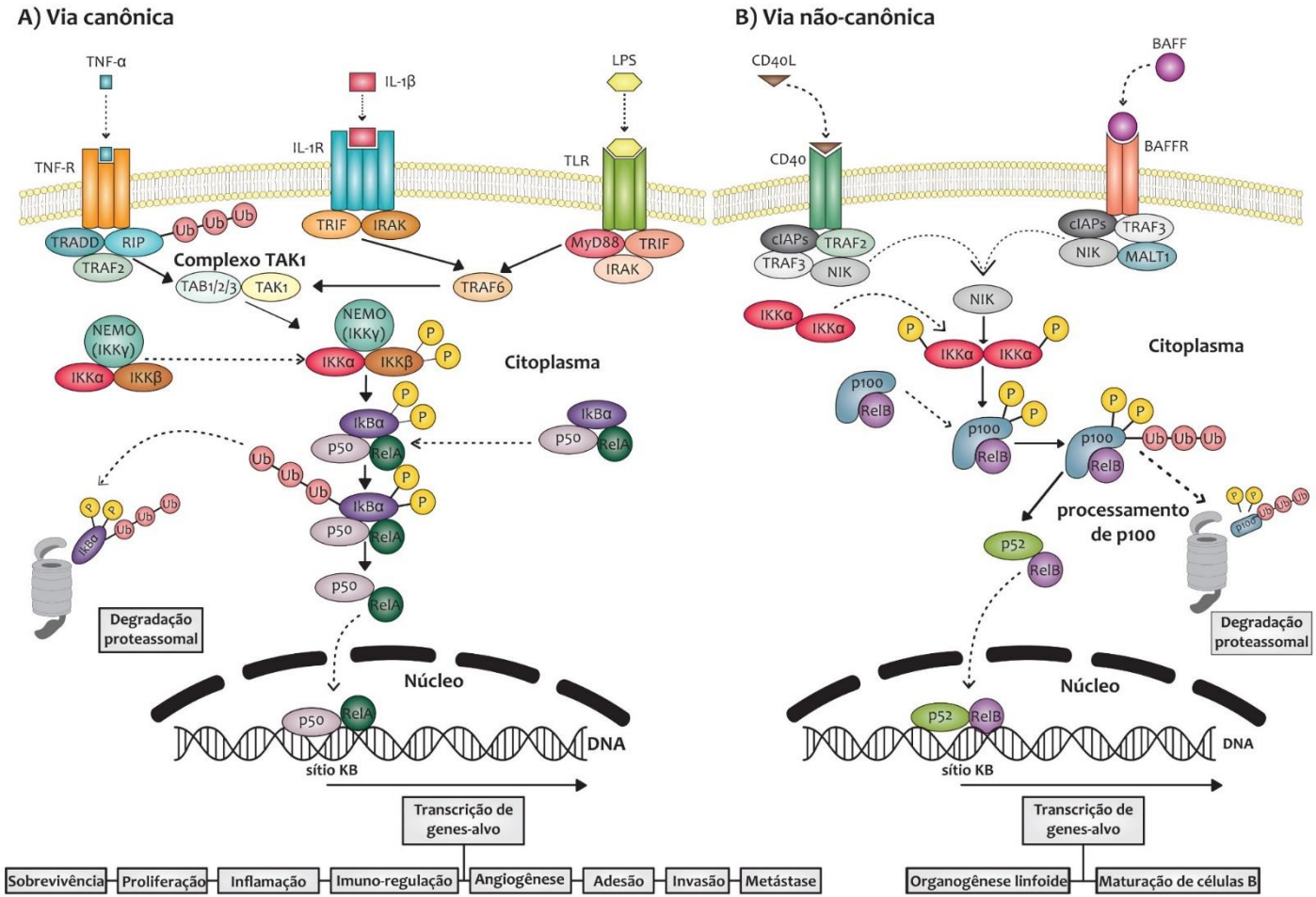
Dentre os genes envolvidos com a ativação da via do NF-kB, têm-se o gene *NFKB1*, o qual apresenta 93 tipos de variações descritas, sendo que destas 2 são polimorfismos de inserção/deleção (indel); e o gene *NFKBIA*, que apresenta 81 alterações, das quais 13 são SNPs (NCBI, 2019). A expressão alterada destes genes pode ter como consequência a alteração dos níveis citoplasmáticos das proteínas da via. Neste contexto, têm-se os polimorfismos rs28362491 de *NFKB1* e rs696 de *NFKBIA*, os quais embora já amplamente estudados, não tiveram os seus respectivos papeis bem estabelecidos no processo tumoral.

O polimorfismo rs28362491 é caracterizado pela inserção/deleção de quatro pares de bases na posição -94 da região promotora (KARBAN et al., 2004) e acredita-se que o alelo contendo inserção possa atuar aumentando a produção da proteína p105/p50, aumentando desta forma a atividade do fator de transcrição NF-kB (Figura 3). Já o polimorfismo rs696 consiste na substituição de uma guanina por uma adenina na posição 2758 na região 3’UTR (PALLAVI; ANOOP; SHOWKET, 2015) e o alelo variante parece estar relacionado com um aumento da estabilidade da ligação com miR-449a, resultando na redução da expressão deste gene, levando à redução dos níveis de IkBa e favorecendo a atividade constitutiva de NF-kB (SONG et al., 2011) (Figura 4).

Infere-se que, nos estágios precoces do câncer, o NF-kB é capaz de inibir o crescimento tumoral, entretanto, o acúmulo de mutações pode levar à dominância das características oncogênicas em detrimento da perda das funções supressoras tumorais deste fator de transcrição (TILBORGHS et al., 2017). Além disso, foi demonstrado que a proteína p50 favoreceu a imunossupressão por reduzir a polarização de macrófagos M1, os quais têm funções relacionadas a morte de microrganismos intracelulares, de células tumorais e à produção de citocinas pró-

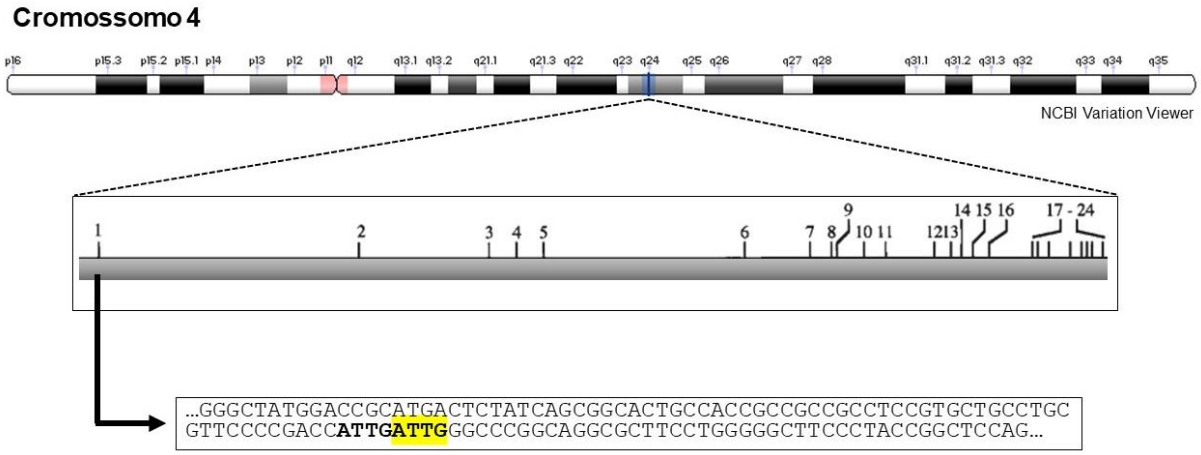
inflamatórias e, induzir a polarização de macrófagos M2, os quais atuam promovendo a angiogênese, dentre outras funções (PORTA et al., 2009). Logo, é possível que a combinação destes dois polimorfismos, os quais interferem diretamente na formação de constituintes da via canônica, possa estar relacionada com um aumento da susceptibilidade para o desenvolvimento de tumores, e esta hipótese levou à realização deste trabalho.

Figura 2 – Vias de transdução de sinais do NF-kB



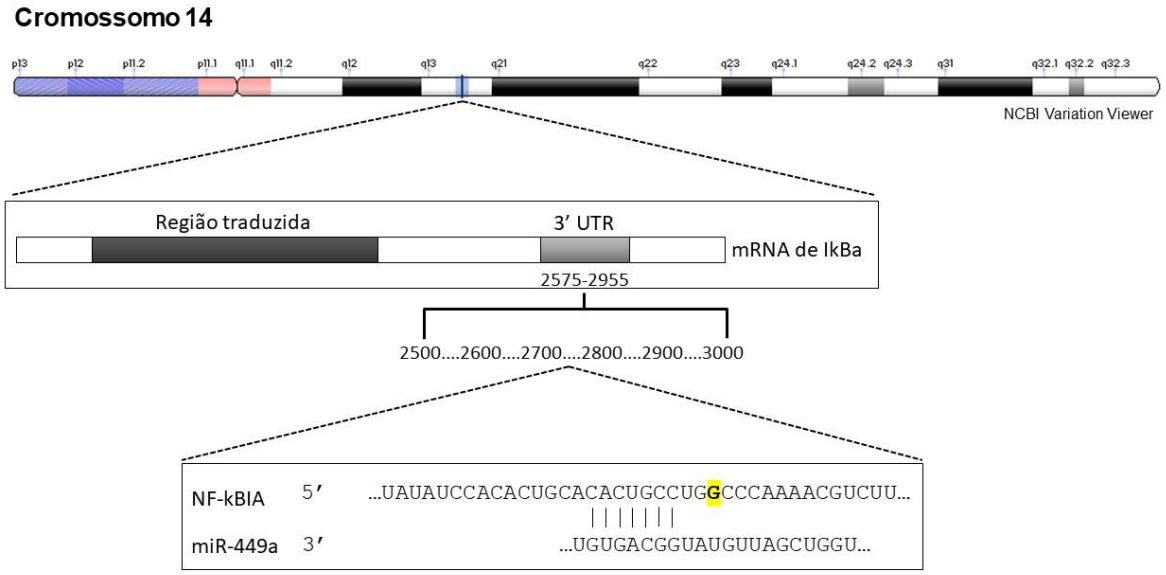
Vias de transdução de sinais de NF-kB. (A) Via canônica: Estímulos como TNF-α, IL-1β e ligantes de TLR levam à ativação de IKK, o qual provoca a fosforilação de IkBα, que ao ser degradada, torna livre o dímero p50/RelA. Este se translocará para o núcleo e está relacionado com a expressão de genes envolvidos na proliferação, metástase, angiogênese, dentre outros. (B) Via não canônica: Por meio de estímulos como CD40L e BAFF, ocorre a ativação de NIK, o qual ativará dímeros de IKKα. Estes, por sua vez, induzirão o processamento de p100, originando p52, que uma vez ligado à RelB, forma um dímero que translocará para o núcleo e está envolvido na organogênese linfoide e no desenvolvimento e sobrevivência de células B. Fonte: Própria autora.

Figura 3 – Localização cromossômica do polimorfismo rs28362491 de *NFKB1*



Presente em 4q24, o polimorfismo rs28362491 consiste em uma inserção de quatro nucleotídeos na posição -94 no promotor do gene *NFKB1*. Fonte: Própria autora.

Figura 4 – Localização cromossômica do polimorfismo rs696 de *NFKBIA*



O polimorfismo rs696 está presente em 14q13 e é um polimorfismo de base única que ocorre devido a alteração de uma guanina por uma adenina na posição 2758 da região 3'UTR do gene *NFKBIA*. Fonte: Própria autora.

2 OBJETIVOS

2.1 Objetivo Geral

O presente trabalho teve por objetivo verificar a influência dos polimorfismos rs28362491 de *NFKB1* e rs696 de *NFKBIA* na infecção por HPV e no desenvolvimento de lesões pré-neoplásicas e neoplásicas em pacientes do sexo feminino atendidas pelos programas de prevenção ao câncer de colo de útero do setor público de saúde da região Norte do Paraná.

2.2 Objetivos Específicos

- Realizar um levantamento bibliográfico sobre o papel dos polimorfismos rs28362491 de *NFKB1* e rs696 de *NFKBIA* no desenvolvimento de tumores baseando-se na literatura para a produção de um artigo de revisão;
- Avaliar o perfil sociodemográfico das mulheres atendidas pelos programas de prevenção ao câncer de colo de útero do Sistema Único de Saúde (SUS) na região metropolitana de Londrina, de acordo com a infecção pelo HPV;
- Comparar as características sociodemográficas, reprodutivas e de comportamento sexual das pacientes com e sem infecção pelo HPV;
- Correlacionar a presença de lesões pré-neoplásicas e neoplásicas em mulheres positivas para a presença do vírus com as variáveis sociodemográficas, reprodutivas e sexuais;
- Avaliar as frequências alélicas e genotípicas dos polimorfismos rs28362491 de *NFKB1* e rs696 de *NFKBIA*;
- Analisar a associação dos polimorfismos rs28362491 de *NFKB1* e rs696 de *NFKBIA* na infecção pelo HPV e no desenvolvimento de lesões pré-malignas e malignas.

3 PRODUÇÃO CIENTÍFICA

3.1 ARTIGO 1

Importance of genetic polymorphisms rs28362491 of *NFKB1* and rs696 of *NFKBIA* in the development of tumors

ABSTRACT

The growth of the world population associated with aging and the action of cancer-causing habits has made it stand out as the main public health problem on a global scale. Although it considered a multifactorial disease, its relationship with chronic infections or unresolved inflammation is well established, placing the Nuclear factor-kappa B (NF-kB) pathway in a prominent position, since this transcription factor acts on more than 200 genes. Therefore, it is evident that alterations, such as polymorphisms that cause functional and/or structural changes, as well as the gene level dysregulation in components of this pathway may be involved in the development of several diseases, among them cancer. Thus, the purpose of this study was to verify the main characteristics of the NF-kB pathway, and later to carry out a bibliographic survey of reports on the genetic polymorphisms rs28362491 of *NFKB1* and rs696 of *NFKBIA* in different tumors and to analyze the main genotypes involved in the risk of tumor development and its characteristics. The main finding is that the insertion variant of *NFKB1* polymorphism is often associated with tumor development, although there are very discrepant results, a fact that was repeated in combination with the low number of studies when *NFKBIA* polymorphism was analyzed. Thus, we conclude that greater efforts are needed to better understand how these alterations acts in different tumor microenvironments.

Key words: NF-kB pathway. Cancer development. Genetic polymorphism.

INTRODUCTION

Considered the major public health problem worldwide, cancer has caused intense concern, mainly due to the aging and expanding world population, as well as the increasing adoption of cancer-causing habits (YANG et al., 2014) such as inadequate eating habits (KIM et al., 2017; PARK et al., 2017), use of hormonal contraceptives (GIERISCH; COEYTAUX; URRUTIA, 2013; XU et al., 2018), tobacco smoking (MARTÍN-SÁNCHEZ et al., 2019), alcohol consumption (VARELA-REY et al., 2011), stressful and sedentary lifestyle (FARAROUEI et al., 2018; WONG et al., 2019), beside others, estimated at around 19 million of new cases by 2020, reaching 29.5 million by 2040 (WHO, 2018). Although the etiology of tumor development has not yet been fully elucidated, it is believed that there is a combination of genetic predisposition and exposure to environmental factors to trigger the pathogenesis, which characterizes cancer as a multifactorial disease (WANG et al., 2016).

It is estimated that at least 25% of all cancers are related to chronic infections and other types of unresolved inflammation (WU et al., 2014). Given that inflammation may be an important risk factor in carcinogenesis, the Nuclear factor-kappa B (NF-kB) assumes a potential role in it, since it acts as a transcription factor of more than 200 genes (WANG et al., 2016), including those responsible for the cytokines TNF-α, IL-1, IL-6 and IL-8, which regulate immune response as well as adhesion molecules, which lead to the recruitment of leukocytes to sites of inflammation, in addition to up-regulate of matrix metalloproteinases (MMPs), which contribute to evasion of cancer cells and control the vascularization of tumors via upregulation of vascular endothelial growth factor (VEGF) and its receptors, contributing for tumor progression (HOESEL; SCHMID, 2013).

