UNIVERSIDADE FEDERAL DE PERNAMBUCO
LABORATÓRIO DE IMUNOPATOLOGIA KEIZO ASAMI
PÓS-GRADUAÇÃO EM BIOLOGIA APLICADA À SAÚDE

CORRELAÇÃO DA INFECÇÃO POR *PAPILLOMAVIRUS HUMANO* (HPV) COM POLIMORFISMOS DE DOIS GENES DE CITOCINAS: FATOR DE NECROSE TUMORAL (TNF) ALFA E INTERLEUCINA (IL) 18 EM PACIENTES COM E SEM LESÃO INTRAEPITELIAL CERVICAL.

MAYARA COSTA MANSUR FERNANDES

Recife

2012

MAYARA COSTA MANSUR FERNANDES

CORRELAÇÃO DA INFECÇÃO POR *PAPILLOMAVIRUS HUMANO* (HPV) COM POLIMORFISMOS DE DOIS GENES DE CITOCINAS: FATOR DE NECROSE TUMORAL (TNF) ALFA E INTERLEUCINA (IL) 18 EM PACIENTES COM E SEM LESÃO INTRAEPITELIAL CERVICAL.

Dissertação apresentada ao Programa de Pós-Graduação em Biologia Aplicada à Saúde – Universidade Federal de Pernambuco, como requisito final para a obtenção do grau de Mestre em Biologia Aplicada à Saúde.

ORIENTADOR: Prof. Dr. Sergio Crovella
CO-ORIENTADOR: Prof. Dr. Paulo Roberto Eleutério de Souza

Recife

2012

Fernandes, Mayara Costa Mansur
Correlação da infecção por *Papillomavírus* humano (HPV) com polimorfismos de dois genes de citocinas: fator de necrose tumoral (TNF) alfa e interleucina (IL) 18 em pacientes com e sem lesão intraepitelial cervical./ . – Recife: O Autor, 2012.

58 folhas : il., fig., tab.
Orientador: Sérgio Crovella
Coorientador: Paulo Roberto Eleutério de Souza
Dissertação (mestrado) – Universidade Federal de Pernambuco, Centro de Ciências Biológicas. Biologia Aplicada à Saúde, 2012.
Inclui bibliografia e anexos

1. Papillomavírus humano 2. Polimorfismo (genética) 3. Câncer de útero I. Título.

616.911 CDD (22.ed.) UFPE/CCB-2012-054

UNIVERSIDADE FEDERAL DE PERNAMBUCO
LABORATÓRIO DE IMUNOPATOLOGIA KEIZO ASAMI
PÓS-GRADUAÇÃO EM BIOLOGIA APLICADA À SAÚDE

CORRELAÇÃO DA INFECÇÃO POR *PAPILLOMAVIRUS HUMANO* (HPV) COM POLIMORFISMOS DE DOIS GENES DE CITOCINAS: FATOR DE NECROSE TUMORAL (TNF) ALFA E INTERLEUCINA (IL) 18 EM PACIENTES COM E SEM LESÃO INTRAEPITELIAL CERVICAL.

Banca Examinadora:

Co-orientador: Prof. Dr. Paulo Roberto Eleutério de Souza
Departamento de Biologia
Universidade Federal Rural de Pernambuco

Prof. Dr. Luiz Cláudio Arraes de Alencar
Laboratório de Imunopatologia Keizo Asami - UFPE

Dr. Rafael Lima Guimarães
Laboratório de Imunopatologia Keizo Asami - UFPE

Recife

2012

RESUMO

O *Papillomavirus* humano (HPV) é responsável por afetar anualmente 500 mil mulheres com câncer cervical invasivo. Fatores de risco podem facilitar a persistência do vírus da cérvice uterina. Polimorfismos genéticos em regiões regulatórias e codificadoras de genes de citocinas estão associadas a patogênese de um vasto número de doenças humanas. Este trabalho objetivou determinar se existe relação entre os polimorfismos existentes na região -G308A do gene *TNFα* e nas regiões -G137C e -C607A do gene *IL18* na susceptibilidade a infecção pelo HPV e na progressão das lesões intraepitelial cervical. O estudo foi realizado com 122 mulheres HPV+ e 132 mulheres HPV- (controle). Os polimorfismos dos genes *TNFα* e *IL18* foram analisados pela técnica *Specific Sequence Polymosphism* (PCR-SSP) e analisadas em gel de agarose a 1,5%. As análises estatísticas para verificar a significância do estudo dos genótipos foram realizadas utilizando o programa BioEstat 5.0. Os resultados mostraram uma prevalência de 49,18% da infecção pelo HPV-16 e 70,49% delas apresentaram lesão cervical de alto grau. Em relação aos polimorfismos houve associação do alelo mutante na região -308A do gene *TNFα* e -607A do gene *IL18* com a susceptibilidade a infecção pelo HPV ($p=0,0008$; $p<0,0001$, respectivamente), mas não foi verificada relação destes genes com a susceptibilidade ao desenvolvimento das lesões ($p>0,05$). Porém não foi encontrada associação significativa em relação a região na região -137 do gene IL-18. Estes resultados sugerem dois possíveis marcadores genéticos de susceptibilidade a infecção pelo HPV na população em estudo e que estes não podem ser usados como marcadores de progressão da lesão cervical.

Palavras Chave: *Papillomavirus Humano,* Neoplasia Intraepitelial Cervical, Polimorfismos de única base, Fator de Necrose tumoral alfa, Interleucina 18.

ABSTRACT

The Human Papillomavirus (HPV) is responsible for affecting anually 500 thousand women with invasive cervical cancer. Risk factors may facilitate the persistence of the virus of the uterine cervix. Genetic polymorphisms in regulatory and coding regions of cytokine genes are associated with the pathogenesis of a large number of human diseases. This study aimed to investigate whether there is a relationship between existing polymorphisms in the region -G308A of the gene *TNFα* and in regions -G137C and C607A of the *IL18* gene in susceptibility to HPV infection and progression of cervical intraepithelial lesions. The study was conducted with 122 women HPV+ and, 132 women HPV- (control). The polymorphisms of the *TNFα* and *IL18* genes were analyzed using the technic of the Specific Sequence Polymosphism (PCR-SSP) and analyzed on agarose gel 1.5%. The Statistical analyzes to check the significance of the study of the genotypes were performed using the BioEstat 5.0 program. The results showed a prevalence of 49.18% of HPV-16 infection and 70.49% of that total had high-grade cervical lesions. Regarding the polymorphisms there was an association of the mutant allele in the region -308A of the gene *TNFα* and -607A of the *IL18* gene with susceptibility to HPV infection ($p = 0.0008$, $p < 0.0001$, respectively), but no relationship was found these genes with susceptibility to the development of lesions ($p > 0.05$). But no significant association was found with regard to the region -137 region of the gene *IL18*. These results suggest two possible genetic markers of susceptibility to HPV infection in the studied population and that these can not be used as markers of progression of cervical injury.

Key-words: Human *Papillomavirus,* Cervical Intraepithelial Neoplasia, Single Nucleotide Polymorphism, Tumour Factor Necrosis alpha, Interleukin 18

LISTA DE ABREVIATURAS E SIGLAS

CCI - Câncer Cervical Invasivo

DNA - Ácido desoxiribonucléico

E- *Early* (precoce)

HPV - Papillomavírus Humano

ICTV - *The International Committee on the Taxonomy of Viruses* (Comitê Internacional de Taxonomia Viral)

IFN - Interferon

IL – Interleucina

L – *Late* (tardia)

MHC - Complexo de Histocompatibilidade Principal

NIC - Neoplasia Intraepitelial Cervical

NK - *Natural killers* (Matadoras Naturais)

SI - Sistema Imunológico

SIL – Lesão Intraepitelial Cervical

SNP - Single nucleotide polymorphism (Polimorfismo de única Base)

Th – Linfócito T *helper* (Linfócito T auxiliary)

TNF - *Tumour Necrosis Factor* (Fator de Necrose Tumoral)

LCR - *Long Control Region* (Região Longa de Controle)

UV – Ultravioleta

LISTA DE FIGURAS

LISTA DE TABELAS

SUMÁRIO

REVISÃO DE LITERATURA

1. Câncer Cervical

O câncer cervical invasivo (CCI), a segunda neoplasia maligna mais comum em todo o mundo, perdendo apenas pelo câncer de mama, é responsável por cerca de 500 mil novos casos anuais, dos quais aproximadamente metade resultam em mortes. A maioria dos casos (em torno de 80%) ocorre em países em desenvolvimento, sendo nesses países o tipo mais incidente nas mulheres. Infecções pelo *Papillomavirus* Humano (HPV) representam cerca de 5% de todos os tipos de cânceres [1,2,3].

Nos Estados Unidos é considerada a infecção transmitida sexualmente mais comum [4], com uma estimativa de 6.2 milhões de novos casos em homens e mulheres, onde 74% dos novos casos ocorrem entre 15 e 24 anos. É altamente prevalente em mulheres sexualmente ativas, sendo responsável por 60% a 80% das infecções em adolescentes neste país [4,5,6,7]. Na Europa, surgem anualmente 33.000 novos casos de câncer, resultado em 15.000 mortes na faixa etária compreendida entre 15-44 anos [8]. No leste europeu a incidência e mortalidade do câncer cervical é ainda maior, comparadas com a Europa Ocidental [9,10].

No Brasil, a ocorrência deste câncer prevista para 2012 é de 17.540 novos casos segundo estimativa bienal do Instituto Nacional do Câncer, com um risco aproximado de 17 casos a cada 100 mil mulheres. Sem considerar os tumores de pele não melanoma, o câncer do colo do útero é o mais incidente na Região Norte (24/100.000). Nas regiões Centro-Oeste (28/100.000) e Nordeste (18/100.000) ocupam a segunda posição mais freqüente, na região

Sudeste (15/100.000) a terceira, e Sul (14/100.000), a quarta posição. Este comportamento evidencia a urbanização e desenvolvimento do Sudeste Brasileiro, que se reflete em maior informação da população com relação aos métodos de prevenção e detecção precoce do CC. A dificuldade de acesso explicaria a alta taxa de incidência de lesões em estádio avançado na região Norte [11].

2. Papillomavírus Humano

2.1 Classificação do HPV

Anteriormente agrupados na família *Papovaviridae* e subfamília *Papillomavirus,* devido à semelhança nas características morfológicas com o gênero Polyomavirus, foram designados pelo Comitê Internacional de Taxonomia de Virus (ICTV) como uma família própria, *Papillomaviridae* [12]. Os papilomavírus foram identificados em uma grande variedade de espécies de vertebrados, além de humanos, sendo específicos para seus respectivos hospedeiros.

A classificação do HPV pode ser feita de diversas maneiras. Estes vírus não são classificados como sorotipos, mas como genótipos com base na seqüência DNA [13,14]. Quanto ao tropismo pela superfície epitelial que tem predileção, são cutaneotrópicos ou mucosotrópicos, e podem induzir a transformação neoplásica dessas células. Nestes, a infecção pelo HPV pode causar verrugas no epitélio cutâneo, enquanto que na região anogenital esses vírus podem causar verrugas genitais e os vários tipos de câncer em homens e mulheres [15].

Podem se classificar também de acordo seu potencial para oncogênese. Baseados em dados filogenéticos e em relação a sua presença em doenças cervicais malignas ou benignas, podem ser classificados como de baixo risco e alto risco. Os de baixo risco (HPV tipo 6, 11, 13, 40, 42-44, 54, 61, 62, 70, 72, 74, 81), incluindo os condilomas genitais, dos quais os tipos 6 e 11 são os mais prevalentes e os de alto risco oncogênico (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, 82) [16,17].