It is established that deregulation of the NF-kB pathway at different levels is involved with several human diseases, such as rheumatoid arthritis, asthma, inflammatory bowel disease (IBD) and ulcerative colitis (LAWRENCE, 2009), as well as cancers. In tumors, mutations, epigenetic alterations or even by pharmacological means the modulation of NF-kB activity occurs, which generally acts aberrantly and varies according to the type of tumor (KINKER et al., 2016). An example of this is the overactivation of NF-kB members that occurs due to chromosome translocations that favor the increase of the expression of the antiapoptotic gene *BCL2*, frequently found in lymphoid tumors (RIEMANN et al., 2006).

In this way, the aim of this study was to make a brief survey about the main characteristics of the NF-kB pathway and, later, a bibliographic survey of the reports of polymorphisms rs28362491 of *NFKB1* and rs696 of *NFKBIA* in different types of tumors. The selection of these genetic polymorphisms for the accomplishment of this review occurred when considering that these consist of alterations in components of the canonical pathway, which are crucial for numerous cellular processes. In addition, although there are several studies that sought to understand their respective roles in several tumor types, the results found were generally very

divergent, making the attempt to understand the involvement of each one of them in the tumor process to be inconclusive.

MATERIALS AND METHODS

The search was performed in the Pubmed/MEDLINE (Medical Literature Analysis and Retrieval System Online) database using the following terms: “NFkB pathway”; “cancer”; “NFKB1 polymorphism”; “rs28362491”; “-94 ins/delATTG”; “NFKBIA polymorphism” and “rs696”. The bibliographical references of articles as well as meta-analysis studies were also analyzed for identification of additional studies.

NF-kB PATHWAY CHARACTERISTICS

NF-kB: genes and proteins

Discovered formerly as a transcription factor which binds to a 10 bp DNA element in kappa immunoglobulin light-chain enhancer in B cells by Sen and Baltimore in 1986 (YANG et al., 2014), NF-kB consists of a pleiotropic transcription factor which is composed by five members in mammals: c-Rel, RelB, RelA (p65), p105/p50, and p100/p52 (FU et al., 2017). *NFKB1* gene mapped on 4q23-q24, is composed of 24 exons and introns varying between 323 and 40000 bp, spanning 156 kb. This gene encodes p105 and p50 proteins, p105 is a non-DNA-binding cytoplasmic molecule whilst p50 is a DNA-binding protein and corresponds to the N-terminus of p105. *NFKB2* gene, mapped on 10q24, encodes p100 and p52 proteins (SUN; ZHANG, 2007). Both p105 and p100 contain ankyrin repeats, which are degraded during the proteasomal processing upon maturation to p50 and p52, respectively (SKAUG; JIANG; CHEN, 2009). *RELA* gene, at 11q12-q13 with 10 exons, encodes RelA; RelB is encoded by *RELB* gene, located in 19q13, and c-Rel is encoded by *REL* gene, located on 2p13-p12 (Table 1).

Table 1 Characteristics of components of NF-kB family

Protein	Gene	Chromosome	Signaling pathway
c-Rel	*REL*	2	canonical
p105/p50	*NFKB1*	4	canonical
RelA	*RELA*	11	canonical
p100/p52	*NFKB2*	10	non-canonical
RelB	*RELB*	19	non-canonical

Although all the NF-kB family members may form homo- and heterodimers, including p50/RelA, p50/RelB, p50/c-Rel, p52/RelA, p52/c-Rel, RelA/RelB, RelA/c-Rel, p50/p50, p52/p52 and RelA/RelA, the major heterodimer is the formed of p50 and RelA subunits (SUN; ZHANG, 2007). Interestingly, because the lack the transactivation domain, the p50 and p52 dimers cannot bind to the elements of gene promoters, acting as transcriptional repressors. However, when they bind to members that have a transactivation domain, such as RelA or RelB, they act as transcriptional activators (TILBORGHS et al., 2017). The ability to form homo- or heterodimers occurs due to the presence of the N-terminal REL-homology domain (RHD). In addition, this RHD are still involved in activities such as DNA binding, nuclear translocation and interaction with IkB proteins (SKAUG; JIANG; CHEN, 2009).

NFKBI: genes and proteins

The inhibitors of NF-kB, called IkB proteins are the most important NF-kB-interacting proteins and are responsible for the retention of NF-kB dimers in the cytoplasm of non-stimulated cells by means the presence of multiple ankyrin repeats which associate with DNA binding domains of the transcription factors, maintaining NF-kB in an inactive state (VALLABHAPURAPU; KARIN, 2009; TILBORGHS et al., 2017). The IkB family is composed of classical proteins including IkBα, IkBβ and IkBε, that act as NF-kB inhibitors and atypical IkB-like proteins IkBζ and Bcl-3, which function as NF-kB coactivators (SKAUG; JIANG; CHEN, 2009). The best characterized proteins is IkBα, a protein that contains 37-kDa and is encoded by the *NFKBIA* gene, which contains 3.5 kb long, with six exons and is located on chromosome 14q13 (SONG et al., 2011; MOROTTI et al., 2016).

NF-kB signaling pathways

The signaling pathways differs according to the NF-kB factors that are activated, and are called canonical or classical pathway, in which occurs involvement of the c-Rel, RelA and p50 factors; and non-canonical or alternative pathway, where the main

factors are the dimers of p52 and RelB (WENIGER; KÜPPERS, 2016). The forms of activation of canonical and non-canonical pathways are represented in Figure 1.

Canonical pathway

Activation of the canonical pathway occurs primarily through the stimulus provided by molecules such as tumor necrosis factor (TNF)-α, interleukin-1β (IL-1β) or Toll-like receptor (TLR) ligands, or yet radiation and stress signals, which lead to the transduction signals that reach the IkB kinase (IKK) complex. This complex is composed by two catalytic subunits (IKKα and IKKβ), as well as of the regulatory subunit NF-kB essential modulator (NEMO or IKKγ). Once activated, this complex will be responsible for the phosphorylation of inhibitor protein NF-kB inhibitor α (IkBα), marking it for subsequent proteasomal degradation (SKAUG; JIANG; CHEN, 2009). Thus, the p50/RelA dimer that was trapped in the cytoplasm by the IkBα becomes free to accumulate in the nucleus and activate the expression of target genes (KINKER et al., 2016). Of course, searching for the termination of NF-kB signaling, one of the first genes to be transcribed is *NFKBIA*, responsible for encoding IkBα, a protein that will promote negative feedback by removing the transcription factor from the nucleus (MOROTTI et al., 2016).

Non-canonical pathway

On the other hand, the non-canonical pathway is triggered by some members of the TNF family, such as CD40L, lymphotoxin, B cell-activating factor (BAFF), receptor activator of NF-kB ligand (RANKL), and TNF-related weak inducer of apoptosis (TWEAK), which promotes the activation of the NF-kB inducing kinase (NIK), which phosphorylates and activates IKKα dimers. In this manner, IKKα through phosphorylation of serine residues contained in the C-terminal ankyrin repeat domain leads to the polyubiquitination of p100. Thus, p100 undergoes partial degradation and there is the release of p52, which binds to RelB. With the increased availability of the p52/RelB free dimer in the cytoplasm, it translocates to the nucleus, allowing the transcription of its target genes, which are mainly involved with lymphoid organogenesis, as well as with the development and survival of B cells (VALLABHAPURAPU; KARIN, 2009; WENIGER; KÜPPERS, 2016).

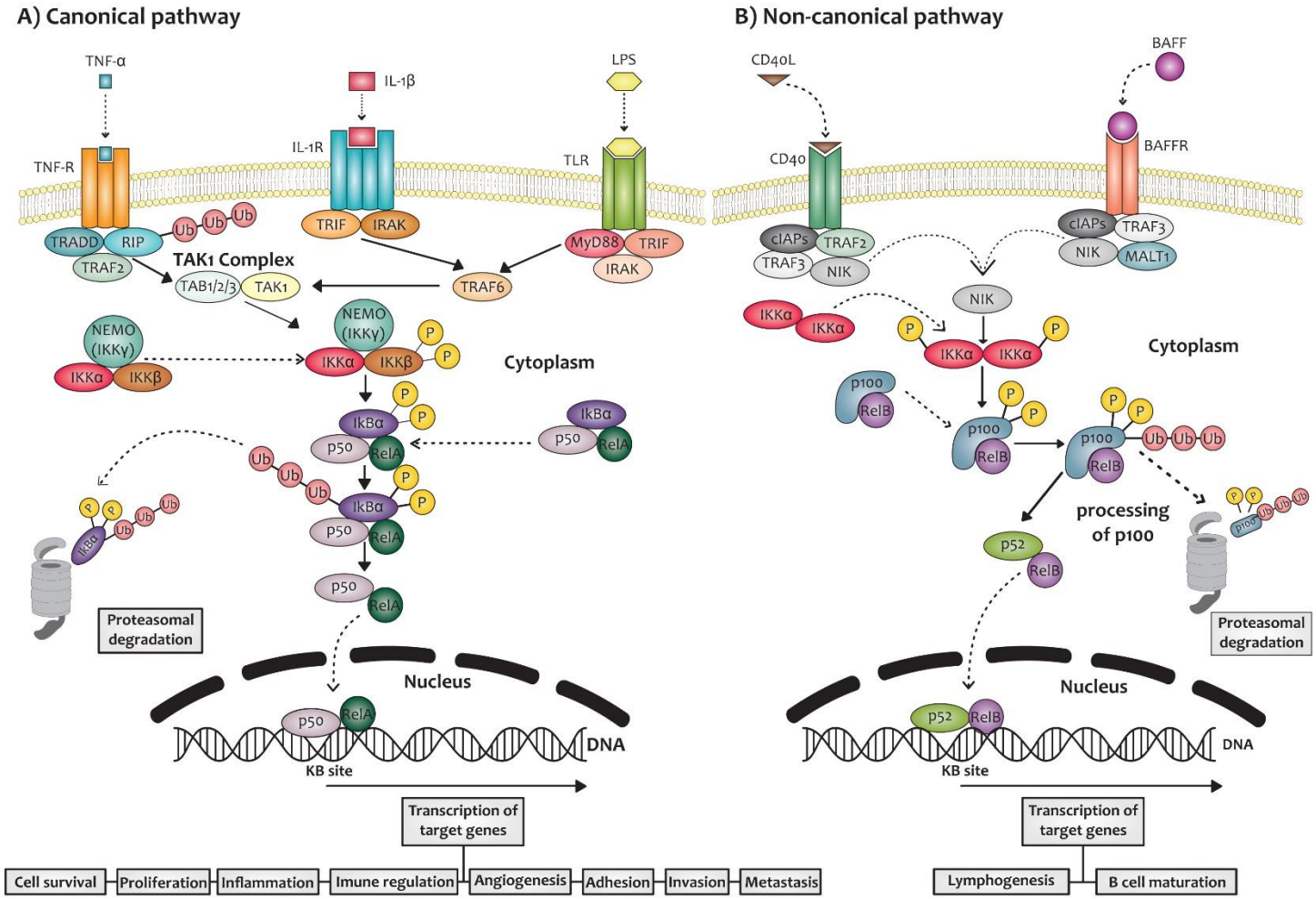


Figure 1 NF-kB signal transduction pathways. (A) Canonical pathway: Stimuli such as TNF-α, IL-1β and TLR ligands lead to the activation of IKK, which causes the phosphorylation of IkBα, which when degraded, free the p50/RelA dimer. It will accumulate in the nucleus and is related to the expression of genes involved in proliferation, metastasis, angiogenesis, among others. (B) Non-canonical pathway: Through stimuli such as CD40L and BAFF, activation of NIK occurs, which will activate IKKα dimers. These, in turn, will induce p100 processing, yielding p52, which once bound to RelB, forms a dimer that will translocate to the nucleus and is involved in lymphoid organogenesis and in the development and survival of B cells.

POLYMORPHISMS OF *NFKB1* AND *NFKBIA* AND TUMORS DEVELOPMENT

Regarding the importance that the NF-kB pathway exerts within the cells through the regulation of genes related to immune response, cell adhesion, proliferation, differentiation, besides apoptosis, metastasis and angiogenesis (ESKANDARI-NASAB et al., 2016; LI et al., 2016), it is evident that the existence of structural or expression alterations in components of this pathway can lead to the loss of cellular homeostasis and favor the increased risk in the development of diseases, such as inflammatory, infectious and autoimmune diseases, as well as the increase in the susceptibility of several cancers (LEONE et al., 2018). Among the genetic mutations that lead to the altered expression of the pathway proteins, there are the polymorphisms of *NFKB1* (rs28362491) and *NFKBIA* (rs696).

The rs28362491 polymorphism of *NFKB1* consists of an insertion/deletion of four bases at position -94 of the promoter region of the gene, located between two putative key promoter regulatory elements. The allele containing the deletion is less able to bind transcription factors, producing lower transcript levels in luciferase reporter systems. Consequently, carriers of the deletion allele have lower intracellular levels of NFKB1 isoforms (p105 and p50) (KARBAN et al., 2004; LEONE et al., 2018), while the allele containing the insertion has been associated with an increased production of NF-kB (LI et al., 2016). The minor allelic frequency (MAF) expected for this polymorphism in the world population is 0.42 for deletion allele as reported for 1000G database (NCBI, 2019).

The exchange of a guanine by an adenine at position 2758 is what characterizes the polymorphism rs696, located in the 3' UTR region of *NFKBIA* gene (PALLAVI; ANOOP; SHOWKET, 2015), whose world MAF is 0.46 for A allele according 1000G (NCBI, 2019). In silico analyses revealed that this variant is located at the miR449a recognition site and ex vivo luciferase data showed that the 2758 A allele appears to favor a greater ability of miR-449a to bind to the 3' UTR *NFKBIA* (SONG et al., 2011). As a result, there is a reduction in the expression of *NFKBIA*, favoring the constitutive activity of NF-kB, which in practice means that individuals carrying the AA genotype, because they have smaller amounts of NF-kB inhibitor, remain with the activity of this prolonged, which is related to the development of inflammatory and infectious diseases and also to tumorigenesis (SIMONIAN et al., 2018).

A total of fifty-two studies that were used in this review are described below, which are organized according to the type of tumor. These studies were carried out on individuals from fourteen nationalities and were published between 2006 and 2018 (Table 2). In addition, the studies that concluded that the polymorphisms rs28362491 and/or rs696 were associated with the risk or protection against the development of tumor are shown in Figure 2.