2.2 Estrutura Viral

Devido à estreita relação com o câncer cervical, estudos são direcionados na determinação das sequências de HPV. Até o momento, mais de 200 tipos de HPV foram identificados baseados no DNA do vírus, apresentando variedades entre os genomas [12].

A família *Papillomaviridae* insere um grupo de pequenos vírus não envelopados, apresentando uma conformação icosaédrica, com uma molécula de DNA circular com uma longa fita dupla [17,18]. O genoma possui menos de 8000 pares de bases, contendo três regiões: genes precoces (do inglês, *early*) e tardios (do inglês, *late*), e entre estas duas regiões está a região reguladora que não codifica genes (LCR de *Long Control Region*) (Figura 1).

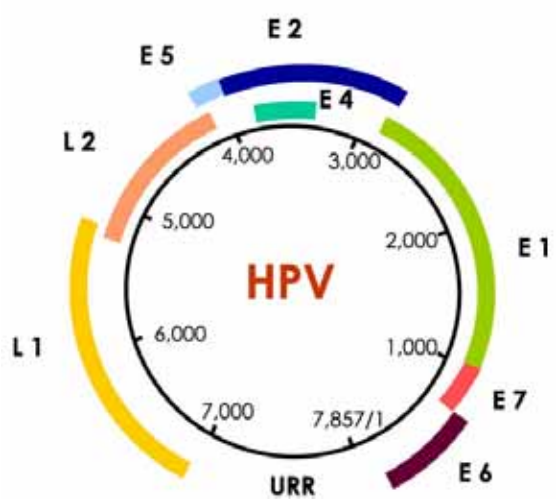

Figura 1. Representação esquemática do genoma do HPV contendo a distribuição das regiões precoce, tardia e regulatória, conforme as unidades de pares de bases cda molécula circular e DNA do vírus (Muñoz *et al.* 2006)

A região *early* é o local onde estão presentes os genes responsáveis pela produção de proteínas envolvidas no controle da replicação do DNA viral (E1, E2, E4, E5, E6 e E7). A região *late* contém os genes L (L1 e L2), responsável pela formação as proteínas do capsídeo protéico viral. Os conjuntos de genes *late e early* são separados por uma região reguladora de cerca de 1000pb que não codifica proteínas, mas contém promotores necessários para a regulação da expressão gênica e origem de replicação do genoma [18].

2.3 Lesões e cânceres causados pelo HPV

A persistência da infecção pelo HPV especialmente com um dos tipos oncogênicos pode causar transformação no epitélio [19,20] (Figura 2). O câncer cervical se desenvolve a partir lesões pré-malignas não-invasivas denominada neoplasia intraepitelial cervical (NIC) ou lesão intraepitelial escamosa (SIL).

Essas lesões pré-malignas são classificados histologicamente em base de atipia progressiva de células epitelias: NIC I corresponde à displasia leve,NIC II displasia moderada e NIC III para ambos displasia acentuada e carcinoma *in situ* [21].

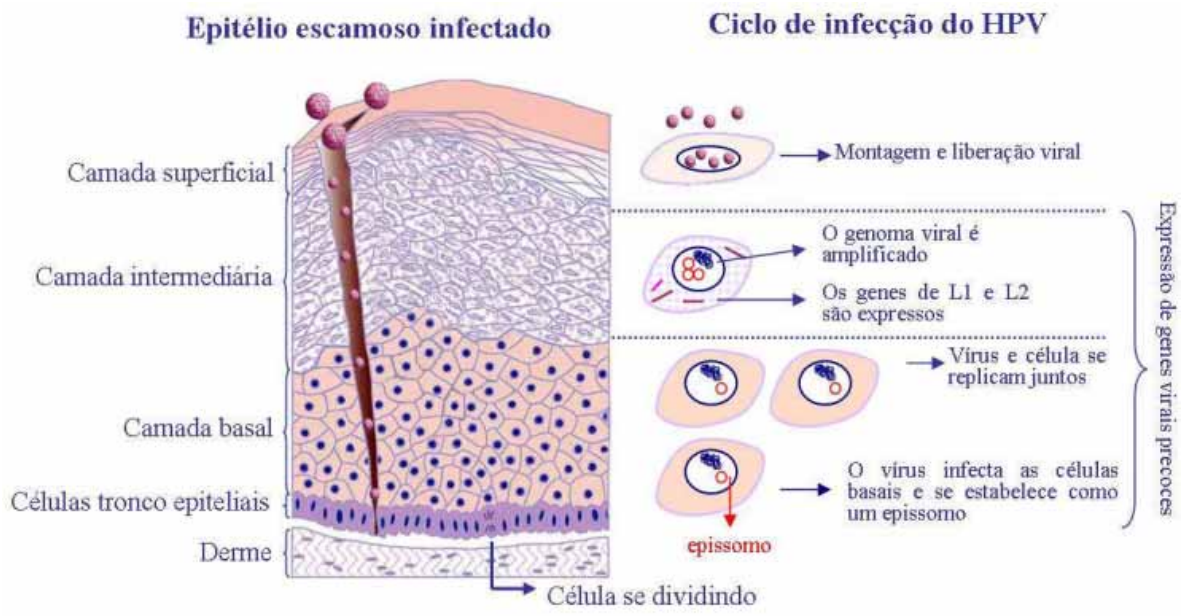

Figura 2. Ciclo de infecção do HPV no epitélio estratificado (Adaptado de Doorbar, 2005)

O risco de progressão de lesões pré-malígnas para formas graves é de 16% e 34% para NIC I e II respectivamente, e 22% para NIC III ou câncer invasivo [22,23]. Na lesão de grau I (NIC I), 60%-75% dos casos não são confirmados no segundo exame, regredindo espontaneamente [21].

O *Papillomavirus* humano é o agente causal principal para o desenvolvimento de 99,7% dos casos de câncer cervical. Em outros tipos de cânceres, estima-se que esse vírus é responsável por 90% dos casos de câncer anal 40% dos cânceres dos órgãos genitais externos (vulva, vagina e

pênis), pelo menos 12% dos cânceres orofaríngeos e pelo menos 3% dos cânceres bucais [12,24].

2.4 Fatores de risco associados com o HPV

A presença de um tipo oncogênico do HPV na região cervical é considerada uma causa necessária para o desenvolvimento da NIC e do CC, mas não exclusivamente. Caracterizada como uma doença multifatorial, o HPV atua em conjunto a cofatores ambientais e fatores imunológicos e genéticos próprios do hospedeiro para que ocorra a progressão para o câncer cervical invasivo[25,26,27,28].

Fatores ambientais relacionados com CC incluem fatores hormonais (uso de contraceptivos orais), multiparidade, coinfecção com outros vírus (herpes simplex 2 - HSV-2) e bactérias ou com outras doenças sexualmente transmissíveis, (*Chlamydia trachomatis* e HIV) e hábitos do hospedeiro como fumo, radiação ultravioleta (UV) e radiação ionizante e os fatores dietéticos. Outros cofatores não-ambientais sendo também considerados incluem os relacionados à resposta imune do hospedeiro e as relacionadas com o próprio vírus, como o genótipo de HPV, coinfecção com outros tipos de HPV, as variantes de HPV, carga viral e integração viral [29].

3. Polimorfismos de Única Base (SNP)

As variações que ocorrem no genoma são em sua maioria formas de polimorfismos de nucleotídeo único (SNP), com uma frequência a cada 300 - 1000 pares de base em todo o genoma e surgem como resultado de defeitos na replicação do DNA. Assim, os SNPs são mutações de ponto que originam

duas formas alélicas. Essas variações nos genes são muito úteis para pesquisa de marcadores genéticos humanos e podem ser usadas para identificar genes de susceptibilidade de doenças humanas complexas [30,31,32].

4. Mecanismos da Resposta Imune

O Sistema Imunológico (SI) é responsável pelo reconhecimento, eliminação e remoção de substâncias estranhas, células mortas e/ou danificadas, além da destruição de células mutantes e cancerosas. É bastante especializado em gerar uma variedade enorme de células e moléculas capazes de reconhecer e eliminar especificamente substâncias que não pertencem naturalmente ao sistema de defesa [33,34].

Várias moléculas solúveis e circulantes ou também aquelas que permanecem aderidas a membranas celulares agem conjuntamente em uma rede dinâmica extraordinariamente adaptável, capaz de produzir uma resposta imune efetora e eficaz contra invasores de estruturas complexas. De acordo com a função exercida, a resposta imune pode ser caracterizada conforme a atividade relacionada em reconhecimento e resposta.

Funcionalmente, a imunidade possui dois caminhos principais envolvidos na eliminação de partículas estranhas: a imunidade inata e a imunidade adquirida, que apresentam aspectos diferentes quanto ao sistema de reconhecimento, células envolvidas e mecanismos de ação.

4.1 Resposta Imune Inata

A imunidade inata é a primeira linha de defesa e está presente na maioria dos organismos. Os receptores das células do sistema imune inato foram desenvolvidos durante o processo evolucionário [34,35].

Fazem parte dessa resposta barreiras físicas epitélios e mucosas e moléculas secretadas em fluidos biológicos como citocinas. Células especializadas nesta resposta são as matadoras naturais (*natural killers*), células dendríticas, macrófagos, linfócitos T citotóxicos e neutrófilos [35,36].

A imunidade inata é ativada por lesão celular ou óbito e se manifesta como uma inflamação, a resposta vascular local da lesão. Durante a inflamação, efetores solúvel e celular imunes inatos são recrutados. Células parenquimatosas são recrutados para o local e fagócitos locais são, então, ativado para secretarem citocinas inflamatórias e moléculas de defesa [14]. É formado por um sistema de reconhecimento com menor especificidade contra patógenos e esses componentes estão previamente formados antes da entrada e contato com a substância estranha [33,34,37].

4.2 Resposta Imune Adaptativa

O sistema imune adaptativo é especifico contra antígenos, necessitando de um primeiro contato com este para ser construída. Agrupa os linfócitos T, que são os grandes efetores dessa resposta e os fagócitos, com as proteínas relacionadas a essas células (complexo principal de histocompatibilidade - MHC e os receptores de células T e B) [33,37].

Quando o hospedeiro tem contato com microorganismo patogênico (vírus ou bactéria, por exemplo), células apresentadoras de antígenos capturam os patógenos invasores e processam internamente seus antígenos. As células T reconhecem antígenos que tenham sido transformadas em peptídeos curtos, vinculado ao Complexo Principal de Histocompatibilidade (MHC), e é apresentado ligado ao receptor de membrana celular [38].

Dois grandes subconjuntos de células T são identificados pelos marcadores de superfície: CD4 ou CD8. Células T CD4 + reconhecem antígenos apresentados por MHC de classe II; as células T CD8 + reconhecem os antígenos apresentados pela classe I do MHC. Células T CD4 + são resultado da ativação na secreção de uma variedade de pequenas proteínas, ou citocinas, que ajudam a regular outras células [39,40].

As citocinas da resposta imune à infecção são geralmente classificadas como imunoestimulante T helper 1 (Th1) ou citocinas do tipo T helper 2 (Th2). Uma resposta Th1 favorecerá a produção de células T citotóxicas efetoras que seria importante na remoção de todas as células infectadas pelo vírus (citocinas tipo Th1- como interferon (IFN), o fator de necrose tumoral (TNF), interleucina (IL) 2, e IL-12, enquanto uma resposta Th2 (IL-4, IL-5, IL-6, IL-8 e IL-10) vai facilitar a estimulação das células B com a geração de anticorpo para neutralização [41].