Table 2 Association studies of the *NFKB1* rs28362491 and *NFKBIA* rs696 polymorphisms in different tumor types

Tumor type	Year	Country	Polymorphism analyzed		Author	Main finding	Genotyping method
			-94 ins/del ATTG (rs28362491)	2758 G>A (rs696)			
Bladder cancer	2010	China	x		Tang	The frequency of ins allele was significantly higher in patients than in control subjects	PCR-PAGE
	2013	China	x		Li	Del/del was associated with increased risk and it	TaqMan
	2016	China	x		Li	Del/del was detected to be associated with a significantly increased risk	TaqMan
Breast cancer	2016	Iran	x		Eskandari-Nasab	Del/del genotype were associated with a reduced risk	AS-PCR
CRC	2006	Germany	x		Riemann	This polymorphism has no effect on risk and course of this disease	Pyrosequencing
	2007	China/ Sweden		x	Gao	GA were related to an increased risk in Chinese individuals and GG may be considered as a prognostic factor for Swedish patients	PCR-RFLP
	2007	China/ Sweden	x		Lewander	Del allele was associated with increased susceptibility to sporadic tumors in the Swedish population	PCR-RFLP
	2010	Denmark	x		Andersen	Del carriers showed an increased risk of disease	TaqMan
	2011	China	x	x	Song	InsATTG variants genotypes (ins/ins + ins/del) combined with GG were associated with an increased risk	PCR-RFLP
	2012	Sweden	x		Ungerbäck	DelATTG variants genotypes (ins/del and del/del) were found to be associated with poorer survival in patients diagnosed with invasive cancer	TaqMan
	2013	Malaysia	x		Mohd Suzairi	Ins/ins was found to significantly increase the risk of this tumor	PCR-RFLP
	2014	Spain	x		Dzhugashvili	Del/del was associated with greater response after the treatment with primary chemoradiation therapy	TaqMan
	2015	Denmark	x		Kopp	Del carriers had an increased risk for the tumor	PCR-KASP
	2017	Brazil	x		Cavalcante	Del/del carriers have more chances of developing this tumor	PCR-CGE
	2017	Brazil	x		Marques	Del allele was associated with more incidents to colon than rectosigmoid tumors	PCR-CGE
	2017	Egypt	x		Youssef	Ins/ins was associated with the risk of developing the tumor in subjects analyzed	PCR-RFLP
	2018	Iran		x	Simonian	AA was more frequent in affected individuals	PCR-RFLP

Continuation of Table 2

Tumor type	Year	Country	Polymorphism analyzed		Author	Main finding	Genotyping method
			-94 ins/del ATTG (rs28362491)	2758 G>A (rs696)			
CSCC	2010	China	x		Zhou	The frequency of ins/ins genotype was higher in affected patients	PCR-PAGE
	2015	India	x	x	Pallavi	Patients with ins/ins and GG genotypes have a high probability of getting persistent infection of HPVs and progress up to the advance stage of disease	PCR-RFLP
ESCC	2013	India	x	x	Umar	No independent role of both polymorphisms in susceptibility of this tumor was found, nor with survival outcome	PCR-PAGE/ PCR-RFLP
Gastric cancer	2009	Taiwan	x		Lo	InsATTG variants were significantly greater in cancer cases	PCR
	2013	Japan	x		Arisawa	Del/del was associated with development of this cancer, it was closely associated with diffuse type	PCR-SSCP
	2014	China	x		Hua	Del allele was significantly higher in affected patients	MassARRAY
	2017	Brazil	x		Cavalcante	Del/del genotype carriers have more chances of developing this tumor	PCR-CGE
GEPC	2009	Turkey	x		Burnik	No significant differences in relation of genotype distribution was detected	PCR-RFLP
HCC	2013	Taiwan	x		Cheng	Ins allele frequency was significantly higher in affected patients	TaqMan
	2014	China	x		Gao	DelATTG variants genotypes were associated with higher risk for this cancer	TaqMan
	2014	China	x	x	Zhang	Del/del genotype increased the frequency of HCC-related mutation in hepatitis B virus, predisposing infected patients to a higher risk of developing this tumor. No associations with the *NFKBIA* polymorphism were found	PCR
HNSCC	2008	Germany	x		Lehnerdt	No association with this polymorphisms was found	Pyrosequencing
	2017	India	x		Gupta	Ins/ins may increase the risk when associated with behavioral factors	PCR-RFLP

Continuation of Table 2

Tumor type	Year	Country	Polymorphism analyzed		Author	Main finding	Genotyping method
			-94 ins/del ATTG (rs28362491)	2758 G>A (rs696)			
Lung cancer	2013	China	x	x	Huang	InsATTG variants genotypes conferred an increased risk of COPD and promoted COPD progression, whereas AA had an increased risk for cancer	TaqMan
	2014	Turkey	x	x	Oltulu	Del/del genotype frequency was higher in affected patients, whereas no association with *NFKBIA* polymorphism was found	PCR-RFLP
	2015	China	x		Wang	Ins allele was significantly associated with the risk of disease	PCR-RFLP
	2015	China	x		Yin	No association was found between the polymorphism and the risk for this cancer	PCR
	2015	China	x		Zhang	DelATTG variants genotypes were associated with significant reduction risk	PCR-RFLP
Melanoma	2007	Sweden	x	x	Bu	The combination of ins/ins and GG genotypes were correlated with melanoma risk	PCR-RFLP
	2016	Brazil	x		Escobar	Ins allele were a risk factor aggravated by homozygosis	PCR-CGE
NPC	2009	China	x		Zhou	Ins allele is associated with increased tumor risk	PCR-PAGE
	2015	China	x	x	Liu	InsATTG variants genotypes and AA conferred an increased tumor risk	TaqMan
Non-solid tumors	2005	United Kingdom		x	Osborne	No significant differences in genotype or allele frequencies between HL cases and controls were found	TaqMan
	2006	Germany	x		Riemann	This polymorphism has no effect on HL risk and course	Pyrosequencing
	2012	Denmark	x		Vangsted	No association with MM risk was found	TaqMan
Ovarian cancer	2011	China	x		Fan	InsATTG variants genotypes increased risk of advanced disease	PCR-CGE
	2013	China	x		Huo	Ins allele was a risk factor for tumor susceptibility in this population	MassARRAY
	2015	China	x		Chen	Ins allele was more frequent in affected patients	MassARRAY
	2015	China	x		Lu	Ins/ins genotype was associated with a significantly increased risk for this tumor	PCR-RFLP
OSCC	2006	Taiwan	x		Lin	Ins allele was more frequent in affected patients older than 50 years	PCR-PAGE
	2012	Taiwan	x		Lin	Ins allele is related to susceptibility to development of tumor when associated with behavioral factors	TaqMan

Continuation of Table 2

Tumor type	Year	Country	Polymorphism analyzed		Author	Main finding	Genotyping method
			-94 ins/del ATTG (rs28362491)	2758 G>A (rs696)			
Osteosarcoma	2015	China	x		Li	InsATTG variants genotypes carriers showed higher risk for this tumor	PCR-RFLP
Prostate cancer	2009	China	x		Zhang	Ins allele frequency was significantly higher in affected patients	PCR-PAGE
	2013	Denmark	x		Kopp	Del allele carriers of had a tendency toward a reduced risk of disease	TaqMan
	2015	China	x	x	Han	DelATTG variants genotypes were associated with a lower tumor risk, whereas association with *NFKBIA* polymorphism was not found	PCR-RFLP
	2016	China	x		Li	No association with the polymorphism was found	TaqMan
Renal cancer	2006	Germany	x		Riemann	This polymorphism has no effect on risk and course of this disease	Pyrosequencing
	2012	China	x		Cai	Ins/ins genotype carriers had an increased risk for this tumor	TaqMan
	2016	China	x		Li	No association with the polymorphism was found	TaqMan
Thyroid cancer	2015	China	x		Wang	DelATTG vatiants genotypes were more frequent in the affected patients	PCR-PAGE

CLL: chronic lymphocytic leukemia; CRC: colorectal cancer; CSCC: cervical squamous cell carcinoma; ESCC: esophageal squamous cell carcinoma; GEPC: gastroenteropancreatic cancer; HCC: hepatocellular cancer; HL: Hodgkin's lymphoma; HNSCC: head and neck squamous cell carcinoma; MM: multiple myeloma; NPC: nasopharyngeal carcinoma; OSCC: oral squamous cell carcinoma; AS-PCR: allele-specific polymerase chain reaction; PCR-CGE: polymerase chain reaction with capillary gel electrophoresis; PCR-KASP: polymerase chain reaction kompetitive allele specific; PCR-PAGE: polymerase chain reaction-polyacrylamide gel electrophoresis; PCR-RFLP: polymerase chain reaction-restriction fragment length polymorphism; PCR-SSCP: polymerase chain reaction-single-strand conformation polymorphism.

Type	Authors	Allele or genotype	Odds ratio	Confidence interval (95%)
Melanoma	Bu et al., 2007	Ins/ins	1.58	1.09 - 2.28
	Escobar et al., 2016	Ins/ins	1.78	1.06 - 3.00
	Bu et al., 2007	GG	1.72	1.21 – 2.44

Type	Authors	Allele or genotype	Odds ratio	Confidence interval (95%)
Lung cancer	Oltulu et al., 2014	Del/del	3.50	1.24 - 9.87
	Wang et al., 2015	Ins	1.37	1.12 - 1.65
	Zhang et al., 2015	Del/del	0.34	0.22 - 0.53
	Huang et al., 2013	AA	1.53	1.30 – 1.80

Type	Authors	Allele or genotype	Odds ratio	Confidence interval (95%)
HCC	Cheng et al., 2013	Ins	2.23	1.32 - 3.77
	Gao et a., 2014	Del variants	1.54	1.04 - 2.28

Type	Authors	Allele or genotype	Odds ratio	Confidence interval (95%)
Osteosarcoma	Li et al., 2015	Ins	1.60	1.04 - 2.47

Type	Authors	Allele or genotype	Odds ratio	Confidence interval (95%)
Colorectal cancer	Lewander et al., 2007	Del/del	6.77	2.34 - 19.63
	Song et al., 2011	Ins variants	1.47	1.14 - 1.86
	Mohd-Suzairi et al., 2013	Ins/ins	2.42	1.24 - 4.73
	Kopp et al., 2015	Del variants	1.17	1.03 - 1.34
	Cavalcante et al., 2017	Del/del	3.73	1.45 - 9.60
	Youssef et al., 2017	Ins/ins	18.28	4.87 - 68.60
	Gao et al., 2007	GA	3.20	1.61 - 6.38
	Song et al., 2011	GG	1.38	1.14 - 1.66
	Simonian et al., 2018	AA	3.46	1.96 - 6.10

Type	Authors	Allele or genotype	Odds ratio	Confidence interval (95%)
Prostate cancer	Zhang et al., 2009	Ins	1.46	1.03 - 2.08
	Han et al., 2015	Del/del	0.60	0.37 - 0.91
	Li et al., 2016	Del/del	1.32	1.14 - 1.52

Type	Authors	Allele or genotype	Odds ratio	Confidence interval (95%)
HNSCC	Gupta et al., 2017	Ins/ins	13.96	4.22 - 46.11
NPC	Zhou et al., 2009	Ins/ins	1.55	1.01 - 2.36
	Liu et al., 2015	Ins variants	1.30	1.09 - 1.55
	Liu et al., 2015	AA	1.41	1.20 – 1.66
OSCC	Lin et al., 2006	Ins	1.78	1.19 - 2.65
	Lin et al., 2012	Ins variants	1.80	1.20 - 2.80
ESCC	Umar et al., 2013	GA + GG	0.52	0.29 – 0.93

Type	Authors	Allele or genotype	Odds ratio	Confidence interval (95%)
Thyroid	Wang et al., 2015	Del variants	1.38	1.03 - 1.85

Type	Authors	Allele or genotype	Odds ratio	Confidence interval (95%)
Breast cancer	Eskandari-Nasab et al., 2016	Del	0.66	0.50 - 0.86

Type	Authors	Allele or genotype	Odds ratio	Confidence interval (95%)
Gastric cancer	Lo et al., 2009	Ins variants	4.52	1.86 - 11.00
	Arisawa et al., 2013	Del/del	2.24	1.33 - 3.75
	Hua et al., 2014	Del	1.41	1.17 - 1.71
	Cavalcante et al., 2017	Del/del	2.92	1.35 - 6.30

Type	Authors	Allele or genotype	Odds ratio	Confidence interval (95%)
Bladder cancer	Tang et al., 2010	Ins	1.46	1.11 - 1.93
	Li et al., 2013	Del/del	1.92	1.42 - 2.59
	Li et al., 2016	Del/del	1.32	1.14 - 1.52

Type	Authors	Allele or genotype	Odds ratio	Confidence interval (95%)
Cervical cancer	Zhou et al., 2010	Ins variants	2.26	1.33 - 3.85
	Pallavi et al., 2015	Ins variants	3.70	2.50 - 5.50
	Pallavi et al., 2015	GA + GG	3.40	2.50 - 5.50
Ovarian cancer	Fan et al., 2011	Ins/ins	2.24	1.22 - 4.13
	Huo et al., 2013	Del	0.63	0.47 - 0.84
	Chen et al., 2015	Ins	1.37	1.13 - 1.65
	Lu et al., 2015	Ins/ins	1.39	1.00 - 1.92

Figure 2 Studies that concluded that polymorphisms rs28362491 and/or rs696 were associated with the risk of tumor development, according to affected site. HCC: hepatocellular cancer; HNSCC: head and neck squamous cell carcinoma; NPC: nasopharyngeal carcinoma; OSCC: oral squamous cell carcinoma.

Bladder Cancer (BC)

It was verified the association of rs28362491 polymorphism with BC in the Chinese population. The del/del genotype in male individuals, over 65 years of age, smokers and with family history was associated with a significant higher risk of carcinoma development. In addition, this polymorphism was also associated with a higher risk of invasive non-muscular, grade 1, single tumor and small size tumor. It was further verified that there was greater expression of *NFKB1* mRNA in tumor tissues of individuals with the homozygous genotype for insertion than in those with deletion allele (LI et al., 2013). Corroborating with this study, other authors also observed the association of the del/del genotype of this polymorphism with BC in the Chinese population (LI et al., 2016). Unlike this, in another study it was verified that the ins/ins genotype was associated with carcinoma risk (TANG et al., 2010).

Breast cancer (BRC)

Only one study sought to evaluate the association of the rs28362491 polymorphism in relation to BRC. This association was evaluated in the Iranian population and it was found that the del/del genotype frequency was decreased in cancer patients. In addition, in inheritance models, the deletion allele had a reduced risk with respect to the tumor. Finally, there were no statistical associations with the clinical characteristics, age, period age and menopause age with the polymorphism (ESKANDARI-NASAB et al., 2016).

Colorectal cancer (CRC)

Several studies have sought a better understanding of the rs28362491 polymorphism action in the context of CRC. A study conducted in the German population found no association between tumor site, grade, lymph node status, occurrence of metastases, pathologic stage or International Union Against Cancer (UICC) stage with the genotypes. In addition, no association between survival and the different *NFKB1* alleles remained unrelated even when only the subgroup of patients with early-stage tumor (UICC stages I and II) was analyzed. The authors concluded that, at least in the population studied, the gene variant should not be considered a predictive marker for risk or disease progression in CRC (RIEMANN et al., 2006).

On the other hand, several studies evidenced that the deletion allele carriers presented an increased risk of developing CRC. It has been demonstrated in Swedish patients that the deletion in homozygosis or heterozygosis was associated with increased susceptibility to the development of CRC, whereas in the Chinese population this association was not observed (LEWANDER et al., 2007). Similar results were found when working with Danish population, in which a higher risk of CRC was observed for those with deletion (ANDERSEN et al., 2010), whereas another study observed that deletion allele carriers had 17% increased risk of CRC compared to homozygous carriers of insertion allele (KOPP et al., 2015). Following the same line, however with the Northern Brazilian population, it was observed that patients with del/del genotype should present more chances of developing CRC in

comparison to other genotypes (CAVALCANTE et al., 2017), whereas in another study was verified an association between deletion allele and anatomical tumor location, being the this allele associated more colon related incidents than rectosigmoid (MARQUES et al., 2017).

Regarding this tumor, it was also observed that, in addition to heterozygosity being significantly associated with CRC susceptibility when compared to ins/ins genotype carriers, patients with invasive CRC whose genotype was del/del presented poorer survival. Thus, authors proposed that rs28362491 polymorphism could be used as a prognostic marker in conjunction with other genetic alterations (UNGERBÄCK et al., 2012). A study developed in Spain, however, demonstrated that the genotype del/del was related to a better response to CRC treatment with preoperative chemoradiation, justified by the decreased inflammatory response observed in patients with del/del genotype, which is possibly associated with a better sensitivity to treatment (DZHUGASHVILI et al., 2014).