4.3. Interleucina 18 (IL-18) e o gene *IL18*

Interleucina (IL) -18 exerce atividade tanto na resposta inata como na imunidade adaptativa [42,43,44,45]. Pertence à superfamília IL-1 decitocinas próinflamatórias e o gene *IL18* está situado na região 11q22.2-22.3 [44].Anteriormente denominada como fator de indução do interferon, devido a identificação desta propriedade [46], sabe-se que tem ação pleiotropica induzindo a expressão de quimiocinas e citocinas, além de moléculas de adesão como a IL-8, TNF-α, VEGF e ICAM-1 [47,48,49,50].

Em cultura de adipócitos humanos pode-se verificar que a IL-18 é secretada pelas células de gordura e a TNFa estimula a expressão de IL-18 e

seus receptores semelhantes aos seus efeitos sobre a inflamação em outros genes relacionados [51].

A IL-18 desempenha um papel primordial na resposta imune, sendo responsável pela diferenciação e ativação das células T helper (Th) em subgrupos, de acordo com o perfil de citocinas [52]. Atua como um indutor para a diferenciação e proliferação de células Th1 na presença de IL-12, estimulando a produção de IFN e TNF nos linfócitos T e natural killer (NK) [52,53,54]. A IL-18 e a IL-12 atuam em sinergismo na ativação de células NK, células B e T [55,56]. Em contrapartida, a IL-18 pode ainda estimular a resposta imune do tipo Th2, na ausência da IL-12 [54,57].

Vários polimorfismos no promotor do gene *IL18* têm sido associados com diferentes doenças inflamatórias e auto-imunes [58-63]. Cinco posições diferentes de polimorfismos de único nucleotídeo na região promotora foram identificados: -656 G / T, -607 C / A, -137 G / C, 113 G / T, e 127 C / T [64]. No entanto, somente em SNPs nas posições -137 e -607 foram confirmados como tendo um impacto sobre a atividade do gene *IL18* em estudos anteriores [65].

4.4 Fator de Necrose Tumoral Alfa (TNF- α) e o gene *TNFα*

O Fator de Necrose Tumoral alfa (TNF-α) é uma potente citocina Th1 próinflamatória liberado principalmente por monócitos e macrófagos estimulados. O gene se encontra dentro do complexo de histocompatibilidade maior no cromossomo 6p21.3 [66,67],e é composto de quatro éxons dispostos ao longo de cerca de 3 kb do DNA [68].

O TNF-α foi originalmente identificado e isolado por duas atividades características: a capacidade de induzir necrose hemorrágica de certos

tumores e da capacidade de induzir caquexia durante os estados de infecção crônica [69]. Em infecções severas, o TNF-α é produzido em larga escala e causa anormalidades sistêmicas clinica e patológica [70].

A principal função fisiológica do TNF-α é estimular o recrutamento de neutrófilos e monócitos para os locais da infecção e ativa essas células para erradicar microrganismos [70]. Possui amplos efeitos biológicos, incluindo proteção contra a infecção, vigilância contra tumores e estimula respostas inflamatórias [68], além de desempenhar um papel essencial no sistema imunológico [71]. O TNF-alfa estimula a síntese de outras citocinas próinflamatórias e a adesão de moléculas. Além disso, tem mostrado mediar a carcinogênese através da indução, proliferação, invasão e metástase de células tumorais [72]. Em situações agudas, a produção local de TNF-α é claramente benéfica. Ele aumenta a expressão de moléculas de adesão no endotélio vascular a fim de permitir que células imunológicas, particularmente os neutrófilos e macrófagos, se desloquem para os sítios onde ocorreram dano tecidual e infecção [69].

Polimorfismos genéticos de citocinas, especialmente em regiões reguladoras, podem estar relacionados com a quantidade de citocinas produzida [73]. O nível de produção desta citocina varia com o genótipo do indivíduo que secreta, podendo produzir níveis baixo, médio ou alto de TNF-α.

Existem vários polimorfismos localizados em diferentes sítios do promotor do gene responsável pela síntese da TNF alfa. Foram identificados na região -1031 (T→C), -863 (C→A), -857 (C→A), -851 (C→T), -419 (G→C), -376 (G→A), - 308 (G→A), -238 (G→A), -162 (G→A) e – 49 (G→A). Polimorfismos

de nucleotídeo único na região -308 e -238 do promotor do gene *TNF* têm sido comumente estudados [69,74,75].

O polimorfismo no -308 supostamente afeta a expressão do gene e têm sido implicados na regulação da transcrição do *TNFα*. Podem ser encontradas numa determinada população duas formas alélicas, onde uma permanece com o alelo G, comum, da guanina ou quando ocorre substituição desse alelo pelo A (adenina) [76,77].

Segundo Cabrera *et al.* (1995) mostra que as células que contém o alelo A aumentam a transcrição de seis a nove vezes mais que aquelas que contém o alelo G [74,75,79]. Outros trabalhos relataram que *in vitro*, o alelo G está relacionado com a reduzida produção de TNF-α, enquanto os indivíduos com alelo A produzem mais TNF-a [74,80,81,82].

OBJETIVOS

Objetivo Geral

Verificar a relação entre os polimorfismos existentes na região (-308 G/A) do gene *TNFα* e nas regiões (-137 G/C) e (-607 C/A) do gene *IL18* com a infecção pelo HPV e com a progressão da lesão intraepitelial cervical.

Objetivos Específicos

- Detectar e genotipar o HPV nas amostras analisadas.
- Determinar o polimorfismo dos genes *TNFα* e *IL18* pela metodologia da *Specific Sequence Polymosphism* (PCR-SSP).
- Associar a presença da infecção pelo HPV com o surgimento de lesões intraepiteliais cervicais.
- Correlacionar os polimorfismos dos genes *TNFα* e *IL18* com susceptibilidade a infecção por HPV e o aparecimento de lesões intraepiteliais.

REFERÊNCIAS BIBLIOGRÁFICAS

[1] Parkin DM, Bray F, Ferlay J, et al. **Global cancer statistics**, 2002. CA Cancer J Clin 2005;55:74–108.

[2] Freeman H, Wingrove B. **Excess Cervical Cancer Mortality: A Marker for Low Access to Health Care in Poor Communities**. NIH Pub. No. 05-5282. Rockville, MD: National Cancer Institute, Center to Reduce Cancer Health Disparities, 2005.

[3] Gargiulo F, De Francesco MA, Schreiber C, Ciravolo G, Salinaro F, B. Valloncini B, Manca N. **Prevalence and distribution of single and multiple HPV infections cytologically abnormal cervical samples from Italian. women** Virus Research 125 (2007) 176–182.

[4] Weinstock H, Berman S, Cates JrW. **Sexually transmitted diseases among American youth: incidence and prevalence estimates**, 2000. Perspect Sex Reprod Health 2004;36(1):6–10.

[5] Dunne EF, Unger ER, Sternberg M, McQuillan G, Swan DC, Patel SS, et al. **Prevalence of HPV infection among females in the United States**. JAMA 2007;297(8):813–9.

[6] Huang CM. **Human papillomavirus and vaccination**. Mayo Clin Proc 2008;83(6):701–6, quiz 706–707.

[7] Moscicki AB. **Impact of HPVinfection in adolescent populations**. J Adolesc Health 2005;37:S3–9.

[8] ECDC (2008) **Guidance for the introduction of HPV vaccines in EU countries**. From http://ecdc.europa.eu/pdf/HPVreport.pdf

[9] Arbyn M, Raifu AO, Autier P et al. **Burden of cervical cancer in Europe: estimates for 2004.** Ann Oncol 2007; 18:1708–1715.

[10] Prymula R, Anca I, André F, Bakir M, Czajka H, Lutsar I, Mészner Z, Salman N, Šimurka P, Usonis V. **Central European Vaccination Advisory Group (CEVAG) guidance statement on recommendations for the introduction of HPV vaccines** Eur J Pediatr (2009) 168:1031–1035

[11] MINISTÉRIO DA SAÚDE. INCA. Estimativa 2012: Incidência de Câncer no Brasil. Rio de Janeiro, 2012. [Acesso em 12/2012]. Disponível em [http://www.inca.gov.br/estimativa/2012/index.asp?ID=5]

[12]Burd EM. **Human Papillomavirus and Cervical Cancer**. Clin. Microbiol. Rev. Jan. 2003, p. 1–17 Vol. 16, No. 1

[13] de Villiers EM, Fauquet C, Broker TR, Bernard HU, zur Hausen H. **Classification of papillomaviruses**. Virology 2004;324:17–27.

[14] Stanley M. **Immunobiology** of HPV and HPV vaccines. Gynecologic Oncology 109 (2008) S15–S21

[15] Bernard HU. **The clinical importance of the nomenclature, evolution and tazonaomy of human papillomaviruses.** J Clin Virol, 32S (2005) S43–S51

[16] Albring L, Brentano JE, Vargas, VRA. **O câncer do colo do útero, o Papilomavírus Humano (HPV) e seus fatores de risco e as mulheres indígenas Guarani: estudo de revisão**. RBAC,2006; 38(2): 87-90.

[17] Magi JC, Brito SEM, Grecco ETO et al. **Prevalência de papilomavirus humano (HPV) anal, genital e oral, em ambulatório geral de coloproctologia**. Rev bras. Coloproct., 2006:26(3): 233-238.

[18] Muñoz N, Castellsagu´e X, de Gonz´alez AB, Gissmann L. **HPV in the etiology of human cancer.** Vaccine 24S3 (2006) S3/1–S3/10

[19] Cogliano V, Baan R, Straif K, Grosse Y, Secretan B, El Ghissassi F. **Carcinogenicity of human papillomaviruses**. Lancet Oncol 2005;6(April (4)):204.

[20] IARC. **IARCmonographs onthe evaluationof carcinogenic risk tohuman.**Human Papillomaviruses 2005:90.

[21] Wright TC, Knrman RJ: **A critical review of the morphologic classification systems of preinvasive lesions of the cervix: The scientific basis for shifting the paradigm.** Papilloma Virus Report 1994:5:175-182.

[22] Piato S. **Tratado de ginecologia**. Porto Alegre: Artes Médicas; 1997.

[23] Brasil. Ministério da Saúde. Instituto Nacional de Câncer. **Neoplasia intra–epitelial cervical – NIC.** Rev Bras Cancerol 2000;46(4):355-7.

[24] Antonsson A, Erfurt C, Hazard K, et al. **Prevalence and type spectrum of human papillomaviruses in healthy skin samples collected in three continents.** J Gen Virol 2003; 84:1881–6.

[25] Bosch FX, Lorincz A, Muñoz N, Meijer CJLM, Shah KV. **The causal relation between human papillomavirus and cervical cancer,** J. Clin. Pathol. 55 (2002) 244–265.

[26] Ylitalo N, Josefsson A, Melbye M, Sorensen P, Frisch M, Andersen PK, Spare ´n P, Gustafsson M, Magnusson P, Ponte ´n J, Gyllensten U, Adami HO. A prospective study showing long-term infection with human papillomavirus 16 before the development of cervical carcinoma in situ, Cancer Res. 60 (2000) 6027–6032.

[27] Santos AM, Sousa H, Catarino R, Pinto D, Pereira D, Vasconcelos A, Matos A, Lopes C, Medeiros R. **TP53 codon 72 polymorphism and risk for cervical cancer in Portugal,** Cancer Genet. Cytogenet. 159 (2005) 143–147.

[28] Craveiro R, Costa S, Pinto D, Salgado L, Carvalho L, Castro C, Bravo I, Lopes C, Silva I, Medeiros R. **TP73 alterations in cervical carcinoma.** Cancer Genet. Cytogenet. 150 (2004) 116— 121.

[29] Xavier Castellsague, F.Xavier Bosch, Nubia Muñoz. **Environmental co-factors in HPV carcinogenesis.** Virus Research 89 (2002) 191-199.

[30] Johnson GCL, Todd JA: **Strategies in complex disease mapping**. Curr Opin Genet Dev 10:330, 2000.