Finally, three papers had conclusions contrary to those presented so far. The first, carried out in the Southern Chinese population, showed that the genotypic distribution between CRC case group and controls differed in both co-dominant (del/del *vs.* ins/ins *vs.* ins/del) and dominant (ins/ins+ins/del *vs*. del/del) genetic models, allowing us to conclude that, in this population, the insertion allele was associated with increased risk of CRC (SONG et al., 2011). The others studies were carried out in the Malaysian and Egyptian populations and both found that the ins/ins genotype was associated with increased risk of CRC (MOHD SUZAIRI et al., 2013; YOUSSEF et al., 2017).

Regarding the involvement of the rs696 polymorphism of *NFKBIA* in CRC, only three studies were found and the results of which were quite different. Developed in two populations, one of them found that Chinese individuals aged ≥ 50 years with GA genotype had a higher risk of developing CRC compared to those with AA genotype, whereas the Swedish patients with GG genotype had a poorer outcome regardless of gender, age, tumor location, Dukes' stage and differentiation (GAO et al., 2007).

Another study concluded that the GG genotype was associated with a significantly increased risk of CRC in a Southern Han Chinese population and demonstrated that G allele decreased mRNA stability and/or translational efficacy and patients with GG genotype had lower levels of IkBa in peritumoral tissues (SONG et al., 2011). Lastly, in Iranian population was demonstrated a significant association of AA genotype with risk of CRC (SIMONIAN et al., 2018).

Cervical squamous cell carcinoma (CSCC)

On the rs28362491 polymorphism is this tumor type only two papers were found. In the first, carried out in the Chinese population, the authors verified that individuals with ins/ins genotype presented higher risk for the development of cervical cancer. It still can be associated with high clinical stage, lymph node status and positive

parametrial invasion (ZHOU et al., 2010). The same result was observed in Indian population, in which the authors observed that cervical cancer patients were found to be more prone of being a carrier of insertion (*NFKB1*) and GG genotype (*NFKBIA*). In this study it was also observed that HPV infected postmenopausal women that had high parity with use of tobacco and were carrier of insertion and GG genotype were more susceptible to develop cervical cancer (PALLAVI; ANOOP; SHOWKET, 2015).

Esophageal squamous cell carcinoma (ESCC)

In the only study with this tumor type that was found, the authors observed no associations of the rs28362491 and rs696 polymorphisms with the overall risk of ESCC in Indian population. There was also no significant effect of these polymorphisms on the survival outcome of ESCC patients. Regarding the tumor location, it was verified that GA+GG genotypes of rs696 polymorphism were associated with reduced risk of upper and lower third esophageal tumors. Finally, the authors also performed the evaluation of the interaction of polymorphisms with environmental risk factors, however, no significant modulation in risk of ESCC was found in tobacco users, smokers and alcohol users (UMAR et al., 2013).

Gastric cancer (GC)

As for this tumor type, a study conducted in Taiwanese population found that the insertion allele and ins/ins genotype were significantly more frequent in GC patients, especially in patients >65 years but not in young patients and the polymorphism did not correlate with clinicopathologic factors, such as sex, tumor location, depth of invasion, tumor cell differentiation, lymph node status; and patient survival (LO et al., 2009). In a study carried out in Japanese population was found association between the del/del genotype and the risk of developing GC, including diffuse type of GC through severe inflammation (ARISAWA et al., 2013). A study in Brazilian population corroborated with this finding, since del/del genotype was associated with a greater chance of developing this type of tumor (CAVALCANTE et al., 2017). On the other hand, in Chinese population was observed that the insertion allele was significantly more present in GC patients than in healthy controls (HUA et al., 2014).

Gastroenteropancreatic cancer (GEPC)

In the only study on this type of tumor, the authors verified the importance and frequency of rs28362491 polymorphism in patients with gastroenteropancreatic neuroendocrine tumor in the Turkish population. It was verified that del/del genotype was less detected in the study group and absent in the subgroup with pancreatic neuroendocrine tumors. However, no significant differences were found for allelic frequency and genotypic distribution between patients and controls (BURNIK; YALÇIN, 2009).

Hepatocellular cancer (HCC)

Regarding the rs28362491 polymorphism in this tumor, it was found that ins/ins genotype may increase the risk of developing HCC in Taiwanese population. When analyzed the clinicopathological characteristics, female patients with HCC and ins/ins

genotype were associated with a lower clinical staging and a reduced tumor size (CHENG et al., 2013). In contrast, a study carried out in Chinese population found that the ins/del and del/del genotypes were associated with a higher risk of this carcinoma. These authors also investigated the effects of rs696 polymorphism in this tumor type, however no significant associations were found (GAO et al., 2014).

Another study also conducted in China provided insights regarding virus-host interactions in hepatitis B virus-induced hepatocarcinogenesis along with rs28362491 and rs696 polymorphisms. It was verified that del/del genotype significantly increased the frequency of HCC-related mutation in hepatitis B virus, predisposing infected patients to a higher risk of developing this tumor. No statistical associations were found related to hepatitis virus mutations and rs696 polymorphism (ZHANG et al., 2014).

Head and neck squamous cell carcinoma (HNSCC)

In this type of tumor, the rs28362491 polymorphism has a not well-established role and was analyzed in only two studies. In a study conducted in India, the ins/ins genotype was observed to be a risk factor to HNSCC when compared to del/del genotype and there is also an association with smoking and tobacco chewing (GUPTA et al., 2017). The other study verified that in German population this polymorphism was not shown as a predictive marker for global survival and relapse-free for this type of tumor, as well as for its different anatomical subsites (LEHNERDT et al., 2008).

Lung cancer (LC)

LC and chronic obstructive pulmonary disease (COPD) are the two major smoking related diseases. A significant association was evidenced between rs28362491 insertion carriers and rs696 AA genotype and risk of COPD and LC in Chinese population, respectively (HUANG et al., 2013). The authors proposed that variant carriers of some IkBα polymorphism have reduced IkBα expression and consequently less suppression of NF-kB which is associated with an increased risk of LC. A study conducted in Turkish population observed that del/del genotype is a risk factor for non-small cell lung cancer (NSLC), whereas rs696 does not confers any risk for this tumor (OLTULU et al., 2014). In agreement with the first study was found in Chinese population that insertion allele was associated with risk of NSLC and a higher frequency of ins/ins and ins/del genotypes was observed in smokers and patients with stage III and IV compared to I and II stages (WANG et al., 2015b). Two other studies carried out in Chinese population obtained different conclusions, while one observed reduced risk for ins/del and del/del genotypes (YIN et al., 2015); the other found no risk at all genotypes of rs28362491 polymorphism in LC development (ZHANG et al., 2015).

Melanoma

Through the analysis of rs28362491 and rs696 polymorphisms in the Swedish population was verified a higher frequency of the ins/ins genotype in patients with

melanoma, males and over 65 years. The del/del genotype, on the other hand, was associated with a higher frequency in tumors less thick and with low Clark stages (I and II), besides being significantly associated with melanomas of intermittent exposure to the sun. Regarding the rs696 polymorphism, the frequency of the GG genotype was higher in patients with melanoma, females and aged 45-65. However, when analyzed in a gender-stratified manner, the GG genotype is significantly higher in men older than 60 years. The frequency of this genotype was also higher in tumors of rarely exposed sites. There were no statistical differences at the transcriptional level for the different rs696 genotypes (BU et al., 2007).

Likewise, it was demonstrated an association between the ins/ins genotype and melanoma in Southern Brazil population. However, no statistical differences were identified regarding histopathological subtype, tumor location, Clark stages and Breslow thickness, gender, age, skin type and other genotypes. In addition, it was also shown that there was a dose effect, in which for each insertion allele the risk of melanoma increased, demonstrating that the genotypes for this polymorphism are significantly associated with melanoma and could be genetic markers of melanoma susceptibility in the studied population (ESCOBAR et al., 2016).

Nasopharyngeal carcinoma (NPC)

In the context of the NPC, ins/del and ins/ins genotypes of rs28362491 polymorphism conferred increased risk of development of this tumor when compared with the del/del genotype. The authors also evaluated the role of rs696 polymorphism and observed that AA genotype increased the risk of NPC by decreasing of IkBα expression due to modulation of miR-449a. The findings indicate that these genetics variants and their synergistic effect may contribute to the NPC predisposition in Chinese population (LIU et al., 2015). These data are in accordance with other study conducted in the same population, which observed that individuals with the insertion allele presented higher risk for NPC development compared with the deletion allele carriers and the risk was higher among homozygous genotype individuals (ZHOU et al., 2009).

Non-solid tumors

It has been previously reported the involvement of NF-kB members in rearrangements [t(10;14(q24;q32)] that cause an overactivation in neoplasms, among them, B and T cell malignancies (FRACCHIOLLA et al., 1993). In this context, the rs28362491 polymorphism in *NFKB1*, being present in the promoter region, probably acts modulating the gene activity and influencing the oncogenesis. An inferred mechanism occurs by increasing the expression of the antiapoptotic gene *BCL2*, which has been shown to be one of the strongest predictor of survival in chronic B-cell lymphocytic leukemia (CLL). Nevertheless, a study conducted in German population found no significant association of this polymorphism with anthropometric or clinical data of B cell CLL patients, showing that laboratory data and progression of CLL were not affected by the genotypes (RIEMANN et al., 2006).

Still aiming that changes in NF-kB pathway genes may lead to changes in the inflammatory response and possibly the risk of disease, a study with Danish individuals analyzed the *NFKB1* gene in multiple myeloma (MM) patients and concluded that there was no association with rs28362491 polymorphism and MM risk (VANGSTED et al., 2012).
A study conducted in the United Kingdom looked for possible changes in the coding region or in the promoter sequence of the *NFKBIA* gene in cases of Hodgkin's lymphoma (HL). For this, the authors evaluated the complete gene in individuals with familial HL but did not find mutations in any case. In addition, they verified and compared the frequency of 4 *NFKBIA* polymorphisms, among them the rs696, but finding no significant differences between cases and controls (OSBORNE et al., 2005).

Ovarian cancer
A few researches have been made about the rs28362491 polymorphism in ovarian cancer and all of them were realized in Chinese population. One of them found a significant increased risk for ovarian cancer development associated to the ins/ins genotype (LU et al., 2015). Significant association for the insertion allele was also observed in advanced ovarian cancer patients (FAN et al., 2011; CHEN; CAI; LIANG, 2015). This probably could happen due to altered *NFKB1* gene expression, since other authors observed that the level of NF-kB (p50) mRNA in cancer tissue was significantly higher in insertion carriers individuals (HUO et al., 2013).

Oral squamous cell carcinoma (OSCC)
Studies about the rs28362491 polymorphism in OSCC are scarce. There are two studies in Chinese population and the results are controversy. In one of them , there was no significant association between the frequency alleles and/or genotypes and risk of OSCC, but after age stratification, there was a higher frequency of the insertion allele in patients older than 50 years. These authors found no difference between *NFKB1* alleles or genotypes in patients with OSCC who exhibited different stages of lymph node metastasis or clinical stage. On the other hand, other study showed that the insertion allele was correlated to OSCC when combined with betel nut chewing and smoking. According to these authors, the increased risk of oral carcinogenesis could be caused by alterations in the affinities between the betel nut and tobacco constituents and the promoter of the polymorphic *NFKB1* gene, which result in changes in NF-kB expression and activity (LIN et al., 2006, 2012).

Osteosarcoma
The only study related to rs28362491 polymorphism and this tumor was developed in Chinese population and was observed that insertion allele carriers showed higher risk of osteosarcoma development. In addition, the authors conducted stratified analyses for combined genotypes with del/del *vs.* ins/del and ins/ins genotypes in osteosarcoma patients according to gender, age, Enneking stage, metastasis and location in order to determine the association between the rs28362491 polymorphism

and this clinicopathological features. The frequency of the insertion allele was significantly higher in metastatic osteosarcoma patients and among them with stage III (LI et al., 2015).

Prostate cancer (PC)

In relation to this tumor type in Chinese population, one study did not find significant associations with rs28362491 polymorphism (LI et al., 2016). Other study involving this polymorphism with PC found that the frequency of the insertion allele in patients was significantly higher compared to the controls. However, the polymorphism no presented significant correlation with clinical or pathological tumor staging (ZHANG et al., 2009).

In Danish population, it was observed that, although not significantly, deletion allele carriers had a tendency toward a reduced risk of cancer. In the analysis of stratification according to the cancer aggressiveness, it was verified that the deletion allele was attributed with a reduced risk to aggressive PC. In addition, it was found that deletion allele carriers individuals had reduced risk of cancer among non-users of nonsteroidal anti-inflammatory drugs (KOPP et al., 2013). In the same way, other study observed that the genotypes containing the deletion allele was associated with a reduced risk of PC. The authors also found that the del/del genotype decreased the risk of cancer in smokers and the elderly in the Chinese population. In addition, it was also investigated the association with rs696 polymorphism, but no significant associations were found (HAN et al., 2015).

Renal cancer

A study conducted in China found no significant associations between this type of tumor and alleles rs28362491 polymorphism under different genetic models (LI et al., 2016). Similarly, a study conducted in the German population also found no association between the genotypic variations of this polymorphism and renal cancer (RIEMANN et al., 2006).

On the other hand, other study conducted in China found that the ins/ins genotype frequency was significantly associated with an increased risk of this tumor. In the stratification analysis by age, gender, smoking habit, alcoholism and body mass index, the association between the ins/ins genotype and renal cancer was significant in younger and nonsmoking individuals. Regarding clinical staging and tumor grade stratification, the insertion allele was significantly associated with localized tumors (stages I and II) and with moderately and poorly differentiated tumors (grades III and IV) (CAI et al., 2012).

Thyroid cancer

A single study that was conducted in the Chinese population sought to verify the association of rs28362491 polymorphism with papillary thyroid carcinoma and was found that the genotypes containing the deletion allele were associated with increased risk of this tumor. In addition, no significant associations were found

regarding age, gender, foci number, lymph node status and tumor grade (WANG et al., 2015a).

CONCLUSION

In view of findings, it was possible to observe many studies related to the polymorphism rs28362491 of *NFKB1* and it concluded that, in general, the insertion allele, whether in homozygosis or heterozygosis, is often associated with the development of several tumor types, although the results presented in the literature are very divergent. On the other hand, there was a lower number of studies that investigated the polymorphism rs696 of *NFKBIA*, which presented quite different conclusions.

The divergence of the results found for the two polymorphisms in question can be justified by some factors: (1) the high complexity found in the NF-kB pathway, which leads to the activation of multiple genes which may be related to distinct responses from a tumorigenic process; (2) ethnicity, which consisted of a very diversified characteristic, although there was a predominance of analyzes performed with the Asian origin individuals, especially of Chinese population. However, even when the results obtained within this population were compared, the results were still quite variable even when the same tumor type was involved, and finally (3) the type of tumor that was analyzed, since the tumor microenvironment is very variable from one tumor to the other, which may have caused the differences found.

Thus, in order to verify the real influence of each polymorphism in the development of the various cancers, it is still necessary to analyze in a thorough way case by case so that it is possible to elucidate the role of these genetic changes in the process of tumor formation.

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3.2 ARTIGO 2

NFKB1/NFKBIA polymorphisms play a protective role against HPV infection

ABSTRACT

Human Papillomavirus (HPV) is the etiological agent of most frequently transmitted infection the anogenital tract. In general, the infection is asymptomatic, however, the persistence of oncogenic types added to the characteristics of the host, such as genetic background, immunological status and behavioral profile, may lead to formation of squamous intraepithelial lesions, which, once untreated, can progress to cervical cancer (CC). The NF-kB pathway plays an important role in controlling the expression of several genes essential to cellular activity, such as those related to immune response, proliferation, differentiation, apoptosis, among others. Thus, the purpose study was to evaluate the influence of rs28362491 polymorphism of *NFKB1* and rs696 of *NFKBIA* on HPV infection and development of pre-neoplastic and neoplastic lesions. Of the 334 patients recruited, 48.8% were HPV infected, and of these, 37.4% presented precursor lesions and 16.8% were diagnosed with CC. It was observed that among the infected women, the majority reported not knowing the virus (*P*=0.038), aged ≤24 years (*P*=0.001), smokers (*P*=0.043), single (*P*=0.007) and reported ≥4 sexual partners during their lifetime (*P*=0.024). When considering the lesions presence, patients with precursor lesions reported having had more sexual partners than other groups (*P*=0.006), whereas CC patients reported having had low education level (*P*=0.004), monthly income <1 minimum wage (*P*=0.001) and ≥5 full-term pregnancies (*P*<0.001). Regarding to the polymorphisms analysis, the combination of homozygous insertion genotype for *NFKB1* (II) and AA genotype for *NFKBIA* showed to be a protective factor against HPV infection [*P*=0.014; OR=0.259 $CI_{95\%}$ (0.088-0.765)], whereas it was not associated with lesion progression and CC development. Thus, the present study demonstrated for the first time that rs28362491 and rs696 polymorphisms may present a protective role against HPV infection in the analyzed population . From this promising finding, it will be possible to begin the search for a better understanding of the mechanisms by which these genetic alterations are involved in protection against HPV.

Key words: NF-kB pathway. NF-kB polymorphism. Cervical cancer. rs28362491. rs696.

INTRODUCTION

Cervical cancer (CC) is the second most prevalent cancer in women worldwide, with an estimative of 570,000 new cases and more than 311,000 deaths annually (WHO, 2018). In Brazil, is the third most common to undertake women, with expected 16,370 new cases for 2018/2019 biennium, representing 8.1% of the total tumors expected for this period (INCA, 2018).

Among the several factors related to CC development, Human Papillomavirus (HPV) infection persistence is mandatory (SAKAMOTO et al., 2018), being also the most frequently diagnosed sexually transmitted infection (WANG et al., 2018). Although the involvement of HPV is already recognized as an etiological factor and, being present in virtually all of CC cases (STEINBACH; RIEMER, 2018), the infection alone is insufficient for tumor development, being necessary the combination of other factors, including smoking habit, alcohol consumption, immune status, number of sexual partners, as well as the genetic background of the host (PALLAVI; ANOOP; SHOWKET, 2015). In this context, mutations in genes of essential inflammatory mediators, such as Nuclear Factor-kappa B (NF-kB) family proteins may play an important role in the pathogenesis and cervical carcinogenesis (UMAR et al., 2013).
NF-kB consists of a pleiotropic transcription factor, which in mammals is composed for five members: c-Rel (*REL*), RelB (*RELB*), RelA/p65 (*RELA*), p50/p105 (*NFKB1*) and p52/p100 (*NFKB2*) (FU et al., 2017). In the cytoplasm of resting cells, NF-kB remains in its inactivated form when associated with inhibitory proteins, such as IkBα which is encoded by *NFKBIA* gene (SONG et al., 2011; LIN et al., 2012). However, due to a wide range of stimuli, such as infections, pro-inflammatory cytokines and growth factors, IkBα is phosphorylated, allowing the release and nuclear translocation of NF-kB. Thus, in the nucleus, NF-kB is capable of binding to DNA promoter regions, inducing cell proliferation and transformation, and preventing the elimination of pre-malignant and malignant cells by upregulation of anti-apoptotic proteins (PALLAVI; ANOOP; SHOWKET, 2015).

Inside cells, the NF-kB pathway regulates several genes related to the immune response, cell adhesion, proliferation, differentiation, apoptosis, metastasis and angiogenesis (LIN et al., 2012; ESKANDARI-NASAB et al., 2016), so alterations on genes encoding components of this pathway may bring important consequences. In this context, two polymorphisms, an insertion/deletion (-94 ins/del ATTG, rs28362491) in *NFKB1*, that is located between two important promoter regulatory elements; and the exchange of a nucleotide on the 3'UTR region of *NFKBIA* (2758 G > A, rs696) (PALLAVI; ANOOP; SHOWKET, 2015), may favor the development of various diseases, such as autoimmune, inflammatory and infectious diseases, in addition to some types of tumors (LEONE et al., 2018). Based on this information, it is possible that the combined effects of these polymorphisms may be associated with susceptibility to cervical tumor development. Therefore, the aim of this study was to

evaluate the prevalence of this polymorphisms, as well as to verify if there is an association of these with HPV infection or with the development of pre-neoplastic and neoplastic lesions in women positive for the presence of HPV DNA.

MATERIALS AND METHODS

Ethical approval

This study was approved by the Institutional Ethics Committee Involving Humans Beings of the State University of Londrina, Londrina-PR, Brazil (CEP/UEL 133/2012; CAAE 05505912.0.0000.5231). The purpose of the study as well as the procedures were explained to all participants, and the written consent was obtained prior to the interview and the material collection.

Sample collection

A total of 334 patients were analyzed in the present study, of whom 308 had their cervical samples collected for convenience at the colposcopy outpatient clinic of the Intermunicipal Health Consortium of the Middle Paranapanema and at two Basic Healthcare Units in Londrina-PR, Brazil, between 2013 and 2018. All the patients were interviewed using a structured questionnaire to collect socio-demographic and sexual behavioral data. Subsequently, the cytobrushes used to collect the cervical secretion were stored in 2 mL of TE Buffer (10 mM Tris-HCl, 1 mM EDTA pH 8.0) at 4ºC until analysis; and peripheral blood was drawn into sterile syringes containing EDTA as anticoagulant and stored at 4ºC until DNA extraction. The remaining 26 samples consisted of cervical tumor tissue embedded in paraffin provided by the Londrina Cancer Hospital, with prior authorization of the patients, who also answered the questionnaire. According to the results of Polymerase Chain Reaction (PCR) and cervical cytology, the patients were stratified based on the presence or absence of the HPV infection and on lesion grade, respectively.

DNA extraction

Genomic DNA was obtained from cervical cytobrushes using DNAzol (Invitrogen Inc., Carlsbad-CA, USA) according to the manufacturer's instructions, and stored at –20ºC until use. Genomic DNA was also extracted from peripheral blood using a Biopur Mini Spin Plus Kit (Biometrix ®, Curitiba-PR, Brazil). Genomic DNA from paraffin embedded tumor samples was obtained using RecoverAll™ Total Nucleic Acid Isolation Kit (Thermo Fisher Scientific, Waltham-MA, USA) according to the manufacturer's instructions. DNA concentration was measured at 260 nm on a NanoDrop 2000c spectrophotometer (Thermo Fisher Scientific), and purity was assessed by the A260/A280 ratio.

HPV detection

HPV detection was performed using the DNA extracted from cervical samples by PCR. For this, were used primers MY09 and MY11, which were designed to amplify a conserved region in the HPV L1 gene (AJ617545.1). Human b-globin gene amplification was performed as an amplification control, using primers GH20 and

PC04. The sequences of primers and other information are contained in Table 1. Reaction conditions were 0.19 mM of dNTPs, 500 nM of each primer, 2 mM of $MgCl_2$, 1X of Buffer (Composition 10X: 200 mM Tris-HCl [pH 8.4], 500 mM KCl), approximately 80 ng of DNA and 1.25 U of Taq polymerase (Invitrogen^{TM}). PCR conditions were an initial cycle of 94ºC for 5 minutes, followed by 37 cycles of 30 seconds at 94ºC, 1 minute at 55ºC, 1 minute at 72ºC and final extension step at 72ºC for 10 minutes. The b-globin amplification was realized under the same conditions of HPV PCR. Reactions without template DNA were used as a negative control to test for contamination, and DNA from HeLa cells, which are stably integrated with HPV18, was used as positive control. The PCR products were submitted to 10% polyacrylamide gel electrophoresis, which was stained with silver nitrate.

Genotyping of *NFKB1* (rs28362491) and *NFKBIA* (rs696) polymorphisms

Genomic DNA from peripheral blood and tumor tissues paraffin embedded were used to detect rs28362491 polymorphism of *NFKB1* and rs696 of *NFKBIA* by Restriction Fragment Length Polymorphism (RFLP)-PCR. For *NFKB1* polymorphism, the primers sequences were designed according to the nucleotide sequence deposited in GenBank (NG_050628.1). In the gene promoter region, there are two ATTG repeats, one of them considered an insertion, which is cleaved in two fragments after the enzymatic restriction (allele I). In the absence of the enzyme restriction site, the PCR product remains with the same size (allele D). For *NFKBIA* polymorphism, the primers sequences were also designed according to GenBank (NC_000014.9) and the reaction products were digested by using the appropriate enzyme, described in Table 1. Reaction conditions for *NFKB1* and *NFKBIA* were 0.1 mM of dNTPs, 0.15 uM of each primer, 1.5 mM of $MgCl_2$, 1X of Buffer (Composition 10X: 200 mM Tris-HCl [pH 8.4], 500 mM KCl), approximately 60 ng of DNA and 1U of Taq polymerase (Invitrogen^{TM}). PCR conditions for *NFKB1* were an initial cycle of 95ºC for 5 minutes, followed by 35 cycles of 30 seconds at 95ºC, 30 seconds at 64ºC, 30 seconds at 72ºC and final extension step at 72ºC for 5 minutes, whereas for *NFKBIA* were an initial cycle of 95ºC for 5 minutes, followed by 35 cycles of 30 seconds at 95ºC, 30 seconds at 62ºC, 30 seconds at 72ºC and final extension step at 72ºC for 10 minutes. The enzymatic reactions for both *NFKB1* and *NFKBIA* occurred with 1U of the respective enzymes at 37ºC for an hour. Like the PCR products, the restriction products for both polymorphisms were subjected to 10% polyacrylamide gel electrophoresis stained with silver nitrate.

Statistical analysis

Women were grouped according the HPV infection status (infected or uninfected). Infected women, subsequently, were sub-grouped according the lesion grade [normal cytology, presence of pre-neoplastic squamous intraepithelial lesions (SIL) or CC]. Continuous data were expressed as median and interquartile range. Comparisons between the groups were performed by the Mann-Whitney test (HPV status) or Kruskal-Wallis test (lesion grade) with Bonferroni adjustment for multiple comparisons. The chi-square test was applied to compare the proportion of women

according socio-demographic and sexual behavioral characteristics within HPV infection status and lesion grade groups. Furthermore, chi-square test was used to verify Hardy-Weinberg equilibrium in these groups. Binary or multinomial logistic regression models were used to assess the association between polymorphisms and HPV infection or lesion grade, respectively. To build the models (adjusted analysis), significant independent variables chosen by chi-square test (P<0.05) were considered and selected using the Backward method. The adjustment variables in the final models were “age” and “sexual partners in past 6 months” for HPV infection analysis, and “marital status”, “number of full-term pregnancies”, “sexual partners in past 6 months” and “sexual partners during lifetime” for lesion grade analysis. The Hosmer-Lemeshow test showed the good quality of model fit when the P-value was greater than or equal to 0.05). Data analysis were conducted by SPSS Statistics 22.0 (SPSS Inc., Chicago-IL, USA). P-value <0.05 was considered statistically significant.

Table 1 PCR and RFLP procedures and products of HPV detection and polymorphisms genotyping

Gene		Primers	PCR product (bp)	Restriction enzyme	Genotype	Restriction products (bp)
L1 (HPV)	FWD	5′-CGTCCMAARGGAWACTGATC-3′ (MY09)	~450	-	-	-
	REV	5′-GCMCAGGGWCATAAYAATGG-3′ (MY11)				
Human b-globin	FWD	5-GAAGAGCCAAGGACAGGTAC-3 (GH20)5-CAACTTCATCCACGTTCACC-3 (PC04)	268	-	-	-
	REV					
NFKB1	FWD	5’-GGGCTATGGACCGCATGAC-3’	124/120	Van91I	II	79, 45
					ID	120, 79, 45
	REV	5’ CTGGAGCCGGTAGGGAAG-3			DD	120
NFKBIA	FWD	5’-GGCTGAAAGAACATGGACTTG-3’	154	BsuRI	GG	118, 36
					GA	154, 118, 36
	REV	5’-GGCTGAAAGAACATGGACTTG-3			AA	154

D: deletion; FWD: forward; I: insertion; REV: reverse.

RESULTS

The patterns of results found for viral detection and genotyping of polymorphisms are shown in Figures 1 and 2. The study population consisted of 334 women, of whom 163 (48.8%) were positive for the virus presence, while 171 (51.2%) were negative for the infection. Infected women were significantly younger than uninfected women, with median age of 39 and interquartile range of 23 years *vs.* 44 (19) years, as shown in Figure 3 (U=11.753.000, *P*=0.013).

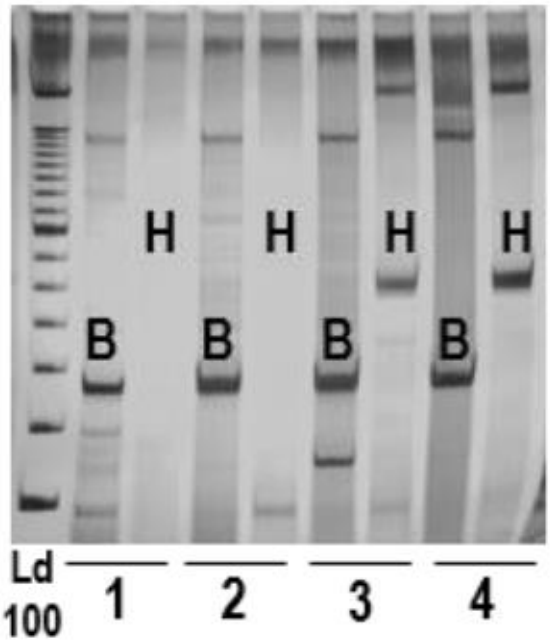

Figure 1 Detection of HPV in 10% polyacrylamide gel (10x10cm) stained with $AgNO_3$. The b-globin gene was used as the amplification control. *Ld100:* ladder 100 bp; *B:* b-globin fragment (268 bp); *H:* HPV fragment (~450 bp). In samples *3* and *4* the presence of HPV was detected, whereas in *1* and *2* the virus was absent.

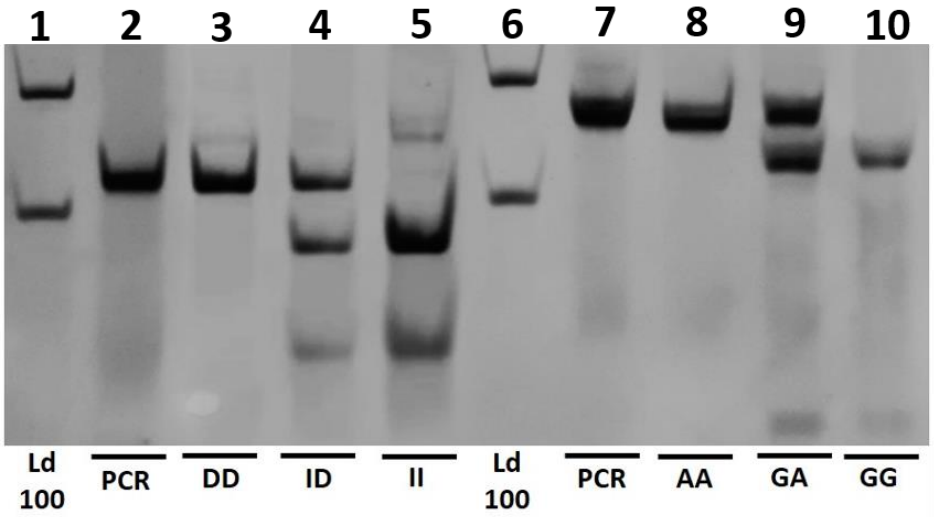

Figure 2 PCR-RFLP for *NFKB1* and *NFKBIA* polymorphisms in 10% polyacrylamide gel stained with $AgNO_3$. *Ld100:* ladder 100 bp by Life Technologies; Lanes *2* and *7* amplification products for *NFKB1* and *NFKBIA*, with 124 bp and 154 bp, respectively. Lanes *3*, *4* and *5* restriction products after digestion with *Van91I* enzyme with genotypes DD (120 bp); ID (120, 79 and 45 bp) and II (79 and 45 bp), respectively. Lanes *8*, *9* and *10* restriction products after digestion with *BsuRI* enzyme with genotypes AA (154 bp); GA (154, 118 and 36 bp) and GG (118 and 36 bp), respectively.