[31] Sachidanandam R, Weissman D, Schmidt SC, Kakol JM, Stein LD, **Marth G, et al. A map of human genome sequence variation containing 1.42 million single nucleotide polymorphisms.** Nature 2001;409:928e33.

[32] Hanchard NA. **Genetic susceptibility and single-nucleotide polymorphism.** Seminars in Fetal & Neonatal Medicine (2005) 10, 283e289

[33] Goldsby, RA.; Kindt, TJ.; Osborne, BA. Kuby **Imunologia**, 4ªed. Ed. Revinter, 2002: 480-495.

[34] Lehner, T. **Innate and adaptive mucosal immunity in protection against HIV infection.** Vaccine, v.21 Suppl 2, Jun 1, p.S68-76. 2003.

[35] Sierra S, Kupfer B. et al. **Basics of the virology of HIV-1 and its replication.** J Clin Virol 2005: 34 (4): 233-44.

[36] Magnadottir B. **Innate Immunity of fish (overview).** Fish Shellfish Immunol, v.20, n.2. Feb, p. 137-151, 2006.

[37] Kindt TJ, Goldsby RA, Osborne BA, Kuby J. **Immunology**. New H. Freeman 2007, XXII, 574, A – 531, G – 512, An – 527, I – 527 p.p.

[38] Inaba K., Metlay JP, Crowley MT, Steinman RM. **Dendritic cells pulsed with protein antigens in vitro can prime antigen-specific, MHC-restricted T cells in situ**. J. Exp. Med. 1990; 172, 631–640

[39] Spellberg B, Edwards Jr JE. **Type 1/Type 2 immunity in infectious diseases.** Clin Infect Dis 2001;32:76–102.

[40] Zur Hausen H. **Papillomaviruses and cancer: from basic studies to clinical application.** Nat Rev, Cancer 2002;2:342–50.

[41] Niedergang F, Didierlaurent A, Kraehenbuhl JP, Sirard JC. **Dendritic cells: the host Achille's heel for mucosal pathogens?** Trends Microbiol 2004;12:79–88.

[42] Okamura H, Tsutsui H, Komatsu M, Yutsudo A, Hakura T, Tanimoto K, Torigoe T. Okura, Y. Nakada, K. Konishi, S. Fukuda, T. Kurimoto, **Cloning of a new cytokine that induces INF-c production by T cells**, Nature 378 (1995) 88–91.

[43] Tsutsui H, Nakanishi K, Higashino K, Okamura H, Miyazawa Y, Kaneda K, **IFN-c-inducing factor upregulates Fas ligand-mediated cytotoxic activity of murine natural killer cell clones**, J. Immunol. 156 (1996) 3967–3973.

[44] Okamura, H, Tsutsui, H Kashiwamura, S, Yoshimoto, T, Nakanishi, K. **Interleukin-18: a novel cytokine that augments both innate and acquired immunity**. Adv. Immunol 1998; 70, 281–312.

[45] Dinarello CA. **IL-18: a TH1-inducing, proinflammatory cytokine and new member of the IL-1 family,** J. Allergy Clin. Immunol. 103 (1999) 11–24.

[46] Parnet P, Garka KE, Bonnert TP, Dower SK, Sims JE. **IL-1Rrp is a novel receptor-like molecule similar to the type I interleukin-1 receptor and its homologues T1/ST2 and IL-1R AcP**. J Biol Chem 1996;271:3967-70.

[47] Kohka H, Yoshino T, Iwagaki H, Sakuma I, Tanimoto T, Matsuo Y, Kurimoto M, Orita K, Akagi T, Tanaka N. **Interleukin-18/interferon-gamma-inducing factor, a novel cytokine, up-regulates ICAM-1 (CD54) expression in KG-1 cells**, J. Leukoc. Biol. 64 (1998) 519–527.

[48] Park CC, Morel JC, Amin MA, Connors MA, Harlow LA, Koch AE. **Evidence of IL-18 as a novel angiogenic mediator**, J.Immunol. 167 (2001) 1644–1653.

[49] Puren AJ, Fantuzzi G, Gu Y, Su MS, Dinarello CA**. Interleukin-18 (IFNgamma-inducing factor) induces IL-8 and IL-1beta via TNFalpha production from non-CD14+ human blood mononuclear cells**, J. Clin. Invest. 101 (1998) 711–721.

[50] Wood S, Wang B, Jenkins JR, Trayhurn P. **The pro-inflammatory cytokine IL-18 is expressed in human adipose tissue and strongly upregulated by TNFa in human adipocytes.** Biochemical and Biophysical Research Communications 337 (2005) 422–429.

[51] Wang B, Jenkins JR, Trayhurn P. **Expression and secretion of inflammation-related adipokines by human adipocytes differentiated in culture: integrated response to TNF-alpha**, Am. J. Physiol. Endocrinol. Metab. 288 (2005) E731–E740

[52] Gracie JA, Robertson SE,McInnes IB. **Interleukin-18.** J Leukoc Biol 2003;73:213–24.

[53] Esfandiari E, McInnes IB, Lindop G, Huang FP, Field M, Komai-Koma M, Wei X, Liew FY. **A proinflammatory role of IL-18 in the development of spontaneous autoimmune disease**. J Immunol 2001;167:5338–47.

[54] Nakanishi K, Yoshimoto T, Tsutsui H, Okamura H. **Interleukin-18 regulates both Th1 and Th2 responses.** Annu Rev Immunol 2001;19:423–74.

[55] Torre D, Speranza F, Giola M, Matteelli A, Tambini R, Biondi G. **Role of Th1 and Th2 cytokines in immune response to uncomplicated Plasmodium falciparum Malaria**. Am Society for Microbiol. 9: 348-351, 2002.

[56] Perkmann T, Winkler H, Graninger W, Kremsner PG, Winkler S. **Circulating levels of the interleukin (IL)-4 receptor and of IL-18 in patients with Plasmodium falciparum malaria**. Cytokine. 29: 153-158, 2005.

[57] Tominaga K, Yoshimoto T, Torigoe K, Kurimoto M, Matsui K, Hada T, Okamura H, Nakanishi K: **IL-12 synergizes with IL-18 or IL-1beta for IFNgamma production from human T cells**. Int Immunol 2000, 12:151-160.

[58] Imboden M, Nicod L, Nieters A, Glaus E, Matyas G, Bircher AJ, Ackermann-Liebrich U, Berger W, Probst-Hensch NM, SAPALDIA Team: **The common Gallele of interleukin-18 single-nucleotide polymorphism is a genetic risk factor for atopic asthma:** The SAPALDIA Cohort Study. Clin Exp Allergy 2006, 36:211-218.

[59] Tiret L, Godefroy T, Lubos E, Nicaud V, Tregouet DA, Barbaux S, Schnabel R, Bickel C, Espinola-Klein C, Poirier O, Perret C, Münzel T, Rupprecht HJ, Lackner K, Cambien F, Blankenberg S, Athero Gene Investigators: **Genetic analysis of the interleukin-18 system highlights the role of the interleukin-18 gene in cardiovascular disease.** Circulation 2005,112:643-650.

[60] Mojtahedi Z, Naeimi S, Farjadian S, Omrani GR, Ghaderi A: **Association of IL-18 promoter polymorphisms with predisposition to Type 1 diabetes**. Diabet Med 2006, 23:235-239.

[61] Sugiura T, Maeno N, Kawaguchi Y, Takei S, Imanaka H, Kawano Y, Terajima-Ichida H, Hara M, Kamatani N: **A promoter haplotype of the interleukin-18 gene is associated with juvenile idiopathic arthritis in the Japanese population.** Arthritis Res Ther 2006, 8:R60.

[62] Tamura K, Fukuda Y, Sashio H, Takeda N, Bamba H, Kosaka T, Fukui S, Sawada K, Tamura K, Satomi M, Yamada T, Yamamura T, Yamamoto Y, Furuyama J, Okamura H, Shimoyama T**. IL18 polymorphism is associated with an increased risk of Crohn's disease**. J Gastroenterol 2002, 37:111-116.

[63] Sivalingam SP, Yoon KH, Koh DR, Fong KY**. Single-nucleotide polymorphisms of the interleukin-18 gene promoter region in rheumatoid arthritis patients: protective effect of AA genotype.** Tissue Antigens 2003, 62:498-504.

[64] Giedraitis V, He B, Huang WX, Hillert J. **Cloning and mutation analysis of the human IL-18 promoter: a possible role of polymorphisms in expression regulation.** J. Neuroimmunol. 2001;112, 146–152.

[65] Kalina U, Ballas K., Koyama N, Kauschat D, Miething C, Arnemann J, Martin H, Hoelzer D, Ottmann OG. **Genomic organization and regulation of the human interleukin-18 gene.** Scand. J. Immunol 2000; 52, 525–530.

[66] Kirkpatrick A., Bidwell J., Van den Brule A.J.C., Meijer C.J.L.M., Pawade J. and Glew S. **TNFa polymorphism frequencies in HPV-associated cervical dysplasia.** Gynecologic Oncology 2004:92: 675–679.

[67] Duarte, I; Santos, A; Sousa, H; Catarino, R; Pinto, D; Matos, A; Pereira, D; Moutinho, J; Canedo, P; Machado, CM; Medeiros, R. **G-308A TNF-α polymorphism is association with na increase risk of invasive cervial câncer.** Biochemical and Biophysical Research Communications 334 (2005) 588-592

[68] Laing KJ,Wang T, Zou J, et al. **Cloning and expression analysis of rainbow trout Oncorhynchus mykiss tumour necrosis factor-alpha.** Eur J Biochem 2001;268:1315–22.

[69] Maqsood, M.E. et al. **Tumor necrosis factor alpha – 308 gene locus promoter polymorphism: An analysis of association with health and disease.** Biochimica et Biophysica Acta 2009: 1792: 163 – 172

[70] Abbas A K, Lichtman A H. **Cellular and Molecular Immunology**. Fifth edition. Saunders. 576pp. 2003

[71] Barth RJ Jr, Camp BJ, Martuscello TA, Dain BJ, Memoli VA. **The cytokine microenvironment of human colon carcinoma. Lymphocyte expression of tumor necrosis factor- á and interleukin-4 predicts improved survival.** Cancer 1996; 78: 1168-1178.

[72] Castelletti, C.H.M. **Possíveis regiões gênicas de interesse para o desenvolvimento do papillomavírus humano**. Tese de Mestrado, Universidade Federal de Pernambuco (UFPE), Brasil, 2006, 99p.

[73] Nishimura M, Mizuta I, Mizuta E, Yamasaki S, Ohtac M, Kaji R, Kuno S.**Tumor necrosis factor gene polymorphisms in patients with sporadic Parkinson's disease.** Neuroscience Letters 2001: 311: 1-4.

[74] Kroeger KM, Carville KS, Abraham LJ. **The 308 tumor necrosisfactor-alpha promoter polymorphism affects transcription.** Mol Immunol 1997;34(5):391– 9.

[75] Wilson AG, Symons JA, McDowell TL, McDevitt HO, Duff GO. **Effects of a polymorphism in the human tumor necrosis factor alpha promoter on transcriptional activation.** Proc Natl Acad Sci U S A 1997;94(7):3195–9.

[76] Bouma G, C' rusius JBA, Oudkerk Pool M, Kolkman JJ, Von Blomberg BM E, Kostense PJ, Giphart MJ, Schreuder GM, Meuwissen SGM and Pena A S. **Secretion of tumour necrosis factor alpha and lymphotoxin alpha in relation to polymorphisms in the TNF genes and HLA-DR alleles relevance for inflammatory bowel disease**. Surtd.I. Imtttutt. 1996: 43, 45&463.

[77] Fernandes APM, Goncalves MAG, Simões RT, Mendes-Junior CT, Duarte G, Donadi EA. **A pilot case–control association study of cytokine polymorphisms in Brazilian women presenting with HPV-related cervical lesions.** European Journal of Obstetrics & Gynecology and Reproductive Biology 2008;140: 241–244

[78] Cabrera, M., M-A. Shaw, C. Sharples et al. **Polymorphism in tumor necrosis factor genes associated with mucocutaneous leishmaniasis**. J. Exp. Med. 1995; 182:1259–1264.

[79] Hildesheim A, Schiffman M, Scott DR et al. **Human leukocyte antigen class I/II alleles and development of human Papillomavirus-related cervical neoplasia: results from a case-control study conducted in the United States.** Cancer Epidemiol Biomarkers Prev 1998;7:1035—41.

[80] Turner DM, Grant SC, Lamb WR, et al. **A genetic marker of high TNF-a production in heart transplant recipients.** Transplantation 1995;60:1113—7.

[81] Braun J, Yin Z, Spiller S et al. **Low secretion of tumour necrosis factor alpha but no other Th1 or Th2 cytokines by peripheral blood mononuclear cells correlates with chronicity in reactive arthritis**. Arthritis Rheum 1999; 42:2039—44.

[82] Chen G, Wilson R, Wang SH, Zheng HZ, Walker JJ, McKillop JH. **Tumour necrosis factor-alpha (TNF-a) gene polymorphism and expression in pre-eclampsia.** Clin Exp Immunol 1996;104:154—9.

Artigo Científico a ser submetido à revista *Virology Journal*, cujo fator de impacto é 2,55

Correlation of the infection by HPV with polymorphisms of two genes of cytokine: Tumor Necrosis Factor (TNF) alpha and Interleukin (IL) 18 in patients with and without Cervical Intraepithelial Lesion.

Mayara C. Mansur Fernandes[1]; Sérgio F. de Lima Júnior[1]; Trícia Ruschelle N. M. Marques[2]; Diêgo Henrique T. de Araújo[2]; Sandra de A. Heráclio[3]; Melânia M. Ramos Amorim[3], Paulo Roberto E. de Souza[2], Sérgio Crovella[1].

[1] Laboratório de Imunopatologia Keizo Asami (LIKA), Universidade Federal de Pernambuco (UFPE), Av. Professor Moraes Rego, Campus Universitário, s/n, 50670-901 Recife, PE, Brasil,

[2] Departamento de Biologia, Universidade Federal Rural de Pernambuco (UFRPE), Rua Dom Manoel de Medeiros, Dois Irmãos, s/n, 52171-900 Recife, PE, Brasil

[3] Instituto de Medicina Integral Professor Fernando Figueira (IMIP), Rua dos Coelhos, nº 300, Boa Vista, 50070-550 Recife, PE, Brasil,

E-mail: Mayara C. Mansur Fernandes* - mayaramansur@gmail.com; Sérgio F. de Lima Júnior - sergioflimajr@hotmail.com; Trícia Ruschelle N. M. Marques - triciaruschelle@hotmail.com; Diêgo Henrique T. de Araújo - diego_digo_bio@hotmail.com; Sandra de A. Heráclio - sandra_heraclio@hotmail.com; Melânia M. Ramos Amorim - melamorim@uol.com.br - Paulo Roberto E. de Souza – prsouza30@gmail.com; Sérgio Crovella - crovelser@gmail.com .

* Corresponding Author

Rua Estevão de Sá, 291, apartamento 204, Várzea, Recife-PE Cep 50740-270.

ABSTRACT

Background: The Human Papillomavirus (HPV) is responsible for affecting anually 500 thousand women with cervical cancer. Risk factors may facilitate the persistence of the virus of the uterine cervix. Genetic polymorphisms in regulatory and coding regions of cytokine genes are associated with the pathogenesis of a large number of human diseases. This study aimed to investigate whether there is a relationship between existing polymorphisms in the region -G308A of the gene *TNFα* and in regions -G137C and C607A of the *IL18* gene in susceptibility to HPV infection and progression of cervical intraepithelial lesions.
Methods: The study was conducted with 122 women HPV+ and, 132 women HPV- (control). The polymorphisms of the *TNFα* and *IL18* genes were analyzed using the technic of the Specific Sequence Polymosphism (PCR-SSP) and analyzed on agarose gel 1.5%. The Statistical analyzes to check the significance of the study of the genotypes were performed using the BioEstat 5.0 program.
Results: The results showed a prevalence of 49.18% of HPV-16 infection and 70.49% of that total had high-grade cervical lesions. Regarding the polymorphisms there was an association of the mutant allele in the region -308A of the gene *TNFα* and -607A of the *IL18* gene with susceptibility to HPV infection ($p = 0.0008$, $p < 0.0001$, respectively), but no relationship was found these genes with susceptibility to the development of lesions ($p > 0.05$). But no significant association was found with regard to the region -137 region of the gene *IL18*.
Conclusion: These results suggest two possible genetic markers of susceptibility to HPV infection in the studied population and that these can not be used as markers of progression of cervical injury.

Key-words: Human *Papillomavirus,* Cervical Intraepithelial Neoplasia, Single Nucleotide Polymorphism, Tumour Factor Necrosis alpha, Interleukin 18

BACKGROUND

The cervical cancer is the second most common maligner neoplasm in the world, losing to the mamma cancer only, it is responsible for about 500 thousand new cases per year, whose approximately half ends in death. Developing countries retain 80% of the new cases, making this cancer the most incident in women. HPV infections represent nearly 5% of all types of cancers [1].

In Brazil, the National Institute of Cancer estimates that in 2012 the occurrence will be 17.540 new cases, with an estimated risk of 17 cases per 100 thousand women [2].

The Human Papillomavirus (HPV), *Papillomaviridae* family, is the main causal etiological agent for the development of 99.7% of cervical carcinogenesis cases worldwide. The presence of one oncogenic type of HPV in the cervical region is considered the central cause not isolated to the development of Cervical Intraepithelial Neoplasm (CIN) and Invasive Cervical Cancer (ICC) [3].

The persistence of the HPV infection especially with one of those oncogenic types can cause epithelial changes [4,5]. The cervical cancer develops from premalignant not-invasive lesions, called Cervical Intraepithelial Neoplasm (CIN) or Squamous Intraepithelial Lesions (SIL).

The HPV acts together to environmental cofactors and immunological and genetic factors the host own for the occurrence of progression into cervical cancer [6,7,8].

The variations that occur in the genome are mostly caused by a single-nucleotide polymorphisms (SNPs) or point mutations that originate two allelic forms. These genetic variations are considered as very helpful for the human genetic markers research and it can be used to identify susceptibility genes of complex human diseases [8,9].

Cytokine genetic polymorphisms, especially in regulatory regions, can be related with the amount of cytokines produced [10].

The Tumor Necrosis factor-alpha (TNF-α) is a strong cytokine Th1, pro-inflammatory mainly released by stimulated monocytes and macrophages. The gene is found within the major histocompatibility complex in the chromosome 6 (p21.3) [11,12], and consists of four exons disposed throughout the 3kb of the DNA [13].

The principal physiological function of TNF-α is to stimulate the recruitment of neutrophils and monocytes to the infection locations and activate these cells to eradicate microorganism [14]. It has large biologic effects, including protection against the infection, surveillance against tumors and stimulates inflammatory responses [13], besides fulfill an essential role in immune system [15]. The TNF-α stimulates the synthesis of others pro-inflammatory cytokines and the molecular adhesion. Moreover, it has showed to mediate the carcinogenesis through induction, proliferation, invasion and tumor cells metastasis [16]. In acute situations, the local production of TNF-α is clearly beneficial. It increases the expression of adhesion molecules in the vascular endothelium to allow that immunology cells, in particular monocytes and neutrophils, move to the sites where occurred tissue damage and infection [17]. There are many polymorphisms localized in different promoter sites of the gene

responsible for the TNF-α synthesis. The Single nucleotide polymorphisms in region -308 and -238 of the TNF-α gene promoter has been commonly studied [17,18,19].

The polymorphism in -308 affects the gene expression and is involved in the regulation of the TNF-α transcription. Two alleles forms can be found in a particular population, where one remains with the G allele (guanine) or when occurs a replacement of this one by the A allele (adenine) [20,21].

The IL-18 interleukin plays a primordial role in the immune response, being responsible for the differentiation and T helper (T_h) cell activation into subgroups, according with the cytokines profile [24]. Acts like an inductor for the differentiation and proliferation of T_h1 cells in IL-12 presence, stimulating the production of IFN and TNF in T lymphocytes and natural killer (NK) [22,23,24]. The IL-18 and IL-12 act in synergism on the activation of NK cells, B and T cells [25,26]. On the other hand, the IL-18 can still stimulate the immune response of T_h2 type, in the absence of IL-12 [24,27]. It has pleiotropic action inducing the expression of chemokines and cytokines, as well as adhesion molecules like IL-18, TNF-α, VEGF and ICAM-1 [28].

Several polymorphisms in the promoter of *IL18* gene have been associated with different autoimmune and inflammatory diseases [29,30]. Five different positions of single nucleotide polymorphisms in the promoter region were identified: -656 G / T, -607 C / A, -137 G / C, 113 G / T, and 127 C / T [31]. However, only in SNPs at positions -137 and -607 were confirmed as having an impact on the activity of *IL18* gene in previous studies [34].

The role of genes polymorphism of *TNFα* and *IL18* genes on the susceptibility to many infectious diseases has been well documented, but the

functional importance about these polymorphisms is still uncertain. The polymorphism study may help in understanding the progression of cervical disease and HPV viral infection control, also allows a gene therapy according to the genotype of the patient.

Therefore, this study aimed to determine the existing polymorphisms in *TNFα* (-308 G/A), *IL18* (-137 G/C) and (-607 C/A) gene regions in patients infected by HPV and healthy, and correlating these polymorphisms with the level of cervical intraepithelial lesion.

METHODS

Design and study site

In the present work, was carried out a population-based cross-sectional study to compare detection rates of HPV infections and genotyping of existing polymorphism in region -308 of the TNF-alpha gene and regions -137 and -607 of IL-18 gene in patients with or without precursor lesions of the cervix.

The patients were treated at the Women's Laboratory, a member unit of the Public Health Central Laboratory of Pernambuco State (LACEN – Laboratório Central de Saúde Pública do Estado de Pernambuco), at the Ambulatory of Lower Genital tract Pathology (PTGI – Ambulatório de patologia do trato genital inferior) of the Woman Attention Center (Centro de Atenção a mulher) and gynecological oncology infirmary at the Institute of Integral Medicine Professor Fernando Figueira (IMIP – Instituto de Medicina Integral), during the period from October 2008 to November 2009. The study was previously approved by the local Ethics Committee in Research (nº355/08) and all included patients agreed to participate, signing the Terms of free and Informed Consent.

The study was conducted at the Laboratory of Genetics, Biochemistry and DNA sequencing Professor Tania Falcão of the Universidade Federal Rural de Pernambuco.

Study Population

The population studied was composed of women with cancer cytology performed in accredited municipal networks and / or state, presenting with lesions of low and high grade / ASCUS / AGUS uterine cervical cancer is valid for one year and had not been submitted to treatment prior approval of the cervix in the last six months. The control group consisted of healthy women with no history of neoplastic disease.

Collecting the samples

The samples were collected from scraping the cervical region, with the aid of brushes like the suitable cytobrush type. The brushes were immediately placed in maintenance buffer containing 1.5 mL of TE buffer (10 mM Tris-HCl and 1 mM EDTA pH 8.0) and later stored at -20 ° C. The samples were sent to the Laboratory of Biochemical Genetics, and DNA sequencing Professor Tania Falcão from Universidade Federal Rural de Pernambuco where they proceeded the DNA extractions and that were analyzed later.

Genomic DNA extraction

The genomic DNA extraction was made from 500μL of cells scraped from the cervical region using the Nucleospin Tissue ® kit (Macherey-Nagel) following the manufacturer's instructions.

Sample quality

Were used initiators primers PC04 and GH20, with the annealing temperature of 55 ° C, which flank a sequence around 268 bp of the β-globin gene, producing a reaction product to verify the absence of inhibitors and the integrity and quality of extracted DNA.

HPV detection by conventional PCR

For HPV detection was carried out a PCR reaction mixture containing 15μL: 1X enzyme buffer(Tris-HCl 10 mM, KCl 50 mM), 1,5mM MgCl2, 100μM dNTP (dATP, dGTP, dCTP, dTTP), 1pmol/μl of each specific primer (MY09 and MY11), 0.2 U of Taq DNA polymerase and 200 ng of DNA. For this reaction was used the kit "GoTaq ® Hot Start Polymerase" (PROMEGA).

The conditions of cycling for PCR were: 94 ° C for 4 minutes, followed by 40 cycles at 94 ° C for 30 seconds, annealing at 55 ° C for 30 seconds and extension at 72 ° C for 30 seconds, followed by an additional extension at 72° C for 8 minutes. The expected fragment size for this reaction was 450pb.

The negative samples for the first set of primers were submitted to a new amplification reaction with internal primers GP5 + and GP6 +. The reaction was performed to a final volume of 15μl containing 1x PCR buffer, 200μM dNTP, 3.5 mM MgCl2, 1pmol/μl of each specific primer (GP5 + and GP6 +), 1U of Taq DNA polymerase and 200 ng of DNA. For this reaction we used the kit "GoTaq ® Hot Start Polymerase" (PROMEGA).

The protocol of amplification used with the system GP5 + / GP6 + was the following: 94°C for 4 minutes, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 45°C for 2 min and extension at 72°C for 1.5 minutes,

followed by a additional extension at 72°C for 4 minutes in the presence of a band of 150bp with primers GP5 + and GP6 +.

The primers information are presented in Table 1.

HPV typing

The reactions for HPV typing were performed using specific primers for the identification of the most frequent high risk HPV in the world population (16 and 18).

The reaction mixture contained 1X Master Mix Colorless (PROMEGA) and 1μM primer, ~ 200 ng DNA and nuclease-free water in qsp. The conditions for cycling were: 94°C for 5 min, followed by 35 cycles of 95°C for 30 s, 57°C for 30 s and 72°C for 60s, and a final extension of 72°C for 10 min [35]. After amplification was observed the fragment of 499bp to the HPV 16 and 172bp to the HPV 18.

Samples sequencing

After confirmation of positive samples for HPV and typing with specific primers for HPV 16 and HPV 18, sequencing reactions were performed with the other samples not genotyped using the kit DyEnamic ET Dye Terminator Cycle sequencing kit (GE Healthcare) for such 8μl Dye Terminator reagent, 0.5 pmol of primer MY11 and 200nm of the amplicon to a final volume of 20μl.

The sequencing PCR followed the protocol of 30 cycles of amplification: 94 ° C for 20 seconds, 50 ° C for 15 seconds and 60 ° C for 60 seconds. Immediately after the reaction was carried out the purification reaction of samples according to the manufacturer's recommendations.

Then the samples were taken to the automated DNA sequencer MegaBACE 1000 DNA Sequencer. At the end of the process the results were compared with the various genotypes already found through the online platform BLASTn of the National Center for Biotechnology Information (http://blast.ncbi.nlm.nih.gov/Blast.cgi) in order to determine the HPV genotype found in the survey.

TNFα and *IL18* genes polymorphisms analysis

The detection of genotypes of the polymorphisms in *TNFα* and *IL18* genes were made using the technique of polymerase chain reaction (PCR), method based on Specific Sequence Polymosphism (PCR-SSP) described by Perrey and his collaborators in 1999 [38]. The PCR was performed using an ordinary primer (generic) and two special primers of specific sequences for the nitrogenous base present in allele, for each gene region.

The polymorphism identification was performed with two reaction mixtures, differing from each other according to the primer used. Patients who only amplified with the mixture containing the wild primer were considered homozygous normal, whereas those samples that amplified only with the mutant primer were genotyped homozygous polymorphic. When bands were observed in both reaction mixtures, were called heterozygous.

The amplification reaction for analysis of *TNFα* gene polymorphism was performed in a final volume of 15µl. The reaction mixture contained 200 ng of DNA from clinical specimen (vaginal secretion), 1x enzyme tampon Taq Platinum® (Invitrogen Life Technologies), 200 µM deoxynucleotide triphosphate (dNTP), 2.5 mM $MgCl_2$, 0.03 U DNA Taq Platinum DNA polymerase (Invitrogen,

Life Technologies) and 1 pmol / µl aliquot of each of the common and specific primers (G or A).

The reaction conditions were: initial heating of 95°C for 4 min in the denaturation step of the double-stranded DNA, followed by 40 cycles of denaturation at 94°C for 30 seconds, annealing at 60.8°C for 30 seconds and final extension at 72°C for 8 minutes. The reaction was kept at 4°C. The reactions were run in automatic thermal cycler Mastercycler Gradient (Eppendorf).

For detection of *IL18*, in both regions was carried out a reaction mixture for PCR containing Master Mix Colorless PROMEGA 2X, 1µM of allele-specific primers, 4.5 µL of nuclease-free water and the DNA template.

The cycling conditions for the PCR of the *IL18*- 137 G/C were: 94°C for 2 min, followed by 5 cycles at 94°C for 20 seconds and 68°C for 60 seconds, 25 cycles of 94°C for 20 seconds, 62°C for 20 seconds and 72°C for 40 seconds, according to the protocol of J. Arimitsu and his collaborators in 2006 [37].

The cycling conditions for PCR of the IL18 - 607 C/A were: 94°C for 2 min, followed by 7 cycles at 94°C for 20 seconds, 64°C for 60 seconds and 72°C for 40 seconds, 25 cycles of 94°C for 20 seconds, 57°C for 20 seconds and 72°C for 40 seconds.

The expected fragments were 261bp for the *IL18*- 137 G/C and 301bp for the region of - 607 C/A.

Electrophoresis

The samples were applied in agarose gel 1.5% colored with Syber Green and subjected to electrophoretic separation in 1X TBE running buffer with a voltage of 70V and amperage of 100 mA, for approximately 40 min. The separation of the amplification product was visualized in ultraviolet light. The expected PCR fragments were compared with the molecular weight marker 100bp ladder (Promega).

Statistical Analysis

Data analysis was performed using the BioEstat 5.0 software. The study was in the cross-sectional type with independents samples consisting of nominal data (genotype). The influence of each polymorphism on the risk for developing cervical disease was estimated by odds ratio (OR) using a confidence interval of 95% for the parameters.

Allele frequencies were estimated by gene counting method. The Hardy-Weinberg test was used to verify if the studied groups were in balance. The prevalence of different genotypes in patients and controls was analyzed by the χ^2 test and by the Fisher exact test in contingency tables. P-value under or equal to 0.05 were considered statistically significant.

RESULTS

Stratification of samples with lesion

The samples belonging to the group with lesion were stratified according to the degree of cervical intraepithelial neoplasia: 20.49% (25/122) of patients

had low-grade lesions (CIN I), 70.49% (86/122) were with high-grade lesions (CIN II / III) and 9.02% (11/122) had ICC.

HPV typing

The HPV genotyping identified 49.18% (60/122) of patients with HPV 16 infection, 15.57% (19/122) with HPV 18, 16.39% (20/122) were coinfected with both and 18.85% (23/122) were positive for other HPV types and coinfections.

Analysis of the *TNFα* gene polymorphism

The polymorphisms in the promoter region of the *TNFα* gene were calculated to check their relationship with the presence of infection and susceptibility to development of cervical lesions (Table 2).

The low producer GG genotype showed to be very representative in healthy controls (53.03%) when compared the frequency of this genotype in the group of patients according to the degree of cervical lesion (32%, 27.91%, 45.45%).

In Table 2, there is the distribution of genotypic and allelic frequencies to the polymorphism in the -308 region of the *TNFα* gene. There was significant difference regarding to the group with HPV infection compared with the control group (p = 0.0008, p = 0.0011, respectively), but there was no correlation between the polymorphism and susceptibility to development of cervical lesion (p = 0 , 5654, p = 0.6646).

Analysis of the *IL18* gene polymorphism

Regarding to the *IL18* gene were analyzed two regions -137 and -607 (Table 3). In relation the region -137 wasn't observed any statistically significant association for both the genotype frequencies and allele frequencies ($p > 0.05$).

For the region -607 was observed a statistically significant difference regarding the genotype distribution between HPV + individuals and healthy control subjects ($p < 0.0001$). The same was not observed when related to the development of cervical lesion ($p > 0.05$).

DISCUSSION

In the present study it was found high frequency of HPV types of high risk 16 and 18 in accordance with the Brazilian population where these two types are the most prevalents [39]. Another relevant data is associated with socioeconomic status of patients that reflects smaller information of the population with respect to methods of prevention and early detection of ICC. This would explain the high rate of high-grade lesions and cancer (79.51%).

In this article we found an association between the HPV infection and polymorphism in the -308 region of the *TNFα* gene. However, we found no literature on this type of association for polymorphism. But, only one study showed a relationship between this polymorphism and the degree of cervical lesion and other studies have linked this polymorphism with cervical cancer.

Kirkpatrick et al. [11] suggest that low secretor phenotype (GG) present in 95% of patients with CIN 1 are giving protection to severe cervical disease (CIN 2 and 3), but this result was not confirmed since only 32% of the patients had GG genotype. Still in this context, Fernandes et al. [21] found no

relationship between this polymorphism and the presence of cervical lesions in Brazilian women.

As the number of cervical cancer patients in this study was small, it was not possible be performed statistical analysis in relation to this group separately. However, Govan et al. [36] studied two ethnic groups in South Africa, one of mixed and one of black people regarding the association with cervical cancer and found no significant differences in the distribution of these polymorphisms. Despite the works refer to different stages of cervical disease, as they also found no association with cancer, we found no association in relation to lesion progression.

Stanczuk et al. [40] in a study realized with the population of Zimbabwe in cervical cancer patients was verified that low producer genotype (GG) was present in 72% of patients infected by HPV. In this same population the A allele, rarely appeared in 1% of patients and 2% of healthy women. However, no significant differences in alleles distribution between patients with cervical cancer and healthy women.

On the other hand, Duarte et al. [12] in a study made in Portugal in cervical cancer patients, we observed that the AA genotype was more frequent in patients than in controls (3.6% and 1.6% respectively), but these results were not statistically significant , despite the frequency of this allele has slight increase in group with the disease, probably due to reduced number of genotype AA, because this genotype is considered rare. Our results showed, in like manner, a higher percentage of AA genotype in the patients (9.02% - 11/122) than control (3.79% - 5/132).

On our work, we haven't found any statistically significant association when related the polymorphism of the region -137 of the *IL18* and the progression for the lesions. The polymorphism on the -607 region was showed associated with susceptibility to infection and persistence of HPV in the uterine cervix. No studies were found relating the *IL18* polymorphisms with susceptibility to the lesions CC precursors caused by HPV.

Sáenz-López et al. [41] found no statistically significant association between polymorphisms in regions -607 or -137 of the *IL18* gene and the risk of renal cell cancer. However, suggest that the low secretor genotypes of both regions can contribute to the onset of disease and the aggressiveness of the renal tumor installed.

Bushley et al. [42] verified that study conducted in Hawaiian women the presence of at least one variant allele (GC / CC) *IL18* in the region -137 demonstrated a decrease in risk of progression to last stages of ovarian cancer, compared women with the GG genotype. In contrast, the high frequency of G allele was associated with progression and metastasis of ovarian cancer.

Liu et al. [43] found significant differences in genotype distribution and allele polymorphism -137 G / C *IL18* gene between patients and controls. The GC and CC genotypes (-137) were associated with an increased risk of prostate cancer when compared with the GG genotype.

Nikiteas et al. [44] observed that the frequency of the mutant allele (CA / AA) at position -607 of *IL18* in patients with colorectal cancer was significantly higher than in healthy controls, indicating a high risk for developing colorectal cancer.

Migita et al. [45] observed that the presence of the C allele at position -607 (C / C + C / A) was associated with increased risk of hepatocellular carcinoma in patients infected with HBV. On the other hand, the genotype AA of region -607 was associated with risk reduction of HBV infection.

The ethnic diversity of the Brazilians, in Pernambuco state in particular seems to interfere with the frequency of polymorphisms in the population, as verified by Alves-Silva et al. [46]: a mixture of white (about 40%), african-Americans (about 40%) and Amerindian (about 20%).

CONCLUSIONS

In the present study, we can conclude that *TNFα* and *IL18* at position (-607) genes polymorphism were associated with HPV infection, but there were no statistically significant association with progression to cervical intraepithelial neoplasia by this genes. No significant association was found with respect to -137 region of the *IL18* gene.

These results suggest two possible genetic markers of susceptibility to HPV infection in this population and that these can not be used as markers of progression of cervical injury.

CONTRIBUTION OF AUTHORS

MCMF designed the study, coordinated and carried out the experiments and drafted the manuscript; SFLJ participated in the experiments of DNA extraction and sequencing of the samples and performed the data analysis; TRNMM contributed to the clinical diagnosis and contributed in the preparation of manuscript; DHTA contributed to the clinical diagnosis; MMRA SAH and

performed the sample collection; PRES conceived the study, performed the data analysis and reviewed the manuscript; SC corrected the manuscript.

All authors have read and have approved the final manuscript.

CONFLICT OF INTEREST

No conflict of interest to declare.

ABBREVIATIONS

Atypical glandular cells of undetermined significance - AGUS

Atypical squamous cells of undetermined significance - ASCUS

Cervical Cancer CC

Cervical intraepithelial neoplasia - CIN

Deoxynucleotide Triphosphate (dNTP),

Human Papillomavirus -HPV

Interleukin 12 – IL-12

Interleukin 18 - IL-18

Instituto de Medicina Integral Professor Fernando Figueira - IMIP

Major histocompatibility complex – MHC

Natural Killer - NK

Odds Ratio (OR)

Patologia do Trato Genital Inferior - PTGI

Polymerase Chain Reaction - PCR

Specific Sequence Polymosphism – SSP

Single Nucleotide Polimorphism - SNPs

TE (Tris-HCl e EDTA)

Tumor factor Necrosis alpha - TNF-α

T helper cells - Th

ACKNOWLEDGMENTS

This research was supported by The National Council of Scientific and Technological Development (CNPQ), Brazil.

REFERENCES

[1] Parkin DM, Bray F, Ferlay J, et al: **Global cancer statistics, 2002**. CA *Cancer J Clin* 2005, 55:74–108.

[2] BRASIL. INCA - **Instituto Nacional do Câncer. Ministério da Saúde**. Disponível em: <http://www.inca.gov.br/estimativa/2012/index.asp?ID=5>. Acesso em: Dezembro de 2011.

[3] Wolschick NM, Consolaro MEL, Suzuki LE, Boer CG: **Câncer do colo do útero: tecnologias emergentes no diagnóstico, tratamento e prevenção da doença.** *Rev. Bras. Anal. Clin* 2007, 39 (Suppl 2):123-129.

[4] Lowy DR, Howley PM: **Fields virology**. In: *Knipe DM, Howley PM, editors*. Papillomaviruses**.** Philadelphia, USA: Lippincott, Williams and Wilkins; 2001: 2231–64.

[5] Doorbar J: **Molecular biology of papillomavirus infection in the cervical cancer**. *Clin Sci Lon* 2006, 110 (Suppl 5): 525-541.

[6] Ylitalo N, Josefsson A, Melbye M, Sorensen P, Frisch M, Andersen PK, Spare´n P, Gustafsson M, Magnusson P, Ponte´n J, Gyllensten U, Adami H-O: **A prospective studyshowing long-term infection with human papillomavirus 16 before the development of cervical carcinoma in situ**. *Cancer Res* 2000, 60: 6027–6032.

[7] Santos AM, Sousa H, Catarino R, Pinto D, Pereira D, Vasconcelos A, Matos A, Lopes C, Medeiros R: **TP53 codon 72 polymorphism and risk for cervical cancer in Portugal.** *Cancer Genet Cytogenet* 2005, 159: 143–147.

[8] Johnson GCL, Todd JA: **Strategies in complex disease mapping**. *Curr Opin Genet Dev* 2000, 10:330.

[9] Sachidanandam R, Weissman D, Schmidt SC, Kakol JM, Stein LD, Marth G, et al: **A map of human genome sequence variation containing 1.42 million single nucleotide polymorphisms**. *Nature* 2001, 409:928-33.

[10] Nishimura M, Mizuta I, Mizuta E, Yamasaki S, Ohtac M, Kaji R, Kuno S:**Tumor necrosis factor gene polymorphisms in patients with sporadic Parkinson's disease.** *Neuroscience Letters* 2001, 311:1-4.

[11] Kirkpatrick A., Bidwell J., Van den Brule A.J.C., Meijer C.J.L.M., Pawade J. and Glew S: **TNFa polymorphism frequencies in HPV-associated cervical dysplasia**. *Gynecologic Oncology* 2004, 92: 675–679.

[12] Duarte, I; Santos, A; Sousa, H; Catarino, R; Pinto, D; Matos, A; Pereira, D; Moutinho, J; Canedo, P; Machado, CM; Medeiros, R: **G-308A TNF-α polymorphism is association with na increase risk of invasive cervial câncer.** *Biochemical and Biophysical Research Communications* 2005, 334 588-592.

[13] Laing KJ, Wang T, Zou J, et al: **Cloning and expression analysis of rainbow trout Oncorhynchus mykiss tumour necrosis factor-alpha.** *Eur J Biochem* 2001, 268:1315–22.

[14] Abbas AK, Lichtman AH: **Cellular and Molecular Immunology**. Fifth edition. Saunders. 576pp. 2003
[15] Barth RJ Jr, Camp BJ, Martuscello TA, Dain BJ, Memoli VA: **The cytokine microenvironment of human colon carcinoma. Lymphocyte expression of tumor necrosis factor- á and interleukin-4 predicts improved survival.** *Cancer* 1996, 78: 1168-1178.
[16] Castelletti, CHM: **Possíveis regiões gênicas de interesse para o desenvolvimento do papillomavírus humano**. Tese de Mestrado, Universidade Federal de Pernambuco (UFPE), Brasil, 2006, 99p.
[17] Maqsood, ME et al. **Tumor necrosis factor alpha – 308 gene locus promoter polymorphism: An analysis of association with health and disease.** *Biochimica et Biophysica Acta* 2009, 1792:163–172.
[18] Kroeger KM, Carville KS, Abraham LJ: **The 308 tumor necrosisfactor-alpha promoter polymorphism affects transcription.** *Mol Immunol* 1997, 34(Suppl 5):391– 9.
[19] Wilson AG, Symons JA, McDowell TL, McDevitt HO, Duff GO: **Effects of a polymorphism in the human tumor necrosis factor alpha promoter on transcriptional activation.** *Proc Natl Acad Sci* U S A 1997, 94(Suppl7):3195–9.
[20] Bouma G, C' rusius JBA, Oudkerk Pool M, Kolkman JJ, Von Blomberg BM E, Kostense PJ, Giphart MJ, Schreuder GM, Meuwissen SGM and Pena A S: **Secretion of tumour necrosis factor alpha and lymphotoxin alpha in relation to polymorphisms in the TNF genes and HLA-DR alleles relevance for inflammatory bowel disease**. *Surtd.I. Imtttutt*. 1996, 43:45-463.
[21] Fernandes APM, Goncalves MAG, Simões RT, Mendes-Junior CT, Duarte G, Donadi EA: **A pilot case–control association study of cytokine polymorphisms in Brazilian women presenting with HPV-related cervical lesions.** *European Journal of Obstetrics & Gynecology and Reproductive Biology* 2008, 140:241–244.
[22] Gracie JA, Robertson SE, McInnes IB: **Interleukin-18**. *J Leukoc Biol* 2003, 73:213–24.
[23] Esfandiari E, McInnes IB, Lindop G, Huang FP, Field M, Komai- Koma M, Wei X, Liew FY: **A proinflammatory role of IL-18 in the development of spontaneous autoimmune disease**. *J Immunol* 2001, 167:5338–47.
[24] Nakanishi K, Yoshimoto T, Tsutsui H, Okamura H: **Interleukin-18 regulates both Th1 and Th2 responses.** *Annu Rev Immunol* 2001, 19:423–74.
[25] Torre D, Speranza F, Giola M, Matteelli A, Tambini R, Biondi G: **Role of Th1 and Th2 cytokines in immune response to uncomplicated Plasmodium falciparum Malaria.** *Am Society for Microbiol* 2002, 9: 348-351.
[26] Perkmann T, Winkler H, Graninger W, Kremsner PG, Winkler S: **Circulating levels of the interleukin (IL)-4 receptor and of IL-18 in patients with Plasmodium falciparum malaria.** *Cytokine* 2005, 29: 153-158.
[27] Tominaga K, Yoshimoto T, Torigoe K, Kurimoto M, Matsui K, Hada T, Okamura H, Nakanishi K: **IL-12 synergizes with IL-18 or IL-1beta for IFNgamma production from human T cells.** *Int Immunol* 2000, 12:151-160.
[28] Wood S., Wang B, Jenkins JR, Trayhurn P: **The pro-inflammatory cytokine IL-18 is expressed in human adipose tissue and strongly**

upregulated by TNFa in human adipocytes. *Biochemical and Biophysical Research Communications* 2005, 337:422–429.
[29] Tamura K, Fukuda Y, Sashio H, Takeda N, Bamba H, Kosaka T, Fukui S, Sawada K, Tamura K, Satomi M, Yamada T, Yamamura T, Yamamoto Y, Furuyama J, Okamura H, Shimoyama T: **IL18 polymorphism is associated with an increased risk of Crohn's disease.** *J Gastroenterol* 2002, 37:111-116.
[30] Sivalingam SP, Yoon KH, Koh DR, Fong KY: **Single-nucleotide polymorphisms of the interleukin-18 gene promoter region in rheumatoid arthritis patients: protective effect of AA genotype.** *Tissue Antigens* 2003, 62:498-504.
[31] Giedraitis V, He B, Huang WX, Hillert J: **Cloning and mutation analysis of the human IL-18 promoter: a possible role of polymorphisms in expression regulation.** *J Neuroimmunol* 2001, 112:146–152.
[32] Kalina U, Ballas K., Koyama N, Kauschat D, Miething C, Arnemann J, Martin H, Hoelzer D, Ottmann OG: **Genomic organization and regulation of the human interleukin-18 gene.** *Scand J Immunol* 2000, 52:525–530.
[33] Manos MM, Ting Y, Wright DK, Lewis AJ, Broker TR, Wolinsky SM: **Use of polymerase chain reaction amplification for the detection of genital human papillomaviruses.** In: Furth M, Greaves M, editors. *Molecular diagnostics of human cancer*. Cold Spring Harbor: Cold Spring Harbor Laboratory; 1989. p. 209-14. (Cancer Cells Series, 7)
[34] de Roda Husman AM, Walboomers JM, Van Den Brule AJ, Meijer CJ, Snijders PJ: **The use of general primers GP5 and GP6 elongated at their 3' ends with adjacent highly conserved sequences improves human papillomavirus detection by PCR.** *J Gen Virol* 1995, 76 (Suppl 4): 1057-62.
[35] Karlsen F, Kalantari M, Jenkins A, Pettersen E, Kristensen G, Holm R, Johansson B, Hagmar B: **Use of Multiple PCR Primer Sets for Optimal Detection of Human Papillomavirus. Journal of Clinical Microbiology.** *Sept* 1996, 2095–2100.
[36] Govan VA, Constant D, Hoffman M, Williamson A: **The allelic distribution of -308 Tumor Necrosis Factor-alpha gene polymorphism in South African women with cervical cancer and control women.** *BMC Cancer* 2006, 6:24.
[37] Arimitsu J. *et al*. **IL-18 gene polymorphisms affect IL-18 production capability by monocytes:** Biochemical and Biophysical Research Communications 342 (2006) 1413–1416
[38] Perrey C, Turner SJ, Pravica V, Howell WM, Hutchinson IV: **ARMS-PCR methodologies to determine IL-10, TNF-alpha, TNF-beta and TGF-beta 1 gene polymorphisms.** *Transpl Immunol.* 1999, 7(Suppl2):127-8.
[39] Freitas TP, Carmo BB, Paula FDF, Rodrigues LF, Fernandes AP, Fernandes PA: **Molecular detection of HPV 16 and 18 in cervical samples of patients from Belo Horizonte, Minas Gerais, Brazil**. *Rev Inst Med Trop S Paulo* 2007, 49(Suppl 5):297-301.
[40] Stanczuk GA, Sibanda EM, Tswana AS, Bergstrom S: **Polymorphism at the −308-promoter position of the tumor necrosis factor-alpha (TNF-α) gene and cervical cancer**. *Int J Gynecol Cancer* 2003, 13:148-153.
[41] Saenz-López P, Carretero R, Vazquez F, Martin J, SÂnchez E, Tallada M, Garrido F, CÔzar JM, Francisco Ruiz-Cabello F: **Impact of interleukin-18 polymorphisms-607 and -137 on clinical characteristics of renal cell carcinoma patients**. *Human Immunology* 2010, 71:309–313

[42] Bushley AW, Ferrell R, McDuffie K, Terada KY, Carney ME, Thompson PJ, Wilkens LR, Tung K, Ness RB, Goodman, MT. **Polymorphisms of interleukin (IL)-1a, IL-1h, IL-6, IL-10, and IL-18 and the risk of ovarian cancer**. Gynecologic Oncology 2004, 95:672–679.
[43] Liu, Yunguang, Lin, Na, Huang, Li , Xu, Qunqing, Pang, Guangfu, **Genetic polymorphisms of the interleukin-18 gene and risk of prostate cancer, DNA and Cell Biology** 2007, 26(Suppl 8):613-18.
[44] Nikiteas N, Yannopoulos A, Chatzitheofylaktou A And Tsigris C**: Heterozygosity for Interleukin-18 –607 A/C Polymorphism is Associated with Risk for Colorectal Cancer.** Anticancer Research 2007, 27:3849-3854
[45] Migita K, Sawakami-Kobayashi K, Maeda Y, Nakao K, Kondoh S, Sugiura M, Kawasumi R, Segawa O, Tajima H, Machida M, Nakamura M, Yano K, Abiru S, Kawasaki E, Yatsuhashi H, Eguchi K, And Ishibashi H: **Interleukin-18 promoter polymorphisms and the disease progression of Hepatitis B virus-related liver disease.** *Translational research the journal of laboratory and clinical medicine* 2009, 153: 2, 91-96.
[46] Alves-Silva J, da Silva Santos M, Guimarães PE, et al: **The ancestry of Brazilian mtDNA lineages**. *Am J Hum Genet* 2000, 67:444–461.

Table 1. Sequence of primers for identification and typing of HPV used in the study

Type	Primer	Primer Sequence	Length
ß-globina humana	PC04	5' - GAA GAG CCA AAG GAC AGG TAC - 3'	268pb
	GH20	5' - GAA GAG CCA AGG ACA GGT AC - 3'	
MY[1]	MY09	5'-CGT CCM ARR GGA WAC TGA TC-3'	450pb
	MY11	5'-GCM CAG GGW CAT AAY AAT GG-3'	
GP+[2]	GP05+	5'-TTT GTT ACT GTG GTA GAT ACT AC-3'	150pb
	GP06+	5'-GAA AAA TAA ACT GTA AAT CAT ATT C-3'	
HPV16[3]	PR3	5'-GTC AAA AGC CAC TGT GTC CT-3'	499pb
	PR4	5'-CCA TCC ATT ACA TCC CGT AC-3'	
HPV18[3]	PR1	5'-CCG AGC ACG ACA GGA ACG ACT-3'	172pb
	PR2	5'-TCG TTT TCT TCC TCT GAG TCG CTT-3'	
TNF alfa (-308 G/A)[4]	TNF G	5' ATA GGT TTT GAG GGG CAT GG 3'	233pb
	TNF A	5' AAT AGG TTT TGA GGG GCA TGA 3'	
	TNF generic	5' TCT CGG TTT CTT CTC CAT CG 3'	
IL18 (-137 G/C)[5]	IL18 G	5' -CCC-CAA-CTT-TTA-CGG-AAG-AAA-AG-3'	261pb
	IL18 C	5'-CCC-CAA-CTT-TTA-CGG-AAG-AAA-AC-3'	
	IL18 generic	5' -AGG-AGG-GCA-AAA-TGC-ACT-GG-3'	
IL18 (-607 C/A)	IL18 C	5'- TTC TGC ATC TTT ACA GCT GGA G - 3'	301pb
	IL18 A	5' - CAC GGA TAC CAT CAT TAG AAT TTT ATT - 3'	
	IL18 generic	5' - TTC TGC ATC TTT ACA GCT GGA G -3'	

[1]Manos *et al.* [33]; [2]Roda Husman *et al.* [34]; [3]Karlsen *et al.* [35]; [4]Perrey 38]; [5]Arimitsu *et al.* [37].

Table 2. Distribuition genotypic and allelic frequencies of the *TNFG308A* among health controls and patients

Polymorphism	Controls	Patients				p-value OR	p-value OR
			CIN 1	CIN 2/3	ICC	Control X HPV	CIN 1 X CIN 2/3
***TNFα* (-308) Genotypic**							
GG	70 (53,03%)	37 (30,33%)	8 (32%)	24 (27,91%)	5 (45,45%)		
GA	57 (43,18%)	74 (60,65%)	16 (64%)	54 (62,79%)	4 (36,36%)	$X^2 = 14.262$	G = 1.1403
AA	5 (3,79%)	11 (9,02%)	1 (4%)	8 (9,3%)	2 (18,2%)	p = 0.0008	p = 0.5654
Allelic							
G	197 (74,62%)	148 (60,66%)	32 (64%)	102 (59,3%)	14 (63,64%)	p = 0,0011	p = 0,6646
A	67 (25,38%)	96 (39,34%)	18 (36%)	70 (40,7%)	8 (36,36%)	95% IC (1,3070 - 2,79831)	
						OR = 1,9072	

Table 3. Distribuition genotypic and allelic frequencies of the *IL18* among health controls and patients

Polymorphism	Controls	Patients				p-value OR	p-value OR
			CIN	CIN 2/3	ICC	Control X HPV	CIN 1 X CIN 2/3
***IL18* (-137) Genotypic**							
GG	54 (40,91%)	66 (54,1%)	11 (44%)	51 (59,3%)	4 (36,36%)		
GC	69 (52,27%)	50 (40,98%)	11 (44%)	32 (37,21%)	7 (63,64%)	$X^2 = 4.447$	G = 3.2608
CC	9 (6,82%)	6 (4,92%)	3 (12%)	3 (3,49%)	0	p = 0.1082	p = 0.2219
Allelic							
G	177 (67,05%)	182 (74,59%)	33 (66%)	134 (77,91%)	15 (68,18%)	p = 0.0770	p = 0.1259
C	87 (32,95%)	62 (25,41%)	17 (34%)	38 (22,09%)	7 (31,82%)	95% IC (0,4712 - 1,0195) OR = 0,6931	
***IL18* (-607) Genotypic**							
CC	33 (25%)	32 (26,23%)	4 (16%)	25 (29,07%)	3 (27,27%)		
CA	80 (60,61%)	53 (43,44%)	13 (52%)	35 (40,7%)	5 (45,45%)	$X^2 = 37.426$	G = 2.3562
AA	19 (14,39%)	37 (30,33%)	8 (32%)	26 (30,23%)	3 (27,27%)	p < 0.0001	p = 0.5134
Allelic							
C	146 (55,30%)	117 (47,95%)	21 (42%)	85 (49,42%)	11 (50%)	p = 0,1169	p = 0,4451
A	118 (44,70%)	127 (52,05%)	29 (58%)	87 (50,58%)	11 (50%)	95% IC (0,9472 - 1,9044) OR = 1,3430	

ANEXO II

Parecer do Comitê de ética em Pesquisa envolvendo seres humanos do Centro de Ciências da Saúde da Universidade Federal de Pernambuco CEP/CCS/UFPE.

SERVIÇO PÚBLICO FEDERAL
UNIVERSIDADE FEDERAL DE PERNAMBUCO
Comitê de Ética em Pesquisa

Of. N. º 105/2009-CEP/CCS

Recife, 29 de abril de 2009.

Registro do SISNEP FR – 226696
CAAE – 0347.0.172.000-08
Registro CEP/CCS/UFPE Nº 355/08
Titulo: "ASSOCIAÇÃO DO POLIMORFISMO DE ALGUNS GENES RELACIONADOS COM A IMUNIDADE HUMANA E A INFECÇÃO POR CHLAMYDIA TRACHOMATIS EM PACIENTES COM E SEM LESÕES INTRA-EPITELIAIS CERVICAIS".

Pesquisador Responsável: Paulo Roberto Eleutério de Souza

Senhor Pesquisador:

Informamos que o Comitê de Ética em Pesquisa envolvendo seres humanos do Centro de Ciências da Saúde da Universidade Federal de Pernambuco CEP/CCS/UFPE registrou e analisou, de acordo com a Resolução N.º 196/96 do Conselho Nacional de Saúde, o protocolo de pesquisa em epígrafe, aprovando-o e liberando-o para início da coleta de dados em 28 de abril de 2009.

Ressaltamos que o pesquisador responsável deverá apresentar relatório anual da pesquisa.

Atenciosamente,

Prof. Geraldo Bosco Lindoso Couto
Coordenador do CEP/ CCS / UFPE

Ao
Prof. Dr. Paulo Roberto Eleutério de Souza
Laboratório de Imunopatologia Keiso Asami – LIKA/UFPE

Av. Prof. Moraes Rego, s/n Cid. Universitária, 50670-901, Recife - PE, Tel/fax: 81 2126 8588; cepccs@ufpe.br

CONSIDERAÇÕES FINAIS

No presente trabalho foi possível verificar a associação dos polimorfismos dos genes *TNFα* e *IL18* (-607) com a infecção pelo HPV na população pernambucana.

A importância deste estudo deve-se ao fato de ter sido o primeiro a analisar a relação do gene *IL18* (-607) com as lesões cervicais e o câncer cervical invasivo e ampliar o conhecimento da *TNFα* e sua relação com as neoplasias intraepiteliais cervicais, ainda escasso na literatura.

Sugerimos para os próximos trabalhos fazer uma associação a outros cofatores que possam estar envolvidos na infecção viral bem como expandir o perfil das citocinas estudadas de cada paciente e assim, trazer novas informações para a população de Pernambuco – Brasil.