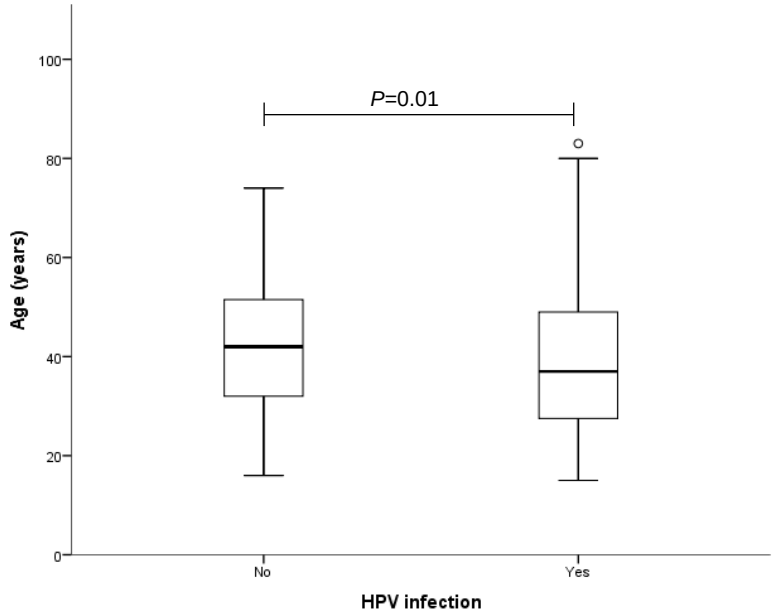

Figure 3 Differences of age distribution according to HPV infection. Circumference represents an outlier. Analysis performed with Mann-Whitney test. Data expressed as median (interquartile range).

Regarding the lesions presence, it was not possible to obtain cytological results of four uninfected and eight HPV-infected patients, so these samples were excluded from analysis regarding lesions. Thus, 167 (100%) of uninfected women presented normal cytology, whereas among those infected by the virus 71 (45.8%) were negative for malignancies; 58 (37.4%) presented some precursor lesion (29.3% presented low grade squamous intraepithelial lesion – LSIL and 70.7% presented high grade squamous intraepithelial lesion - HSIL); and 26 (16.8%) were diagnosed with CC. Considering that HPV involvement is essential for the lesions development and in virtually all cases of CC there is viral integration to the host genome (FERNANDES et al., 2015; SCHIFFMAN et al., 2016; STEINBACH; RIEMER, 2018), for further analysis involving lesions, only infected women were considered.

The women were different in age among the groups of lesion grade ($H_{(df=2)}$=25.1, *P*<0.001), in which cervical cancer group [54 (28)] were older than SIL [36 (12), *P*<0.001] and normal cytology [37 (14), *P*<0.001] (Figure 4). No differences were found in the distribution among the groups when the age of menarche (*P*=0.282) and the age of the first sexual intercourse (*P*=0.056) were considered.

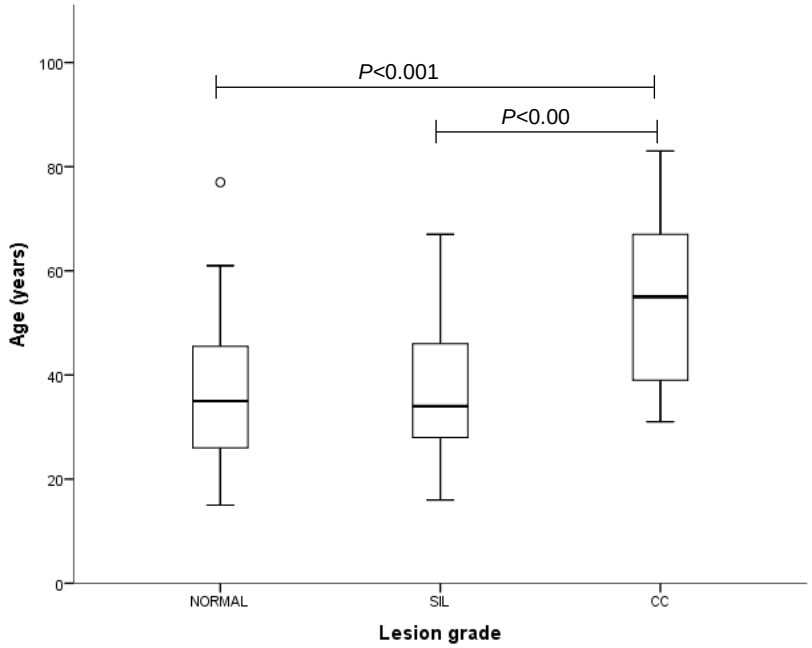

Figure 4 Differences of age distribution according to lesions grade. Circumference represents an outlier. Analysis performed with Kruskal-Wallis test with Bonferroni post-test. Data expressed as median (interquartile range). CC: cervical cancer; SIL: squamous intraepithelial lesion.

Population characteristics

Information about socio-demographic and sexual behavioral characteristics according to HPV infection are in Tables 2 and 3, which show that the viral presence was proportionally more frequent among who declared that did not know the virus (*P*=0.038) and among those were 24 years old or younger (*P*=0.001) when compared with the uninfected group. In addition, HPV was more common in women who reported smoking habit (*P*=0.043), unmarried (*P*=0.007), and those who reported having had ≥4 sexual partners during lifetime (*P*=0.024) and who had no sexual partners in past 6 months prior to the sample collection (*P*=0.001).

Table 2 Socio-demographic data of HPV-uninfected and HPV-infected women

Variable	HPV-uninfected		HPV-infected		*P* value*
	n= 171**	%	n= 163**	%	
Knowledge of HPV					**0.038**
No	32	18.7	50	30.7	
Have ever heard	96	56.1	76	46.6	
Yes	43	25.2	37	22.7	
Knowledge about transmission forms					0.255
No	78	45.6	84	51.5	
Yes	93	54.4	78	47.9	
Missing data	0	0	1	0.6	
Age range (years)					**0.001**
≤ 24	10	5.8	29	17.8	
25-34	42	24.6	41	25.2	
35-44	42	24.6	39	23.9	
45-54	49	28.6	22	13.5	
≥ 55	28	16.4	32	19.6	
Ethnicity					0.968
Caucasian	88	51.5	84	51.5	
Non-Caucasian	81	47.3	78	47.9	
Missing data	2	1.2	1	0.6	
Smoking status					**0.043**
No	131	76.6	109	66.9	
Yes	27	15.8	44	27.0	
Ex-smoker	13	7.6	10	6.1	
Marital status					**0.007**
Married/Civil partner	127	74.3	94	57.7	
Single	14	8.2	30	18.4	
Divorced	22	12.9	26	15.9	
Widowed	8	4.6	13	8.0	
Education [a]					0.249
Incomplete elementary	51	29.8	65	39.9	
Complete elementary	20	11.7	21	12.9	
Incomplete secondary	23	13.5	17	10.4	
Complete secondary	56	32.7	48	29.4	
Incomplete higher education	6	3.5	6	3.7	
Complete higher education	13	7.6	5	3.1	
Missing data	2	1.2	1	0.6	
Monthly income [b]					0.456
< 1 minimum wage	81	47.4	84	51.5	
1 - 3 minimum wages	81	47.4	68	41.7	
> 3 minimum wages	7	4.0	10	6.2	
Missing data	2	1.2	1	0.6	
Cases of CC in the family					0.855
No	151	88.3	142	87.1	
Yes	20	11.7	20	12.3	
Missing data	0	0	1	0.6	

[a] Based on Brazilian educational system
[b] Based on Brazilian minimum wage (approximately US$245.00)
*By two-sided χ^2 test, with $P<0.05$ considered significant
**Some patients did not know or did not want to respond, therefore depending on the socio-demographic characteristic analyzed not all 334 patients were included.

Table 3 Behavioral characteristics of HPV-uninfected and HPV-infected women

Variable	HPV-uninfected		HPV-infected		*P* value*
	n= 171**	%	n= 163**	%	
Hormonal contraceptive use					0.743
No	120	70.2	111	68.1	
Yes	51	29.8	51	31.3	
Missing data	0	0	1	0.6	
Condom use					0.605
No	151	88.3	140	85.9	
Yes	20	11.7	22	13.5	
Missing data	0	0	1	0.6	
Age at menarche (years)					0.278
≤ 11	37	21.6	38	23.3	
12	34	19.9	44	27.0	
13	45	26.3	31	19.0	
≥ 14	53	31.0	50	30.7	
Missing data	2	1.2	0	0	
Age at first sexual intercourse (years)					0.157
≤ 17	85	49.7	93	57.1	
≥ 18	85	49.7	68	41.7	
Missing data	1	0.6	2	1.2	
Number of full-term pregnancies					0.384
0	16	9.4	26	15.9	
1	24	14.0	28	17.2	
2	54	31.6	39	23.9	
3	39	22.8	35	21.5	
4	17	9.9	16	9.8	
≥ 5	21	12.3	19	11.7	
Parturition					0.344
No	16	9.4	26	15.9	
Natural birth	70	40.9	64	39.3	
Cesarean birth	52	30.4	35	21.5	
Both	33	19.3	35	21,5	
Missing data	0	0	3	1.8	
Abortion					0.561
No	121	70.8	103	63.2	
Yes	34	19.9	34	20.9	
Not applicable [a]	16	9.3	26	15.9	
Sexual partners during lifetime					**0.024**
1	63	36.8	43	26.4	
2-3	63	36.8	54	33.1	
≥ 4	45	26.4	64	39.3	
Missing Data	0	0	2	1.2	
Sexual partners in past 6 months					**0.001**
0	23	13.5	46	28.2	
≥ 1	148	86.5	117	71.8	
Last preventive examination					0.926
No	7	4.1	7	4.3	
Yes	162	94.7	154	94.5	
Missing data	2	1.2	2	1.2	

*By two-sided χ^2 test, with $P<0.05$ considered significant

**Some patients did not know or did not want to respond, therefore depending on the socio-demographic characteristic analyzed not all 334 patients were included.

[a]Women who had not being pregnant

When the lesion degree was analyzed and considering the normal diagnosis as reference (Tables 4 and 5), it was observed that the majority of patients who had CC did not know the forms of HPV transmission (*P*=0.016), were older than 55 years at the time of diagnosis (*P*<0.001), proportionally had a higher number of widows (*P*=0.001), in general presented incomplete education (*P*=0.004), as well as monthly income lower than 1 minimum wage (*P*=0.001). In addition, the CC group had a lower number of cesarean deliveries when compared to the group of women without lesion (*P*=0.014). Finally, patients diagnosed with precursor lesions reported having had a greater number of sexual partners during the lifetime (*P*=0.006), while the number of sexual partners in the past 6 months was lower in the CC group compared to the others (*P*<0.001).

NFKB1 and *NFKBIA* polymorphisms and HPV infection

It was found that both the *NFKB1* and *NFKBIA* polymorphisms were in Hardy-Weinberg equilibrium (HWE) (*P*>0.05) in infected and uninfected women. For the first polymorphism, among uninfected women, 65 (38.0%) had genotype II, whereas 76 (44.5%) and 30 (17.5%) had genotype ID and DD, respectively. Among infected women, 54 (33.1%) had genotype II; 86 (52.8%) had genotype ID and 23 (14.1%) had genotype DD. There were no differences in genotype distribution for this polymorphism between groups, regardless of the genotype models considered (codominant, dominant, recessive or over dominant).

For the *NFKBIA* polymorphism, the genotype distribution among uninfected women was as follows: 57 (33.3%) had GG genotype; 73 (42.7%) had GA genotype and 41 (24.0%) had AA genotype. For infected women, the genotype distribution was: 58 (35.6%), 79 (48.5%) and 26 (15.9%) for GG, GA and AA, respectively. For this polymorphism there were also no differences regarding the genotype distribution between the groups, even when different genotype models were used (codominant, dominant, recessive or over dominant).

When the combination of genotypes of the polymorphisms was analyzed, the protective role of the II/AA genotypes was evidenced (*P*=0.014) (Table 6).

NFKB1 and *NFKBIA* polymorphisms and development of precursor lesions and cervical cancer

It was verified that when the lesion grade was considered, both polymorphisms remained in HWE and, based on a multinomial logistic regression model adjusted for marital status, number of full-term pregnancies, sexual partners in past 6 months and during lifetime, no differences in the *NFKB1* and *NFKBIA* polymorphisms genotypes distribution were found. Also, no differences were observed between the combination of genotypes II/AA when compared to the other possible combinations of genotypes (Table 7).

Table 4 Socio-demographic data of HPV-infected women according to lesions

Variable	Degree of lesion						*P* value*
	NL		SIL		CC		
	n= 71**	%	n= 58**	%	n= 26**	%	
Knowledge of HPV							0.493
No	20	28.2	17	29.3	12	46.2	
Have ever heard	32	45.1	28	48.3	9	34.6	
Yes	19	26.7	13	22.4	5	19.2	
Knowledge about transmission forms							**0.016**
No	28	39.4	29	50.0	19	73.1	
Yes	42	59.2	29	50.0	7	26.9	
Missing data	1	1.4	0	0	0	0	
Age range (years)							**< 0.001**
≤ 24	13	18.3	12	20.7	0	0	
25-34	20	28.2	18	31.0	2	7.7	
35-44	18	25.3	13	22.4	7	26.9	
45-54	9	12.7	10	17.3	3	11.5	
≥ 55	11	15.5	5	8.6	14	53.9	
Ethnicity							0.156
Caucasian	40	56.3	24	41.4	16	61.5	
Non-Caucasian	31	43.7	33	56.9	10	38.5	
Missing data	0	0	1	1.7	0	0	
Smoking status							0.078
No	53	74.7	34	58.6	14	53.8	
Yes	15	21.1	21	36.2	8	30.8	
Ex-smoker	3	4.2	3	5.2	4	15.4	
Marital status							**0.001**
Married/Civil partner	43	60.6	34	58.6	12	46.2	
Single	15	21.1	13	22.4	0	0	
Divorced	10	14.1	9	15.5	7	26.9	
Widowed	3	4.2	2	3.5	7	26.9	
Education [a]							**0.004**
Incomplete elementary	20	28.2	22	37.9	20	76.9	
Complete elementary	10	14.1	6	10.4	4	15.5	
Incomplete secondary	8	11.3	9	15.5	0	0	
Complete secondary	27	38.0	16	27.6	1	3.8	
Incomplete higher education	3	4.2	3	5.2	0	0	
Complete higher education	3	4.2	1	1.7	1	3.8	
Missing data	0	0	1	1.7	0	0	
Monthly income [b]							**0.001**
< 1 minimum wage	29	40.8	31	53.5	21	80.8	
1 - 3 minimum wages	33	46.5	26	44.8	4	15.4	
> 3 minimum wages	9	12.7	0	0	1	3.8	
Missing data	0	0	1	1.7	0	0	
Cases of CC in the family							0.611
No	60	84.5	53	91.4	23	88.5	
Yes	10	14.1	5	8.6	3	11.5	
Missing data	1	1.4	0	0	0	0	

NL = No Lesion; SIL = Squamous Intraepithelial Lesions; CC = Cervical Cancer

[a] Based on Brazilian educational system

[b] Based on Brazilian minimum wage (approximately US$245.00)

*By two-sided χ^2 test, with $P<0.05$ considered significant

**Some patients did not know or did not want to respond, therefore not all 155 patients were included depending on the socio-demographic characteristic analyzed

Table 5 Behavioral characteristics of HPV-infected women according to lesions

Variable	Lesion grade						*P* value*
	NL		SIL		CC		
	n= 71**	%	n= 58**	%	n= 26**	%	
Hormonal contraceptive use							0.086
No	52	73.2	34	58.6	20	76.9	
Yes	19	26.8	24	41.4	5	19.2	
Missing data	0	0	0	0	1	3.9	
Condom use							0.308
No	60	84.5	49	84.5	24	92.4	
Yes	11	15.5	9	15.5	1	3.8	
Missing data	0	0	0	0	1	3.8	
Age at menarche (years)							0.594
≤ 11	16	22.5	16	27.6	5	19.2	
12	21	29.6	16	27.6	7	26.9	
13	11	15.5	14	24.1	4	15.4	
≥ 14	23	32.4	12	20.7	10	38.5	
Age at first sexual intercourse (years)							0.176
≤ 17	38	53.5	39	67.3	13	50.0	
≥ 18	33	46.5	18	31.0	12	46.2	
Missing data	0	0	1	1.7	1	3.8	
Number of full-term pregnancies							**< 0.001**
0	15	21.2	9	15.5	0	0	
1	19	26.8	7	12.1	1	3.8	
2	17	23.9	14	24.1	6	23.1	
3	12	16.9	15	25.9	6	23.1	
4	4	5.6	9	15.5	3	11.5	
≥ 5	4	5.6	4	6.9	10	38.5	
Parturition							**0.014**
No	15	21.1	9	15.5	0	0	
Natural birth	20	28.2	27	46.6	14	53.8	
Cesarean birth	22	31.0	10	17.2	2	7.7	
Both	12	16.9	11	19.0	10	38.5	
Missing data	2	2.8	1	1.7	0	0	
Abortion							0.154
No	47	66.2	36	62.1	17	65.4	
Yes	9	12.7	13	22.4	9	34.6	
Not applicable [a]	15	21.1	9	15.5	0	0	
Sexual partners during lifetime							**0.006**
1	24	33.8	8	13.8	10	38.5	
2-3	16	22.5	27	46.5	4	15.4	
≥ 4	30	42.3	23	39.7	11	42.3	
Missing Data	1	1.4	0	0	1	3.8	
Sexual partners in past 6 months							**< 0.001**
0	10	14.1	10	17.2	25	96.2	
≥ 1	61	85.9	48	82.8	1	3.8	
Last preventive examination							0.117
No	5	7.1	0	0	1	3.8	
Yes	65	91.5	58	100.0	24	92.4	
Missing data	1	1.4	0	0	1	3.8	

*By two-sided χ^2 test, with $P<0.05$ considered significant

**Some patients did not know or did not want to respond, therefore not all 155 patients were included depending on the sexual behavioral characteristic analyzed

[a]Women who had not being pregnant NL: No lesion; SIL: Squamous intraepithelial lesion; CC: cervical cancer

Table 6 Distribution of *NFKB1* rs28362491 and *NFKBIA* rs696 polymorphisms genotypes by HPV infection

Genotype	HPV-uninfected		HPV-infected		*P* value*	Odds ratio and $CI_{95\%}$
	n= 171[a]	%	n= 163[b]	%		
rs28362491						
Codominant model						
II	65	38.0	54	33.1	1.000	Reference
ID	76	44.5	86	52.8	0.121	1.490 (0.900 – 2.469)
DD	30	17.5	23	14.1	0.620	0.839 (0.418 – 1.683)
Dominant model						
II	65	38.0	54	33.1	1.000	Reference
DD+ID	106	62.0	109	66.9	0.290	1.292 (0.804 – 2.078)
Recessive model						
II+ID	141	82.5	140	85.9	1.000	Reference
DD	30	17.5	23	14.1	0.217	0.670 (0.355 – 1.265)
Over dominant model						
II+DD	95	55.6	77	47.2	1.000	Reference
ID	76	44.4	86	52.8	0.055	1.570 (0.990 – 2.490)
Allele frequency						
I	206	60.2	194	59.5	1.000	Reference
D	136	39.8	132	40.5	0.848	0.970 (0.712 – 1.322)
rs696						
Codominant model						
GG	57	33.3	58	35.5	1.000	Reference
GA	73	42.7	79	48.5	0.852	1.050 (0.629 – 1.751)
AA	41	24.0	26	16.0	0.253	0.683 (0.355 – 1.314)
Dominant model						
GG	57	33.3	58	35.6	1.000	Reference
AA+GA	114	66.7	105	64.4	0.753	0.926 (0.574 – 1.495)
Recessive model						
GG+GA	130	76.0	137	84.0	1.000	Reference
AA	41	24.0	26	16.0	0.171	0.664 (0.370 – 1.193)
Over dominant model						
GG+AA	98	57.3	84	51.5	1.000	Reference
GA	73	42.7	79	48.5	0.431	1.202 (0.761 – 1.898)
Allele frequency						
G	187	54.7	195	59.8	1.000	Reference
A	155	45.3	131	40.2	0.180	1.234 (0.907 – 1.677)
rs28362491/rs696[1]						
Other genotypes	153	89.5	158	96.9	1.000	Reference
II/AA	18	10.5	5	3.1	**0.014**	**0.259 (0.088-0.765)**

*Binary logistic regression, with "uninfected group" as reference and $P<0.05$ considered significant. Adjusted values for age and sexual partners in past 6 months

[1] Combined rs28362491 (*NFKB1*) and rs696 (*NFKBIA*) polymorphisms genotypes

[a] Hardy-Weinberg equilibrium χ^2: *NFKB1*= 0.889, *P*> 0.05; *NFKBIA*= 3.287, *P*>0.05

[b] Hardy-Weinberg equilibrium χ^2: *NFKB1*= 1.458, *P*>0.05; *NFKBIA*= 0.011; *P*>0.05

I: insertion; D: deletion

Table 7 Distribution of rs28362491 (*NFKB1*) and rs696 (*NFKBIA*) genotypes by lesion grade

Genotype	NL		SIL				CC			
	n= 71	%	n= 58	%	P value*	Odds ratio ($CI_{95\%}$)	n= 26	%	*P* value*	Odds ratio ($CI_{95\%}$)
rs28362491										
Codominant model										
II	22	31.0	19	32.8	1.000	Reference	9	34.6	1.000	Reference
ID	38	53.5	33	56.9	0.554	0.656 (0.163 – 2.647)	12	46.2	0.127	9.472 (0.526 – 170.560)
DD	11	15.5	6	10.3	0.957	1.025 (0.428 – 2.452)	5	19.2	0.776	0.724 (0.078 – 6.689)
Dominant model										
II	22	31.0	19	32.8	1.000	Reference	9	34.6	1.000	Reference
DD+ID	49	69.0	39	67.2	0.896	0.945(0.403 – 2.216)	17	65.4	0.546	1.812 (0.263 – 12.481)
Recessive model										
II+ID	60	84.5	52	89.7	1.000	Reference	21	80.8	1.000	Reference
DD	11	15.5	6	10.3	0.496	0.647 (0.185 – 2.266)	5	19.2	0.087	10.886 (0.707 – 167.671)
Over dominant model										
II+DD	33	46.5	25	43.1	1.000	Reference	14	53.8	1.000	Reference
ID	38	53.5	33	56.9	0.685	1.176 (0.537 – 2.575)	12	46.2	0.398	0.412 (0.053 – 3.219)
Allele frequency										
I	82	57.8	71	61.2	1.000	Reference	30	57.7	1.000	Reference
D	60	42.2	45	38.8	0.574	1.154 (0.700 – 1.904)	22	42.3	0.995	0,998 (0.524 – 1.898)
rs696										
Codominant model										
GG	26	36.6	20	34.5	1.000	Reference	9	34.6	1.000	Reference
GA	32	45.1	33	56.9	0.352	0.536 (0.144 – 1.994)	12	46.2	0.650	0.556 (0.044 – 7.016)
AA	13	18.3	5	8.6	0.180	1.794 (0.763 – 4.218)	5	19.2	0.119	12.572 (0.522 – 302.826)
Dominant model										
GG	26	36.6	20	34.5	1.000	Reference	9	34.6	1.000	Reference
AA + GA	45	63.4	38	65.5	0.409	1.403 (0.628 – 3.136)	17	65.4	0.406	2.423(0.300 – 19.565)
Recessive model										
GG + GA	58	81.7	53	91.4	1.000	Reference	21	80.8	1.000	Reference
AA	13	18.3	5	8.6	0.141	0.395 (0.115 – 1.359)	5	19.2	0.280	0.275 (0.026 – 2.863)
Over dominant model										
GG + AA	39	54.9	25	43.1	1.000	Reference	14	53.8	1.000	Reference
GA	32	45.1	**33**	56.9	**0.076**	2.069 (0.927 – 4.618)	12	46.2	0.085	14.549 (0.688 – 307.643)
Allele frequency										
G	84	59.2	73	62.9	1.000	Reference	30	57.7	1.000	Reference
A	58	40.8	43	37.1	0.537	1.172 (0.708 – 1.940)	22	42.3	0.855	0.942 (0.494 – 1.793)
rs28362491/rs696[1]										
Other genotypes	68	95.8	57	98.3	1.000	Reference	25	96.2	1.000	Reference
II/AA	3	4.2	1	1.7	0.412	0.363 (0.032 – 4.091)	1	3.8	0.885	1.328 (0.028 – 65.562)

*Multinomial logistic regression with "No lesion group" as reference and *P*<0.05 considered significant. Adjusted values for marital status, number of full-term pregnancies, sexual partners in past 6 months and sexual partners during lifetime

[1] Combined rs28362491 (*NFKB1*) and rs696 (*NFKBIA*) polymorphisms genotypes

I: insertion; D: deletion; NL: No lesion; SIL: Squamous intraepithelial lesion; CC: cervical cancer

DISCUSSION

It is well established that infection with high-risk types of HPV is an important and necessary etiological factor for the onset and progression of cervical disorders and invasive CC. However, only the presence of the virus is insufficient for this outcome, requiring several other factors, both intrinsic and extrinsic, which will be decisive for development of lesions. In this context, this study was the first performed in the Brazilian population that aimed to analyze the influence of *NFKB1* and *NFKBIA* polymorphisms on HPV infection, as well as on the development of pre-neoplastic and CC.

The socio-demographic and sexual behavior profiles of the patients showed that HPV was associated with women who were not aware of the virus, whereas the lesions development was associated with the lack of information about the virus transmission forms. These findings corroborate with our previous data (OKUYAMA et al., 2018; TRUGILO et al., 2018) and suggest that the lack of knowledge about the virus and the prevention forms prevent women from avoiding exposure, which predisposes them to infection and the lesions progression.

HPV infection was also associated with age younger than 24 years. Indeed, young age is already an independent factor associated with HPV infection well established in previous studies (SANJOSÉ et al., 2007; NUNES et al., 2014). Young women are more vulnerable not only to HPV but also to other pathogens due to various mechanisms, such as cervical ectopy, an anatomical feature that causes exposure of the ectocervix columnar epithelium. In addition, cervical immaturity and inadequate production of protective cervical mucus also increase susceptibility to HPV infection, as well as sexual activity that is generally higher in this age (BURCHELL et al., 2006; COSER et al., 2016).

The relationship of tobacco smoking with HPV infection was verified in this study and it was also observed previously, when it was demonstrated for the first time that the main carcinogenic constituent of the cigarette smoke, benzo[a]pyrene (BaP), acts by promoting the synthesis of high levels of virion in productive HPV-infected cell lines (ALAM et al., 2008). Before that, evidence was found that carcinogenic components of the cigarette, such as polynuclear aromatic hydrocarbons (PAHs), could somehow be transported to the cervix since BaP and its metabolites were found in the cervical mucus of female smokers (MELIKIAN et al., 1999). Later, the presence of BaP was associated with direct carcinogenic effects through the transformation of DNA in cervical epithelium cells, that since affected by BaP showed increased proliferation and decreased terminal differentiation (MOORE et al., 2001). The presence of BaP was also associated with immunosuppression, since smoking interferes with HPV clearance by impairing the immune response by decreasing the number of CD4 T lymphocytes and Langerhans cells, as well as reducing the activity of natural killers cells (SO et al., 2018).

It was also found that the HPV-infected group had a higher number of single women, a result that was in agreement with the findings of previous study (ZIDI et al., 2018) and which may be related to the greater number of sexual partners during lifetime declared by these patients, a factor that favors the attainment of HPV infection (TASIC et al., 2018), unlike the married patients, who by having fixed sexual partner tend to limit the chances of infection. When considered the lesions grade, having 4 sexual partners or more during lifetime also contributed to the development and worsening of cervical intraepithelial lesions.

A peculiar association between the number of sexual partners in the past 6 months and HPV infection was found, which indicated that, proportionally, HPV-infected patients had a lower number of sexual partners compared to the uninfected group. However, when investigating this data in relation to the presence of lesions, it was found that this association was only maintained among patients with CC, which mostly reported not having had sexual partners in the past 6 months that preceded the diagnosis, indicating that possibly at this time these women were already manifesting clinical symptoms of the tumor, which prevented them from having sex.

No association was found between the infection and the age of the first sexual intercourse, as previously described (COSER et al., 2016). In relation to the use of oral contraceptives (OCs), no significant differences were found between the groups, differing from other studies, which indicated that the use of these drugs leads to the persistence of oncogenic HPVs infections (XU et al., 2018) and the prolonged use acts as a cofactor increasing the risk of CC among women who are HPV-DNA carriers (CASTELLSAGUÉ; BOSCH; MUÑOZ, 2002).

The development of CC was associated with the age of the patients and is related to the moment of diagnosis, which tends to occur in women older than 55 years, contributing disproportionately to CC mortality, primarily as a result of more advanced disease at diagnosis (WAGGONER, 2003). This age range is included in a second peak of HPV prevalence, which can occur for two reasons: (1) hormonal changes resulting from menopause, which leads to impaired immune response and can induce reactivation of existing, perhaps latent, HPV infections that were replicating at a very low, undetectable rate; (2) changes in the sexual behavior of women and their partners in middle age (SANJOSÉ et al., 2007). These statements would support what was found in this study, in which it was observed in the CC group a higher proportion of women over 55 and widows.

Several studies have shown that low level of education is a significant risk factor for the development of cervical lesions (WONG; LOKE; CHAN, 2011; GARGANO et al., 2012; TAO et al., 2014). Educational level is directly related to the level of health education, since poorly educated individuals have difficulty in understanding information and advice. In addition, it influences their behavior in relation to the prevention of diseases and health problems (DA SILVA et al., 2017), as well as may be associated with lack of knowledge about virus transmission forms reported by CC

group. Thus, educational level possibly acts as marker of a combination of characteristics such as inadequate screening, early age of first sexual intercourse and first pregnancy, high parity, sexual behavior and possible co-infection with other infectious agents in addition to HPV infection (TASIC et al., 2018). In addition, the significant difference between the groups according to lesion grade in terms of monthly income demonstrates a direct link with education level, and is in line with a global tendency in which there is an increased vulnerability among women in poverty, due to the limited access to information and health services presented by them (COSER et al., 2016).

It was observed that proportionally there was a higher number of full-term pregnancies among the patients who developed CC. This result was in accordance with previously findings that demonstrated that nulliparous women had a lower risk of squamous-cell carcinoma of the cervix than those who had already given birth, and among the latter, a clear trend towards increasing risk with increasing number of full-term pregnancies emerged (MUÑOZ et al., 2002). Different hypotheses have been elaborated that try to describe possible biological mechanisms for the association between parity and cervical neoplasia. The first, the hormonal one, is based on the effects induced by the OCs, it is believed that the hormonal influences play an important role in the HPV carcinogenesis. The traumatic hypothesis is based on the fact that high parity may increase the risk of CC, since it maintains the transformation zone in the exocervix for many years; and the last one, the immunological hypothesis leads us to believe that high levels of progesterone in conjunction with high estrogen levels, downregulate cell-mediated immunity that could be harmful to the fetus, but are essential for clearance of HPV infection, influencing the risk of the virus persistence or progression (CASTELLSAGUÉ; BOSCH; MUÑOZ, 2002; BANURA et al., 2008).

Curiously, data on parturition show that there was a higher number of cesarean deliveries among the patients with normal diagnosis in relation to CC patients. This information, associated with the higher age and the higher number of full-term pregnancies observed in the last group, allow us to infer that possibly this association occurred due to the historical moment and perhaps the geographical location and/or social status of these patients as pregnant women, since when the cesarean became known, was more restricted to women in better economic situations.

Although no association was found between smoking status and the development of cervical lesions, it was already evidenced the synergistic action of cigarette smoking and HPV for the development of SIL and CC (CASTELLSAGUÉ; BOSCH; MUÑOZ, 2002) and was found that the risk of high grade cervical disease was higher for current-smokers than never-smokers, increasing with the number of cigarettes smoked per day and with increased duration of smoking (XU et al., 2018). Furthermore, in this study, condom use was not evidenced as a protective factor for

lesion development, in contrast to previously published reports (WONG; LOKE; CHAN, 2011; GARGANO et al., 2012; TASIC et al., 2018).
Among the intrinsic factors of the host, there are the genetic factors, which act as non-modifiable elements and influence the individual susceptibility to cancers. Under these circumstances, genetic polymorphisms especially those located in promoter region of the genes, but not limited to them, could potentially enhance or reduce transcriptional efficiency of the corresponding genes (TAN; ANKATHIL, 2015). In this sense, the polymorphisms in the NF-kB pathway were analyzed, searching for possible associations with HPV infection or, with the development of pre-neoplastic lesions and progression to CC, since this transcription factor is necessary to maintain the normal function of the immune system and its inadequate activation can mediate inflammation and tumorigenesis (CHENG et al., 2013).

No significant associations were found between the polymorphisms and HPV infection in the different genetic models analyzed. However, it was found that the combination of genotypes II of *NFKB1* and AA of *NFKBIA* demonstrated a protective effect against HPV infection. It was verified that the allele of *NFKB1* containing the deletion is less able to bind transcription factors, producing lower transcript levels in luciferase reporter systems, demonstrating that carriers of the deletion allele have lower intra-cellular levels of NFKB1 (p50) when compared with the increased activity provided by increased production of NF-kB transcription factor observed in carriers of insertion for this polymorphism (LI et al., 2016; LEONE et al., 2018). In contrast, ex vivo luciferase data evidenced that 2758 A allele of rs696 *NFKBIA* polymorphism thermodynamically favor stronger binding capacity of miR-449a with 3'UTR region, resulting in reduced expression of this gene (SIMONIAN et al., 2018). Thus, the variants of both polymorphisms probably act synergistically favoring the prolonged constitutive activity of NF-kB, leading us to infer that it acts optimally in the case of HPV infection. However, this combination has not been shown to exert effects on the development of lesions and progression to cancer, unlike what has been reported in the literature, that the prolonged activation of NF-kB is related to the propensity of chronic inflammatory diseases, such as rheumatoid arthritis and inflammatory bowel disease (TILBORGHS et al., 2017), as well as to tumorigenesis (OSBORNE et al., 2005; CHENG et al., 2013; CHEN et al., 2015; ESKANDARI-NASAB et al., 2016).

When considering the presence of lesions, no difference was found between the genotypes, regardless of the genetic model used. This result differed from those found in India and China, sites in which a higher frequency of genotype II was found in cases of CC (ZHOU et al., 2009; PALLAVI; ANOOP; SHOWKET, 2015). However, the Brazilian population presented DD genotype being related to an increased chances of developing gastric and colorectal cancers (CAVALCANTE et al., 2017), while the same genotype and the allele D were associated with reduced risk of breast cancer in the Iranian population (ESKANDARI-NASAB et al., 2016).

In addition, no differences were observed between the genotypes of *NFKBIA* with the development of SIL and progression to CC in the different genetic models addressed. Again, our results differed from those found in India, where was found that GG genotype as being at greater risk for CC (PALLAVI; ANOOP; SHOWKET, 2015). In relation to this polymorphism, was also observed a variation among populations, since while in the Chinese population the GA genotype was related to the increased risk for colorectal cancer, in the Swedish population the GG genotype was associated with a poor prognosis for the same disease and other study verified in the Chinese population that the AA variant led to an increased risk for nasopharyngeal carcinoma (GAO et al., 2007; LIU et al., 2015).

Based on the comparison of results, it was observed that for both polymorphisms, the carcinogenic effects varied according to the ethnicity and tumor type analyzed. The first factor is relevant when it comes to the Brazilian population, given the absence of a well-defined ethnic group that characterizes it due to the miscegenation process (LINS et al., 2010). Finally, in a manner similar to that described in a previous study about the polymorphism of *NFKB1*, we consider that possibly none of the polymorphisms studied exerts a direct carcinogenic effect, but may increase susceptibility if there is exposure to some factor, such as smoking or HPV infection itself in the case of CC, and this susceptibility varies according to studied population (LEWANDER et al., 2007).

In conclusion, this study confirmed the involvement of some factors as facilitators of HPV infection and the development of cervical lesions, such as age, smoking and number of sexual partners. It was found that in general, women who are infected with HPV and who develop the lesions are not aware of HPV and its forms of transmission, confirming the need to invest in programs to control and prevent sexually transmitted infections for low-income populations. The main limitation or our study was the low number of samples, which may have influenced the statistical power of our analysis. It was also verified that, although no differences were found between the genotypes of both polymorphisms when analyzed in an individual way through different models, the combination of II/AA genotypes exerted a protective role against HPV infection and appeared not to be involved in the progression of the lesions as well as in the CC development. Considering that this was the first study in Brazilian population that sought to understand as changes in the NF-kB pathway may affect the cervical microenvironment in the presence of HPV infection, future studies are important to determine as the rs28362491 polymorphism of *NFKB1* and rs696 of *NFKBIA* interfere in HPV infection and if, under some circumstance, it can influence the progression of lesions.

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4 CONCLUSÕES E CONSIDERAÇÕES FINAIS

Por meio da revisão bibliográfica presente neste trabalho, tornou-se possível o entendimento das principais características estruturais e de funcionamento da via do NF-kB, além de como os polimorfismos rs28362491 de *NFKB1* e rs696 de *NFKBIA* atuam em diferentes tipos de tumores. Podemos concluir que, ambos polimorfismos se comportam de formas distintas conforme o tipo de tumor e a etnia predominante na população analisada, podendo variar até mesmo dentro de uma mesma população, não nos permitindo afirmar qual alelo ou qual genótipo está relacionado à proteção ou ao aumento do risco contra tumores de forma geral, sendo necessário analisar caso a caso o microambiente tumoral em questão.

Na etapa experimental, foi observado que aproximadamente metade da população estudada apresentou DNA viral, das quais 54,2% apresentaram algum grau de lesão. Ao responderem ao questionário sociodemográfico e comportamental, a maioria das mulheres acometidas declarou nunca ter ouvido falar do vírus; enquanto que as pacientes diagnosticadas com CC relataram um baixo grau de escolaridade e baixa renda mensal. Outras associações encontradas em relação à infecção e ao desenvolvimento de lesões também corroboraram estudos anteriores, como os efeitos causados pelos componentes do cigarro; as características biológicas que favorecem a infecção e o desenvolvimento de lesões, tais como idade e número de gestações, respectivamente; e comportamentais, como número de parceiros sexuais. Por outro lado, quanto à análise dos polimorfismos, apresentamos um resultado inédito, o qual revelou que quando combinados, os genótipos II e AA parecem exercer papel contra a infecção pelo HPV. No entanto, de que forma isso acontece ainda precisa ser melhor esclarecido.

Por fim, apesar do baixo número amostral, o qual pode ser considerado como principal limitação deste estudo, as informações obtidas confirmam que a parcela da população em condições financeiras menos favorecidas está mais vulnerável à infecção e, consequentemente, à desenvolver este tipo de tumor, demonstrando a importância do investimento em programas de controle e prevenção a infecções sexualmente transmissíveis voltados para populações de risco. Além disso, estudos que visem entender de que forma os polimorfismos analisados podem interferir no microambiente cervical seriam de grande relevância, tendo em vista os resultados promissores destes biomarcadores em outros tipos tumorais.

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ANEXOS

ANEXO A

Autorização do Comitê de Ética em pesquisa Envolvendo Seres Humanos

Universidade Estadual de Londrina

PARANÁ
GOVERNO DO ESTADO

COMITÊ DE ÉTICA EM PESQUISA ENVOLVENDO SERES HUMANOS
Universidade Estadual de Londrina
Registro CONEP 5231

Parecer CEP/UEL:	133/2012
CAAE:	05505912.0.0000.5231
Processo:	19275/2012
Pesquisador(a):	Karen Brajão de Oliveira
Unidade/Órgão:	CCB – Departamento de Ciências Patológicas

Prezado(a) Senhor(a):

O "**Comitê de Ética em Pesquisa Envolvendo Seres Humanos da Universidade Estadual de Londrina**" (Registro CONEP 5231) – de acordo com as orientações da Resolução 196/96 do Conselho Nacional de Saúde/MS e Resoluções Complementares, avaliou o projeto:

"PREVALÊNCIA E GENOTIPAGEM DE HPV E SUA POSSÍVEL ASSOCIAÇÃO COM OS GENES DE CITOCINAS, QUIMIOCINAS E SEUS RECEPTORES EM NÍVEL DE DNA, RNA E PROTEÍNA: implicações no microambiente tumoral."

Situação do Projeto: **Aprovado**

Informamos que deverá ser comunicada, por escrito, qualquer modificação que ocorra no desenvolvimento da pesquisa, bem como deverá ser encaminhado ao CEP/UEL relatório final da pesquisa, conforme prevê a Resolução 196/96 do Conselho Nacional de Saúde/MS e Resoluções Complementares.

Londrina, 28 de agosto de 2012.

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ANEXO B
Termo de Consentimento Livre e Esclarecido

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

"Prevalência e genotipagem de HPV e sua possível associação com os genes de citocinas, quimiocinas e seus receptores em nível de DNA, RNA e proteína: implicações no microambiente tumoral."

Prezado(a) Senhor(a):

Gostaríamos de convidá-lo (a) a participar da pesquisa **"Prevalência e genotipagem de HPV e sua possível associação com os genes de citocinas, quimiocinas e seus receptores em nível de DNA, RNA e proteína: implicações no microambiente tumoral."**, realizada no **"Laboratório de Genética Molecular e Imunologia, Departamento de Ciências Patológicas da Universidade Estadual de Londrina".** O objetivo da pesquisa é avaliar a presença do vírus em mulheres atendidas em programas de prevenção ao câncer cervical do setor público de saúde da região norte do Paraná, por meio de metodologia específica e sensível, visando também à associação de dados demográficos, para análise dos fatores de risco que contribuem para a exposição da população ao vírus, bem como os determinantes de sua manutenção. Adicionalmente objetiva-se compreender o papel do sistema imune no controle e iniciação tumoral, bem como na sua formação, crescimento e progressão, em especial avaliar a interação tumor-hospedeiro em pacientes portadoras do vírus HPV e no desenvolvimento do câncer cervical. A sua participação é muito importante e ela se daria da seguinte forma: **doação de 5mL de sangue periférico coletado por punção venosa e doação do swab cérvico-vaginal utilizado para confecção das lâminas para o exame preventivo para análises moleculares, bem como responder um questionário sociodemográfico.** Gostaríamos de esclarecer que sua participação é totalmente voluntária, podendo você: recusar-se a participar, ou mesmo desistir a qualquer momento sem que isto acarrete qualquer ônus ou prejuízo à sua pessoa. Informamos ainda que as informações serão utilizadas somente para os fins desta pesquisa e serão tratadas com o mais absoluto sigilo e confidencialidade, de modo a preservar a sua identidade.

As amostras biológicas (sangue periférico e secreção cérvico-vaginal) serão utilizados para extração de DNA e RNA para análises moleculares e imunológicas. Estes materiais serão obtidos em pequenas quantidades portanto não haverá sobra de material biológico.

Os benefícios esperados são a detecção precoce do vírus HPV em mulheres atendidas em programas de prevenção ao câncer de colo de útero do setor público de saúde da região norte do Paraná. Informamos que a paciente que se dispor a participar do projeto não sofrerá desconfortos nem riscos à saúde, não havendo qualquer prejuízo às mesmas. Informamos que a senhora não pagará nem será remunerada por sua participação. Garantimos, no entanto, que todas as despesas decorrentes da pesquisa serão ressarcidas, quando devidas e decorrentes especificamente de sua participação na pesquisa.

Caso você tenha dúvidas ou necessite de maiores esclarecimentos pode nos contactar **Karen Brajão de Oliveira, Laboratório de Genética Molecular e Imunologia, Departamento de Ciências Patológicas, Universidade Estadual de Londrina, 3371-4267, karen.brajao@uel.br),** ou procurar o Comitê de Ética em Pesquisa Envolvendo Seres Humanos da Universidade Estadual de Londrina, na Avenida Robert Kock, nº 60, ou no telefone 33712490. Este termo deverá ser preenchido em duas vias de igual teor, sendo uma delas, devidamente preenchida e assinada entregue a você.

Londrina, ___ de _____de 201__.

Pesquisador Responsável _____
Profª. Drª. Karen Brajão de Oliveira
RG: 6.538.742-5

_____ (nome por extenso do sujeito de pesquisa), tendo sido devidamente esclarecido sobre os procedimentos da pesquisa, concordo em participar **voluntariamente** da pesquisa descrita acima.

Assinatura (ou impressão dactiloscópica):_____
Data:_____

ANEXO C
Questionário Socioepidemiológico

Nº LAB

QUESTIONÁRIO SOCIOEPIDEMIOLÓGICO

Data: ____/____/_____ Reg. Nº _____

1. Conhece o HPV???
( ) Nunca ouvi falar
( ) Já ouvi falar mas não sei o que é
( ) Conheço

2. Idade _____ anos DN, _____

3. Etnia: _____
Branca / parda / negra / asiática / indígena

4. Sua renda mensal (em salário mínimo) é de?
( ) Até 1 Salário ( ) De 1 à 3 salários
( ) De 3 à 5 salários ( ) De 5 à 7 salários
( ) De 7 à 10 salários

5. Você fuma?
( ) Não ( ) Sim Tempo: _____

6. Qual o seu grau de escolaridade?
( ) Fundamental Incompleto
( ) Fundamental Completo
( ) Médio Incompleto ( ) Médio completo
( ) Superior incompleto ( ) Sup. completo

7. Estado Civil:
( ) Solteira ( ) Casada
( ) Divorciada ( ) Viúva

8. Qual sua profissão?

9. Faz o uso de algum método contraceptivo?
( ) Não ( ) Sim Qual:

10. Tipo de Parto:
( ) Normal ( ) Cesária

11. Nº de gestações: _____

12. Números de Partos:
( ) Nenhum ( ) Um
( ) Dois ( ) Três
( ) Quatro ou mais

13. Idade da 1ª relação sexual: anos
14. Idade da 1ª menstruação: _____ anos

15. Número de parceiros sexuais durante a vida:

_____

16. Número de parceiros sexuais nos últimos 6 meses:

17. Já realizou outros exames preventivos?
( ) Sim ( ) Não

18. Exames de prevenção realizados no passado apresentaram algum tipo de alteração?
( ) Sim ( ) Não
( ) Não me lembro
Em caso de resposta "Sim" favor descrever a alteração:

_____

19. Já contraiu alguma infecção ginecológica
( ) Não ( ) Sim ( ) não sei informar
Em caso de resposta ``SIM``, se possível descrever qual:

20. Já esteve infectada pelo HPV?
( ) Sim ( ) Não ( ) Não sei informar

21. Conhece as formas de transmissão ou formas de contrair o vírus?
( ) Não ( ) Sim Qual ou quais:

22. Existem casos de câncer de colo de útero em sua família?
( ) Sim ( ) Não
Em caso de resposta ``SIM`` descrever o grau de parentesco:

_____

Pesquisador: