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Estudo comparativo entre a citologia convencional versus citologia em meio líquido e avaliação do diagnóstico das doenças sexualmente transmissíveis em nível de Saúde Pública

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Tese apresentada ao Programa de Pós-Graduação em Ciências Biológicas da Universidade Federal de Pernambuco, como pré-requisito para a obtenção do título de Doutora em Ciências Biológicas, área de concentração Biotecnologia.

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Co-orientadora: Prof. Dr. Paulo Roberto Eleutério de Souza (UFRPE)

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Ao Deus Eterno, imortal, invisível, mas real, autor da minha vida.

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“As letras e a ciência só tomarão seu verdadeiro lugar na obra do desenvolvimento humano no dia em que, livres de toda a servidão mercenária, forem exclusivamente pelos que as amam e para os que as amam.”

Piotr Kropotkine
1842 // 1921

RESUMO

O presente estudo avaliou o desempenho da metodologia citológica de base líquida (*ThinPrep*-TP) com o da citologia convencional de Papanicolaou (CC) no diagnóstico de alterações citopatológicas e de resultados insatisfatórios, sob a visão de um serviço público no estado de Pernambuco (LACEN-PE) e da Fundação Oncocentro de São Paulo (FOSP-SP). Também foram avaliadas as associações entre a presença de coinfecções genitais dos subtipos de *Human papillomavirus* (HPV) com *Chlamydia trachomatis* (CT) e/ou outras microfloras, com os diferentes estágios de alterações cervicais das pacientes. A população do estudo foi de 525 mulheres na faixa etária dos 18-65 anos, atendidas por demanda espontânea, pelas Unidades Básicas de Saúde no Estado de Pernambuco, no período de abril a novembro de 2011. Um questionário padronizado com informações sobre características sociodemográficas, sexuais, reprodutivas e de hábitos (tabagismo, consumo de bebidas alcoólicas e uso de drogas) foram obtidos de todas as pacientes do estudo. A presença de DNA do HPV e da CT foram diagnósticadas através da reação em cadeia da polimerase (PCR) e o exame citológico foi realizado para detecção das demais infecções. Para avaliar a relação das infecções genitais com a presença de alterações intraepiteliais cervicais, foi utilizado o teste exato de Fisher. Os resultados mostraram que 11,05% das pacientes tinham menos de 25 anos, 30,86% eram solteiras, 6,86% tiveram mais de 5 parceiros sexuais, 44% não faziam uso de métodos contraceptivos, 38,85% eram usuárias de álcool, 24,38% eram fumantes e 3,24% haviam consumido drogas. Além disso, 42,01% tinham queixas ginecológicas; e 12,19% história pregressa de doenças sexualmente transmissíveis (DST). Quando comparadas a eficiência das técnicas TP e CC sob a visão do LACEN-PE, observou-se que as duas metodologias avaliadas apresentaram fraca concordância entre os métodos (*k=0,19;*95%IC(0,11-0,26); *p*<<0,001). A metodologia TP reduziu a taxa de resultados insatisfatórios de 4,38% para 1,71% ($\times^2$= 5,28; p=0,02), e o número de alterações citopatológicas diagnosticadas aumentaram de 2,47% para 3,04%. Porém sob a avaliação da FOSP-SP, as duas metodologias apresentaram concordância (*k=0,39;*95%IC(0,29-0,50); *p*<<0,001). A metodologia TP apresentou taxas insatisfatórias e praticamente semelhantes de 3,20% para 3,60% ($\times^2$= 5,00; p=0,17), e de alterações citopatológicas de 5,60% para 4,20%. Em 87 casos, foram observadas alterações cervicais por uma das metodologias utilizadas; destas, em 83,91% foram detectados CT e 82,76% foram positivas para HPV, ocorrendo coinfecção em 65 casos (74,7%). Em 93,1% dos casos houveram alterações colposcópicas, observando-se uma associação estatisticamente significativa entre coinfecção HPV-CT e presença de lesão (p=0.037). Outras microfloras encontradas foram *Gardnerella vaginalis* (35,6%), cocos (18,4%), *Candida sp* (9,2%), *Trichomonas vaginalis* (6,9%), *Lactobacillus sp* (4,6%) e herpesvírus (1,15%). Flora mista (coinfecção por várias microfloras – com exceção de CT simultaneamente) ocorreram em 41,38% dos casos. No entanto, nenhuma associação da presença desses microrganismos não-virais e os herpesvírus com a gravidade das lesões intraepiteliais foram encontradas. Os subtipos de HPV mais frequentes foram 16 e 31 (34,3% e 17,15%, respectivamente). Porém nas lesões de maior gravidade, os mais prevalentes foram 16 e 18. Desta forma, concluimos que este estudo demonstra a superioridade da metodologia TP no diagnóstico citológico das amostras cervicais o que poderá contribuir na diminuição de possíveis perdas por repetição citológica e seguimento das pacientes. É importante chamar atenção para as infecções genitais, em especial a CT, que devem sem investigadas e tratadas adequadamente, haja vista, que coinfecções com o HPV estão associadas ao favorecimento de lesões cervicais, e podem evoluir a graus mais avançados.

Palavras-chaves: Citologia Papanicolaou; Citologia em meio liquido; *ThinPrep*; HPV infecção, lesão cervical; coinfecção genital.

ABSTRACT

This study evaluated the performance of cytological methodology net basis (*ThinPrep*-TP) with the conventional Pap cytology (CC) in the diagnosis of cytopathological findings and unsatisfactory results under the vision of a public service in the state of Pernambuco (LACEN -PE) and Oncocentro Foundation of São Paulo (FOSP-SP). We also evaluated whether there is an association between the presence of genital co-infections of *human papillomavirus* subtypes (HPV) with *Chlamydia trachomatis* (CT) and or other microflora, with the different stages of cervical abnormalities of patients. The study population of 525 women between the ages of 18-65 years, assisted by spontaneous demand, the Basic Health Units in the State of Pernambuco, in the period from April to November 2011. A standardized questionnaire with information on sociodemographic characteristics, sexual, reproductive habits (such as smoking, alcohol consumption and drug use) were obtained from all study patients. The presence of HPV DNA and CT were both diagnosed by polymerase chain reaction (PCR) and cytological examination was performed to detect other infections. To evaluate the relationship of genital infections with the presence of cervical intraepithelial changes, Fisher's exact test was used. The results showed that 11.05% of patients were under 25yrs, 30.86% were single, 6.86% had more than five sexual partners, 44% did not use contraception, 38.85% were users of alcohol, 24.38% smokers and 3.24% had used drugs before. Moreover, gynecological complaints were 42.01%; and 12.19% history of STD. When comparing the efficiency of two techniques used in diagóstico of cervical abnormalities in the view of LACEN-PE, it was observed that the two methodologies evaluated showed poor agreement between the methods ($k = 0.19$; 95% CI (0,11- 0.26), $p << 0.001$). TP method reduced the rate of unsatisfactory 4.38% to 1.71% ($5.28 = 2 \times p = 0.02$) and the number of cytopathological changes diagnosed increased 2.47% to 3 04%. But in the assessment of FOSP-SP, the two methodologies showed reasonable agreement ($k = 0.39$; 95% CI (0.29 to 0.50); $p << 0.001$) .The TP methodology showed unsatisfactory results virtually rates similar 3.20% to 3.60% ($\times 2 = 5.00$; $p = 0.17$), and cytological changes from 5.60% to 4.20%. In 87 cases, cervical abnormalities were observed by any of the methods used, and of these, 83.91% were detected CT, and 82.76% were positive for HPV, occurring co-infection in 65 cases (74.7%). In 93.1% of cases there were colposcopic changes, observing a statistically significant association between co-infection HPV-CT and presence of lesions ($p = 0.037$). Other microflora were found *Gardnerella vaginalis* (35.6%), coconut (18.4%), *Candida sp* (9.2%), *Trichomonas vaginalis* (6.9%), *Lactobacillus sp* (4.6%) and herpesviruses (1.15%). Mixed flora (co-infection with various microflora - CT exception with both) occurred in 41.38% of cases. However, no association between the presence of non-viral organisms and herpesviruses with the severity of intraepithelial lesions were found. The most common HPV subtypes there were 16 and 31 (34.3% and 17.15%, respectively). But in more severe injuries, the most prevalent were 16 and 18. Thus, we conclude that this study demonstrates the superiority of TP methodology in the cytological diagnosis of cervical samples which could contribute to decrease possible losses by repetition and cytologic follow-up of patients . It is important to draw attention to the genital infections, especially CT, which are not investigated and dealt with appropriately, given that co-infections with HPV are associated with the favoring of cervical lesions, and can progress to more advanced degrees.

***Keywords**:* Papanicolaou Cytology; Liquid-based cytology; *ThinPrep*; HPV infection; cervical lesion; genital coinfection.

LISTA DE FIGURAS

LISTA DE TABELAS

LISTA DE ABREVIATURAS

a.C.: antes de Cristo

ASC-H: Atipias escamosa indeterminada sugestiva de alto grau

ASCUS: Atipia escamosa indeterminada

ATP: Adenosina Trifosfato

CBL: Citologia em base líquida

CC: Câncer cervical

CDC: Centro de Controle de Doenças (EUA)

CEP-UFPE: Comissão de Ética em Pesquisa da UFPE

CLUE CELL: célula guia característico da *Gardnerella vaginalis*

CML: Citologia em meio líquido

CNPq: Conselho Nacional de Desenvolvimento Científico e Tecnológico

CP: Citologia de Papanicolaou

CT: *Chlamydia trachomatis*

DNA: ácido desoxirribonucléico

dNTP: Os quarto nucleotídeos do DNA: Adenina, Citosina, Guanina e Timina

DST: Doença sexualmente transmissível

E: Região precoce

E1: Gene de codificação da proteína viral precoce 1

E2: Gene de codificação da proteína viral precoce 2

E2F: Fator ativador da transcrição em eucariontes

E4: Gene de codificação da proteína viral precoce 4

E5: Gene de codificação da proteína viral precoce 5

E6: Gene de codificação da proteína viral precoce 6

E7: Gene de codificação da proteína viral precoce 7

EUA: Estados Unidos da América

FDA: Food and Drug Administration

FOSP: Fundação Oncocentro de São Paulo

HA: atividade hemaglutinante (do inglês *haemagglutinating activity*)

HCl: ácido clorídrico

HeLa: linhagem celular de carcinoma cervical humano

HIV: Vírus da imunodeficiência humana

HPV: *Human Papillomavirus*

HSP 60: *Heat shoch proteins 60*

IC: Intervalo de Confiança

IARC: International Agency for Research on Cancer

ICTV: International Committee on the Taxonomy of Viruses

IF: fator de impacto (do inglês *impact factor*)

IFCPC: International Federation for Cervical Pathology and Colposcopy

IgG: Imunoglobulina G

IgM: Imunoglobulina M

IL: interleucina

INCA: Instituto Nacional do Câncer

INFECÇÃO POR HPV: presença do vírus na região anogenital diagnosticada pela pesquisa do DNA-HPV por reação em cadeia de polimerase (PCR)

JEC: Junção escamocolunar

K: Kappa

KCl: Cloreto de potássio

L: Região tardia

L1: Região tardia 1

LACEN: Laboratório Central de Saúde Púplica de Pernambuco

LBC: Líquid based cytology

LIEBG: Lesão intraepitelial de baixo grau

LIEAG: Lesão intraepitelial de alto grau

LILACS: Centro Latino-Americano e do Caribe de Informação em Ciências da Saúde

MEQ: Monitoramento Externo de Qualidade

$MgCl_2$: Cloreto de magnésio

MHC: moléculas de histocompatibilidade

mL: Mililitros

mm: Milímetros

mM: Milimolar

NCI: Instituto Nacional do Câncer (do inglês *National Cancer Institute*)

NIC: Neoplasia intraepitelial cervical

OMS: Organização Mundial da Saúde

***P*:** Teste chi quadrado

PAG: Página

PAP TEST: citologia oncótica obtida através da coleta em solução sobrenadante líquida

PB: Pares de bases

PCR: Reação em cadeia de polimerase

PCT: Proteinas do choque térmico

p16: Proteína de 16kDa inibidora de CDK (CDKI) da família Ink

p53: Proteína supressora tumoral

ppm: partes por milhão

ppt: partes por trilhão

pRb: produto gênico do Retinoblastoma

Primers: seguimentos de ácido nucléico

PTGI: Patologia do trato genital inferior

QT: Quimioterapia

RAD: Radioterapia

RIP: Proteína inativadora de ribossomo (do inglês, *ribossome-inactivating protein*)

RNAse.: Enzima de degradação do RNA

SBTGI: Sociedade Brasileira do Trato Genital Inferior

SCIELO: Scientific Electronic Library Online

sp: Espécie

SUS : Sitema Único de Saúde

ThinPrep: Método automatizado de citologia em meio líquido que permite a deposição do material coletado em única camada

Th1: célula T helper 1

TM: Temperatura de Melting específica

TNF: fator de necrose tumoral (do inglês *tumour necrosis factor*)

UFRPE: Universidade Federal Rural de Pernambuco

URL: Região reguladora não codificante

URR: Região reguladora não codificante

ul: Microlitro

uM: Micromol

VB: vaginose bacteriana

ZT: Zona de transformação

WHO: Organização Mundial da Saúde (do inglês *World Health Organization*)

SUMÁRIO

CAPÍTULO I

1 INTRODUÇÃO

O carcinoma do colo uterino é o segundo tipo de câncer mais frequente em mulheres no mundo e a terceira maior causa de morte por câncer em mulheres (WHO, 2013; MS, 2014). Configurando-se como um importante problema de saúde pública, segundo as últimas estimativas mundiais no ano de 2012 foram atribuídos 527.000 novos casos de câncer cervical e 265.000 mortes relacionadas a essa neoplasia a cada ano, com 85% dos casos nos países em desenvolvimento (Ministério da Saúde, 2012; INCA, 2014). A inexistência ou a baixa eficiência dos programas de prevenção (rastreamento citológico) é apontada como a provável causa da elevada incidência do câncer de colo nesses países (Ministério da Saúde, 2013; WHO, 2013; WHO, 2003; INCA, 2014).

No Brasil, considerando o ano de 2014, a expectativa é de 15.590 casos novos de câncer do colo do útero, com um risco estimado de 15,33 casos para cada 100 mil mulheres. Diferentemente dos outros tipos de cânceres humanos, o câncer cervical é uma doença evitável, devido a sua lenta evolução, com longo período desde o desenvolvimento de lesões precursoras ao surgimento do câncer (INCA/Ministério da Saúde, 2014).

O teste de Papanicolaou é a principal estratégia utilizada nos programas de controle do câncer do colo do útero. No Brasil, o Ministério da Saúde determina que o exame citológico de Papanicolaou (CP) seja realizado prioritariamente em mulheres com idade entre 25 a 64 anos (INCA/Ministério da Saúde, 2014).

Sugerido em 1941, por um médico grego Geórgios Papanicolaou, como uma ferramenta para a detecção precoce do câncer do colo do útero. A CP é considerada um método eficiente, de fácil aplicabilidade, pois tem a habilidade de identificar lesões precursoras do câncer do colo do útero, que neste momento são tratáveis (TAVARES *et al.*, 2007). Porém, apesar de todo reconhecimento do método, este ainda apresenta altas taxas de falsos-negativos devido a sua oscilação na sensibilidade (BERNSTEIN *et al.*, 2001), cujas taxas podem variar de 2% a 50% (BERGERON *et al.*, 2000; FERRAZ *et al.*, 2005; TAVARES *et al.*, 2007). Segundo uma meta-análise realizada por FAHEY *et al.* (1995) a sensibilidade da CP é de 58% (variando de 11 a 99%), com especificidade de 68% (variando de 14 a 97%).

O Estado de Pernambuco - Brasil se destaca por apresentar elevados índices de exames insatisfatórios. Dos 185 municípios existentes, 77 municípios, ou seja, 41,62%

apresentaram amostras insatisfatórias acima de 5% durante o ano de 2013. O Laboratório Central de Saúde Pública do Estado de Pernambuco- LACEN-PE, é o local onde converge o grande quantitativo destes exames. No ano de 2011, tivemos no LACEN-PE, uma incidência de 8,16%, ou seja, 9.367 exames foram dados como insatisfatórios, e baixas taxas de exames com alterações citopatológicas (0,6-1,5%), o que pode representar baixa qualidade da rede laboratorial do Estado (INCA/ DATASUS, 2013).

Na década de 90 foi desenvolvida uma nova metodologia para realização da citopatologia do colo uterino: a citologia em meio líquido. Esta metodologia foi introduzida como uma alternativa à utilização do método convencional, com o propósito de melhora na especificidade e qualidade da amostra na lâmina. Como neste método é feita uma suspensão de células, cria-se a possibilidade de fazer outros testes que não apenas a leitura da lâmina no microscópio ótico, na coloração de Papanicolaou (DAVEY*et al.,* 2006; BEERMAN *et al.*, 2009).

A citologia em meio líquido Thin Prep ® (TP) foi aprovada em 1996 pela FDA (United States Food and Drugs Administration) e existem evidências suficientes que este método reduz a proporção de amostras insatisfatórias. No entanto, ainda restam dúvidas quanto sua eficácia, também em relação ao método convencional, na detecção precoce de câncer do colo do útero (CAMPAGNOLI *et al.,* 2005; GIRIANELLI *et al.*, 2007; FDA, 2014).

Esta técnica vem sendo estudada e, gradativamente, está substituindo a CP nos programas de controle do câncer de colo uterino de alguns países (PAYNE *et al.,* 2000; HOELUND *et al.,* 2003;). Seu surgimento se deu objetivando viabilizar a leitura das lâminas por computadores, para atender às demandas de escrutínio computadorizado (PEREIRA *et al.,* 2003; STABILE *et al.,* 2012). O intuito era melhorar a sensibilidade, através do uso de uma monocamada de células para facilitar o diagnóstico do citopatologista, com melhor preservação celular e a possibilidade de realizar estudos de pesquisa do DNA do *Papillomavírus Humano* (HPV) e da *Chlamydia trachomatis* (BERNSTEIN *et al.,* 2001; ABULAFIA *et al.,* 2003; BIDUS *et al.,* 2006; RONCO, 2006; TAKEI *et al.,* 2006; STABILE *et al.,* 2012). Vários estudos evidenciam que a infecção pelo HPV é a principal causa de câncer de colo uterino, assim como, a participação de outros cofatores de origem infecciosa, como a *C. trachomatis*, podem contribuir na evolução e intensificação da lesão cervical.

A presente tese foi realizada com o objetivo de comparar as taxas de alterações citopalógicas e de resultados insatisfatórios por duas metodologias (Citologia Convencional de Papanicolaou e a Citologia em Meio líquido-ThinPrep ®) em amostras cervicais de mulheres atendidas pelo Sistema Único de Saúde do Estado de Pernambuco e analisadas pelo Laboratório Central de Saúde Pública (LACEN-PE) e Fundação Oncocentro de São Paulo (FOSP-SP); assim como avaliar as características clínicas, biológicas e demográficas dessas pacientes; e determinar associação entre a presença de coinfecções genitais dos subtipos de HPV com *C. trachomatis* e outras microfloras nos diferentes estágios de alterações cervicais, pela técnica da PCR, fazendo-se a concordância entre os diagnósticos. Como justificativa de:

- Demonstrar a eficácia no diagnóstico do câncer de colo uterino e justificar o custo/benefício da citologia em meio líquido em nível de Saúde Pública no Estado de Pernambuco.
- Melhorar os índices exames citológicos insatisfatórios no estado de Pernambuco.
- Melhorar a qualidade dos diagnósticos de lesão de alto e baixo grau, principalmente glandular, além da praticidade na coleta e possibilidade de rastreamento de doenças sexualmente transmitidas (DST) dentro da mesma amostra coletada.
- Substituir a citologia convencional pela citologia em meio líquido em nível de "*scrinning*" na rotina de saúde pública do Estado de Pernambuco.

Esta análise faz parte de um estudo piloto no Estado de Pernambuco com objetivo principal justificar a implantação da Citologia em meio líquido em nível de saúde pública para os municípios mais distantes e com maior demanda reprimida no Estado de Pernambuco, sugerindo possíveis medidas e/ou introdução de metodologias, que ajudem a trazer melhorias nestes indicadores da qualidade da rede de saúde pública do Estado, tendo em vista o controle e seguimento do diagnóstico do câncer de colo de útero.

2 OBJETIVOS

2.1 GERAL

Determinar o nível de concordância entre a citologia convencional de Papanicolaou e citologia em meio líquido (ThinPrep) para o rastreio do câncer de colo uterino em nível de saúde pública, além de pesquisar as Doenças Sexualmente Transmissíveis (DST), *Human papillomavirus* (HPV) *e Clhamydia trachomatis*.

2.2 ESPECÍFICOS

2.2.1 Determinar a concordância dos diagnósticos entre citologia convencional X citologia em meio líquido, avaliados por dois centros de referências (LACEN-PE e FOSP-SP).
2.2.2 Obter os índices de insatisfatórios e de alterações citopatológicas pelas duas metodologias;
2.2.3 Determinar a prevalência dos sub-tipos do HPV de alto risco oncogênico (16,18,31 e 33), infecção pela *C. trachomatis* e outras microfloras, e avaliar a concordância entre os diagnósticos;
2.2.4 Relacionar as características: biológicas (cor, idade); sócio-demográficas (escolaridade, raça, nível sócio-econômico, estado civil, procedência e religião); reprodutivas (idade da primeira relação sexual, número de parceiros, relação sexual sem proteção, paridade, história de DST prévia e uso de método contraceptivo) e hábitos (tabagismo, etilismo, uso de drogas) com os diagnósticos dos exames;
2.2.5 Comparar os achados clínicos e laboratoriais.
2.2.6 Responder ao estudo piloto no Estado de Pernambuco para implantação da citologia em meio líquido em nível de saúde pública.

3 REVISÃO BIBLIOGRÁFICA

3.1 O CÂNCER CERVICAL

A palavra câncer vem do grego *karkínos*, que quer dizer caranguejo, e foi utilizada pela primeira vez por Hipócrates, o pai da medicina, que viveu entre 460 e 377 a. C. O câncer não é uma doença nova. O fato de ter sido detectado em múmias egípcias comprova que ele já comprometia o homem há mais de 3 mil anos antes de Cristo. Atualmente, câncer é o nome geral dado a um conjunto de mais de 100 doenças heterogêneas que afetam drasticamente a humanidade, e que tem em comum o crescimento desordenado de células, que tendem a invadir tecidos e órgãos vizinhos. (CERQUEIRA, 2000; DESCH *et al.,* 2002; KLUG *et al.,* 2002; THOMAS, 2003; INCA, 2012).

O câncer é comumente descrito como uma doença de mutações em genes que regulam caminhos essenciais da função celular levando a um crescimento exagerado e descontrolado das células do tecido. Estas mutações podem ocorrer devido à ação de agentes carcinogênicos. No entanto, evidências epidemiológicas e clínicas apontam para o importante, porém multifacetado, papel do hospedeiro (LIEHR *et al.,* 2000; FERNANDES JÚNIOR *et al,* 2000; KLUG *et al.*, 2002; THOMAS *et al.*, 2003; WEST *et al.,* 2003; HOUGHTON *et al.,* 2005; HANAHAN *et al.,* 2011).

Atualmente, o câncer de uma forma geral, é um dos problemas de saúde pública que o sistema de saúde brasileiro enfrenta mais complexos, dada a sua magnitude epidemiológica, social e econômica. Afeta os seres humanos com alta frequência e contribui significativamente para a morbidade e mortalidade globais. Ressalta-se que pelo menos um terço dos novos casos de câncer que ocorre anualmente no mundo poderia ser prevenido (MARTINKOVA *et al.,* 2009; INCA, 2012).

Outro fato digno de atenção é que a carga global de câncer continua a aumentar, em grande parte por causa do envelhecimento e crescimento da população mundial, ao lado de uma crescente adoção de comportamentos do hospedeiro, que estão diretamente associados aos de câncer de colo uterino, como: o início precoce das relações sexuais, vários parceiros sexuais ao longo da vida, alta paridade em partos não cirúrgicos, tabagismo, baixo nível socioeconômico, uso de anticoncepcional oral prolongado, deficiências nutricionais, co-infecção com outras doenças sexualmente transmissíveis como a infecção pelo vírus da imunodeficiência humana (HIV), *Chlamydia trachomatis*

e outros tipos de *Human papillomavirus* (HPV), além da influência de hormônios endógenos, fatores genéticos e outros fatores relacionados à resposta imune (COELHO *et al.*, 2008; WOODMAN *et al.*, 2007; MUÑOZ el al, 2003).

3.2 SITUAÇÃO ATUAL DO DIAGNÓSTICO DO CÂNCER DO COLO UTERINO NO ESTADO DE PERNAMBUCO

Segundo o Instituto Nacional do Câncer (INCA/Ministério da Saúde, 2014), no Brasil, são esperados 15.590 casos novos de câncer de colo útero para o ano de 2014. A região Nordeste, é a segunda região mais frequente, ou seja, são 18,79 por 100 mil habitantes.

Para o Estado de Pernambuco, estima-se em 2014, uma taxa bruta de incidência de 20,47 casos novos para 100 mil habitantes para o câncer de colo uterino, ou seja, são 970 casos novos para cada 100 mil habitantes. Em Recife, são 180 casos novos por 100 mil habitantes ao ano, ou seja, uma taxa bruta de 20,43. A incidência de câncer do colo do útero evidencia-se na faixa etária de 20 a 29 anos e o risco aumenta rapidamente até atingir seu pico, geralmente na faixa etária de 45 a 49 anos. Ao mesmo tempo, é o câncer que apresenta maior potencial de prevenção e cura quando diagnosticado precocemente (WHO, 2013; INCA/Ministério da Saúde, 2014).

Durante o ano de 2013, no Estado de Pernambuco foram processados 517.895 exames citopatológicos convencional em toda a rede credenciada ao SUS. A Unidade do Laboratório da Mulher, pertencente à rede de laboratórios ligados ao Laboratório Central do Estado de Pernambuco (LACEN-PE), hoje é responsável pela realização do controle de qualidade da rede credenciada ao SUS. Porém durante os anos de 2011 a 2013, era responsável pelo processamento de 30% destes exames, ou seja, eram cerca 170.000 (cento e setenta mil) exames/ano na área de Citopatologia e Histopatologia, com demanda mensal média de 14.000 (quatorze mil) exames citopatológicos/mês pelo método convencional de Papanicolaou, com atendimento direto a rede de saúde pública e convênio com 144 (cento e quarenta e quatro) municípios do Estado de Pernambuco (Ministério da Saúde/DATASUS, 2013).

Estas amostras, processadas neste serviço, representam a avaliação laboratorial do estado, pois, são as representações exatas, da qualidade técnica da coleta, processamento e análise dos exames citopatológicos, e representam a amostragem de 144 municpíos dos 185 existentes (Ministério da Saúde/DATASUS, 2013).

A avaliação do material citolopatológico da rede de Saúde Pública do Estado de Pernambuco, assinala elevados índices de exames insatisfatórios, decorrentes de coletas inadequados e demora de envio de material, com tempo médio superior a 10 (dez) dias corridos para serem processados. Os diagnósticos destas lesões são feitos pela citologia oncótica convencional de Papanicolaou, colposcopia e anatomopatológico. O diagnóstico clínico em geral não ocorre, pois estas mulheres em geral são assintomáticas. A citologia oncótica cérvico-vaginal convencional representa um bom teste para rastreamento desta neoplasia, apresentando uma sensibilidade (50-95%) e especificidade (97-100%), porém quando não bem coletada, fixada e acondicionada, pode representar grandes perdas em amostras insatisfatórias, repercutindo na qualidade do diagnóstico do câncer cervical e proporcionar altos custos com tratamento, uma vez que o diagnóstico primário não foi bem realizado (LAPIN *et al.*,2000).

3.3 INFECÇÃO PELO *Human papillomavirus* (HPV)

3.3.1. Agente Etiológico

Evidências epidemiológicas e laboratoriais sugerem que em 99,7% dos carcinomas cervicais tem sido detectada a presença da infecção pelo HPV (BOSCH *et al.*,1995; COELHO *et al.*, 2008; MARTINS *et al.*, 2012).

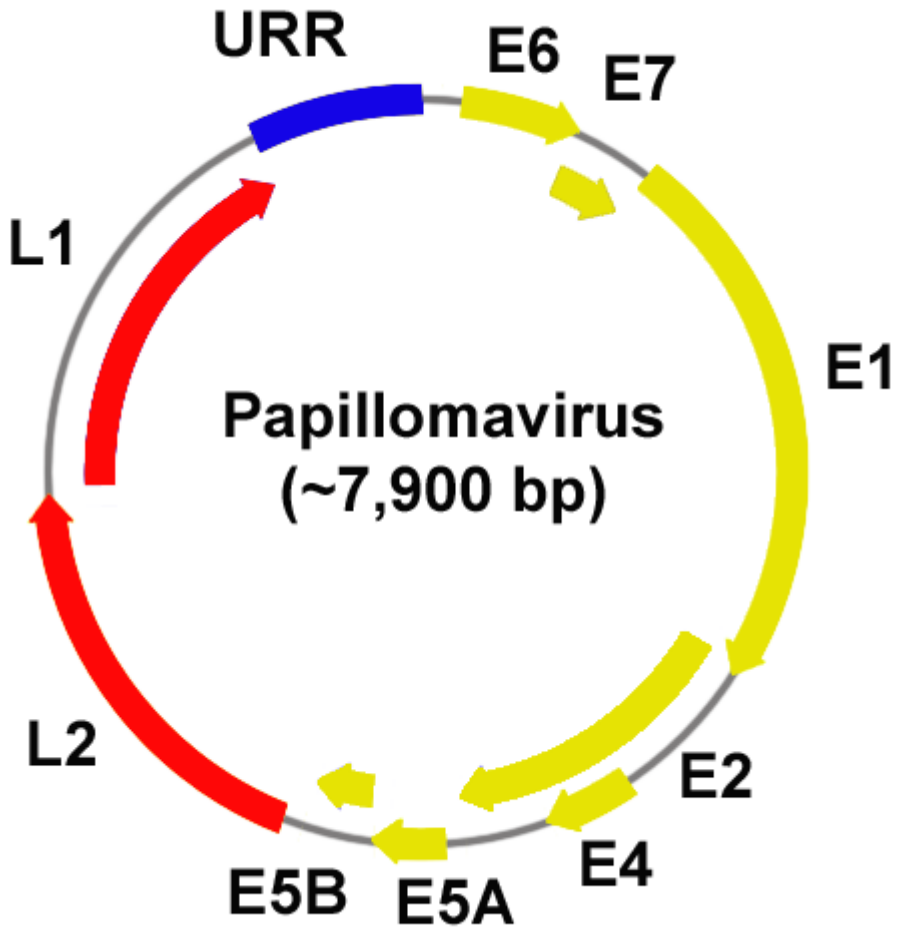
Os *Papillomavirus* formam um gênero que, juntamente com os *Polyomavirus*, constituem a família do *Papovaviridae*. O nome oficial da espécie é representado pelo nome do hospedeiro em itálico seguido do nome do gênero, por exemplo, *Bovine papillomavirus, Ovine papillomavirus, Human papillomavirus* (HPV). Segundo o *International Committee on Taxonomy of Viruses* (ICVT) este nome é derivado em parte do latim, *papilla* significando mamilo ou pústula, e do grego, sufixo *Omã*, que significa tumor, ou seja, o tumor que forma mamilos ou papilas (ICTV, 2013). Na língua portuguesa existem controvérsias da nomenclatura a ser utilizada nos textos em relação a esse vírus. Assim, optou-se por usar o nome oficial da espécie.

O HPV é um alphapapillomavirus assinalado com o número 00.099.0.02 no ICVT. É um DNA vírus que tem tropismo pelo tecido epitelial escamoso e glandular não cultivável, de dupla fita, pequeno, circular, não envelopado com capsídio icosaédrico de 72 capsômeros e um genoma contendo 8.000 pares de bases nitrogenadas. A organização genômica dos *Human papillomavirus* (**Figura 1**) é

semelhante e apresentam uma região precoce (E), uma região tardia (L) e uma região reguladora não codificante (URR) (ICTV, 2013).

Reconhecem-se mais de 130 tipos de HPV associados a diferentes manifestações clínicas. Os tipos de HPV distinguem-se pela seqüência dos ácidos nucléicos de E6, E7 e L1. Destes, aproximadamente 40 tipos estão associados à infecção do trato genital inferior (vulva, vagina, colo, região perineal, perianal e canal anal) (OGUMMODEDE *et al.*, 2007).

Figura 1 - Organização do genoma do papillomavirus humano
E = Região Precoce; L = Região Tardia; URR = Região Reguladora não Codificante



FONTE:http://www.intechopen.com/books/dna-replication-current-advances/oncogenic-aspects-of-hpv-infections-of-the-female-genital-tract [04-11-2014]

3.3.2 Infecção por *Human papillomavirus* (HPV)

A infecção pelo HPV é estabelecida pela penetração dos vírions por uma escara qualquer na superfície epitelial que permita o acesso às células-tronco basais multipotentes. A exposição destas células permite a penetração das partículas virais e a infecção por HPV é iniciada. Essa escara pode ser ocasionada ao menor trauma, mesmo durante a relação sexual e, permite o acesso viral ao epitélio. A entrada viral na célula parece ser via um receptor intermediário de endocitose, dependente de clatrina, porém muito do mecanismo de entrada do vírus na célula ainda permanece sem elucidação (DAY *et al*., 2003; THOMISON III *et al.,* 2008; MONSONEGO *et al.*, 2010).

A porta de entrada para infecção por HPV no epitélio cérvico-vaginal, de modo geral, é a junção escamo-colunar, onde a transmissão do HPV acontece na grande maioria dos casos pela via sexual, mas pode também acontecer pela via materno-fetal ou por fômites e a partir daí pode haver comprometimento do epitélio estratificado e também do endocervical (SILVA FILHO *el al*, 2000; CASON *et al.*, 2005; MEDEIROS *et al.*, 2005; CAVALCANTI *et al.*, 2006).

No início da infecção viral, em uma fase dita produtiva, coordenada pelas proteínas E1 e E2, que são amplamente expressas, acontece uma multiplicação limitada do genoma viral, nas células jovens do epitélio, durante a fase S do ciclo celular, seguida por uma fase de conservação de genomas virais que acontecem nas células basais e suprabasais do epitélio, para manutenção do número de genoma viral. Estes genomas são então distribuídos às células filhas, da mesma maneira que o DNA celular, à medida que acontecem as divisões celulares. Para o amadurecimento celular, fatores implicados neste processo são liberados (SILVA FILHO *et al.*, 2000; CASON *et al.*, 2005; MEDEIROS *et al.*, 2005; CAVALCANTI *et al.*, 2006).

Estes fatores ativam também os genes tardios do vírus, que levarão à expressão dos genes L1 e L2 nas camadas celulares maduras e mais externas do epitélio, responsáveis pela formação do capsídeo que envolve o genoma viral, havendo então a produção de novos vírions completos (SILVA FILHO *et al.*, 2000; CASON *et al.*, 2005; MEDEIROS *et al.*, 2005; CAVALCANTI *et al.*, 2006).

A liberação de partículas virais completas acontece em conjunto com o processo de descamação do epitélio, e tornam a mucosa muito infectante. Nestas etapas do desenvolvimento viral, quase não há a expressão dos genes virais E6 e E7, responsáveis pela progressão tumoral celular e o DNA viral permanece na forma

episssomal, não integrado (SILVA FILHO *et al.*, 2000; CASON *et al.*, 2005; MEDEIROS *et al.*, 2005; CAVALCANTI *et al.*, 2006).

As proteínas E6 e E7 do HPV somente são expressas em grande quantidade quando não há mais a ação de E1 e E2, ou seja, quando não acontece mais a multiplicação viral, e para tal expressão, é necessário que ocorra a integração do genoma viral ao DNA celular, fenômeno associado aos HPV de alto risco (SILVA FILHO *et al.*, 2000; CASON *et al.*, 2005; MEDEIROS *et al.*, 2005; CAVALCANTI *et al.*, 2006).

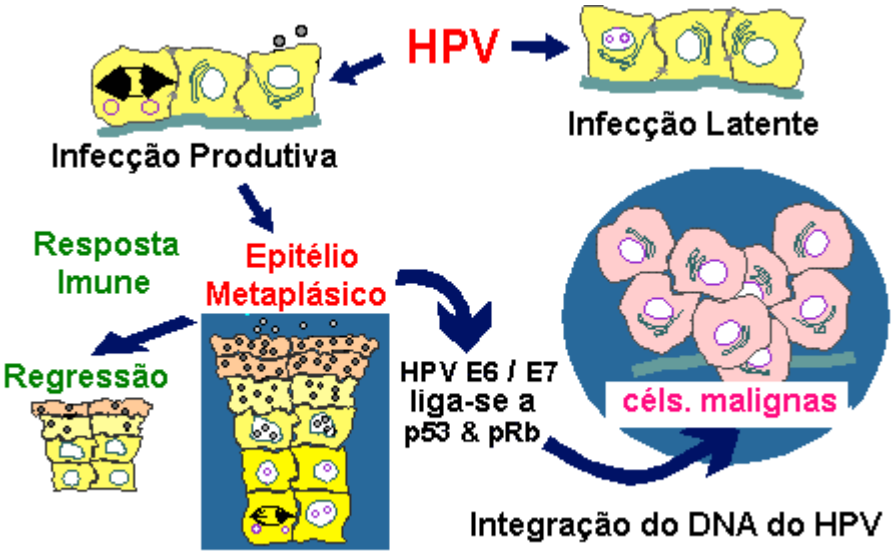
Os HPV **(figura 2)** são classificados em baixo e alto risco em função do papel transformante da região precoce do seu genoma (Região E) (MUNGER *et al*,2004). Com a expressão destas proteínas liberadas, acontece a perda da regulação do ciclo celular. Em certo número de vírus a presença da proteína E6 na região precoce facilita a degradação da proteína p53, supressora tumoral, enquanto a proteína E7 liga-se ao produto gênico do retinoblastoma (pRb) e proteínas responsáveis pela regulação do ciclo celular. Este processo de transformação celular leva ao desbloqueio do ciclo celular e instabilidade genética que impedem a apoptose, causando a imortalidade celular (BIBBO *et al.*, 1998; BRENNA *et al.*, 2003; MUNGER *et al.*,2004; CAVALCANTI *et al.*, 2006; COELHO, *et al.*, 2008; MONSONEGO *et al.*, 2010; MARTINS *et al.*, 2012).

A resposta imunológica pode ter uma ação efetiva e promover o total *clearence* viral, que é o que acontece na maioria dos casos de infecção por HPV, porém, em alguns casos, esta infecção torna-se latente, assintomática, somente identificada por exames biomoleculares. Esta infecção latente, tanto pode tornar-se novamente ativa devido a uma imunodepressão como evoluir para uma progressão tumoral do epitélio, observada em casos de infecção por HPV de alto risco (DOUVIER, *et al.*, 2004; GARCIA-VALLVÉ *et al.*, 2005; WOODMAN *et al.*, 2007; COELHO *et al.*, 2008; MONSONEGO *et al.*, 2010). Os indivíduos acometidos pela infecção assintomática ou latente podem ter a cura dentro de um ano, 70% dos casos; ou dentro de um período de dois anos, 90% dos casos (CAVALCANTI *et al.*, 2006).

O mesmo indivíduo pode desenvolver infecções por vários tipos de HPV ao longo do tempo, principalmente pacientes jovens sexualmente ativos e imunodeprimidos (CAMPOS *et al.*, 2005; JIN-KYOUNG *et al.*,2008). Vale salientar que a infecção por um tipo de HPV não isenta uma co-infecção por outro tipo e que, há evidências de que múltiplos tipos infectando o epitélio agem de forma sinérgica e

conduzem a um pior prognóstico, mesmo quando um dos HPV envolvidos é de baixo risco (ROSSEAU *et al.*, 2003a; ROSSEAU *et al.*, 2003b; TROTTIER *et al.*, 2006).

Figura 2 - Representação esquemática da infecção por HPV



http://web.stanford.edu/group/virus/papilloma/2004goglincarnevale/Papilloma/Cancer_files/image001.gif [novembro de 2014].

A taxa de incidência de infecção por mês nesses pacientes é de 1,3% com positividade acumulada de 38% após 12 meses (FRANCO *et al.*,1999). A infecção é auto-limitada com percentual de até 85,3% de regressão nos casos de infecção pelos tipos oncogênicos (JIN-KYOUNG *et al.*,2008). Das mulheres infectadas 3% a 10% desenvolvem infecções persistentes ao longo dos anos, constituindo grupo de risco para progressão neoplásica (SCHIFFMAN *et al.*, 1993; SCHLECH *et al.*, 2001).

O HPV de alto risco após infectar a célula integra-se ao genoma do hospedeiro e encontram-se associados a 98% dos carcinomas do colo e 90% das lesões pré-invasivas também conhecidas como lesões intraepiteliais de baixo (LIEBG) e alto grau (LIEAG) (ICTV, 2013). Enquanto que o HPV de baixo risco infecta as células localizando-se no núcleo na forma episssomal sendo fortemente associados aos condilomas anogenitais com baixo potencial transformante.

Apesar da infecção por HPV ser necessária para o desenvolvimento de câncer cervical, isolada ela não é suficiente para tal evento. Além da indução da carcinogênese

pelas proteínas virais os papéis genético, hormonal e imunológico do hospedeiro também são importantes (KADISH *et al.,* 2002).

Estudos de biologia molecular permitem identificar novos tipos de HPV quando a diferença de seqüência de nucleotídeo em E6 e L1 é de 5%, nas partículas virais em que a diferença de seqüência é apenas de 2% classificamos este DNA HPV de variante de um mesmo tipo de HPV. Em Brasília, a variante do HPV 16 de maior prevalência é a européia (CRUZ *et al.*,2004; ÂNGULO *et al.*,2007).

Conhecer as características biomoleculares dos vírus tem implicações diretas nas estratégias de prevenção inclusive no desenvolvimento de vacinas (BOSCH *et al.*,1995). Os principais subtipos de HPV do grupo de baixo risco são 6, 11, 42, 43 e 44. Os do grupo de alto risco são principalmente os tipos 16, 18, 31,33, 34, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68 e 70 (MARTINS *et al,* 2005; CAVALCANTI *et al.,* 2006).

As manifestações da infecção por HPV dependem da localização das lesões e do tipo do vírus. Em geral, as lesões anogenitais surgem na pele e superfície mucosa com localização multicêntrica (JACYNTO *et al.*,2005). A infecção pelo HPV pode se apresentar na forma clínica (condiloma acuminado ou verrugas), identificada facilmente durante a inspeção, na forma subclínica, identificada pela colposcopia, citologia e histopatologia e na forma latente (presença do vírus em epitélio normal), não detectado pelas técnicas convencionais de diagnóstico, apenas os métodos de biologia molecular identificam o DNA viral (NORONHA *et al.*,2005).

3.3.3 Patogênese

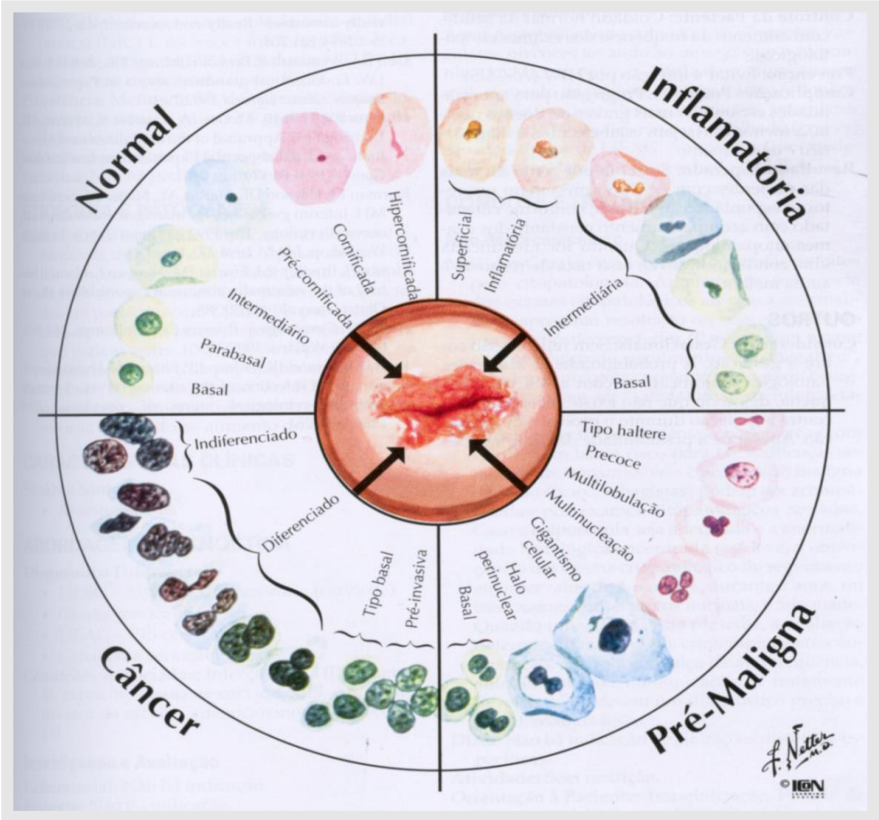
O primeiro relato da presença de alterações celulares cervicais associadas a um agente infeccioso foi em 1933 (FLETCHER *et al.*,1983). Na década de 70 estabeleceu-se forte associação entre a presença de alterações celulares virais às anormalidades epiteliais precursoras do câncer cervical (MEISELS *et al.,* 1977). O desenvolvimento da genética e biologia molecular fortaleceu as evidências epidemiológicas com a utilização de técnicas laboratoriais que permitiram a identificação do DNA do HPV (GISSMANN *et al.,* 1976). Entretanto, a associação do HPV com o câncer cervical só foi reconhecida nos anos 90 (SCHIFFMAN *et al.*,1993).

O período de incubação da infecção costuma ser de três meses podendo chegar até dois anos (FRANCO *et al.*,1999). Dados obtidos a partir de estudos experimentais e epidemiológicos mostraram que em 99,7% dos carcinomas cervicais tem sido detectada

a presença da infecção pelo HPV e destes, 70% representados pelos tipos 16 e 18 (BOSCH *et al.*,1995; COELHO *et al.*,2008; MARTINS *et al.*,2012).

Outros estudos de coorte têm demonstrado que a presença e persistência do DNA-HPV são necessárias para o desenvolvimento de neoplasia na região anogenital (SCHLECHT*et al.*,2008). Existe um consenso de que as neoplasias invasivas do trato genital inferior são em geral precedidas por uma longa fase de doença pré-invasiva (**Figura 3**) ou precursora, período em que várias alterações celulares ocorrem limitadas às camadas do epitélio sem ultrapassar a membrana basal (MUNGER *et al.*,2004) A maioria das infecções genitais pelo HPV regridem dentro de 2 anos, e somente uma minoria das mulheres desenvolvem uma infecção persistente que poderá eventualmente causar neoplasia intraepitelial cervical (NIC). Desta forma, apesar da infecção pelos tipos virais de alto risco oncogênico (HR-HPV) ser necessária, esta não é suficiente para iniciar o processo de malignização de células do colo do útero (YLITALO *et al.,* 2000). Outros fatores podem estar envolvidos neste processo, tais como, a participação de cofatores imunológicos, ambientais e genéticos (CRAVEIRO *et al.*, 2004; SANTOS *et al.*, 2005).

Figura 3 - Representação dos estágios citológicos evolutivos do câncer do colo uterino



FONTE: MAIA, 2008

As lesões precursoras são estratificadas a partir de critérios morfológicos com a finalidade de definir grupos de acordo com seu comportamento biológico, o que permite aos clínicos escolher a metodologia mais adequada para tratamento e seguimento. Dessa forma, as classificações das lesões precursoras sofreram mudanças ao longo dos anos, desde o termo clássico de displasia sugerido pela Organização Mundial de Saúde, passando pelo termo neoplasia intraepitelial da *IARC* task force (RICHART, 1998) e atualmente recebe a denominação de lesão intraepitelial pelo Sistema Bethesda (SOLOMON, 2002).

Os países do continente americano passaram a adotar a classificação do Sistema Bethesda (2001), de lesão intraepitelial de baixo grau (LIEBG) e lesão intraepitelial de alto grau (LIEAG) para descrever as anormalidades citológicas precursoras. Na terminologia histológica, o Sistema Bethesda utiliza as mesmas denominações empregadas para as lesões intraepiteliais usadas na citologia e faz uma combinação com outras classificações.

O termo lesão, empregado no Sistema Bethesda, substituiu o termo neoplasia, por ter sido considerado que a associação de qualquer um desses graus morfológicos da doença não indica, necessariamente, um processo neoplásico. O Sistema Bethesda é aplicado nos Estados Unidos, tanto para a citologia como para a histologia. No entanto, na maioria dos países, a terminologia neoplasia intraepitelial cervical (NIC) permanecem mais comuns para os relatórios histopatológicos (SALVIA *et al.*,2004).

Um estudo de coorte realizado em São Paulo encontrou que a lesão de baixo grau cervical pode evoluir em 11% dos casos para lesão de alto grau ou desaparecer espontaneamente em 57% dos casos (FRANCO *et al.*,1999***).*** Uma lesão intraepitelial de alto grau pode apresentar uma taxa de até 33% de regressão espontânea e até 17% de progressão para câncer invasivo. A LIEAG é o último estágio antes do câncer, sendo considerado por alguns autores como uma fase de transição (RONCO *et al.*,2006).

Estudos epidemiológicos sugerem outros fatores associados á neoplasia cervical: fatores físicos (traumas), químicos (trimetilamina, derivados do fumo, cocaína), infecciosos (herpes genital simples, *Chlamydia trachomatis*), nutricionais (deficiência vitamina A), contraceptivos hormonais e fatores do hospedeiro (estado imune sistêmico e local) exercendo papel de fundamental importância na promoção e persistência das anormalidades (PALEFSKY *et al.*,1999; MINKOFF *et al.,* 2008). Posteriormente, também foram incorporados como cofatores associadas à neoplasia cervical, a religião e a obesidade (MCCREE *et al.,* 2003; BHURGRI *et al.,* 2007).

3.3.4 Diagnóstico

A lesão clínica causada pelo HPV pode ser diagnosticada por meio do exame físico. O diagnóstico da lesão subclínica é realizado por colposcopia, citologia e histopatologia. A associação desses três métodos fornece o diagnóstico, não apenas da infecção viral, mas também o grau de comprometimento do epitélio, fator primordial no manejo clínico (RICHART, 1998). Políticas públicas de prevenção do câncer cervical foram implantadas usando o exame citológico como método de rastreamento para detectar as lesões pré-invasivas e na presença de citologia com alterações compatíveis com lesão intra-epitelial de baixo e/ou alto grau, a paciente é submetida à genitoscopia e biópsia de eventual área suspeita (RAMA *et al.,* 2008).

Os estudos que estabeleceram relação entre lesão intra-epitelial escamosa com HPV e câncer cervical foram realizados utilizando o exame histopatológico como padrão ouro (RONCO *et al.,* 2006). Nesses estudos, comparando os critérios citomorfológicos das lesões induzidas pelo HPV, com pesquisa do DNA do HPV e resultado histopatológico, observou-se que a atipia coilocitótica isolada é o achado citológico mais específico para o diagnóstico da LIEBG. As atipias nucleares discretas e esboço de coilocitose não tiveram valor para conclusão diagnóstica (SALVIA *et al.*, 2004). No entanto, isto não é consenso entre os estudiosos, alguns valorizam o somatório das discretas alterações como dado importante para o diagnóstico, visto que a célula coilocitótica tem elevada especificidade, porém baixa sensibilidade (KANESHIMA *et al.*,2005).

Os métodos utilizados de rastreabilidade no nível de saúde pública é a citologia convencional de Papanicolaou (Pap test) e a citologia em meio líquido (CML), porém os métodos mais sensíveis e específicos de diagnóstico virológico incluem técnicas de biologia molecular como ensaio de captura hibrida para detectar ácidos nucléicos do HPV ou a reação em cadeia de polimerase (SALVIA *et al.,* 2004). Vários estudos utilizando a técnica de reação em cadeia de polimerase (PCR) relatam uma prevalência de 7% a 14% de HPV em mulheres assintomáticas com citologia normal. Quando analisados materiais de neoplasias pré-invasivas e câncer invasivo a presença do vírus pode ser detectada em 80% a 100% dos casos (LAPIN *et al.,* 2000; DERCHAIN *et al.,* 2005). Outros estudos apontam que o método de reação em cadeia da polimerase é mais sensível que a captura hibrida, apresentando elevada taxa de detecção em lesões de alto

grau e carcinoma e, também, oferece a vantagem da genotipagem (SALVIA *et al.*,2004; CHACON *et al.*, 2007***)***.

3.4 *Chlamydia trachomatis*

A infecção por *Chlamydia trachomatis* tem sido reconhecida como um dos maiores problemas de saúde pública (WHO, 2005; OMS, 2013; Ministério da Saúde, 2014). Dados da Organização Mundial de Saúde (OMS) mostram que as doenças sexualmente transmissíveis (DST) são a segunda enfermidade que mais acomete as mulheres entre 15 e 44 anos nos países em desenvolvimento. São 499 milhões de casos novos de DST no mundo (OMS, 2013). Hoje, a infecção pela *C. trachomatis* é considerada como uma das DST mais freqüentes em todo o mundo (WHO, 2005; CDC, 2005; Ministério da Saúde, 2013; OMS, 2014). Ocorrem cerca de 50 milhões de casos novos por ano no mundo (OMS, 2013), e no Brasil não existem estudos documentando a situação global da infecção pela *C. trachomatis*. Estão disponíveis apenas estudos isolados em populações específicas, mas que mostram a importância dessa infecção silenciosa em nosso meio (CODES *et al.*, 2002; Ministério da Saúde, 2013).

Na maioria das mulheres (70% a 75%) e em mais de 50% dos homens essas infecções cursam de forma assintomática (FRIAS *et al.*, 2001; WHO, 2005; OMS, 2013). Assim, esse microorganismo pode ser considerado bem adaptado ao ser humano, uma vez que consegue multiplicar-se sem causar respostas exacerbadas do organismo, daí as dificuldades para o seu diagnóstico (FRIAS *et al.*, 2001; WHO, 2013; Ministério da Saúde, 2014). Esta peculiaridade retarda o tratamento, permitindo que os casos de infecção genital se propaguem ao trato genital superior, causando endometrites e salpingites (MELLES *et al.*, 2000; FRIAS *et al.*, 2001; OMS, 2013).

Nos serviços públicos brasileiros, são raros os locais oferecendo sistematicamente a pesquisa da *C. trachomatis*. Nos serviços privados, normalmente só se pesquisa essa infecção em casos sintomáticos ou quando um dos parceiros sexuais está acometido. Mesmo nessas situações, a pesquisa da *C. trachomatis* ainda não faz parte da rotina da maioria dos ginecologistas, urologistas ou médicos que atendem DST, apesar da sua importância e sua possível relação com o câncer de colo uterino (CODES *et al.*,2002; CODES *et al.*,2006; Ministério da Saúde, 2013).

Diversos trabalhos comprovam a correlação entre agentes infecciosos e o aumento de risco de desenvolvimento do câncer cervical (KOSKELA *et al.*, 2000;

BERKER *et al.,* 2011). Alguns estudos, bem como estudos de caso-controle, têm sugerido que agentes como a *C. trachomatis* (KOSKELA *et al.*,2000; BARROS *et al.,* 2007; OLIVEIRA *et al.,* 2008; SERACENI *et al.,* 2014; SILVA *et al.,* 2014; TAVARES *et al.,* 2014); *Trichomonas vaginalis* (GRAM *et al.,* 1992); *Gardnerella vaginalis* (KLOMP *et al.*,2008) e o vírus da imunodeficiência humana (HIV) (McKENZIE *et al.,* 2010), também podem estar envolvidos como cofatores da carcinogênese cervical.

Neste contexto e em conformidade com outros trabalhos, a *C. trachomatis* esteve bem presente nas mulheres com alterações intra-epiteliais cervicais (83,90%), associado à presença do HPV em 82,8%, detectado através do método da PCR, o que se torna bem evidente esta inter-relação (BARROS *et al.,* 2007; OLIVEIRA *et al.,* 2008; BHATLA *et al.,* 2011; SERACENI *et al.,* 2014; TAVARES *et al.,* 2014).

O agente etiológico nas neoplasias do trato genital inferior da mulher, segundo evidências clínicas, biomoleculares e epidemiológicas, é o HPV que irá agir no alvo suscetível: as células metaplásicas imaturas ou células basais do epitélio pavimentoso (PAAVONEM *et al.,* 2001; FINAN *et al.,* 2002; SILVA *et al.,* 2014). Entretanto, são necessários cofatores de promoção complementares favorecedores da instalação do agente HPV (PAAVONEM *et al.,* 2001; SMITH *et al.,* 2002; SERACENI *et al.,* 2014), como o antecedente de DST (SMITH *et al.,* 2002)**,** incluindo-se a *C. trachomatis* (Ministério da Saúde, 2013).

Sabe-se que a *C. trachomatis* necessita do crescimento intracelular obrigatório utilizando-se do aparato enzimático da célula do hospedeiro para a produção de ATP, e a sua replicação invariavelmente determina a morte desta célula, sendo sempre considerada patogênica (FRIAS *et al.,* 2001; Ministério da Saúde/DST, 2013). Atualmente, o mecanismo pelo qual esse patógeno induz a inflamação e dano tecidual é apenas parcialmente conhecido (DI FELICE *et al.*,2005; SILVA *et al.,* 2014).

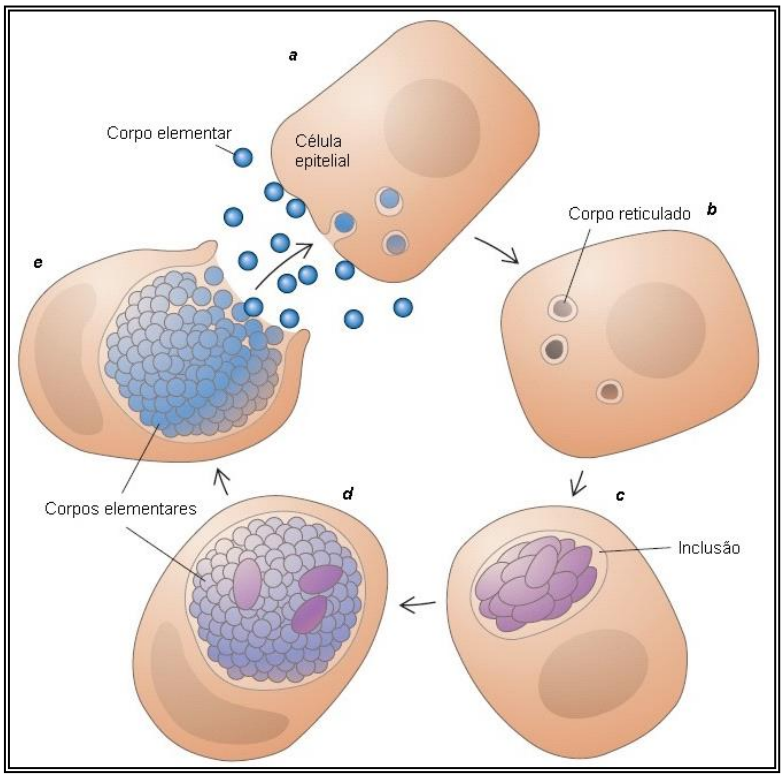
Há forte evidência epidemiológica que sugere que o HPV e a *C. trachomatis*, em particular os sorotipos de D a K, desempenham um papel central na etiologia da neoplasia intra-epitelial cervical e subsequentemente no carcinoma cervical (TAMIM *et al.,* 2002).

A infecção por *C. trachomatis* **(Figura 4)** parece aumentar a suscetibilidade à infecção por HPV em um nível basal por facilitar o acesso às células epiteliais basais por micro-abrasões ou por alterar as características das células epiteliais, aumentando a carga viral da infecção e facilitando a persistência. O processo infeccioso depende

basicamente da capacidade de defesa do organismo e da agressividade do microorganismo. O embate entre estes dois fatores pode determinar o bloqueio do processo na sua fase inicial ou o desencadeamento do processo infeccioso de modo ameno ou com graus variáveis da gravidade clínica. (PAAVONEM *et al.,* 2001; Ministério da Saúde /DST, 2013; SILVA *et al.,* 2014)

Figura 4 - Ciclo de vida da *Chlamydia trachomatis*

(a) As *C. trachomatis* são transmitidas como uma forma extracelular não replicante conhecida como corpo elementar. O corpo elementar adere e é fagocitado por célula epitelial hospedeira; (b) Uma vez dentro da célula epitelial, o corpo elementar se transforma em um organismo intracelular replicante – o corpo reticulado; (c) Corpos reticulados se dividem por fissão binária junto aos vacúolos ligados à membrana, que são denominados inclusões; (d) Decorridas aproximadamente 35-40 horas, a inclusão se rompe e os corpos elementares são liberados para infectar células epiteliais adjacentes ou serem transmitidos a outros hospedeiros.



FONTE: Site: http://www.medicinanet.com.br/m/conteudos/acp-medicine/4883/doencas_causadas_por_clamidia_%E2%80%93_walter_e_stamm.htm [06-10-2014]

Alternativamente, a infecção concorrente por *C. trachomatis* pode impedir a resolução da infecção por HPV através de uma indução de um padrão da resposta imune do tipo humoral. Além disto, a infecção por *C. trachomatis* tem sido associada à hiperplasia de células de reserva e metaplasia, processos relacionados a carcinogênese cervical. A hipótese de que a infecção com *C. trachomatis* interfere no curso da infecção por HPV precocemente é sustentada pelo fato de que DNA de *C. trachomatis* é detectado em esfregaço cervical realizado anos antes do diagnóstico de câncer (SAMOFF *et al.,* 2005; BARROS *et al.,* 2007; SERACENI *et al.,* 2014; TAVARES *et al.,*2014).

A modulação da resposta imune humoral pela *C. trachomatis*, certamente facilita ainda mais os mecanismos de evasão desenvolvidos pelo próprio HPV como, a menor expressão de genes virais para reconhecimento imunológico, dificultando assim o reconhecimento pelas células imunocompetentes, diminuição da expressão de moléculas de histocompatibilidade (MHC) de classe I e II, superexpressão da proteína oncogênica viral E7 que inibe a apresentação de antígenos pelas células dendríticas, menor suscetibilidade das células infectadas à lise mediada por linfócitos T citotóxicos, além da evasão dos efeitos de inibição do crescimento provocado pelas citocinas (SMITT *et al.,* 2002; TAMIM *et al.,* 2002; SILVA *et al.,* 2014).

Por outro lado, acredita-se ainda que uma modulação da resposta imune e/ou precipitação de uma resposta inflamatória favoreça a uma subseqüente infecção por *C. trachomatis*, aumentando a taxa de infectividade por *C. trachomatis* em mulheres HPV positivas (TAMIM *et al.,* 2002; SILVA *et al.,* 2014). Neste mesmo raciocínio, a resposta inflamatória provocada pela *C. trachomatis*, associados a outros agentes infeciosos, como a *G. vaginallis*, *Candida albicans* e *T. vaginalis*, provocam uma desestruturação maior local, como é sugeridos em outros estudos (BARROS *et al.,* 2007; KLOMP *et al.,* 2008; BECKER *et al.,* 2011; MARTINS *et al.,* 2012; SERACENI *et al.,* 2014; SILVA *et al.,* 2014).

Outra explicação plausível seria que a infecção persistente por *C. trachomatis* tem papel facilitador na carcinogênese cervical, decorrente de reações de hipersensibilidade tardia, devido a um provável antígeno relacionado à sensibilização da HSP-60 (proteína de choque térmico, HSP, do inglês *heat shock proteins*), pertencente à classe das HSP. Essas proteínas sintetizadas pelas *C. trachomatis* têm ação anti-apoptótica durante a infecção persistente, facilitando a atuação das oncoproteínas em

células simultaneamente infectadas por HPV de alto risco dos tipos 16, 18 (DI FELICE *et al.*, 2005; SERACENI *et al.*, 2014).

A HSP-60 possui grande semelhança com as HSP humanas, assim as proteínas sintetizadas pelas *C. trachomatis* poderiam sensibilizar os linfócitos a responderem de forma cruzada com as HSP humanas, e a expressão desses antígenos nas células dos tecidos do hospedeiro poderia induzir uma resposta imunológica contra as células expressoras, resultando na destruição dessas células (DI FELICE *et al.*, 2005; SERACENI *et al.*, 2014). Entretanto, a magnitude da associação entre *C. trachomatis* e lesões intraepiteliais cervicais persiste por ser bem esclarecida. Porém, percebe-se que a concentração de anticorpos séricos contra HSP das *C. trachomatis* guarda correlação com a intensidade do dano, e quanto maior o dano causado à célula justificar-se-ia a associação da *C. trachomatis* com as lesões precursoras da cérvice (DI FELICE *et al.*, 2005). Outros fatores de risco também vêm sendo admitidos a esta associação, como tabagismo (MCINTYRE-SELTMAN *et al.*, 2005) e nível de instrução (GOTZ *et al.*, 2005). Por outro lado, a história pregressa de qualquer DST é um indicador importante de variáveis ligadas ao comportamento sexual, incluindo a possibilidade de contágio tanto pela *C. trachomatis* como pelo HPV (OLIVEIRA *et al.*, 2008).

TAMIM *et al.* (2002) em um estudo de prevalência de HPV e *C. trachomatis* em 129 mulheres observaram que a taxa de infecção por *C. trachomatis* é maior em mulheres HPV positivas e estas apresentaram também, taxas maiores de citologia anormal. SAMOFF *et al.* (2005) encontraram uma prevalência do HPV de 78% e da *C. trachomatis* de 65% em adolescentes sexualmente ativas entre 13 e 19 anos.

A prevalência de DNA de *C. trachomatis* foi significativamente maior (47%) em mulheres com lesão intraepitelial de alto grau (HSIL), quando comparada à observada em mulheres com citologia normal (11%) (GOLIJOW *et al.*, 2005).

Desta forma, acredita-se que a evolução maligna em geral requer que o vírus seja de médio/alto risco, a lesão apresente alta carga viral e que haja a presença de alguns cofatores (imunodepressão, tabagismo, outras DST atuando sinergicamente sobre o epitélio metaplásico ou sobre as células de reserva deste epitélio. A coitarca e a primeira gestação em idade cada vez menor, onde é freqüente o achado de ectopia cervical, associado à multiplicidade de parceiros, aumentariam o risco de exposição das células cervicais à ação mutagênica direta e interação mais prolongada com um ou mais tipos de HPV (TAMIM *et al.*, 2002; WEST *et al.*, 2003; SERACENI *et al*, 2014).

3.4 VAGINOSE BACTERIANA (*Gardnerella vaginalis/ Mobiluncus sp)*

Atualmente, estes dois microrganismos são agrupados pela Nomenclatura Brasileira para Laudos Citológicos com a denominação de bacilos supracitoplasmáticos (sugestivos de *Gardnerella vaginalis/ Mobiluncus sp*), por ser difícil a distinção no exame de Papanicolaou com relação a indicação morfológica destes agentes microbianos após coloração e fixação, porém igualmente importantes e que, de modo geral, respondem aos mesmos tratamentos (BRASIL/MS, 2006).

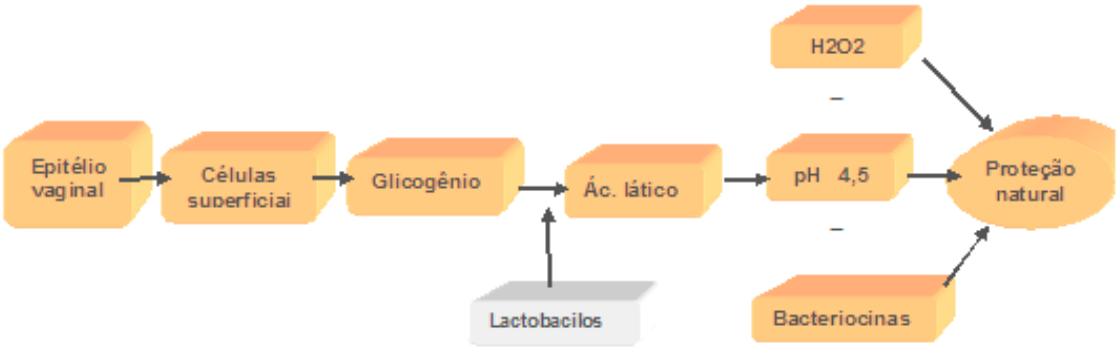
No Brasil, junto com a Trichomoníase e a Candidíase, correspondem a 24,30% dos casos de infecções vaginais, sendo a vaginose bacteriana presente em 14,37 - 16,00%, a Trichomoníase em 4,20 - 4,61% e a Candidíase em 3,69 - 3,05%. (SILVA FILHO *et al.*, 2000)

A vaginose bacteriana é uma alteração na flora vaginal normal, muito comum nas mulheres do período reprodutivo, e tem como característica a diminuição ou ausência de *Lactobacillus* da microbiota vaginal e predominância de outras bactérias de potencial patogênico (KLOMP *et al.*, 2008; BECKER *et al.*, 2011).

É considerada por muitos autores a causa mais comum de infecções vaginais e endocervicais, na maioria dos casos sem infiltrado leucocitário importante e não raramente encontrada durante a gravidez. Essa mudança contribui para elevação do pH e produção de aminas que levam ao aparecimento de sinais e sintomas da vaginose (MIKAMO *et al.*, 1999; SILVA FILHO *et al.*, 2000; SOLOMON *et al.*, 2005; KOSS *et al.*, 2006; KARANI *et al.*, 2007).

O estrógeno, em mulheres em fase reprodutiva, faz com que o epitélio vaginal se torne maduro e se diferencie em células superficiais ricas em glicogênio. Os lactobacilos de Doderlein promovem a metabolização deste glicogênio em ácido láctico, conferindo um pH menor que 4,5 à vagina. Este pH ácido, juntamente com o Peróxido de Hidrogênio (H2O2) e bacteriocinas, também produzidos pelos lactobacilos, conferem a proteção natural da vagina, inibindo o crescimento de microrganismos (MURTA EF *et al.*, 2005)**(Figura 5)**. Entre os microrganismos anaeróbios isolados da secreção vaginal de mulheres que possuem a vaginose bacteriana, os de maior frequência são: *Gardnerella vaginalis*, *Mobilluncus sp*, *Peptostreptococcus*, *Prevotella sp* e *Porphyromona sp.* (ALBORGHETTI G. *et al.*, 2007).

Figura 5 - Proteção Natural da Vagina



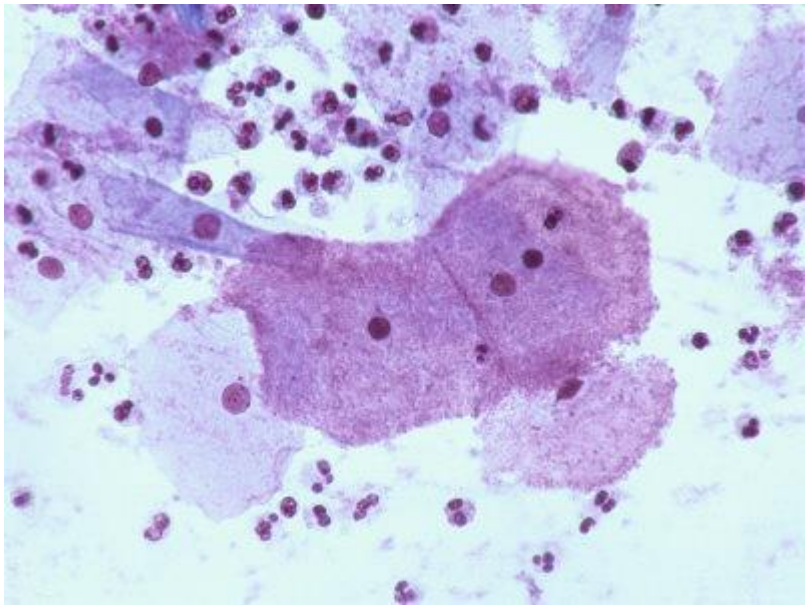
Fonte: GUERRA NETO, 2011

A *G. vaginalis*, antes chamada de *Haemophilus vaginalis*, é uma bactéria em formato de bastão, sendo Gram negativa ou Gram variável e quando corada pela técnica de Papanicolaou se apresenta em azul (**Figura 6)**. Ela pode estar aderida à superfície das células escamosas de forma parcial ou total (*clue cells*), ou seja, células do epitélio vaginal cobertas com bactérias gram variáveis, de modo que os bordos das células epiteliais perdem a definição, com ausência ou com raros leucócitos (CARVALHO MG, 2005). Possui como principal sinal clínico a secreção vaginal abundante, de coloração acinzentada grumoso ou bolhoso, em quantidade variável, com forte odor de aminas, especialmente no Ph acima de 4,5. (SILVA FILHO *et al*, 2000; KOSS *et al*, 2006; KARANI *et al.*, 2007). O teste das aminas, com hidróxido de potássio a 10%, geralmente é positivo, devido à volatilização de aminas do conteúdo vaginal, resultando num forte odor de peixe ou amônia. O exame a fresco do conteúdo vaginal revela a presença das "*clue cells*" e a bacterioscopia pela técnica de Gram evidencia diminuição acentuada de lactobacilos e polimorfonucleares, com numerosos cocobacilos ou bacilos gram-negativos, como a *G. vaginalis* (KONEMAN *et al.*, 2001; KOSS *et al.*, 2006; KARANI *et al.*, 2007).

O motivo exato para a diminuição destes lactobacilos ainda não é conhecido, mas existem alguns fatores como: gravidez, menopausa, alteração do pH vaginal (que acontece na ejaculação ou no uso de duchas), cirurgias, elevado número de parceiros sexuais, utilização de dispositivo intrauterino (DIU), uso de espermicidas, antibióticos de largo espectro, maus hábitos de higiene, diminuição de uma resposta imune vaginal, entre outros, que podem alterar o ecossistema vaginal levando à infecções pelos agentes

que habitualmente fazem parte da flora normal (KOSS *et al*, 2006; GOMPEAL *et al*, 1997).

Figura 6 - Gardnerella vaginalis (clue-cells) – Citologia de Papanicolaou (100X).



FONTE: Site: http://yeztli.com/v-infecciones-vaginales/gardnerella-vaginalis-clue-cells-pap-smear-x10/ [02-11-2014]

A *G. vaginalis* produz ácidos orgânicos, principalmente o ácido acético, utilizados pelas bactérias anaeróbias para sua proliferação. Tais bactérias multiplicadas produzem maior quantidade de aminopeptidases que formarão aminas aromáticas, sendo as principais a putrecina, cadaverina e trimetilamina. Estas aminas, em presença de pH elevado, rapidamente se volatilizam ocasionando o cheiro característico nas portadoras da doença, de "peixe podre". O odor se torna mais acentuado em contato com o sêmen e ao final da menstruação, pois ambos causam alcalinização da vagina, liberando as aminas voláteis. (GIRALDO *et al.*, 2007). Tanto os ácidos como as aminas são citotóxicos, causando esfoliação das células epiteliais e, por conseguinte, o corrimento vaginal contendo as características das células indicadoras ou "*clue cells*". (MIKAMO *et al.*, 1999; SILVA FILHO *et al.*, 2000; SOLOMON *et al.*, 2005; KOSS *et al.*, 2006; KARANI *et al.*, 2007).

A literatura sugere a hipótese de que o ambiente cérvico-vaginal desempenha um determinante papel na suscetibilidade à infecção pelo HPV, pois mulheres com infecção por *Gardnerella vaginalis* apresentam a flora lactobacilar normal alterada. A hipótese no estudo está baseada na consideração de que a infecção por HPV está associada a um

aumento do risco de desenvolver anormalidades escamosas, sendo favorecida pelo ambiente pobre em lactobacilos (KLOMP *et al.,* 2008; BECKER *et al.,* 2011) .

A inflamação do epitélio do colo do útero também tem sido reconhecida como um dos cofatores predisponentes à carcinogênese cervical, pois a perturbação da flora vaginal é conhecida por aumentar o risco de aquisição da infecção por HPV, com uma associação significativamente alta com as lesões pré-neoplásicas (OR, 10,3; 95%, IC :6,6-16,1) (KLOMP *et al.*,2008).

3.5 CITOLOGIA ONCÓTICA CONVENCIONAL (PAPANICOLAOU)

A citologia oncótica convencional é uma técnica bem conhecida e utilizada há mais de cinquenta anos, sendo empregada com sucesso na prevenção e rastreio do câncer do colo do útero. George Nicholas Papanicolaou (1883-1962) introduziu a técnica e até hoje é reconhecido pela solidez dos conceitos e utilidade na prática médica (COELHO *et al.,* 2008; DIAS *et al.*, 2008).

Antes da publicação da técnica, em 1941, Papanicolaou havia abandonado seu experimento, por não ter demonstrado relevância significativa. Então, após uma revisão com o ginecologista Herbert F. Traut, publicaram o trabalho no American Journal of Obstetrics and Gynecology, intitulado "The diagnostic value of vaginal smears in carcinoma of the uterus", que culminou na publicação da monografia dos dois autores em 1943 e consolidou os fundamentos da citopatologia ginecológica. Desde então, este exame tem sido utilizado como ferramenta de prevenção e rastreio do câncer de colo uterino, detectando precocemente lesões pré-cancerosas do colo uterino e diminuindo significantemente as taxas de incidência e mortalidade desta neoplasia, principalmente nos países desenvolvidos (MICHALAS *et al.,* 2000; BERNSTEIN *et al.,* 2001; COELHO *et al.,* 2008).

Este teste é hoje utilizado em toda a rede de saúde pública brasileira. Apesar da reconhecida efetividade do método, este ainda apresenta altas taxas de resultados falso-negativos. A grande oscilação nas taxas de sensibilidade, que podem variar de 2% a 90% demonstra a vulnerabilidade do procedimento, susceptível particularmente a falhas nas técnicas de colheita das amostras e preparação dos esfregaços, assim como também à subjetividade na interpretação dos achados citológicos (BERGERON *et al.*,2000; BERNSTEIN *et al.*,2001; FERRAZ *et al.*,2005; TAVARES *et al.*,2007; Ministério da Saúde, 2012).

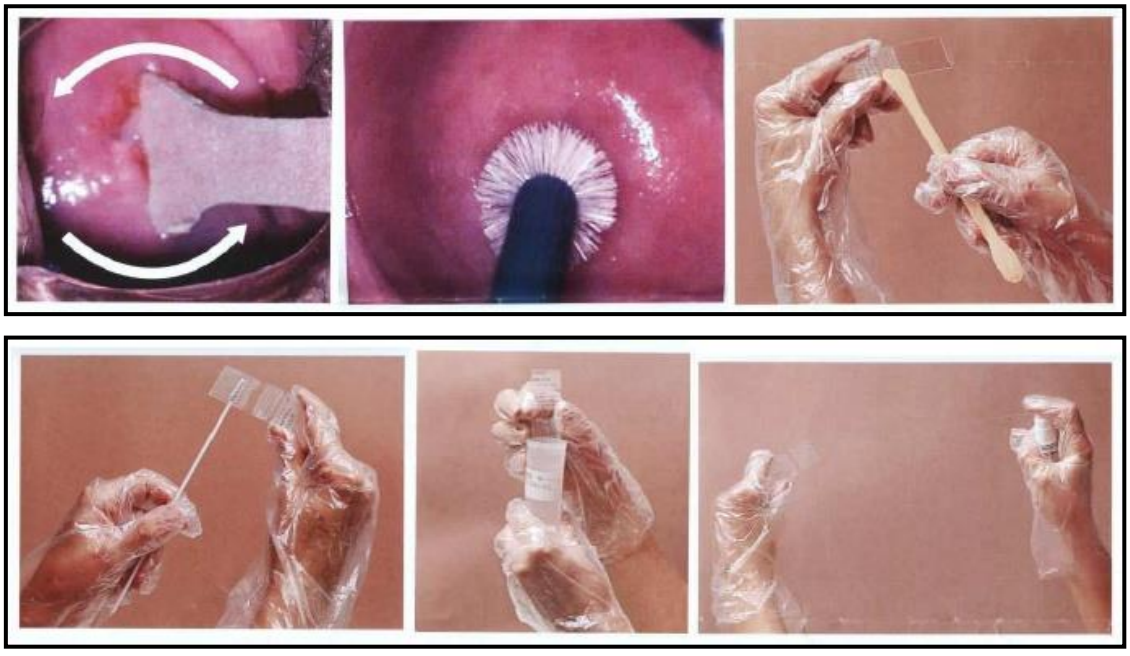
3.6.1 Procedimento técnico

A qualidade desta técnica está diretamente relacionada ao modo como é feita a coleta e o esfregaço. Deve-se fazer o uso combinado de espátula de Ayres e a escova endocervical para obtenção de melhores resultados. O exame se inicia com a introdução do espéculo bivalve na vagina em sentido longitudinal-oblíquo, afastando os pequenos lábios e imprimindo um trajeto direcionado ao mesmo tempo em que se gira o instrumento para o sentido transversal. (Ministério da Saúde, 2012)

Depois de introduzido e aberto, faz-se a coleta com a parte maior da espátula colocada no orifício cervical, girando 360° a fim de coletar as células de toda superfície da zona de transição (junção escamocolunar [JEC] e ectocérvice). A escova endocervical deve ser empregada posteriormente à espátula, especialmente nos casos em que a JEC se localiza internamente no canal endocervical, fazendo-se o giro de 360º, para melhor representação celular. (Ministério da Saúde, 2012)

O material coletado deve então ser espalhado e fixado imediatamente sobre a lâmina, de maneira delicada e uniforme (**Figura 7**), evitando-se a formação de artefatos para reduzir a possibilidade de erros na análise microscópica (BOON *et al.*, 1989; PLEWKA *et al.*, 2007).

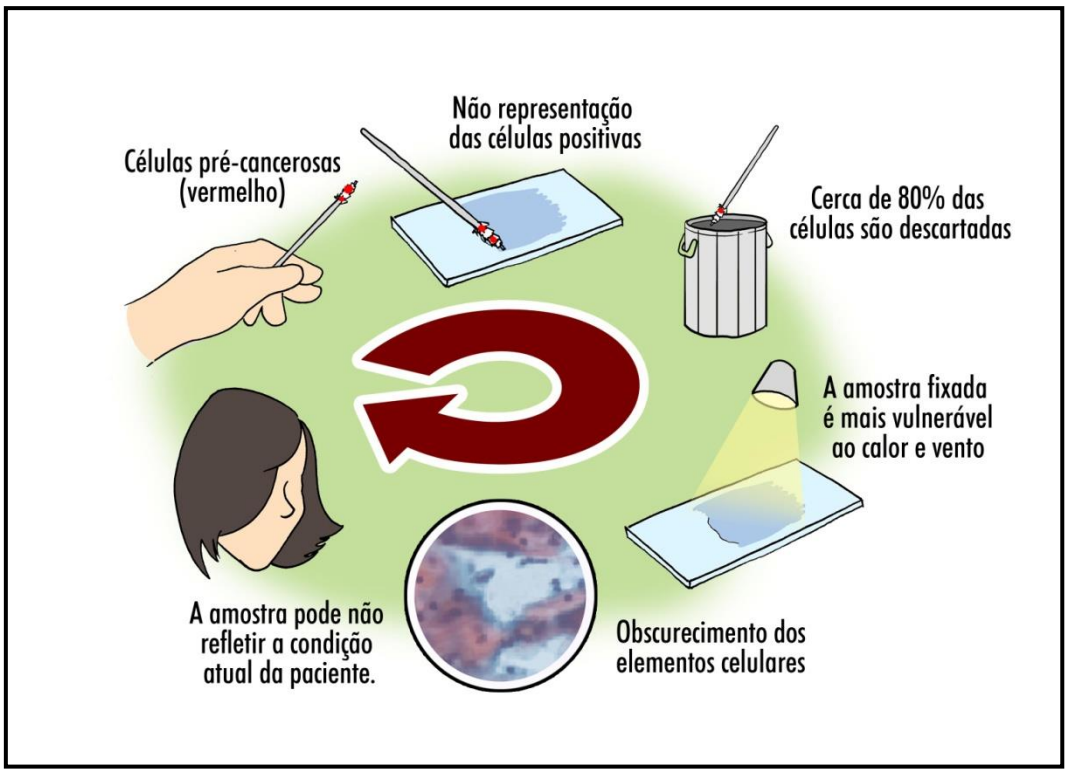
Figura 7 - Coleta, extensão e fixação do material cervical no método convencional



FONTE: RUSSO, 2008

Em uma coleta adequada de material cérvico-vaginal, são obtidas entre 600 mil e 1,2 milhão de células epiteliais. Apenas 20% dessas células vão para a superfície da lâmina, sendo o restante, aderido à espátula e escova, e descartado após a coleta e transporte para a lâmina **(Figura 8**). A retirada do muco e restos celulares da superfície cervical, com um swab de celulose antes da coleta da amostra, aumenta a sensibilidade do exame (HUTCHINSON *et al.,* 1999; OBWEGESER *et al.,* 2001).

Figura 8 - Representação esquemática da coleta da citologia convencional



FONTE: MINISTÉRIO DA SAÚDE- Caderno de Referência 1: Citopatologia Ginecológica- Brasília- DF. 2012

Segundo BEERMAN *et al.* (2009), a citologia convencional possui grande índice de resultados falso-positivos e falso-negativos. SIEBERS *et al.* (2010) consideram um teste de qualidade inferior, devido aos resultados falso-positivos e falso-negativos. Além disso, declaram que isso ocorre devido à má qualidade da amostra (obscurecimento por sangue, inflamação, má fixação celular, e distribuição não homogênea das células), erros na interpretação e detecção. CELIK *et al.*(2008) constataram que a taxa de resultados falso-negativos podem chegar a 50% na citologia convencional, em estudo feito em Hong Kong com dados de quase 191.581exames.

Também relataram que tal método possui sensibilidade que varia entre 30% e 87% e especificidade que pode variar entre 86% e 100%.

O fato de ser uma metodologia menos sensível e mais trabalhosa, em relação a outros métodos, impulsionou a pesquisa para o desenvolvimento uma nova tecnologia de rastreamento que servisse como alternativa ou coadjuvante à detecção precoce do câncer do colo do útero: a citologia em meio líquido (WHITLOCK *et al.,* 2011).

3.7 CITOLOGIA EM MEIO LÍQUIDO

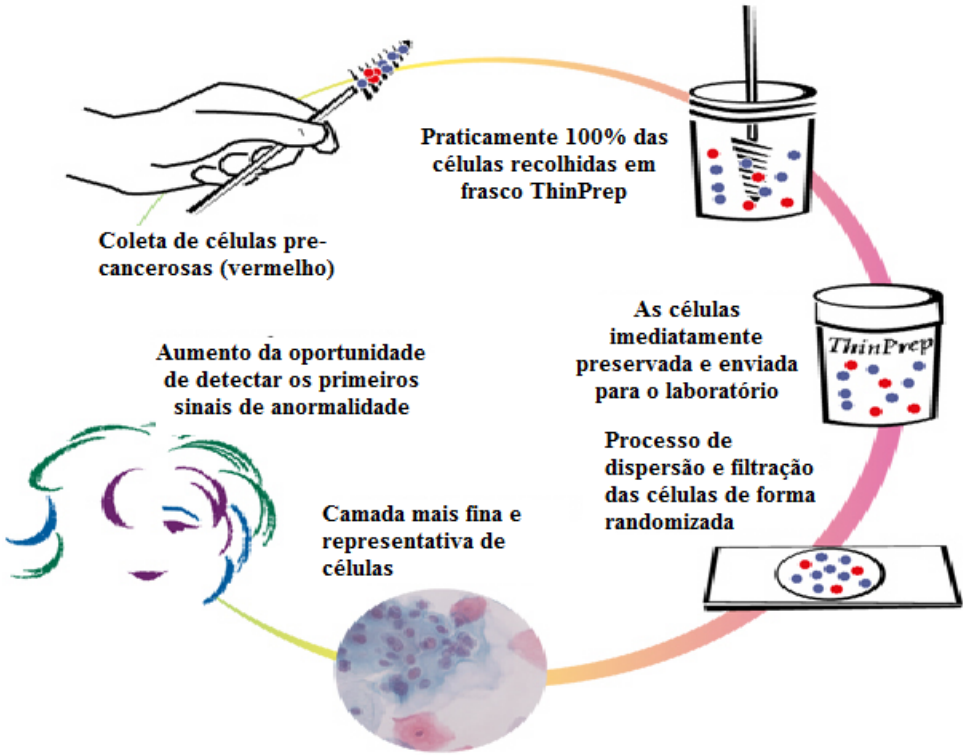
Na década de 90 foi desenvolvida e implantada uma nova metodologia para a realização da citopatologia do colo uterino: a citologia em meio líquido (CML) (PAYNE *et al.,* 2000; ALVES *et al.,* 2003; HOELUND *et al.,* 2003;). O seu surgimento se deu devido ao empenho de viabilizar a "leitura" dos espécimes por computadores que exige o menor número possível de artefatos e sobreposições celulares (PEREIRA *et al.,* 2003),

Assim, surgiu a CML para atender às demandas de escrutínio computadorizado e também com o intuito de melhorar a sensibilidade diagnóstica da citologia. Esse objetivo é alcançado pela maior facilidade na identificação das anormalidades pelo citopatologista, devido à apresentação em monocamada das células e a sua melhor preservação, pois consiste em um método em que as células cervicais são imersas em um líquido conservante antes do processamento da amostra. Tal metodologia retém, através de uma película de filtro, hemácias e células inflamatórias, além de evitar artefatos como excesso de muco, ressecamento provocado pelo ar e sobreposição celular (CAMATA *et al.,* 2010). Outro aspecto importante é a possibilidade de realizar testes de biologia molecular para a detecção do DNA do HPV e outros micro-organismos como a *Chlamydia trachomatis* e a *Neisseria gonorrhoeae*, no mesmo material encaminhado para o estudo citológico (LEE *et al.,*1997; AUSTIN *et al.,*1998; BERSTEIN *et al.,*2001; ABULAFIA *et al.,*2003; BIDUS *et al.,*2006; RONCO *et al.,*2006; TAKEI *et al.,*2006; Ministério da Saúde, 2012).

O procedimento técnico da CML consiste na suspensão e centrifugação de células provenientes do material colhido em líquido fixador, obtendo-se a seguir uma fina camada de células sobre a lâmina (**Figura 9**). Daí o porquê do método ser também conhecido como citologia de monocamada ou camada fina (BERNSTEIN *et al.,* 2001; OBWEGSER *et al.,* 2001; ALVES *et al.,*2003; HOELUND, *et al.,* 2003; MELAMED *et*

al., 2003; PEREIRA *et al.*,2003; McGOOGAN *et al.*, 2004; SCHLEDERMANN *et al.*, 2004; HAYAMA *et al.*, 2005).

Figura 9 - Representação esquemática da coleta da citologia em meio líquido



FONTE: Site : http://www.thinprep.com/ [03-11-2014]

A citologia de meio líquido é utilizada em vários países e está substituindo gradativamente a citologia convencional (CC) nos programas de controle do câncer de colo uterino, como acontece nos E.U.A e na Inglaterra (PAYNE *et al.*, 2000; HOELUND *et al.*,2003).

Novas tecnologias como a CML, de forma automatizada, como o "ThinPrep", permite o estabelecimento de padrões na coleta, preparo e coloração das amostras o que garante uma melhora na qualidade dos testes, pois reduzem as variáveis do processo e da interferência humana (ALVES *et al.*,2003; PEREIRA*et al.*,2003; SCHLEDERMANN *et al.*,2004; HAYAMA *et al.*,2005).

Na citologia em base líquida a área de leitura é reduzida em até 81% e como ocorre à eliminação dos interferentes que normalmente obscurecem a amostra, permite um ganho de cerca de 50% no tempo de "leitura", chegando alguns autores como SASS *et al.* (2004), a apontarem uma melhoria de 73% na produtividade do laboratório. Quando auxiliados por equipamentos que fazem o rastreamento por guia

computadorizado, os técnicos em citopatologia passam a ter uma produtividade ainda superior, podendo avaliar até 170 lâminas por dia de trabalho.

Uma grande meta-análise de 25 estudos prospectivos, incluindo mais de 500 mil mulheres relatam que o ThinPrep aumentou a detecção de LSIL e HSIL, mas as conclusões foram severamente limitadas pela falta de um padrão de referência e de grande heterogeneidade entre as populações de estudo (BERNSTEIN *et al.*, 2001). Outros estudos relatam que o ThinPrep foi significativamente mais sensível que o esfregaço convencional na detecção de HSIL e câncer, com baixos índices de resultados insatisfatórios e com taxas de sensibilidade de 92,9 e 100% vs 77,8% e 90,9%, respectivamente (*p<0,001)* (HUTCHINSON *et al,*1999; BAKER *et al*, 2002). Essa evidência sugere que o ThinPrep é melhor na detecção do câncer cervical (LEE *et al.,*1997; ASHFAQ *et al.,* 1999; BERNSTEIN *et al.,*2001; BAKER *et al.,*2002; ABULAFIA *et al.,* 2003; TAKEI *et al.,* 2006; BIDUS *et al.,*2006; MOOSA *et al.,* 2014; MACHARIA *et al.*, 2014).

As seguintes vantagens são atribuídas a CML (ALVES *et al.,*2003; PEREIRA *et al.,*2003; SCHLEDERMANN *et al.,*2004; HAYAMA *et al.,*2005):

- melhor preservação celular
- melhor distribuição das células analisadas
- redução de muco, exsudato inflamatório e hemácias
- redução do tempo de "leitura"
- obtenção de preparações adicionais da amostra sem a necessidade de nova coleta de material
- utilização possível de resíduos para testes de biologia molecular para vírus como o HPV e outros microorganismos patogênicos entre eles a *Chlamydia trachomatis* e a *Neisseria gonorrhoeae*
- menor percentual de amostras insatisfatórias para a avaliação

Quanto às desvantagens da CML, compreendem:

- maior consumo de tempo no processamento técnico (em alguns métodos não automatizados)
- maior custo
- necessidade de adaptação profissional à nova técnica

Para efeito comparativo, as desvantagens da citologia convencional correspondem a:

- desperdício de aproximadamente 80% do material coletado (permanece aderido à escova, sendo depois descartado). (**figura 8**)
- maior número de células para analisar (aproximadamente 300 mil células)
- distribuição irregular das células com sobreposição
- dependência da habilidade do profissional na confecção dos esfregaços e na sua pronta fixação (falta de padronização)
- maior percentual de amostras insatisfatórias para a avaliação
- repetição mais freqüente na colheita de material devido ao maior percentual de insatisfatórios
- Impossibilidade para teste adicional de biologia molecular;
- baixa produtividade do laboratório.

Em amostras cervico-uterinas e vaginais a CML tem sido referida como método de desempenho superior por proporcionar melhor representação celular, com sensibilidade aumentada na detecção de lesões, em comparação com o preparado convencional (PAYNE *et al.,* 2000; ALVES *et al.*,2003; BERGERON *et al.*,2003; HOELUND *et al.*,2003; KLINKHAMER *et al.*,2003; Ministério da Saúde, 2012; MACHARIA *et al.,* 2014; MOOSA *et al.,* 2014). Há vários autores que mostram uma maior sensibilidade diagnóstica nos casos de "ASC" (alterações em células escamosas de significado indeterminado), enquanto outros atribuem uma melhor efetividade no diagnóstico de lesões de alto grau e glandulares. Contudo, não há um consenso nessa matéria (Ministério da Saúde, 2012) e prova disso é o trabalho recente de JESDAPATARAKUL (2011), que através do estudo comparativo entre os métodos não observou melhoria significativa no diagnóstico pela CML

A CML pode ser realizada através de métodos automatizados e não automatizados. Entre os automatizados, os mais utilizados são o "ThinPrep" (Hologic, Inc., Marlborough, MA) e o "SurePath" (Becton, Dickinson and Company, Franklin Lakes, NJ). Os métodos não automatizados incluem o "Liqui-Prep", "Gel hidroalcoólico", entre outros.

3.7.1 “ThinPrep”

A expressão que em português significa "preparado fino". Aprovado em 1996 pela FDA, foi o primeiro exame Papanicolaou em meio líquido aceito pela instituição e é o mais utilizado pelos laboratórios de citologia hoje em dia, juntamente com o concorrente AutoCyte® . Este método permite a deposição do material coletado em uma única camada, ou seja, camada tão fina quanto à própria espessura de uma célula.

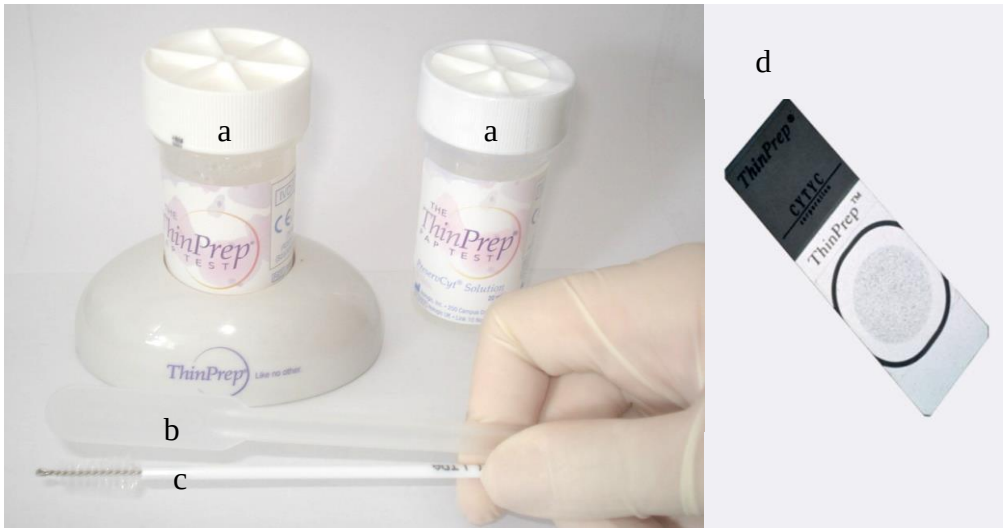
Em comparação com a citologia convencional, existem evidências suficientes que este método reduz a proporção de amostras insatisfatórias. No entanto, ainda restam dúvidas quanto sua eficácia, também em relação ao método convencional, na detecção precoce de câncer do colo do útero (FDA, 1996; GIRIANELLI *et al.*, 2007).

O “Kit” para a coleta do material cérvico-vaginal pelo método “ThinPrep” inclui: frasco com líquido conservante para receber as células coletadas do colo uterino (**Figura 10a**), espátula plástica com a superfície lisa, que impede a adesão das células, devido a tratamento anti-estático (**Figura 10b**); escova endocervical com ponta protegida para evitar sangramento no momento da colheita celular (**Figura 10c**); lâmina de vidro especialmente transparente com área determinada para depósito das células, garantindo uniformidade e regularidade, além do processador computadorizado que prepara o material coletado, de modo a garantir que a amostra que vai para a lâmina, represente de fato o real estado celular do colo uterino de cada paciente (**Figura 10d)**.

A coleta do material cérvico-vaginal para o “ThinPrep” é semelhante à coleta pelo método convencional, alterando apenas o instrumento utilizado e a quantidade de giros feitos para a obtenção da amostra. Ao invés da espátula de Ayres e a escova cervical, é utilizada uma escova especial, fabricada especialmente para o método**.** Neste caso, a escova é introduzida quase que completamente no canal endocervical, ficando apenas as cerdas mais proximais em contado com o orifício externo e superfície do colo. Procede-se girando no mesmo sentido cerca de cinco vezes para posteriormente fazer o destaque de sua extremidade e depósito no frasco com o meio líquido específico para análise **(Figura 11**).

Figura 10 - Kit de coleta da citologia em meio líquido “ThinPrep”

(a) frasco com líquido conservante (Thin Prep); (b) espátula plástica; (c) escova endocervical com ponta protegida; (d) lâmina de vidro.



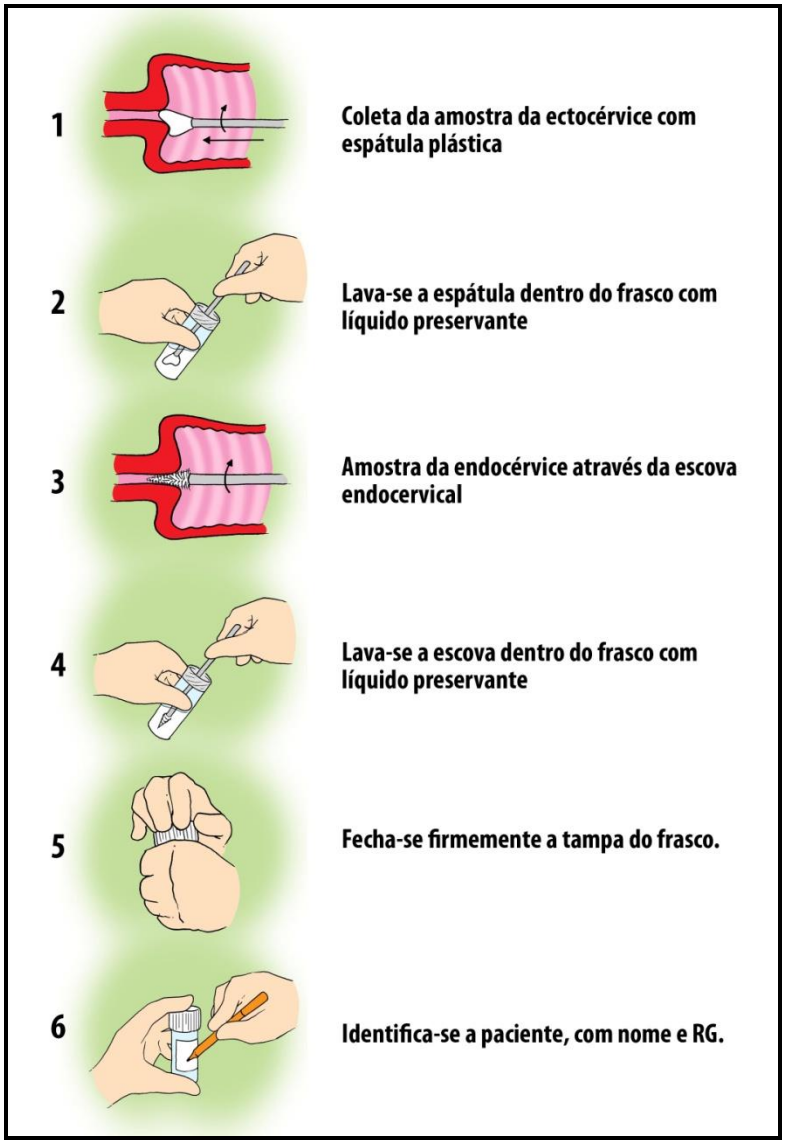
FONTE: MINISTÉRIO DA SAÚDE- Caderno de Referência 1: Citopatologia Ginecológica- Brasília- DF. 2012

Figura 11 - Escova e frasco fabricados pela ThinPrep ®

FONTE: KELUARGA. Disponível em: http://cervarix.blogspot.com.br/2010/05/thin-prep-pap-smear.html>. Acesso em novembro,2014.

A coleta e a fixação das amostras cérvico-vaginal é efetuada através das etapas apresentadas na F**igura 12**.

Figura 12 - Representação das etapas da colheita da amostra da citologia em meio líquido ThinPrep

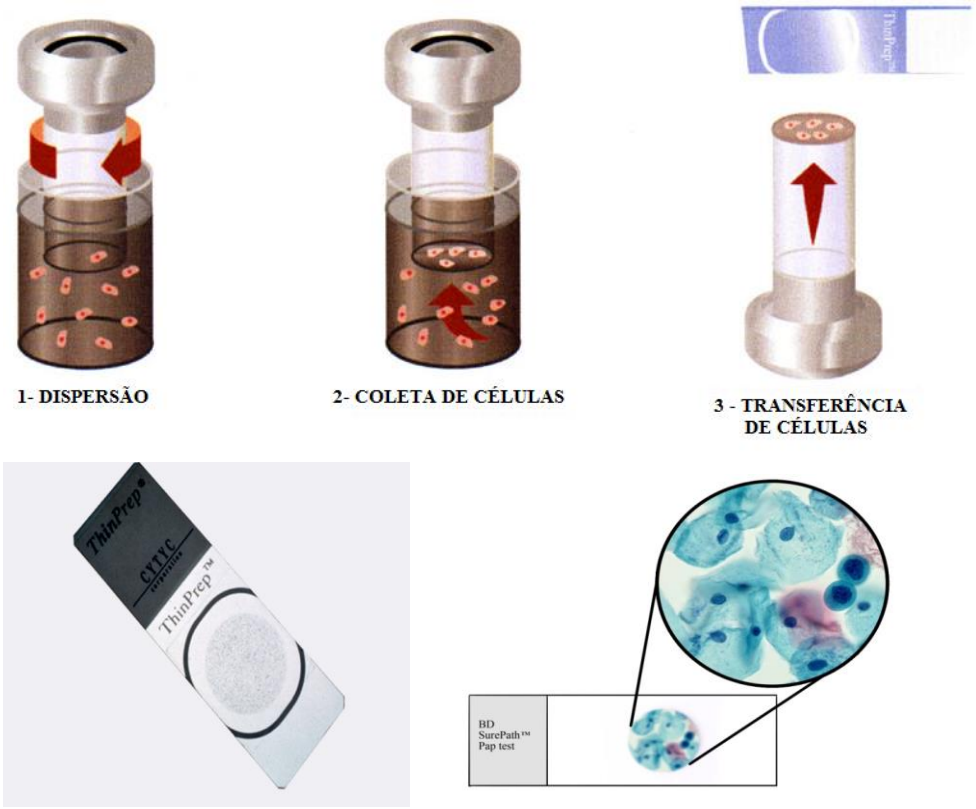


FONTE: MINISTÉRIO DA SAÚDE- Caderno de Referência 1: Citopatologia Ginecológica- Brasília- DF. 2012.

Para a confecção da lâmina para o exame, o ThinPrep ® System se utiliza um sistema de centrifugação com filtros e vácuo, preparando a lâmina por impressão do material coletado (**Figura 13 e 14**). Depois de pronta, a lâmina pode ser processada pelo ThinPrep ® Imaging System . Trata-se de um sistema computadorizado de leitura

de lâminas, o qual identifica 22 campos de interesse com a maior probabilidade de conter células anormais, posteriormente examinados pelo citologista (FDA, 2003; PLEWKA *et al.*, 2007; DAVEY*et al.*,2007).

Figura 13 - Representação automatizada do processamento da citologia em meio líquido Thin Prep

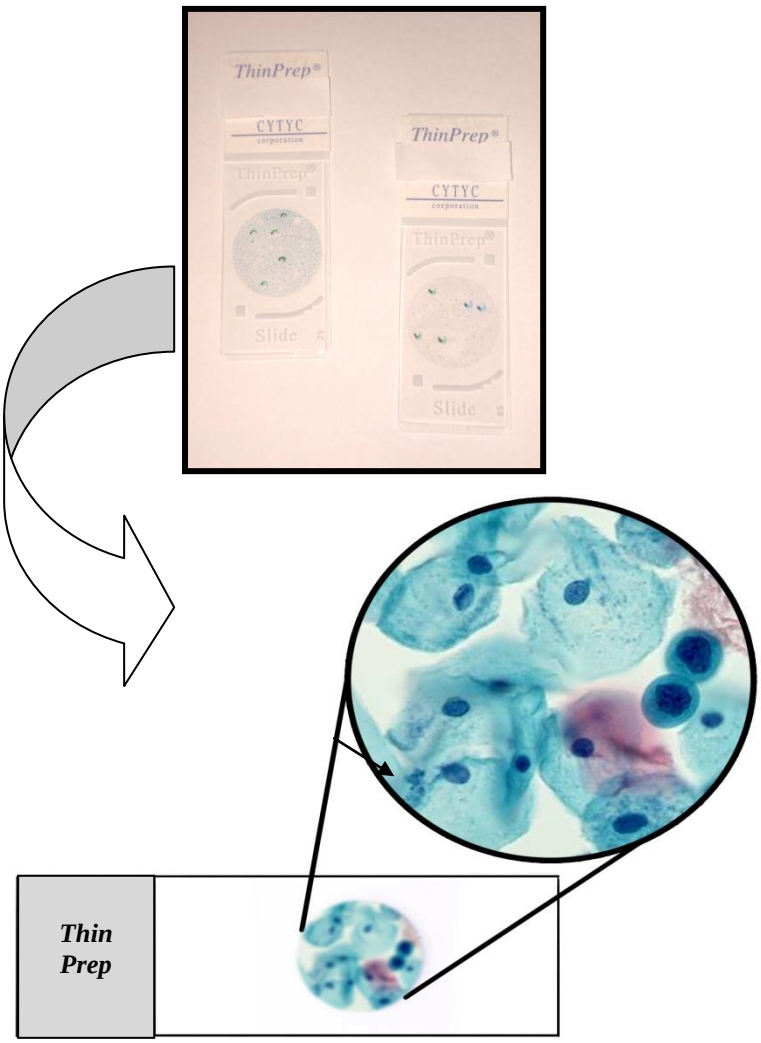


FONTE: MINISTÉRIO DA SAÚDE- Caderno de Referência 1: Citopatologia Ginecológica- Brasília- DF. 2012.

3.7.2 A execução do exame

No sistema ThinPrep as células coletadas do colo uterino não são esfregadas em uma lâmina, e sim depositadas em um frasco contendo meio líquido preservante a base de metanol **(Figuras 10 e 11).** Após a coleta da amostra, o frasco é então encaminhado para o laboratório, onde a amostra é processada eletronicamente (processador eletrônico de lâminas T2000 ou T5000), passando pelas etapas de dispersão, coleta das células e transferência das células para a lâmina **(Figuras 13, 14 e 15).**

Figura 14 - Preparações citológicas obtidas através do método "ThinPrep"



FONTE: MINISTÉRIO DA SAÚDE- Caderno de Referência 1: Citopatologia Ginecológica- Brasília- DF. 2012.

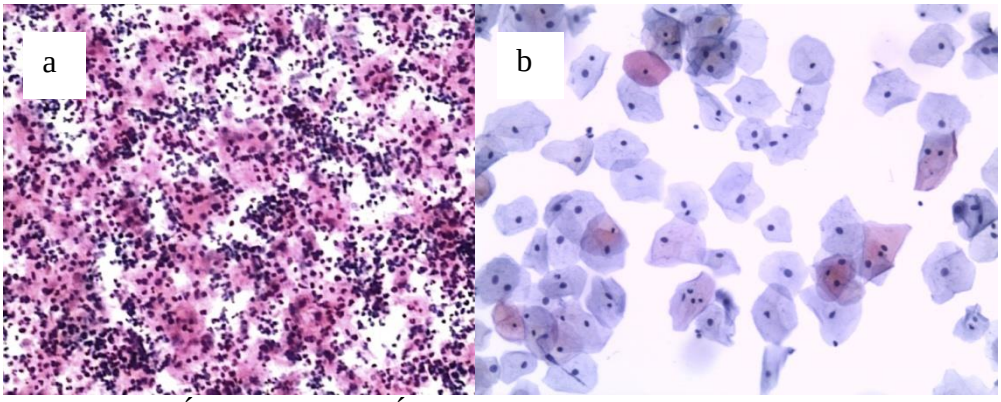
Figura15 - Processador eletrônico de lâminas T2000.

FONTE: Site : http://www.thinprep.com/ [03-11-2014]

Depois de pronta, a lâmina é processada pelo ThinPrep ® Imaging System que tem por finalidade identificar campos de interesse com a maior probabilidade de conter células anormais, facilitando a leitura e melhorando a qualidade de análise das amostras pelo citopatologista (FDA, 2003; DAVEY*et al.*, 2007; PLEWKA, 2007).

Esta etapa, não requer ser obrigatoriamente automatizada, podendo o citopatologista realizar somente com o microscópio óptico. A grande vantagem, é que com esta metodologia, o campo de análise é menor, o material de fundo fica sem os interferentes, o que ocorre um ganho no tempo útil de análise e qualidade diagnóstica (**Figura 16**).

Figura 16 - Representações microscópicas das preparações citológicas
(a) citologia de Papanicolaou convencional; (b) citológica em meio líquido pelo método "ThinPrep".



FONTE: MINISTÉRIO DA SAÚDE- Caderno de Referência 1: Citopatologia Ginecológica- Brasília- DF. 2012.

3.8. EXTRAÇÃO DE DNA GENÔMICO HUMANO E VIRAL

As amostras de secreção vaginal coletadas em tubos da citologia em meio líquido, baseado em metanol, foram submetidas a procedimento de extração do DNA genômico utilizando o kit de extração de DNA "GenomicPrep Blood DNA isolation kit (Ameshan Bioscience)" de acordo com as instruções do fabricante.

3.8.1. Metodologia para diagnóstico da infecção viral

Para detecção do HPV foram realizado uma mistura de reação contendo: 1X Tampão da enzima (Tris-HCl 10 mM, KCl 50 mM); 3,0 mM $MgCl_2$; 200 µM, dNTPs,

0,6 pmol de cada primer; e 2U da Taq Platinum (invitrogen). Foram utilizando os primers consenso MY 09(5'-CGT CCM ARR GGA WAC TGA TC-3') e MY 11 (5'-GCM CAG GGW CAT AAY AAT GG-3') e os primers mais internos GP5+ e GP6+, cujas sequências são dadas a seguir: GP5+ (5'-TTTGTTACTGTGGTAGATACTAC-3') e GP6+ (5'-GAAAAATAAACTGTAAATCATATTC-3'), Como controle da reação foram utilizados primers iniciadores do gene da β-globina PC04 e GH20, que produzem um produto de reação em torno de 268 pb. Este procedimento tem por finalidade garantir o resultado negativo para HPV, pois quando não se obtém a banda correspondente ao produto da amplificado é necessário que se tenha certeza que a reação funcionou adequadamente. Esta confirmação se faz pela presença da banda correspondente ao produto de amplificação do gene da beta-globina.

As condições de ciclagem para a PCR foram: 95ºC por 10 min, seguidos por 40 ciclos a 95ºC por 30s, 57ºC por 1 min e 72ºC por 1 min, com um passo de extensão final a 72ºC por 10 min.

Aos produtos amplificados, foi adicionado 1/10 do tampão de corrida de gel de agarose, Syber Green (Loading gel Syber Green, LGC). Foi preparado um gel de agarose a 1,5%. As amostras foram aplicadas no gel de agarose e submetidas a separação em tampão de corrida 1X TAE (Tris, Acetato e EDTA) com voltagem de 90V e amperagem de 35mA, durante aproximadamente 30 min.

Os resultados foram determinados após a corrida em eletroforese, quando o gel de agarose foi exposto a luz ultravioleta e posteriormente fotografado. A presença de uma banda de aproximadamente 450 pb confirmava o diagnóstico positivo para HPV usando os pares de primers MY09 e MY11, ou de 200pb quando usados os primers GP5/6+.

A amplificação consiste na desnaturação inicial por 5 minutos seguida por 40 ciclos de 1 minuto a 94º C, 1 minuto a 55º C e 1 minuto a 72º C. O produto da PCR é submetido á eletroforese em gel de agarose 1,2%, corado com brometo de etídio (0,5 mg/ml) e examinado sob luz ultravioleta.

O resultado padrão ouro de PCR será considerado quando um dos testes com os *primers* MY09/11 ou GP5+GP6+ detectarem DNA de HPV (450pb ou 250 pb, respectivamente). Além disso, foram utilizados primers específicos para detecção dos HR- HPVs (16, 18, 31 e 33).

3.9. PCR EM TEMPO REAL PARA A *Chlamydia trachomatis*

A quantificação destes ácidos nucléicos é possível porque se reproduzem valores durante a fase exponencial da reação. O ponto que detecta o ciclo na qual a reação atinge o limiar da fase exponencial é denominado de "Cycle Threshold". Este ponto permite a quantificação exata e reprodutível baseada na fluorescência. A emissão dos compostos fluorescentes gera um sinal que aumenta na proporção direta da quantidade de produtos da PCR.

Sendo assim, os valores da fluorescência são gravados durante cada ciclo e representam a quantidade de produto amplificado. O SYBR Green é um corante que se liga à alça menor do DNA dupla fita. Quando o corante SYBR Green liga a dupla fita de DNA, a intensidade da emissão fluorescente aumenta. Quanto mais amplicons de dupla fita forem produzidos, o sinal do corante irá aumentar. A PCR em tempo real requer uma plataforma de instrumentação que contém um termociclador com sistema ótico para a excitação da fluorescência e coleção da emissão; e um computador com um software para aquisição de dados e análise final da reação (VALONES *et al*., 2009; CHENG, ZHANG *et al*., 2004; WILHELM, PINGOUD *et al*., 2003).

A análise da curva de Melting, uma tecnologia revolucionária patenteada pela Roche Applied Science, baseia-se na adição de um corante ou sonda de oligonucleotídeo seqüência-específica, marcados com fluorescência durante as fases da reação em cadeia da polimerase (PCR). Após a PCR, uma "curva de melting" é gerada por um aquecimento lento e gradativo da dupla fita amplicom/corante (heteroduplex) medindo a mudança na fluorescência que resulta quando a sonda desnatura, ou "melts", fora do amplicom. Cada DNA tem sua temperatura de Melting específica (TM), que é definida como a temperatura em que 50% de DNA torna-se fita simples. Estas temperaturas são determinadas pelo comprimento da fita dupla de DNA e o grau de complementaridade entre as fitas. Caso a fluorescência do corante seja monitorada continuamente através dos ciclos de temperatura, a desnaturação do produto pode ser observada como uma rápida perda de fluorescência próxima à temperatura de desnaturação (VALONES *et al*., 2009; CHENG, ZHANG *et al*., 2004; WILHELM, PINGOUD *et al*., 2003).

A peculiaridade da PCR em tempo real é que o processo da amplificação é monitorizado em tempo real usando técnicas de fluorescência, sem necessidade de uma

manipulação pós-PCR (VALONES *et al*., 2009; CHENG, ZHANG *et al*., 2004; WILHELM, PINGOUD *et al*., 2003).

Vários estudos evidenciam que a PCR em tempo real é mais sensível para as cervicites e uretrites que a cultura (FINAN *et al.,* 2002), detectando com maior rapidez pequenas quantidades de ácidos nucléicos em amostras clínicas, independente da forma de apresentação (SMITH *et al.,*2002; GOTZ *et al*., 2005; FARIVAR *et al.,* 2012).

A *Chlamydia trachomatis* possui 15 sorotipos diferentes, os quais são responsáveis por doenças diversas. Os sorotipos L1, L2 e L3 são responsáveis pela linfogranuloma venéreo; os sorotipos A, B, Ba e C pelo tracoma e; os D,E,F,G,H,I,J e K pela conjuntivite de inclusão, uretrites, cervicites, salpingites e pneumonias do recém-nascido (TELES *et al.,*1997; PAAVONEM *et al.,* 2001).

A PCR detecta com rapidez pequenas quantidades de ácidos nucléicos em amostras clínicas. São capazes de detectar até uma única partícula plasmídica de *C. trachomati* (VALONES *et al*., 2009; CHENG, ZHANG *et al*., 2004; WILHELM, PINGOUD *et al*., 2003).

A reação da PCR consiste na obtenção de milhares de cópias de um segmento de DNA a partir de primers (iniciadores) de uma seqüência de DNA-alvo (VALONES *et al*., 2009; CHENG, ZHANG *et al*., 2004; WILHELM, PINGOUD *et al*., 2003). Os primers definem as regiões de DNA a serem amplificadas e a especificidade da técnica (VALONES *et al*., 2009). A sensibilidade destes testes de amplificação do DNA é em torno de 20% maior do que as demais técnicas de cultura, imunofluorescência direta e enzimoimunoensaio.

Embora mais caros, aumentam a capacidade de diagnóstico desta infecção, com sensibilidade de 98,0 a 99,9% (VALONES *et al*., 2009). Os *primers* utilizados em nosso estudo é específico para a *C. trachomatis*, havendo quantificação da positividade, porém não permite a tipagem dos sorotipos.

Estudos anteriores mostram que é necessário avaliar o impacto do diagnóstico de infecção por *C. trachomatis* em pacientes sem alteração da citologia oncótica, com a finalidade de determinar se vale à pena utilizar PCR em tempo real de rotina na população geral ou de risco para infecção por *C. trachomatis,* ou se alternativamente seria mais interessante utilizar a IMF-direta. Analisar a presença de sintomas e as possíveis implicações da infecção por *C. trachomatis* sobre o futuro reprodutivo é importante no sentido de elaborar uma estratégia de rastreamento. Embora o tratamento da infecção por *C. trachomatis* seja relativamente simples e de baixo custo, uma

positividade de até 40% implicaria no tratamento de muitas mulheres, sem a comprovação de efeito benéfico em termos de prevenção de obstrução tubária e infertilidade.

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CAPÍTULO II

Artigo 1: Comparative study between conventional Pap cytology × liquid-based *ThinPrep*® Pap Test cytology in the State of Pernambuco, Brazil

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Comparison of conventional Papanicolaou cytology samples with liquid-based cervical cytology samples from women in Pernambuco, Brazil

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Abstract

In the present study, we compared the performance of a ThinPrep cytological method with the conventional Papanicolaou test for diagnosis of cytopathological changes, with regard to unsatisfactory results achieved at the Central Public Health Laboratory of the State of Pernambuco. A population-based, cross-sectional study was performed with women aged 18 to 65 years, who spontaneously sought gynecological services in Public Health Units in the State of Pernambuco, Northeast Brazil, between April and November 2011. All patients in the study were given a standardized questionnaire on socio-demographics, sexual characteristics, reproductive practices, and habits. A total of 525 patients were assessed by the two methods (11.05% were under the age of 25 years, 30.86% were single, 4.4% had had more than 5 sexual partners, 44% were not using contraception, 38.85% were users of alcohol, 24.38% were smokers, 3.24% had consumed drugs previously, 42.01% had gynecological complaints, and 12.19% had an early history of sexually transmitted diseases). The two methods showed poor correlation (k=0.19; 95%CI=0.11-0.26; $P<0.001$). The ThinPrep method reduced the rate of unsatisfactory results from 4.38% to 1.71% (χ^2=5.28; P=0.02), and the number of cytopathological changes diagnosed increased from 2.47% to 3.04%. This study confirmed that adopting the ThinPrep method for diagnosis of cervical cytological samples was an improvement over the conventional method. Furthermore, this method may reduce possible losses from cytological resampling and reduce obstacles to patient follow-up, improving the quality of the public health system in the State of Pernambuco, Northeast Brazil.

Key words: Papanicolaou cytology; Liquid-based cytology; ThinPrep

Introduction

Cervical carcinoma is the second most frequent cancer type and the third leading cause of death by cancer in women worldwide (1). Thus, it becomes an important public health problem. According to the latest global estimates, there were 527,000 new cases and 265,000 cervical cancer related deaths in 2012, with 85% of total cases located in developing countries. The absence or low effectiveness of prevention programs (cytological screening) is singled out as the likely cause of the high incidence of cervical cancer in these countries (1).

As is widely known, human papilloma virus (HPV) infection is a prerequisite necessary but not sufficient cause of cervical lesions, implying that the combination of host, bacterial, environmental and genetic factors, along with persistent infection with high-risk HPV strains, may be considered as cofactors rather than independent factors. The main prevention tool against cervical cancer is cytological screening, and vaccination against HPV, which has high efficacy for prevention of HPV infection and its associated lesions; but barriers to vaccination include costs, limited vaccine availability, and lack of vaccine awareness (2).

In Brazil, 15,590 new cases of cervical cancer were expected in 2014, with an estimated risk of 15.33 cases for every 100,000 women. Unlike other types of human cancers, cervical cancer is a preventable disease, due to its slow progression, with a long period from the

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development of precursor lesions to the emergence of neoplasia (3).

The Papanicolaou cytological examination (conventional Pap test, CP), developed by the Greek doctor Geórgios Papanicolaou in 1941 as a tool for early detection of cervical cancer, is the main strategy used in control programs of cervical cancer. In Brazil, the Ministry of Health has determined that the CP should be performed primarily in women aged 25 to 64 years (2).

The CP is considered an efficient and easy-to-apply methodology, as it has the ability to identify precursor lesions of cervical cancer while they are still treatable (2). However, despite its well-known methodology, the CP has high rates of false-negatives due to its oscillation in sensitivity (4), and those rates can vary from 2% to 50% (2,5,6). In a meta-analysis study conducted by Fahey et al. (7), sensitivity of the CP was found to be 58% (ranging from 11% to 99%), with a specificity of 68% (ranging from 14% to 97%).

In the 1990s, a new methodology was developed for the collection and preparation of cervical cytological samples for screening, a liquid-based cytology called the ThinPrep® Pap test (Cytyc Corporation, USA). Approved in 1996 by the United States Food and Drug Administration, ThinPrep was introduced as an alternative to using the conventional method, with the purpose of improving the screening of atypical cells, cervical cancer, or its precursor lesions [low-grade squamous intraepithelial lesions (LSIL) and high-grade squamous intraepithelial lesions (HSIL)]. The aim was to improve sensitivity, because it permits the use of a monolayer of cells to facilitate the diagnosis by the cytopathologist, with better cellular preservation and the possibility to carry out molecular biology testing, making possible, for example, HPV and *Chlamydia trachomatis* DNA detection (4,8-12). This technique has been widely adopted and is gradually replacing the CP in control programs of cervical cancer in some countries (13,14).

There is sufficient evidence that the ThinPrep method reduces the proportion of unsatisfactory samples. However, there are still doubts about its effectiveness, as is also true for the conventional method, in the early detection of cervical cancer (15-17).

The State of Pernambuco (Northeast Brazil) stands out because of its high rates of unsatisfactory tests: of 185 municipalities, 77 (41.62%) had rates of unsatisfactory results above 5% during the year 2013 (18). The Central Public Health Laboratory of the State of Pernambuco (LACEN-PE) is the laboratory where a great number of statewide samples are processed and examined. Thus, the present study was conducted to compare the rates of cytopathological changes and unsatisfactory results of the two methodologies (CP cytology and liquid-based ThinPrep cytology) in cervical samples of women served by the Public Health Units of the State of Pernambuco and analyzed by LACEN-PE, and we also evaluated clinical, biological, and sociodemographic characteristics of these patients.

Material and Methods

A population-based cross-sectional study was conducted with women aged between 18 and 64 years, attended by spontaneous demand at Public Health Units of the State of Pernambuco, Brazil, during the period between April and November 2011. These units make up 63.24% of the public health care system in the State of Pernambuco, which is divided into 185 municipalities with basic health units in five regions as follows: Recife (19 units), Limoeiro (31 units), Palmares (22 units), Caruaru (32 units), and Arcoverde (13 units). On average these units serve 60 patients each week, and more than 70% of them are of low socioeconomic status.

Cervical smear samples were collected by various professionals (nurses, cytotechnologists, and cytopathologists) at different basic health centers. All cytological slides were then referred to LACEN-PE, a public reference center for female genital diseases in Pernambuco, Brazil, where the study was carried out. The slides were screened by local cytotechnologists who stained them for the Papanicolaou technique, and were classified by one of the 16 local cytopathologists according to international norms of standardization (Bethesda System 2001/ adapted by the Brazilian Society of Cytopathology). Patients who had undergone radiation treatment or chemotherapy for invasive cervical neoplasia and/or who had been subjected to oncotic cytology collection within the last 3 months before recruitment were excluded from the study.

A double-blind study was designed as follows: paired specimens were subjected to both CP and ThinPrep, permitting the evaluation of the two methodologies. The collection of cervical-vaginal material for the method of liquid-based preparation was similar to the collection of material for the conventional method, changing only the instrument used and the number of turns made for obtaining the sample.

In one-half of the samples, the material was initially transferred to the conventional frosted slide with an endocervical brush and Ayres spatula and fixed with polyethylene glycol spray, and then new samples were collected and agitated in the supernatant of the liquid medium for the ThinPrep Pap test, according to the manufacturer's instructions (15). In the other half, the endocervical brush was first agitated in the supernatant of the liquid medium, and then immediately a new brush and spatula were used for conventional cytology. Samples were transported at the end of the day to the LACEN-PE, where they were kept under refrigeration (between 5 and 8°C) awaiting analysis for a maximum of 1 month.

Information on sociodemographics, sexual characteristics, reproductive practices, and habits of patients (such as smoking, alcohol consumption, and drug use) was obtained from all patients on a standardized questionnaire.

The study was approved in advance by the Ethics Committee for Research of the Universidade Federal de Pernambuco (#105/09-CCS), and all patients signed an informed consent form and filled out the questionnaires.

The sample size was calculated using the STATCALC program of Epi-Info 3.5 for Windows (USA), based on prevalence data from the literature. For statistics, the chi-square test of association (Pearson) was used at a significance level of 5%, and the kappa and McNemar tests were also used to better evaluate discrepancies using the statistical software (Epi-Info version 5.0 or higher) with double-entry.

Cohen's kappa test for agreement between paired data measurements was performed to assess the level of agreement between the results of the two methodologies applied to the same samples (paired data). If a level of low agreement was observed, then a McNemar test was applied to verify which results were responsible for the low agreement. Therefore, we compared the sensitivity of the ThinPrep method taking conventional cytology results in the same individual as a reference.

Results

In this study, cervical samples from 525 women were included and analyzed at the LACEN-PE. The distribution of sociodemographics, sexual characteristics, reproductive practices, and habits of patients is shown in Table 1. This sample consisted of a majority of women older than 25 years (86.7%), with elementary-level schooling (52.2%; 8 years or less of study), predominantly from urban areas (71.1%). Regarding ethnicity, 47.6% self-reported being mulatto. In addition, the majority of the women reported being married or otherwise in a stable relationship (69.1%). The majority of women reported having five or less total number of sexual partners (93.5%). Although previous sexually transmitted infections (STI) episodes were not reported for the women before being recruited for the present study (87.8%), some had previously complained about genital discharge, bleeding, or rashes (42.1%). Regarding contraceptive methods, some women reported not using any method whatsoever (31.8%). Other women reported surgical sterilization (tubal ligation: 31.8%, hysterectomy: 6.3%). Only 5.3% of the women reported the frequent use of condoms, and 8.6% used a combination of oral contraceptive pills. Only 0.8% of the women reported being in menopause.

It was observed that 5.9% were smokers (11.6% reported past use), 20.0% reported frequent alcohol use, and only one woman (0.2%) reported past use of illicit drugs (marijuana).

The results of the diagnostic interpretations between the two methodologies, ThinPrep and CP, are shown in Table 2. Evaluation of agreement between methodologies through Cohen's kappa test resulted in a measure of weak agreement between the two methods, kappa=19%. The low level of agreement was due to an increase in the percentage of normal diagnoses and altered diagnoses using the ThinPrep methodology (from 34.7% to 48.8% and from 2.4% to 3.0%, respectively) and a reduction in the number of inflammatory and unsatisfactory diagnoses (from 58.5% to 46.5% and from 4.4% to 1.70%, respectively; $P<0.01$ for both analyses; Table 2).

Under a dichotomous classification (satisfactory or unsatisfactory), we observed that the ThinPrep method was better than conventional cytology for diagnostic definition, because all 23 samples with unsatisfactory results by CP had defined diagnostics using the ThinPrep protocol. In other words, the conventional test had 4.4% (23/525) unsatisfactory results, *vs* only 1.7% (9/525) unsatisfactory results from the ThinPrep test (McNemar $\chi^2=5.28$, $P=0.02$ for both analyses; Table 3). Finally, no significant differences were observed in the detection of altered cytology between the tests carried out. A total of 13 samples were detected by CP (2.47%) and 16 by ThinPrep (3.04%, $\chi^2=0.24$; $P=0.63$; Table 4).

Discussion

Bezerra et al. (19) and Amaral et al. (20) relate early onset of sexual activity, multiple sexual partners, use of oral contraceptives, smoking, nutritional deficiency, and immunological state as important risk factors for the development of neoplastic lesions. Analyzing the sociodemographics, sexual characteristics, reproductive practices, and habits of patients (such as smoking, alcohol consumption, and drug use), it was observed that users of the Public Health System in the State of Pernambuco (SUS-PE) are young women, of a low educational level, who use few methods of protection, both from the reproductive point of view and for prevention of STIs. Dunne et al. (21) found a higher prevalence of HPV infection among women who have lower education, are unmarried, belong to certain racial or ethnic groups, and have lower socioeconomic status. We speculate that the Public Health Service outreach for cervical cancer screening is greater among women over 25 years of age and among women living in a geographic area with greater access to public services that promote prevention and recovery of health.

Several authors have demonstrated and confirmed the advantages of ThinPrep in relation to CP for cervical exams (8,22-25). ThinPrep has been referred to as the method of superior performance because it provides better cellular representation, with increased sensitivity for the detection of lesions, compared with conventional methodology (5,8,14,24-28). Several authors have shown that this greater diagnostic sensitivity applies to high-grade and glandular lesions (26-28).

Table 1. Biological, demographic, reproductive, clinical-gynecological characteristics and habits of 525 patients who underwent conventional cytology evaluation and liquid-based ThinPrep evaluation in the State of Pernambuco, 2011.

Characteristic	n (%)	Characteristic	n (%)
Age		Gynecological complaint	
≤25 years old	60 (11.4)	(discharge/genital bleeding/rash)	
>25 years old	455 (86.7)	Yes	221 (42.1)
Not reported	10 (1.9)	No	304 (57.9)
Origin		Episode of STI	
Urban	373 (71.1)	Yes	64 (12.2)
Rural	133 (25.3)	No	461 (87.8)
Not reported	19 (3.6)	Contraceptive methods use and fertility status	
Race		Condom	28 (5.3)
White	160 (30.5)	Combined oral contraceptive pills	45 (8.6)
Black	98 (18.7)	Intrauterine device (IUD)	3 (0.6)
Mulatto	250 (47.6)	Contraceptive injection	8 (1.5)
Indigenous	2 (0.4)	Method combination	10 (1.9)
Not reported	15 (2.8)	Others/not reported	61 (11.6)
Education		No use	166 (31.6)
≤8 years	274 (52.2)	Tubal ligation	167 (31.8)
>8 years	164 (31.2)	Hysterectomy	33 (6.3)
Not reported	87 (16.6)	Women in menopause	4 (0.8)
Marital status		Alcohol use	
Without partner (single/other)	152 (29.0)	Yes	105 (20.0)
With partner (married/stable relationship)	363 (69.1)	No	321 (61.1)
Not reported	10 (1.9)	Past use	56 (10.7)
Age at first sexual relation		Not reported	43 (8.2)
≤14 years old	84 (16.0)	Smoking	
>14 years old	429 (81.7)	Yes	31 (5.9)
Not reported	12 (2.3)	No	397 (75.6)
Parity		Past use	61 (11.6)
≤2	237 (45.1)	Not reported	36 (6.9)
>2	248 (47.2)	Illicit drugs use	
Not reported	40 (7.7)	Yes	0 (0.0)
Total number of sexual partners		No	508 (96.7)
≤5	491 (93.5)	Past use	1 (0.2)
>5	23 (4.4)	Not reported	16 (3.1)
Not reported	11 (2.1)		

STI: sexually transmitted infection.

Table 2. Comparison of the diagnostic interpretations evaluated by LACEN of 525 patients obtained through conventional cytology evaluation and liquid-based ThinPrep evaluation in the State of Pernambuco, 2011.

Diagnosis by LACEN (n total=525)	Conventional cytology n (%)	Liquid-based cytology n (%)	Cohen's *K* (95%CI)	P
Normal[a]	182 (34.7)	256 (48.8)		
Inflammatory	307 (58.5)	244 (46.5)	0.19 (0.11-0.26)	<0.01
Altered[b]	13 (2.40)	16 (3.00)		
Absence of diagnosis (unsatisfactory)	23 (4.40)	9 (1.70)		

LACEN: Laboratório Central de Saúde Pública do Estado de Pernambuco, Brazil. [a]Including normal and atrophied cytologies. [b]Altered: ASCUS/ASC-H/LSIL/HSIL/CA *in situ*. ASCUS: atypical squamous cells of undetermined significance; ASCH: atypical squamous cells without high-grade lesion; LSIL/HSIL/CA: low-grade squamous intraepithelial lesion/high-grade squamous intraepithelial lesion/cervical cancer.

Table 3. Comparison of unsatisfactory cytological result rates of 525 patients who underwent conventional cytology and liquid-based ThinPrep methodology, evaluated by LACEN in the State of Pernambuco, 2011.

Conventional cytology	Liquid-based cytology		Total	McNemar (χ^2)	P
	Unsatisfactory	Diagnosed			
Unsatisfactory	0	23	23		
Diagnosed[a]	9	493	502	5.28	0.02
Total	9	516	525		

LACEN: Laboratório Central de Saúde Pública do Estado de Pernambuco, Brazil. [a]Including normal, inflammatory, atrophied and altered cytologies (ASCUS/ASC-H/LSIL/HSIL/CA) *in situ*. ASCUS: atypical squamous cells of undetermined significance; ASCH: atypical squamous cells without high-grade lesion; LSIL/HSIL/CA: low-grade squamous intraepithelial lesion/high-grade squamous intraepithelial lesion/cervical cancer.

Other authors note that ThinPrep allows better preservation and cellular disposition, allowing for better diagnostic interpretation, due to reduction of the presence of mucus, inflammatory exudates, and erythrocytes; reduction in reading time, in addition to enabling the processing of additional samples without the need to call in the patient for new material collection; and allowing the use of residual samples for molecular biology testing of viruses such as HPV (4,26) and of various other associated pathogenic organisms (e.g., *C. trachomatis*, *Neisseria gonorrhoeae*, etc.). However, the disadvantages identified are related to the higher cost and the need to train professionals in the new technique.

In most studies comparing the two methods, ThinPrep increases the quality of the results by reducing the number of cases classified as unsatisfactory (27-32). Cheung et al. (31) found a reduction in the rate of unsatisfactory results with the ThinPrep method, from 0.48% to 0.32%. Similarly, in the current study, there was a reduction in the rate of unsatisfactory results from 4.4% to 1.70% ($P < 0.01$; Table 2).

In the present study, the percentage of inflammatory results with the CP method was 58.47% *vs* 46.47% with ThinPrep. However, there was an increase in the detection of altered cells by ThinPrep (3.04% *vs* 2.47%). Khalbuss et al. (33) detected a greater number of blood cells and inflammatory cells using CP. These data can be explained by considering that, for liquid-based ThinPrep, because the reading area is reduced by up to 81% and interference that normally obscures the samples is eliminated, there is an increase of about 50% in reading time and improvements of up to 73% in lab productivity (8,23).

Data from the literature were controversial when reporting satisfactory diagnosis rate comparisons between CP and ThinPrep, although some studies did not show statistically significant differences (13,31-33). Khalbuss et al. (33) and Cheung et al. (31) did not detect any significant difference between the two methods. In contrast, Coste et al. (34) detected an increase in satisfactory results using CP (91%) compared with ThinPrep (87%). The same was observed by Stabile et al. (8), where quality was measured according to the presence of elements of the squamocolumnar junction. These researchers detected an increase in the number of diagnoses with CP compared with liquid cytology (93% *vs* 84%, respectively). Corroborating with these last two studies, our results showed an increase in the percentage of satisfactory results using the ThinPrep method (98.3% *vs* 95.6%; P=0.02).

Agreement between the ThinPrep and CP methods, in a meta-analysis conducted by Abulafia et al. (10) using 17 articles selected from the literature in the period between 1990 and 2002, found agreement among 89% of the cases based on a classification with five levels of diagnosis (negative, atypical, LSIL, HSIL, and carcinoma).

In the present analysis, despite the increase in the number of cytopathological changes detected by ThinPrep (3.04% *vs* 2.47%), this difference was not significant (P=0.63; Table 4). Similarly, Davey et al. (35) conducted a

Table 4. Comparison of altered result rates of 525 patients who underwent conventional cytology and liquid-based ThinPrep methodology in the State of Pernambuco, 2011.

Conventional cytology	Liquid-based cytology		Total	McNemar (χ^2)	P
	Altered	Unaltered			
Altered	6	7	13		
Unaltered	10	502	512	0.24	0.63
Total	16	509	525		

Altered/positive: ASCUS/ASC-H/LSIL/HSIL/CA *in situ*. ASCUS: atypical squamous cells of undetermined significance; ASCH: atypical squamous cells without high-grade lesion; LSIL/HSIL/CA: low-grade squamous intraepithelial lesion/high-grade squamous intraepithelial lesion/cervical cancer.

meta-analysis study of 56 studies and did not detect any difference between the performances of the two techniques. Jesdapatarakul et al. (36) also did not observe any significant improvement in the diagnosis when comparing both methods. In contrast, Stabile et al. (8) detected 3% atypical diagnoses with CP *vs* 10% with liquid-based cytology. Cheung et al. (31) detected an increase in the number of diagnoses by ThinPrep compared with CP (3.74% *vs* 3.19% for atypical squamous cells of undetermined significance (ASCUS) and 1.67% *vs* 1.01% for LSIL).

A limiting factor of this study is that it was not possible to assess the specificity and sensitivity of the techniques analyzed, because not all the patients were evaluated by histopathology, which is used as the gold standard in cytology. However, results of some previous studies regarding the sensitivity and specificity of the conventional and liquid-medium methods were summarized in a meta-analysis conducted by Abulafia et al. (10). In that study, they found 68% general sensitivity for the CP and 76% for ThinPrep. However, the difference was statistically significant in only two studies. The same was observed in relation to specificity, which was 79% for CP and 86% for ThinPrep, a difference that was not significant in most cases. In another study, conducted by Coste et al. (34), the sensitivity of conventional cytology ranged from 57% to 74%, while liquid-based cytology ranged from 61% to 73%. The specificity ranged from 91% to 96% for CP and 90% to 95% for ThinPrep. However, diagnoses using liquid-based cytology reported more ASCUS-type abnormalities. Similarly, Arbyn et al. (37), following a systematic review and meta-analysis of 109 studies of various designs, noted that liquid-based cytology did not provide significant differences in sensitivity and specificity.

It is important to stress that the collection of material was performed by various professionals in different health centers, which may be related to false-negative results. Even though guidelines for such procedures were applied to the laboratories, there was no way to ensure effective standardization of the quality of the process when executed manually. According to recommendations in the Executive Summary of the National Program for Control of Cervical Cancer in 2010 (38), published by the Pan-American Health Organization, in order to maintain quality control standards, a laboratory must have a minimum production of 15,000 exams/year. In Brazil, among the laboratories that provided services for Public Health Units in 2008, only 15% of a total of 1116 laboratories presented production above this threshold.

Given the results obtained from the population of this study, it is possible to conclude that liquid-based cytology offered an improvement in cytological diagnosis and contributed to a decrease in the number of unsatisfactory results in the reports from the Public Health network in the State of Pernambuco, Brazil. Our results show that around 5% of the women would not have a correct diagnosis through CP. In principle, 5% seems low, but considering that Pernambuco has a high prevalence of HPV infection, it is possible that a majority of this 5% of women may already have lesions or even altered cytology, but their clinicians would not know because of unsatisfactory methodologies.

Thus, although liquid-based cytology is more expensive, its widespread introduction to the routine of the Pernambuco public health network will allow the establishment of standards in collection, preparation, and staining of samples that will guarantee an improvement in the quality of testing and diagnostics, as well as reduce possible losses from cytological repetition, and support additional investigation of STIs in the population of this area, in the State of Pernambuco, Brazil.

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Capítulo III

Artigo 2: Incidence of *human papilomavirus* and other sexually transmitted agentes in Brazilian women with cervical atypia from Public Health Care Service of Pernambuco, Brazil

Artigo *submetido* ao periódico ***Molecular Biology Reports*** **(IF: 1,95)**

Molecular Biology Reports

Incidence of human papillomavirus and other sexually transmitted agents in Brazilian women with cervical atypia from Public Health Care Service of Pernambuco, Brazil.

--Manuscript Draft--

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Full Title:	Incidence of human papillomavirus and other sexually transmitted agents in Brazilian women with cervical atypia from Public Health Care Service of Pernambuco, Brazil.
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Abstract:	To evaluate the occurrence of possible association between the presence of high risk types of human papillomavirus (HR-HPV) with Chlamydia trachomatis (CT) co-infection and other micro-floras with the different stages of cervical alterations of patients of the public health service in the State of Pernambuco-Brazil. The population of study comprised by 87 women, who underwent routine colposcopy and were diagnosed with cytopathological changes of varying degrees analyzed by the Central Public Health Laboratory. The polymerase chain reaction method on a cervical brush specimen was used to detect both agents. Of the 87 women enrolled in this study, 73 (83.91%) were positive for CT infection and 72 (82.76%) to HPV infection with co-infections in 65 cases (74.7%). Of these, 93.1% had colposcopic alterations. There was a statistically significant association between HPV-CT co-infection and presence of lesions (p=0.037). Other micro-floras found include Gardnerella vaginalis with 31 (35.63%) cases, Coccos sp. 16 (18.39%), Candida sp. 8 (9.19%), Trichomonas vaginalis 6 (6.90%), Lactobacillus sp. 4 (4.6%) and herpes virus 1 (1.15%). Mixed Flora (co-infection by multiple micro-floras - with the exception of simultaneous CT) occurred in 36 (41.4%) cases. However, no association of the presence of these non-viral microorganisms and herpes viruses was found with the severity of intra-epithelial lesions (p>0.05). Genital infections, in particular of Chlamydia trachomatis, should be investigated and dealt with appropriately, given that co-infections with HPV are associated with increased likelihood of low grade lesions, which can develop into more serious lesions, such as carcinoma of the cervix.
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Incidence of human papillomavirus and other sexually transmitted agents in Brazilian women with cervical atypia from Public Health Care Service of Pernambuco, Brazil.

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Abstract

To evaluate the occurrence of possible association between the presence of high risk types of human papillomavirus (HR-HPV) with *Chlamydia trachomatis* (CT) co-infection and other micro-floras with the different stages of cervical alterations of patients of the public health service in the State of Pernambuco-Brazil. The population of study comprised by 87 women, who underwent routine colposcopy and were diagnosed with cytopathological changes of varying degrees analyzed by the Central Public Health Laboratory. The polymerase chain reaction method on a cervical brush specimen was used to detect both agents. Of the 87 women enrolled in this study, 73 (83.91%) were positive for CT infection and 72 (82.76%) to HPV infection with co-infections in 65 cases (74.7%). Of these, 93.1% had colposcopic alterations. There was a statistically significant association between HPV-CT co-infection and presence of lesions (p=0.037). Other micro-floras found include *Gardnerella vaginalis* with 31 (35.63%) cases, *Coccos* sp. 16 (18.39%), *Candida* sp. 8 (9.19%), *Trichomonas vaginalis* 6 (6.90%), *Lactobacillus* sp. 4 (4.6%) and herpes virus 1 (1.15%). Mixed Flora (co-infection by multiple micro-floras – with the exception of simultaneous CT) occurred in 36 (41.4%) cases. However, no association of the presence of these non-viral microorganisms and herpes viruses was found with the severity of intra-epithelial lesions (p>0.05). Genital infections, in particular of *Chlamydia trachomatis*, should be investigated and dealt with appropriately, given that co-infections with HPV are associated with increased likelihood of low grade lesions, which can develop into more serious lesions, such as carcinoma of the cervix.

Keywords: HPV infection, cervical lesion, genital co-infections

Introduction

Cervical cancer is a serious public health problem among women worldwide. There are about 500,000 new cases diagnosed each year, second in frequency only to breast cancer. It has the third highest mortality rate among women with cancer, being associated with 270,000 deaths annually. About 80% of cases occur in developing countries, where in some regions it is the most common cancer among women [1,2,3].

In Brazil, the estimate was of 15,590 new cases in 2014 (18 per 100,000 women), corresponding to the third most common neoplasia in the country. Its peak incidence is among women from 40 to 60 years of age, with only a small percentage of occurrence in those younger than 30 years. A striking feature of cervical cancer, throughout the world, is its consistent association with low socioeconomic level, i.e. with the groups that have greater social vulnerability [4,5,6].

In Pernambuco, a state in northeastern Brazil, the incidence is higher than the national estimate, with approximately 22.73 cases per 100,000 women. This is a statistic of particular concern because it is estimated that only 30% of women undergo preventive examinations at least three times in their life, which results in diagnoses in advanced stage of the disease, as is the case in around 70% of the cases [7].

The development of cervical cancer, in most cases, progresses slowly through detectable and treatable preclinical phases. It is estimated that 44% of the cases of precursor lesions of cervical cancer are *in situ*, i.e. the lesion is still localized, a stage in which early diagnosis together with proper treatment can result in a practically 100% chance of cure [4].

Data obtained from experimental and epidemiological studies have shown that human papillomavirus (HPV) is detected in 99.7% of cervical carcinomas. Of this total, 70% of the infections are by types 16 and 18 [8,9,10]. Most genital HPV infections regress within 2 years and only a minority of women develop a persistent infection that could eventually cause cervical intra-epithelial neoplasia (CIN). In this way, while infection by viral high-risk oncogenic types (HR-HPV) is necessary it is not sufficient to begin the process of malignant transformation of cells of the cervix [9,11]. Other factors may be

involved in this process, such as the participation of environmental, genetic or immunological co-factors [9,12].

Among these factors, it is suspected that co-infection with other sexually transmitted diseases (STDs), such as human immunodeficiency virus (HIV), *Chlamydia thrachomatis*, herpesvirus and other types of HPV are related with the appearance of cervical lesions [12,13,14,15].

The persistent and simultaneous presence of several infectious agents could lead to chronic inflammation, which is regarded as important in the pathogenesis of several types of cancer, since about 90% of cases of cervical neoplasms occur from pre-cancerous intra-epithelial lesions, which might have originally been induced by viral infections [12].

The present study aimed to analyze the prevalence of various infectious agents of the genitals, particularly the co-infection of *Chlamydia trachomatis* (CT) with human papillomavirus (HPV), in cervical cytological alterations of patients in the public health service in the State of Pernambuco, northeastern Brazil.

Materials and Methods

A population-based, cross-sectional study was carried out included 87 women aged 18 to 64 years, who were found to have lesions of varying degrees based on conventional cytological Papanicolaou exams. The women were diagnosed by oncotic cytology analyzed by the Central Public Health Laboratory in the State of Pernambuco (LACEN) - Brazil, in the period from April to November 2011. All the women who underwent colposcopy examinations and/or cervical biopsy (if necessary) were included in the study. Women who had undergone radiation treatment or chemotherapy for invasive cervical neoplasia were excluded from the study.

A standardized questionnaire was used to interview the subjects regarding their clinical history, sexual behavior, cultural habits and socio-economic and living conditions. After providing informed consent, all women were examined by a gynecologist. The study was approved in advance by the Committee of Ethics in Research of the Health Sciences Center of the Federal University of Pernambuco – CCS/UFPE (No. 105/09).

The colposcopic examination was performed according to the Classification of Nomenclature of the International Federation for Cervical Pathology and Colposcopy, IFCPC-2011. Using a colposcopy device (DF Vasconcelos, model CP-M2220), colposcopic images were analyzed after placement of 0.9% physiological saline in the cervix and vagina. Subsequently, a solution of 3% acetic acid was used and, finally, Lugol solution was used for the Schiller test. The following data were considered atypical: aceto-white epithelium, dotted, mosaic, leukoplakia, negative iodine zone and atypical vessels, following international standards (IFCPC-2011). Biopsies were performed for histopathological studies using a Gaylor-Medina caliper.

Cytopathological changes were classified according to international convention (Bethesda System 2001 adapted by the Brazilian Society of Cytopathology - BSC).

The samples were screened for the presence of HPV using the standard nested PCR approach consisting of the MY09/11 primer set (primary PCR) and GP5+/6+ primer set (secondary PCR) as previously described by Tavares et al. (2014) [15].

The detection of CT infection was realized by the methodology of Real Time PCR using the apparatus Line-gene K Real-time PCR Detection System (Bioer Technology) as previously described by Tavares et al. (2014) [15].

Statistical analysis was done using STATCALC program of Epi-Info 3.5 for Windows. Cytology was reported according to the Bethesda System. Association of outcome and exposure was tested with Fischer exact test and exposure to more than two categories with the non parametric trend chi-square-test. $P<0.05$ was considered significant; the Kappa test and McNemar test were also chosen to better evaluate discrepancies using the statistical software, EPI-INFO version 5.0 or higher, with double-entry. Initially, tables were prepared with the frequency distribution for categorical variables, and for quantitative variables, measures of central tendency (mean, median) and dispersion (standard deviation, percentage) were used.

To assess whether there was an association between co-infections of HPV and CT with the different stages of cervical lesions, the Fisher exact test was used at a significance level of 5%. Odds ratios (OR) and their confidence intervals of 95% (95% CI) were calculated for each test.

Logistic regression analysis was performed to assess the significance of the differences in paired data, for example, comparing the sensitivity of conventional cytology with ThinPrep cytology in the same individual.

Results

The mean age of the 87 women selected for this study was 36.4 ± 11.9 years (mean ± standard deviation). The demographic characteristics, as well as the reproductive and sexual history of the 87 women are shown in Table 1. Of these, 52.9% had less than eight years of schooling. Most women (80.5%) were of urban origin and 65.5% considered themselves of black or mulatto race.

More than half of the women selected (62.1%) were married or in a civil union; 37.9% had a previous history of sexually transmitted diseases (STD) and 41.38% did not use any contraceptive method. Only 14.9% were smokers, 33.3% were alcohol drinking and 1.15% had a prior history of drug use [Table 1].

Table 2 highlights the distribution of cytological alterations detected by the conventional cytological Papanicolaou test. It was found that 42.5% (37/87) of the patients had low-grade squamous intra-epithelial lesions (LSIL), 40.2% (35/87) had a pattern of atypical squamous cells of undetermined significance (ASCUS) and 17.3% (15/87) had alterations of greater severity, distributed as follows: 8.05% with high-grade intra-epithelial lesions (HSIL), 6.9% with atypical squamous cells which could be high-grade lesion (ASC-H), 1.15% with atypical glandular cells of undetermined significance (AGUS) and 1.15% with carcinoma.

In Table 3, the distribution of genital infections and STDs are shown in the 87 patients with cervical alterations detected by the Papanicolaou test. For non-viral flora, with the exception of infection by *Chlamydia trachomatis* (CT), the most prevalent STD was *Gardnerella vaginalis* with 35.63% (31/87), followed by *Coccos* sp. 18.39% (16/87), *Candida* sp. 9.19% (8/87), *Thrichomonas vaginallis* 6.9% (6/87), *Lactobacillus* sp. 4.6% (4/87) and herpes virus 1.15% (1/87). In addition, mixed flora (co-infection by multiple micro-floras – with the exception of simultaneous CT) was detected in 36 patients

(41.4%). However, for the above infections, no association was detected with the degree of cervical intra-epithelial lesion ($p>0.05$).

A prevalence of HPV infection of 82.76% (72/87) was detected among the selected patients. Of these, 43 (59.72%) showed high-grade lesions, seven (9.72%) had only HPV infections and the remaining 36 (83.7%) were co-infected with HPV-CT. Of the 29 women with low-grade lesions, all were co-infected with HPV-CT [Table 3].

The incidence of infection by CT was 83.91% (73/87). Of these, 93.1% had colposcopic alterations and 68.96% had histopathological concordance. The most prevalent subtypes were HPV16 in 48.6% of the cases and HPV31 in 22.2% [Table 4]. A statistically significant association was observed between the presence of infection by CT with the presence of low-grade lesions ($p=0.036$) when compared to the presence of high-grade lesions. Co-infection with HPV-CT was found in 89.04% (65/73) of the cases and there was a significant difference when comparing co-infection with HPV-CT and the degree of intra-epithelial cervical lesions ($p=0.037$).

Discussion

Research in relation to the etiology of cervical cancer has achieved substantial progress in the last two decades, both in scientific and operational terms. For several years, the epidemiological profile of women with cervical cancer was recognized as suggestive of sexually transmitted disease (STD). However, in the State of Pernambuco, as is also true for other states of Brazil, the coverage of services for prevention of cervical cancer is still deficient.

In the year 2013, 41.62% of the 185 municipalities in the State of Pernambuco presented unsatisfactory cytological exams with $\geq 5\%$ contributing to the poor quality of oncological analysis [7]. In this study, we investigated associations between HPV and other sexually transmitted infections in female residents of the State of Pernambuco, northeastern Brazil, with different grades of cervical intra-epithelial lesions.

It was verified that 52.9% of the women had less than eight years of schooling, 80.5% lived in urban settings, 62.1% were married or in a civil union; 37.9% had a previous history of STD and 41.38% did not use any contraceptive method. Only 14.94% were smokers, 33.3% alcohol drinkers and 1.15% had already experienced the use of drugs. Previous studies have shown that a low level of schooling, early initial experience of sexual intercourse, high numbers of sexual partners, smoking and previous contact with some type of STD can partly explain the incidence of cervical cancer in this group [12,17,18].

Franco *et al.* (1999) [19] observed cervical cancer about three times more frequently among women who smoked, making smoking an independent risk factor related to the intensity and duration of the addiction. This fact can be explained because smoking leads to a reduction in the defenses of immunological tissue, by decreasing the amount of T4 lymphocytes, "Natural Killer" cells (NK) and Langerhans cells of the uterine cervix. Confirming this hypothesis, Rama *et al.* (2008) [18], observed that women from the ages of 35 to 44 years, in civil unions and ex-smokers were associated with protection from HPV infection.

In addition to the risk factors reported above, the long-term use of oral contraceptives and number of births have been associated with cervical cancer as determinants of the persistence of HPV infection [5,6,12,17,18].

Several infectious agents, such as type 2 herpes simples virus, *Treponema pallidum*, *Neisseria gonorrhoeae*, *Gardnerella vaginalis*, and *Chlamydia trachomatis* have been proposed as co-factors in cervical carcinogenesis [12,13,20,21,22]. In our study, in relation to non-viral flora, *Gardnerella vaginalis* was the most frequent in women (35.6%), followed by *Coccos* sp. (19.5%), *Candida* sp. (9.2%), *Thrichomonas vaginallis* (5.7%), *Lactobacillus* sp. (4.6%) and herpes virus (1.2%). On the other hand, mixed flora infections (co-infection by multiple micro-floras – with the exception of simultaneous CT) were detected in 41.4% of the patients analyzed. Nonetheless, no association of the presence of such non-viral microorganisms and the herpes virus was found with the severity of intra-epithelial lesions [Table 2].

Inflammation of cervical epithelium has also been recognized as one of the predisposing co-factors of cervical carcinogenesis, because disturbance of the vaginal flora is known to increase the risk of

acquiring HPV infections, with a significantly high association with pre-neoplastic lesions (OR, 10.3; 95% CI: 6.6-16.1) [22]. These data suggest that the local cervicovaginal environment plays a decisive role in susceptibility to infection HPV, since women cervical *G. vaginalis* infection likely to have an altered *Lactobacillus*-poor vaginal flora with an increased risk of acquiring HPV infection [22].

The prevalence of HPV infection in women varies between 2 and 44%, is high among young women and appears to decrease with increasing age. On the other hand, several studies have shown a spike in the prevalence of infection in women under 25 years and another after 55 years [6]. Among young women, generally speaking, there is a widely-recognized biological vulnerability to sexually transmitted infections [17], and cervical ectopia, also frequent at this developmental stage, leads to metaplasia, as a natural repair mechanism, promoting the transmission of HPV and other microorganisms [17]. As described by Baseman *et al.* (2005) [6] there is no doubt that persistent, high-risk HPV infections represent a necessary cause for cervical cancer because HPV infections occur as soon as sexual activity starts and are transient. HPV positive women older than 30 years most likely represent cases of persistent infections and are under greater risk for intra-epithelial neoplasia and invasive cancer [6,13].

The fact that a high rate of HPV infection (82.8%) was found in this study can be explained because only women showing cervical intra-epithelial lesions were analyzed, in addition to the previous history of STD associated with the presence of cytopathological alterations. The most prevalent HPV subtypes found in this study were 16 and 31, with 48.6% and 22.2%, respectively [Table 4]. These data corroborate the findings of a previous study performed by Tavares *et al* (2014) [15] which also detected high frequencies of these subtypes in patients with histopathological alterations. In this study, in cases of low-grade lesions, the most prevalent subtypes were 16 and 31 (40.54% and 18.91%, respectively), whereas in cases of high-grade lesions and carcinoma, the most prevalent subtypes were 16 and 18 (71.42% and 50%, respectively).

In this analysis, there was a high frequency of *Chlamydia trachomatis* infection in patients with cervical squamous intra-epithelial lesions (83.90%). Co-infections of HPV-CT occurred in 65 cases. Of these, colposcopic alterations were found in 93.1%, and histopathological concordance was found in 68.96%. Infection by CT was more common in low-grade lesions ($p=0.036$) when compared to the presence of high-grade lesions. The same was observed in co-infection with HPV-CT for the presence of

cervical lesions compared the presence of only HPV infection (p=0.0368), agreeing with results previously described in the literature [13,15,21,23].

The literature offers strong epidemiological evidence that suggests that HPV and *Chlamydia trachomatis*, especially the D to K serotypes, play a central role in the etiology of cervical intra-epithelial neoplasia and subsequently cervical carcinoma [24]. *Chlamydia trachomatis* infection appears to increase susceptibility to infection by HPV in a basal level by facilitating access to the basal epithelial cells through micro-abrasions or by altering the characteristics of the epithelial cells, increasing the viral load of the infection and facilitating its persistence [21].

Tamim *et al.* (2002) [24], in a study of the prevalence of HPV and *Chlamydia trachomatis* in 129 women, observed that the rate of infection by *Chlamydia trachomatis* was higher in HPV-positive women and these also showed higher rates of abnormal cytology. Samoff *et al.* (2005) [25], found a prevalence of 78% HPV and 65% *Chlamydia trachomatis* infections among sexually active adolescents between the ages of 13 and 19 years. Golijow *et al.* (2004) [27], showed that the prevalence of *Chlamydia trachomatis* DNA was significantly higher in women with high-grade intra-epithelial lesion (HSIL) (47%) when compared to that in women with normal cytology (11%).

The hypothesis that infection with *Chlamydia trachomatis* interferes in the course of precocious HPV infection is supported by the fact that *Chlamydia trachomatis* DNA can be detected in cervical smears done years before the diagnosis of cancer [13,15,25].

Concurrent infection by *Chlamydia trachomatis* can impede HPV infection by inducing a pattern of humoral type immune response. In addition, *Chlamydia trachomatis* infection has been linked to hyperplasia of reserve cells and metaplasia, processes related to cervical carcinogenesis. On the other hand, it is believed that a modulation of immune response and/or precipitation of an inflammatory response favors a subsequent infection by *Chlamydia trachomatis*, increasing the rate of infectivity by *Chlamydia trachomatis* in HPV-positive women [24]. Using this same reasoning, the inflammatory response provoked by CT, associated with other infectious agents, such as *Gardnerella vaginallis*, *Candida albicans* and *Trichomonas vaginalis*, causes major local disruption, as has been suggested in other studies [12,13,22,26].

In a study conducted by Golijow *et al.* (2005) [27], the prevalence of co-infection by HPV and *Chlamydia trachomatis* increased with increasing severity of cervical neoplasia and women positive for *Chlamydia trachomatis* DNA, presenting a higher risk of having LSIL and HSIL after controlling for age and positivity for HPV.

It is believed that development of malignancy generally requires that the virus is of medium/high risk, lesions have high viral load and that the presence of some co-factors exist (immuno-depression, smoking, other STD), acting synergistically on the metaplastic epithelium or reserve cells of the epithelium. With the ever earlier ages of first sexual intercourse and pregnancy, where the finding of cervical ectopia is frequent, associated with the multiplicity of partners, an increase in the risk of exposure of cervical cells to direct mutagenic action and prolonged interaction with one or more types of HPV would be expected [26,28].

Changes that have been observed in the sexual behavior of women in the last thirty years have had implications on the epidemiology of the disease [9].

Conclusion

The genital infections in particular those caused by *Chlamydia trachomatis*, HPV and *Gardnerella vaginalis*, among other, should continue to be investigated and dealt with appropriately, given their direct association with pre-neoplastic lesions and action of the HPV virus.

Abbreviations

HR-HPV- high risk types of human papillomavirus

CT -*Chlamydia trachomatis*

LACEN- Central Public Health Laboratory

CIN- cervical intra-epithelial neoplasia

STD- sexually transmitted diseases

HIV- human immunodeficiency virus

HPV- human papillomavirus

IFCPC- International Federation for Cervical Pathology and Colposcopy

LSIL- low-grade squamous intra-epithelial lesions

ASCUS- atypical squamous cells of undetermined significance

ASC-H- atypical squamous cells that allow not exclude a high-grade lesion

HSIL- high-grade intra-epithelial lesions

AGUS- atypical glandular cells of undetermined significance

Competing interests

The researchers and advisors of the study state that there are no conflicts of interest to the survey.

Authors' contributions

Conception and design: MOLPC, PRES; Collection and assembly of the data: SAH, VLA; Analysis and interpretation of the data: MOLPC, PRES, AVCC; Statistical expertise: AVCC; Drafting of the manuscript: MOLPC, PRES, AVCC, MTSC.

All authors read and approved the manuscript before submission.

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Table 1
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Table 1. Biological, socio-demographic and habit characteristics of 87 patients with cervical lesions from Pernambuco.

Characteristics	n=87	%
Origin		
Rural	17	19.5
Urban	70	80.5
Race		
White	29	33.3
Black/ Mulatto	57	65.5
Others	1	1.2
Education (years)		
$\geq$ 8 years	41	47.1
< 8 years	46	52.9
Marital status		
With partner (married/civil union)	54	62.1
Without partner (single/others)	33	37.9
Previous episode of STD		
Yes	33	37.93
No	54	62.07
Smokers		
Yes	13	14.9
No	74	85.1
Drug users		
Yes	1	1.1
No	86	98.9

Table 2
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Table 2. Cervical alterations detected by conventional cytology smear in 87 patients from State of Pernambuco.

Cytopathological Alterations	n (%)
ASC-US	35 (40.25)
LSIL	37 (42.50)
HSIL	07 (08.05)
ASC-H	06 (06.90)
AGUS	01 (01.15)
Carcinoma	01 (01.15)
TOTAL	87 100%

* ASC-US (Atypical squamous cells of undetermined significance, possibly not neoplastic); LSIL (low-grade intra-epithelial lesions); HSIL (high-grade intra-epithelial lesions); ASC-H (atypical squamous cells which could possibly be high-grade lesion); AGUS (atypical glandular cells of undetermined significance).

Table 3
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Table 3. Distribution of genital infections and STDs in 87 patients with cervical lesions from State of Pernambuco.

Infectious agent	Cervical lesions of		Total (%)	OR (95% IC)	P
	High-grade** n=50 (%)	Low-grade* n=37 (%)			
Lactobacillus sp.					
Present	3 (6.0)	1 (2.7)	4 (4.6)		
Absent	47 (94.0)	36 (97.3)	83 (95.4)	2.28 (0.17-123.85)	0.63
Coccos sp.					
Present	12 (24.0)	5 (13.5)	17 (19.5)		
Absent	38 (76.0)	32 (86.5)	70 (80.5)	2.00 (0.58-8.06)	0.28
Gardnerella vaginalis					
Present	15 (30.0)	16 (43.2)	31 (35.6)		
Absent	35 (70.0)	21 (56.8)	56 (64.4)	0.57 (0.21-1.50)	0.26
Trichomonas vaginallis					
Present	4 (8.0)	1 (2.7)	5 (5.7)		
Absent	46 (92.0)	36 (97.3)	82 (94.3)	3.10 (0.29-158.25)	0.39
Herpes virus					
Present	0 (0.0)	1 (2.7)	1 (1.2)	-	
Absent	50 (100.0)	36 (97.3)	86 (98.8)		0.43
Candida sp.					
Present	6 (12.0)	2 (5.4)	8 (9.2)	2.36 (0.39-25.36)	0.46
Absent	44 (88.0)	35 (94.6)	79 (90.8)		
Mixed flora					
Present	20 (40.0)	16 (43.2)	36 (41.4)		
Absent	30 (60.0)	21 (56.8)	51 (58.6)	0.88 (0.33-2.27)	0.83
Chlamydia trachomatis (CT)					
Present	38 (76.0)	35 (94.6)	73 (83.9)		
Absent	12 (24.0)	2 (5.4)	14 (16.1)	0.18 (0.02-0.92)	**0.036**
HPV and CT simultaneously					
Present	36 (83.7)	29 (100.0)	65 (90.3)		
Absent	7 (16.3)	0 (0.0)	7 (9.7)	-	**0.037**

* Low-grade includes ASC-US (atypical squamous cells of undetermined significance, possibly not neoplastic) and LSIL (low-grade intra-epithelial lesions)

** High-grade includes HSIL (high-grade intra-epithelial lesions); ASC-H (atypical squamous cells which could possibly be high-grade lesion); AGUS (atypical glandular cells of undetermined significance) and carcinoma.

Table 4
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Table 4.Incidence of HR-HPV subtypes in 87 patients with cervical lesions from State of Pernambuco.

HPV subtype	Cervical lesions of		Total of infections by subtype (%)
	High-grade (n=36)	Low-grade (n=29)	
Subtype 16	20	15	35 (48.6)
Subtype 18	6	4	10 (13.9)
Subtype 31	9	7	16 (22.2)
Subtype 33	6	5	11 (15.3)

Capítulo IV

Artigo 3: Comparative analyze between Liquid-based cervical cytology and Conventionally Cytology under the two Public Health reference Centers in Brazil.

Artigo *submetido* ao periódico ***Molecular Biology Reports*** **(IF: 1,95)**

Molecular Biology Reports

Comparative analyze between Liquid-based cervical cytology and Conventionally Cytology under the two Public Health reference Centers in Brazil.

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Abstract:	The goal of this study was to compare the performance of the ThinPrep, a liquid-based cytological methodology (LBC), with the conventional Papanicolaou (Pap) smear cytology (CP) for the detection of cytopathological changes, on two Public Health reference centers in Brazil (LACEN-PE and FOSP). Cervical smears were taken from 525 women referred to colposcopy clinic over a 8-month period. There was poor agreement between the two methods on diagnoses performed by the LACEN-PE team ($k = 0.1$; $p << 0.01$). On the other hand, the procedures by FOSP team showed reasonable agreement ($k = 0.39$; $p << 0.01$). The proportion of diagnostic absence (unsatisfactory results) was significantly less frequent in LBC (1.7%) compared to 4.4% for CP on LACEN-PE routine ($p<<0.01$). In contrast, the FOSP team found similar results for both methodologies ($p>0.05$). Regarding the ability to detect cytological changes, both methods were equally good in both centers. It was also observed a decrease in the number of inflammatory cells of 58.5% to 46.5% by LACEN-PE team when using the LBC. However, the FOSP team detected an increment (78.1% on CP to 81.5% on LBC). The most frequent microbiota in both LACEN-PE and FOSP evaluations were Lactobacillus sp or mixed with Coccus sp. We conclude that there is need for exchanges of experience and protocols between the two centers to better standardize the readings specimens and criteria of inflammatory and normal cytology diagnosis.
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Avaliação e interpretação de possíveis causas de erros da CC x LBC.

Comparative analyze between Liquid-based cervical cytology and Conventionally Cytology under the two Public Health reference Centers in Brazil.

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ABSTRACT

The goal of this study was to compare the performance of the ThinPrep, a liquid-based cytological methodology (LBC), with the conventional Papanicolaou (Pap) smear cytology (CP) for the detection of cytopathological changes, on two Public Health reference centers in Brazil (LACEN-PE and FOSP). Cervical smears were taken from 525 women referred to colposcopy clinic over a 8-month period. There was poor agreement between the two methods on diagnoses performed by the LACEN-PE team ($k = 0.1$; $p << 0.01$). On the other hand, the procedures by FOSP team showed reasonable agreement ($k = 0.39$; $p << 0.01$). The proportion of diagnostic absence (unsatisfactory results) was significantly less frequent in LBC (1.7%) compared to 4.4% for CP on LACEN-PE routine ($p<<0.01$). In contrast, the FOSP team found similar results for both methodologies ($p>0.05$). Regarding the ability to detect cytological changes, both methods were equally good in both centers. It was also observed a decrease in the number of inflammatory cells of 58.5% to 46.5% by LACEN-PE team when using the LBC. However, the FOSP team detected an increment (78.1% on CP to 81.5% on LBC). The most frequent microbiota in both LACEN-PE and FOSP evaluations were *Lactobacillus sp* or mixed with *Coccus sp.* We conclude that there is need for exchanges of experience and protocols between the two centers to better standardize the readings specimens and criteria of inflammatory and normal cytology diagnosis.

KEYWORDS: Papanicolaou smear; Cytopathological diagnosis; public healthcare; cervical cancer.

Avaliação e interpretação de possíveis causas de erros da CC x LBC.

Introduction

The cytopathology has been used for over 50 years with successful employment in diagnosis of cervical cancer, as well as other non-gynecological diseases [1-2]. In Brazil, data from Instituto Nacional do Câncer (Cancer National Institute, INCA) estimated 15.590 new cases of cervical cancer in 2014. In the State of Pernambuco, Northeast Brazil, there is an estimated incidence rate of 20.67 new cases for 100,000 people and 970 new cases per 100,000 people [3].

In some populations, the incidence of cervical cancer has a bimodal distribution, a first peak at around age 20 and 29 years, and a second peak around age 45–50 years. At the same time, this is a cancer that has great potential for prevention and is curable when diagnosed early [4-5].

During 2013, 517,895 conventional cytopathology lab tests were performed in Pernambuco state through the Brazilian public healthcare system (SUS)-accredited laboratories. Among the 185 municipalities, 76 (41.08%) had absence of diagnostic (unsatisfactory results) frequency above 5% [3]. The Women Unit Laboratories, a laboratory network linked to Pernambuco Central Laboratory (LACEN-PE), is currently responsible for carrying out the quality control of SUS-accredited laboratories. In 2011, for example, there were 9367 unsatisfactory tests, corresponding to 8.16% of all performed lab tests. Furthermore, we detected a low number of abnormal findings on cytopathological examination (0.6-1.5%), which may represent low quality of cytopathological lab tests services in the state of Pernambuco.

The conventional Papanicolaou (Pap) smear cytology test is the most widely used test in the Brazilian public healthcare system. It is as a tool for early detection of cervical abnormalities through cellular morphology observation. This methodology is considered efficient, fast, low-cost and with able to identify cervical cancer precursor lesions, which is a treatable cancer when diagnosed early, resulting in a significant decrease in mortality chance [1-2]. The constant repetition of the test at intervals of one to five years, risk population coverage and quality control procedures are the reasons for the Pap test wide adoption on public health screenings [6-8]. Despite the perceived effectiveness of the method, the accuracy of the Pap cytology has been questioned due to high rates of false-negative results [6]. The large variation in sensitivity rates (2% to 90%) demonstrates the vulnerability of the procedure, particularly susceptible to failure

of the sampling techniques and preparation of smears, as well as to the subjectivity in cytology findings interpretation [9].

The aim of this study was to compare the performance of conventional Pap smear cytology (CP) test with the ThinPrep liquid-based cytology method (LBC), when used by two reference public healthcare services in Brazil (LACEN-PE and Fundação Oncocentro, São Paulo state - FOSP). The obtained results will be used to seek further standardization of procedures, to help bring improvements in quality of diagnosis and cervical cancer follow-up on the Pernambuco public healthcare network.

Methods

We performed a cross-sectional population-based study with women aged 18-64 years, assisted by spontaneous demand in Public Health Units in the State of Pernambuco, Brazil, from April to November 2011. The study excluded women who underwent radiotherapy (RAD) or chemotherapy (QT) for cervical invasive cancer and / or have done collecting cytology within three months before recruitment..

A double-blind study was designed to evaluate the performance of the two applied cytological methods: CP and LBC for paired specimens (each sample was evaluated by the two methods). Additionally, the samples were further evaluated by pathologists teams of two reference centers in Brazil, Central Public Health Laboratory of Pernambuco (LACEN-PE) and Oncocentro Foundation of São Paulo-SP (FOSP-SP).

The specimens were stained by the Papanicolaou technique, and classified according to international standard standards (Bethesda System 2001 / adaptation by the Brazilian Society of Cytopathology-SBC) in both centers. The collection of cervicovaginal materials by the method of cytology in liquid medium was similar to that collected by the conventional method, by changing the instrument used and the amount of turns made to obtain the sample.

In half the samples, the material was initially transferred to the conventional matte blade spatula and endocervical brush Ayres, polyethylene glycol and fixed spray, and then made a new collection and stirred in the supernatant liquid through the ThinPrep ®Pap Test [10] and in the other, the endocervical brush was first stirred in the supernatant of the liquid medium, and soon after, took up new brush and spatula to conventional cytology.

The samples were transported cervicacis the close of business the Central Public Health Laboratory of the State of Pernambuco (PE-LACEN) where they were refrigerated (between 5 and 8 ° C) for analysis by maximum time of one month.

The study was approved by the Research Ethics Committee of UFPE No. 105/09-CCS and all patients included agreed to participate by signing the Informed Consent and Informed and questionnaires were completed.

Methodology performance comparison was performed through Kappa test. The McNemar and Pearson chi-square tests were used to better assess the eventual disagreements between methodologies. All statistical tests were performed trough Epi Info version 5.0 or higher, with double entry and a significance level of 5%.

Results

Five hundred and twenty-five patients met the criteria for the diagnostic standard for the study. The mean age of the women was 32.5 years (DP± 5). The comparisons between CP and LBC smears are shown in Tables 1 and 2 (LACEN-PE and FOSP-SP, respectively). There was low level of agreement between two methodologies when evaluated by LACEN-PE team (Cohen's kappa test = 0:19; 95% CI = 0.11-0.2; $p << 0.01$), whereas for FOSP-SP team there was a reasonable agreement (Cohen's kappa test = 0.39; 95% CI = 0.29 to 0.50; $p << 0.01$).

In the evaluation of LACEN-PE team, the LBC smear tended to improve the performance of cytological diagnosis, since all 23 samples with unsatisfactory results by CP had defined diagnostics by using the LBC (McNemar test $\chi 2 = 5.28$; $p = 0.02$). On the other hand, of the 502 samples diagnosed by LBC only 9 (9/502) failed to establish the diagnosis by CP. Thus, there was a decrease of unsatisfactory rate of 4.38% CP to 1.7% with LBC in this analysis [Table 1]. Both methods agreed in reporting cytopathological findings, showing no statistical difference between detection rates (McNemar test $\chi 2 = 0.24$; $p = 0.63$). The classified as inflammatory cytology were present in 58.5% of cases (307/525) by CP, while the LBC were present in 46.5% of cases (244/525), indicating that the LBC reduced the interfering inflammatory by improving specimen qualitty and reducing cell morphology interference.

In relation to the FOSP-SP team, CP and LBC were equally good for detecting cytological changes ($x^2 = 5.00$; $p = 0.17$). Conventional cytology showed 3.20% (17/525) of unsatisfactory results, against 3.60% for LBC (19/525) [Table 2].

Abnormality findings also showed equally good concordance (5.60% CP versus 4.20% LBC). The inflammatory cytology was present in 78.1% of cases (410/525) by CP, whereas in LBC cases observed 81.5% (428/525), indicating that the São Paulo criteria inflammatory cytology should also be better assessed (Table 2).

Regarding microbiota detection, in the evaluation of LACEN-PE, the most prevalent flora found by CP, was *Lactobacillus sp + Coccus ssp* - (191/525) as mixed flora, or *Coccus ssp* (108/525) and *Lactobacillus sp* (106/525) followed by *Gardnerella vaginalis* (78/525) and *Candida sp* (32/525), as individual flora. The similar prevalence was observed during LBC evaluating [Table 3]. The most present flora by the FOSP CP evaluation were *Lactobaccilus sp* (281/525), followed by *Cocos sp* (44/525), *Gardnerella vaginalis* (50/525) and *Candida sp* (13/525) as individual flora, with also a somewhat similar distribution observed during LBC evaluation (Table 3).

Discussion

The introduction of liquid based cytology public health in Pernambuco State, Northeast of Brazil, may allow the establishment of better standards in the collection, preparation and staining of cervical samples. This would ensure an improvement in the quality of CP analysis, since it reduces the process variably and human interference. With this technique, all the specimens could be processed with assured quality, through an accurate and fast reading [11-12].

This statement was also observed in our study findings, since we noted the improvement in the diagnostics, by reducing the number of unsatisfactory reports (Table 1). Similar results are found by other studies [13-19] suggesting that in both cervico-uterine and vaginal samples the LBC has been reported as a method superior performance providing better representation cell with increased sensitivity for detecting lesions compared to the conventional preparation.

The great challenge of Pap cytology is the standardization of sample processing. Difficulty arises from the need for manual intervention and the involvement of various professionals in various stages of the conventional methodology. Despite the training of these professionals, they are susceptible to many variables, such as the turnover of supervisor personnel in key steps of the process [16-18]. Although there are guidelines for such a procedure, there is no guarantee of simple and effective way to quality and standardization of the process when performed manually. The processing and reading

of the samples, according to the Executive Summary of the National Cervical Cancer Control Program Uterus published in 2010, OPAS (Pan American Health Organization) recommends that to maintain the quality standards, a laboratory needs production of at least 15,000 scans / year. In Brazil, between laboratories that provided services to the Single Health System (SUS) in 2008, only 15% of a total of 1,116 units had production above this threshold.

High rates of false-negative (27.3%) and false positive (12.5%) results were found when conventional cytology was used as a screening method in the National Program Against Cervical Cancer [20]. False-negative results are mainly due to collection errors, scrutiny and subjective interpretation of diagnosis. EJERSBO *et al* (2006)[21] report that, to prevent false-negative results, it is important the training and continuing education of the tellers. MAEDA *et al* (2004)[22] showed in their studies the same concern, and the need strategies such as monitoring of procurement procedures, fixation, transport cytological material, internal and external quality programs, training / retraining, and review laboratory indicated by competent body or reference laboratory in the region.

Since errors during sample collection is responsible for 20-39% of false-negative results [9], LBC adoption would greatly minimize problems during the collection, by removing interfering material from samples. Therefore, we surmise that the LBC is as a facilitator, bringing convenience to cytology diagnostic process, since it reduce the scanning area of up to 81% and eliminates interfering materials that normally obscure the sample, which allows a gain of about 50% at the reading time, and improvements of up to 73% in laboratory productivity [12].

Although the ability to detect cytological abnormalities for both methods were equally good in the two centers, as the LACEN-PE team used LBC, there was a reduction in the number of unsatisfactory results of 4.38% with CP to 1.71% with LBC, as noted previously. However, the same was not observed by the FOSP team, where both methodologies had similar results. This means that the adoption of LBC by LACEN-PE and associated with an internal quality control programs in routine laboratory would bring great improvement for diagnostic reporting.

Furthermore, we observed that there was an individual variability in the diagnosis of inflammatory cytology and normal cytology. There was a decrease in the number of inflammatory cells of 58.5% the CP to 46.5% in LBC, on analysis by

LACEN-PE team. However, research conducted by the team of FOSP-SP, shows an increase in the number of cells classified as inflammatory, in both methods, 78.1% (410/525) and 81.5% (428/525) respectively, CP and LBC [Table 2].

The primary goal of cervicovaginal cytology is the detection of precancerous lesions of the cervix, although the method enables the recognition and evaluation of the intensity of the inflammatory processes. In many of these conditions can also establish the causal agent [23]. Ours results showed difference in the evaluation of prevalence of the vaginal microflora between the two reference centers (Table 3). This analysis shows the need for better standardization of diagnostic and protocols of cytomorphological inflammatory and normal cytology criteria.

Conclusion

The determination and pattern definitions proved to be the milestone in the definition and diagnosis for both methodologies and services.

Therefore, although the liquid medium cytology have shown improvements in setting standards in the collection, preparation and staining of the samples, in the view of LACEN-PE, a much greater need for the standardization of specimens implementations readings was observed that the currently being used. Possibly an exchange of experiences and protocols between the two centers can promote improvements in these indicators.

Abbreviations

CC - conventional cytology Papanicolaou

LBC- ThinPrep liquid-based cytology

FOSP-SP- the Oncocentro Foundation of Stade of São Paulo

LACEN-PE- Central Public Health Laboratory of the State of Pernambuco

OPAS - Pan American Health Organization

QT- Chemotherapy

RAD- Radiotherapy

Competing interests

The researchers and advisors of the study state that there are no conflicts of interest to the survey.

Authors' contributions

Conception and design: MOLPC, PRES; Collection and assembly of the data: LCPBC, VLA; Analysis and interpretation of the data: MOLPC, PRES, AVCC, LCPBC; Statistical expertise: AVCC; Drafting of the manuscript: MOLPC, PRES, AVCC, MTSC.

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Table 1
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Table 1. Results of diagnostic interpretations evaluated by LACEN *, of 525 patients who underwent the methodology of conventional cytology and Pap cytology based liquid - ThinPrep® in the state of Pernambuco, 2011.

LACEN-PE (n=525)	Conventional Cytology n (%)	Cytology based-liquid (CBL) n (%)	*K* (IC95%)	*p*
Normal**	182 (34,7)	256 (48,8)		
Inflammatory	307 (58,5)	244 (46,5)	0,19	<<0,01
Altered ***	13 (2,40)	16 (3,00)	(0,11-0,26)	
Absense of diagnostic (unsatisfactory)	23 (4,40)	9 (1,70)		

* Central Laboratory of Public Health of the State of Pernambuco- Brazil

** Includes normal cytology and atrophy.

***ALTERADO: ASC-US/ASC-H/LSIL/HSIL/ CA in situ

Table 2
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Table 2. Results of diagnostic interpretations evaluated by FOSP-SP *, of 525 patients who underwent the methodology of conventional cytology and based-liquid - ThinPrep® in the state of Pernambuco, 2011.

FOSP-SP (n=525)	Conventional cytology (CP) n (%)	Cytology based-liquid (CBL) n (%)	*K* (IC95%)	*p*
Normal**	69 (13,1)	56 (10,7)		
Inflammatory	410 (78,1)	428 (81,5)	0,39	<<0,01
Altered ***	29 (5,60)	22 (4,20)	(029-0,50)	
Absense of diagnostic (unsatisfactory)	17 (3,20)	19 (3,60)		

* Oncocentro Foundation of Sao Paulo- Brazil

** Includes normal cytology and atrophy.

***ALTERADO: ASC-US/ASC-H/LSIL/HSIL/ CA in situ

Table 3
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Table 3. Results of Diagnostic Interpretations of vaginal flora by Cytology Methodology Conventional Pap and Cytology based-liquid- ThinPrep® by LACEN-PE* and FOSP-SP**, of the 525 patients analyzed.

Microflora	LACEN-PE		FOSP-SP	
	CP	CBL	CP	CBL
Candida Albicans	32	30	13	17
Clhamdydia Trachomatis	0	1	0	0
Coccus spp	108	174	44	48
Gardenerella vaginalis	78	76	50	53
Herpesvirus	0	0	0	0
Lactobacillus spp	106	83	281	247
Mista (Lactobacillus spp + Coccus spp)	191	178	93	89
Trichomonas spp	8	1	5	1
Não identificada	42	13	57	87

CP= Conventional Papanicolaou cytology
CBL= Cytology based-liquid
LACEN-PE= Central Laboratory of Public Health of the State of Pernambuco- Brazil
FOSP-SP = Oncocentro Foundation of Sao Paulo-Brazil

Capítulo V

CONCLUSÕES

- A metodologia ThinPrep apresentou melhora na qualidade do diagnóstico citológico das amostras cervicais e contribuiu na diminuição do número de resultados insatisfatórios nos laudos da rede de Saúde Pública do Estado de Pernambuco- Brasil.

- A introdução da citologia em meio líquido na rotina da rede pública do Estado de Pernambuco, permitirá o estabelecimento de padrões na coleta, preparo e coloração das amostras, além de diminuir possíveis perdas por repetição citológica, e possibilita a investigação das DST na população desta região.

- A Citologia Convencional e a Citologia em Meio Líquido demonstraram que são igualmente boas para detectar alterações citopatológicas.

- A avaliação pelas duas metodologias feito pela Fundação Oncocentro de São Paulo (FOSP-SP) apresentaram razoável concordância, entretanto sob a avaliação do LACEN-PE, apresentaram fraca concordância.

- Os testes avaliados pelo LACEN-PE, quanto ao padrão das alterações citopatológicas e diagnóstico de exames insatisfatórios, foram melhores com a utilização da metodologia *ThinPrep*, assim como os critérios citomorfológicos dos processos inflamatórios e normais, foram melhores estabelecidos e avaliados. Possivelmente uma troca de experiências e protocolos entre os dois centros pudesse ser de utilidade para estabelecer padrões de rotina e diagnósticos, e assim contribuir na melhorias dos indicadores no Estado de Pernambuco.

- Foi observado possível correlação entre o epitélio inflamado e agentes infecciosos, com as alterações cervicais, sendo estas alterações mais prevalentes em mulheres mais jovens, menos instruídas, com história pregressa de DST, e não usuárias de métodos anticonceptivos.

- As infecções genitais, em especial a *Chlamydia trachomatis*, devem sem investigadas e tratadas adequadamente, haja vista, que co-infecções com o HPV estão associadas ao favorecimento de lesões de baixo-grau, e podem evoluir a lesões mais graves, como o carcinoma de colo de útero.

- A *Gardenerella vaginalis*, por si só, não é um agente causal do processo inflamatório, entretanto o seu aumento populacional acompanha a redução da concentração de lactobacilos e o aumento de agentes anaeróbios obrigatórios, que desencadeiam a inflamação, favorecem ao desencadeamento das lesões cervicais.

- Os subtipos de HPV mais prevalentes na rede pública do Estado de Pernambuco foram o HPV16 (48,6%) e o HPV 31 (22,2%).

- As infecções genitais, em especial a *Chlamydia trachomatis*, o vírus do HPV e outras infecções genitais, como a *Gardnerella vaginalis*, devem sem investigadas e tratadas adequadamente, haja vista, a sua associação direta no favorecimento das lesões pré-neoplásicas, e no favorecimento da atuação do vírus do HPV.

COMENTÁRIOS IMPORTANTES:

- Apesar da introdução de diferentes tecnologias com propostas inovadoras, visando melhoria na rotina do diagnóstico citológico, o teste de Papanicolaou Convencional ainda é um método de EXCELÊNCIA.

- Além da efetividade diagnóstica, pesa muito a favor da Citologia Convencional de Papanicolaou o seu baixo custo, propiciando a sua aplicação em larga escala nos programas de Saúde Pública.

Capítulo VI

ANEXOS

Brazilian Journal of medical and biological research

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INSTRUCTIONS TO AUTHORS

SUBMIT A MANUSCRIPT

How to submit a manuscript to the BJMBR

Complete and detailed Guidelines for Authors

Scope and policy

The purpose of the BRAZILIAN JOURNAL OF MEDICAL AND BIOLOGICAL RESEARCH is to publish the results of original experimental research that contribute significantly to knowledge in medical and biological sciences. Preference will be given to manuscripts that develop new concepts or experimental approaches and are not merely repositories of data. Papers that report negative results require special justification for publication. Methodological papers shall be considered for publication provided they describe new principles or a significant improvement of an existing method.

Papers that will not be accepted for publication

- Studies on people not approved by an accredited Ethics Committee or without written informed consent from the subject or legal guardian.
- Studies on animals not approved by an accredited Ethics and Animal Care Committee.
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confirm previously reported results.
- Manuscripts that describe the pharmacodynamics, bioavailability and toxicity of drugs in people or animals.
- Manuscripts that deal with transcultural adaptation and validation of instruments of measurements.
- Manuscripts that translate a text published in another language and validate it on local patients.
- Manuscripts that use questionnaires translated from the language of another country and their validation in local patients.
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The authors are responsible for "publication charges" of all accepted papers. Publication charges will be billed to the Corresponding Author when the paper is accepted.

The charge is **R$2.200,00** /paper for Brazilian authors and **US$1.400,00** /paper for authors outside Brazil and is independent of the length of the paper.

The Journal does not provide reprints to corresponding authors. They will receive a CD containing the issue in which the paper is published. There is no charge for figures in color. Please contact Reinaldo de Souza (bjournal@fmrp.usp.br) if you have any questions.

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The Brazilian Journal of Medical and Biological Research is a peer-reviewed electronic journal published monthly by the Associação Brasileira de Divulgação Científica (ABDC).

Submission of a manuscript to the Brazilian Journal implies that the data have not been published previously and will not be submitted for publication elsewhere while the manuscript is under review.

The following represent "prior publication": any printed material in excess of 500 words describing results or methods of a submitted/in press manuscript; published tables or illustrations that duplicate the content of a manuscript; electronic manuscripts or posters available via the Internet. When part of the material in a manuscript has been presented as a preliminary communication or in an unrefereed symposium, this should be cited as a footnote on the title page and a copy should accompany the submitted manuscript.

Manuscript Submission

The cover letter should contain the following information:

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- If a version of the manuscript has been previously submitted for publication to another journal, include comments from the peer reviewers and indicate how the authors have responded to these comments.
- Papers in the area of Clinical Investigation should include a statement indicating that the protocol has been approved by the Hospital Ethics Committee (Hospital with which at least one of the authors is associated) and that written informed consent was obtained from all participants.
- Animal experimentation should be carried out according to institutional guidelines for experimental use of animals.
- The authors should obtain written permission to reproduce figures and tables from other sources.

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The Brazilian Journal are organized into sections. Authors should specify in the cover letter the specific section in which they prefer to publish their paper.

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- Blood, immunology and organ transplantation
- Cardiovascular, respiratory and sport medicine
- Digestive system
- Endocrine diseases, nutrition and metabolism
- Environmental factors of diseases
- Health care and community medicine
- Infectious agents and diseases
- Kidney and extracellular environment
- Neonatal medicine, growth and development
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- Reproductive medicine
- Skeletal, muscle and nervous systems
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Full-length Paper.

Each manuscript should clearly state its objective or hypothesis; the experimental design and methods used (including the study setting and time period, patients or participants with inclusion and exclusion criteria, or data sources and how these were selected for the study); the essential features of any interventions; the main outcome measures; the main results of the study, and a section placing the results in the context of published literature.

The manuscript should contain:

- an abstract of no more than 250 words
- no more than 6 key words
- a running title to be used as a page heading, which should not exceed 60 letters and spaces
- the text should be divided into separate sections (Introduction, Material and Methods, Results, Discussion), without a separate section for conclusions

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Short Communication

A short communication is **a report on a single subject**, which should be concise but definitive. The scope of this section is intended to be wide and to encompass methodology and experimental data on subjects of interest to the readers of the Journal.

The manuscript should contain:

- abstract of no more than 250 words
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- a running title to be used as a page heading, which should not exceed 60 letters and spaces
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- no more 20 references (without exceptions)
- no more than three illustrations (figures and/or tables)

Review Article

A review article should provide a synthetic and critical analysis of a relevant area and should not be merely a chronological description of the literature. A review article by investigators who have made substantial contributions to a specific area in medical and biological sciences will be published by invitation of the Editors. However, an outline of a review article may be submitted to the Editors without prior consultation. If it is judged appropriate for the Journal, the author(s) will be invited to prepare the article for peer review. A minireview is focused on a restricted part of a subject normally covered in a review article.

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- a rare case that is particularly useful to demonstrate a mechanism or a difficulty in diagnosis
- new diagnostic method
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- a text that demonstrates relevant findings and is well documented and without ambiguity.

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- abstract of no more than 250 words
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- the text may be divided into sections with appropriate titles and subtitles.
- no more 20 references (without exceptions)
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An overview does not contain unpublished data. It presents the point of view of the author(s) in a less rigorous form than in a regular review or minireview and is of interest to the general reader.
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The main characteristic of research papers in the area of Cell Biology is the emphasis on the integration at the cellular level of biochemical, molecular, genetic, physiological, and pathological information. This section considers manuscripts dealing with either prokaryotic or eukaryotic biological systems at any developmental stage. Papers on all aspects of cellular structure and function are considered to be within the scope of Cell Biology by the BJMBR. The Editors encourage submission of manuscripts defining cell biology as an area of convergence of several other research fields, especially manuscripts providing insights into the cellular basis of immunology, neurobiology, microbial pathology, developmental biology, and disease. Manuscripts containing purely descriptive observations will not be published. Manuscripts reporting new techniques will be published only when adequately validated and judged by the Editors to represent a significant advance.

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- Papers should describe the separation of the crude material into fractions (not necessarily into homogeneous materials) with the fractions containing biological activity identified clearly in the separation scheme. Phytochemical studies should be accompanied by biological tests. A survey of pharmacological activity of plant extracts or teas will not be considered for publication.
- In addition to the demonstration of activity in one or more biological system, experiments must be performed attempting to provide information concerning the mechanism(s) of action of the substance(s) being tested.
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- **The Journal does not publish toxicological**

studies.

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Only those persons who contributed directly to the intellectual content of the paper should be listed as authors. Authors should meet all of the following criteria, thereby allowing persons named as authors to take public responsibility for the content of the paper.

- Conceived, planned and carried out the experiments that led to the paper or interpreted the data it presents, or both.
- Wrote the paper, or reviewed successive versions.
- Approved the final version.
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Manuscripts should be submitted in English. Authors are requested to use American spelling, except, of course, for references whose titles should appear exactly as published. Guidance on grammar, punctuation, and scientific writing can be found in the following sources: Scientific Style and Format: The CSE Manual for Authors, Editors, and Publishers. 7th edn. Rockefeller University Press, Reston, 2006; Medical Style and Format. Huth EJ (Editor). ISI Press, Philadelphia, 1987, Marketed by Williams & Wilkins, Baltimore, MD. The Brazilian Journal of Medical and Biological Research follows the reference format of the Uniform Requirements for Manuscripts Submitted to Biomedical Journals, which can be found on the website of the National Library of Medicine (**http://www.nlm.nih.gov/bsd/uniform_requirements.html**).
Text Format The text of a manuscript can only be accepted as a Microsoft Word

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- Report all measurements in Système International, SI (**http://physics.nist.gov/cuu/Units**) and standard units where applicable (see below).
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- The length of the manuscript and the number of tables and figures must be kept to a minimum.
- Ensure that all references are cited in the text.
- Generic names must be used for all drugs. Instruments may be referred to by proprietary name; the name and country or electronic address of the manufacturer should be given in parentheses in the text.

Footnotes. Text footnotes, if unavoidable, should be numbered consecutively in superscript in the manuscript and written on a separate page following the abstract.

Headings in text

- Position all headings flush with the left margin.
- Keep headings short (three or four words).
- Use only three types of headings in the text. Clearly indicate the type of level of headings by using the following typographic conventions.
    - First-level: Only the 1st letter of the 1st word is capitalized, font size 11**, bold type**.
    - Second-level: Only the 1st letter of the 1st word is capitalized, font size 9**, bold type**.
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Abbreviations and symbols

- Explain all abbreviations in the text, figure and table legends when they first appear. Keep the number of abbreviations to a minimum.
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- s for second
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- h for hour
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- m for meter
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- 5 mM rather than 5 x 10-3 M or 0.005 M

Title page.
The title page should contain the following information:

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- Initials and last name(s) of author(s) (matched with superscript numbers identifying institutions).
- Institution(s) (Department, Faculty, University, city, state, country) of each author (in Portuguese if authors are from Brazil).
- Acknowledgment of research grants and fellowships (agency and grant number).
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Abstract

- Since abstracts are published separately by Information Services, they should contain sufficient hard data to be evaluated by the reader.
- The abstract should briefly and clearly present the problem, experimental approach, new results as quantitative data if possible, and conclusions.
- The abstract should not exceed 250 words and should be written as a single paragraph double-spaced on a separate page following the title page.
- Abbreviations should be kept to a minimum and must be defined at

first citation.
- If the use of a reference is unavoidable, the full citation should be given within the abstract.
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Introduction. This should state the purpose of the investigation, relationship to other work in the field, and justification for undertaking the research. An extensive listing or review of the literature is not recommended.

Material and Methods. Sufficient information should be provided in the text or by referring to papers in generally available journals to permit the work to be repeated and to determine the suitability of the methods used for the objectives of the research.

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Acknowledgments. When appropriate, briefly acknowledge technical assistance, advice and contributions from colleagues to the research. Financial support for the research and fellowships should be acknowledged on the title page.

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- Tables must be submitted in word (.doc) or Excel (.xls).
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- Tables must have a concise and descriptive title.
- All explanatory information should be given in a footnote below the table. Footnotes should be used to explain abbreviations and provide statistical information.
- All abbreviations must be defined in this footnote, even if they are explained in the text.
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- Vertical and diagonal lines should not be used in

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- Tables in Excel must be cell-based; do not use picture elements, text boxes, tabs, or returns in tables.

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Brazilian Journal of Medical and Biological Research encourages authors to use figures where this will increase the clarity of an article. The use of color figures in articles is free of charge. The following guidelines must be observed when preparing figures. Failure to do so is likely to delay acceptance and publication of the article.

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- Each figure should be closely cropped to minimize the amount of white space surrounding the illustration. Cropping figures improves accuracy when placing the figure in combination with other

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- PowerPoint (suitable for diagrams and/or images, figures must be a single page)
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- Details of the magnification should be given as a magnification bar.
- Details of any stains used and the method of preparation the sample should be given in the figure legend or in the Methods section.
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- The type of camera, photographic software and details of any subsequent image manipulation should be given in the article text.

References

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The Brazilian Journal of Medical and Biological Research follows the reference format of the Uniform Requirements for Manuscripts Submitted to Biomedical Journals, which can be found on the website of the National Library of Medicine (**http://www.nlm.nih.gov/bsd/uniform_requirements.html**). Use the Medline journal abbreviations and follow the reference style shown on the Website noted above, with several exceptions. See below for details. If the author uses the program "Reference Manager", copy the file containing the **style of the Brazilian Journal of Medical and Biological Research <http://bjournal.com.br/Brazilian%20Journal%20of%20Medical%20and%20Biological%20Research.zip>** and place it in the folder of "Styles". When submiting the manuscript, send the file produced in Reference Manager (".rmd" and ".rmx") as an attachment.
The following information must be given in the list of references:
Standard article. Up to the first 6 authors followed by et al., Title, Journal (abbreviation), Year, Volume, Complete Pages.

- Xu J, Liu M, Liu J, Caniggia I, Post M. Mechanical strain induces constitutive and regulated secretion of glycosaminoglycans and proteoglycans in fetal lung cells. *J Cell Sci* 1996; 109 (Pt 6): 1605-1613.
- Poirier P, Lemieux I, Mauriege P, Dewailly E, Blanchet C, Bergeron J, et al. Impact of waist circumference on the relationship between blood pressure and insulin: the Quebec Health Survey. *Hypertension* 2005; 45: 363-367.
- The Cardiac Society of Australia and New Zealand. Clinical exercise stress testing. Safety and performance guidelines. *Med J Australia* 1996; 164: 282-284.

Abstract. Up to the first 6 authors followed by et al., Title, Journal (abbreviation), Year, Volume, Complete Pages (Abstract).

- Lima SM, Bonci DM, Grotzner SR, Ribeiro CA, Ventura DF. Loss of amacrine cells in MeHg-treated retinae in a tropical fish.*Invest Ophthalmol Vis Sci* 2003; 44: E-5172 (Abstract).

Article accepted for publication but not yet published. Up to the first 6 authors followed by et al., Title, Journal (abbreviation), Year of expected publication, (in press) at the end of the citation.

- Janiszewski M, Lopes LR, Carmo AO, Pedro MA, Brandes RP, Santos CXC, et al. Regulation of NAD(P)H oxidase by associated protein disulfide isomerase in vascular smooth muscle cells. *J Biol Chem* 2005 (in press).

"**Unpublished results", "Personal communication" and "Submitted papers"**. Reference should appear in the text with the individual name(s) and initials and not in the reference list.

- (Santos CS, da-Silva GB, Martins LT, unpublished results).
- It is assumed that the author has obtained written permission from the

source when "personal communication" is cited.

Book, whole. Authors, Book title, Edition, City, Publisher, Year.

- Norman IJ, Redfern SJ. *Mental health care for elderly people*. New York: Churchill Livingstone; 1996.

Book, chapter. Authors, Chapter Title, Editors, Book title, Edition, City, Publisher, Year, Pages of citation.

- Kintzios SE. What do we know about cancer and its therapy? In: Kintzios SE, Barberaki MG (Editors), *Plants that fight cancer*. New York: CRC Press; 2004. p 1-14.
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- National Commission on Sleep Disorders Research. *Wake up America: a national sleep alert*. Washington: Government Printing Office; 1993.

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- Joselevitch C. Visão no ultravioleta em Carassius auratus (Ostariophysi, Cypriformes, Cyprinidae): estudo eletrofisiológico do sistema cone - células horizontais. [Master's thesis]. São Paulo: Instituto de Psicologia, USP; 1999.

Conference, Symposium Proceedings. Cite papers only from published proceedings.

- Hejzlar RM, Diogo PA. The use of water quality modelling for optimising operation of a drinking water reservoir. *Proceedings of the International Conference Fluid Mechanics and Hydrology*. 1999 Jun 23-26; Prague. Prague: Institute of Hydrodynamics AS CR; 1999. p 475-482.

Electronic citations (Online Journals). Ensure that URLs are active and available.

- American Academy of Ophthalmology. Diabetic retinopathy disease severity scale. *Am Acad Ophthalmol*http://www.aao.org/education/library/recommendations/international_dr.cfm; 2005.
- Simon JA, Hudes ES. Relationship of ascorbic acid to blood lead levels. *JAMA* http://jama.ama-assn.org/cgi/content/abstract/281/24/2289; 1999.

Internet communication. Ensure that URLs are active and available. Provide

DOI, if available.

- Developmental toxicology. http://www.devtox.org/nomenclature/organ.php. Accessed June 27, 2005.
- CAPES Statistics. http://www.capes.gov.br/capes/portal. Accessed March 16, 2006.
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- *Physician's Desk Reference (PDR)*. Release 2003.1AX. [CD-ROM]. Montvale: Thomson PDR; 2003.

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- Dean AG, Dean JA, Coulombier D, Brendel KA, Smith DC, Burton AH, et al. *Epi info, version 6.04: a word processing database and statistics program for public health on IBM-compatible microcomputers*. [Computer program]. Atlanta: Centers of Disease Control and Prevention; 1998.
- *Statistical Package for the Social Sciences (SPSS)*. Version 12.0. [Computer program]. Chicago: SPSS Inc.; 2006.

Patent

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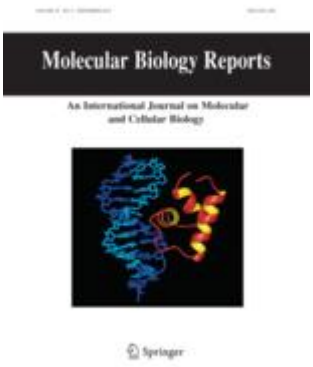

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An International Journal on Molecular and Cellular Biology

Editor-in-Chief: Nilanjana **Maulik**
ISSN: 0301-4851 (print version)
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Journal no. 11033

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Guidelines for Short Communication Articles

1. Article Length: 2,500 words or less

2. References: 25 or less

3. Results and Discussion sections may be combined

4. Figures and/or Tables: 3 or less

5. Article must contain clinical significance

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Max Word Count: 250 words
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II. Methods and Results: Brief explanation of experimental procedure and presentation of significant results. Include sample sizes as well as animal species if applicable.
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The main text of the review article should be divided into sections. Examples for headings include:

I. Background

II. Recent Studies and Results

III. Points of Dispute or Unanswered Questions

IV. Potential Research/Future

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II. Recent Studies and Results

III. Points of Dispute or Unanswered Questions

IV. Potential Research/Future

The majority of the review should focus on the most recent findings and their implications. Points of dispute or controversial ideas should not be overlooked or ignored. Authors should consider the future direction for further research as well as provide information for studies that are currently going on.

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- Online document

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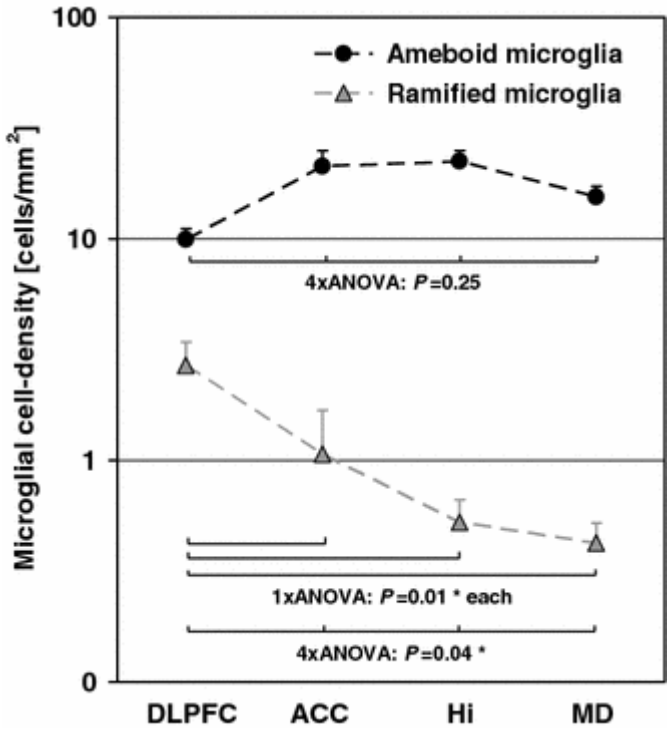

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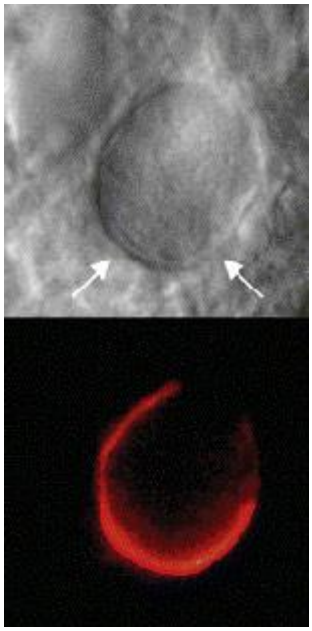

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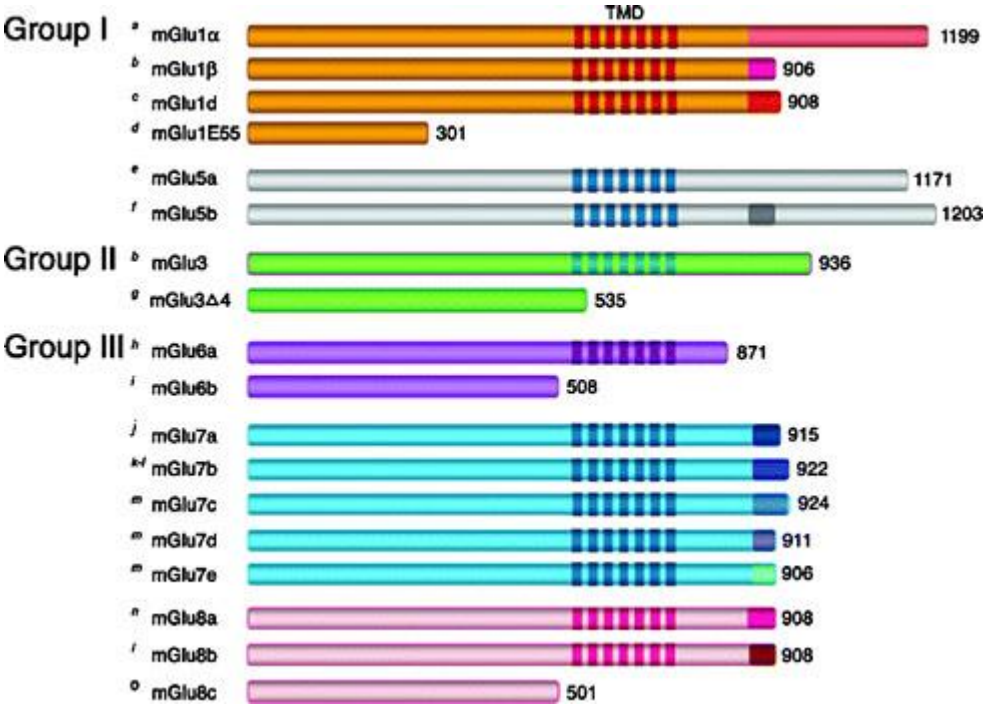

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Pesquisador Responsável: Paulo Roberto Eleutério de Souza

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

ESTUDO COMPARATIVO E DE EFICÁCIA ENTRE CITOLOGIA CONVENCIONAL X CITOLOGIA EM MEIO LÍQUIDO E AVALIAÇÃO DO DIAGNÓSTICO E SEGUIMENTO DAS DST A NÍVEL DE SAÚDE PÚBLICA NO ESTADO DE PERNAMBUCO

LISTA DE CHECAGEM

Nome _____

Registro ☐☐☐☐☐☐

Critérios de Inclusão

Mulheres que procurarem a Unidade Móvel do LACEN. 1. ☐ Sim 2. ☐ Não

Faixa etária 18 a 60 anos 1. ☐ Sim 2. ☐ Não

Critérios de Exclusão

Radioterapia ou quimioterapia para Neoplasia invasiva pélvica 1. ☐ Sim 2. ☐ Não

Realização de citologia oncótica dentro do prazo de 3(três) meses. 1. ☐ Sim 2. ☐ Não

CONCLUSÃO

☐ Elegível ☐ Não elegível

CONCORDA EM PARTICIPAR

1. ☐ Sim 2. ☐ Não

FORMULÁRIO

ESTUDO COMPARATIVO E DE EFICÁCIA ENTRE CITOLOGIA CONVENCIONAL X CITOLOGIA EM MEIO LÍQUIDO E AVALIAÇÃO DO DIAGNÓSTICO E SEGUIMENTO DAS DST A NÍVEL DE SAÚDE PÚBLICA NO ESTADO DE PERNAMBUCO

Número do formulário: ☐☐☐

Pesquisador _____

Local _____

Data da coleta de dados ___/___/____

Data da 1ª. Revisão ___/___/_____ Data da Digitação ___/___/____

Data da 2ª. Revisão ___/___/_____

Data da 3ª. Revisão___/___/_____

I. *IDENTIFICAÇÃO (ETIQUETA)*

Nome _____ Registro ☐☐☐☐☐☐

(preencher a lápis)

Data da admissão ___/___/_____ Data de nascimento ___/___/_____

Endereço: _____

Rua Nº. Bairro Cidade

Telefone: _____

II. *CARACTERÍSTICAS BIOLÓGICAS*

Idade (anos) ☐☐

Cor 1. ☐ Branca 2. ☐ Negra 3. ☐ Parda 4. ☐ Indígena 5. ☐ Outras

Peso (kg) ☐☐.☐☐☐

Altura (m) ☐.☐☐

IMC ☐☐.☐

III. *CARACTERÍSTICAS SÓCIO-DEMOGRÁFICAS*

Renda per capita (total em R$) ☐☐☐☐☐.☐☐

Escolaridade (anos completos estudados e aprovados) ☐☐

Procedência 1. ☐ Zona rural 2. ☐ Zona urbana

Procedência _____

1. ☐ Solteiro 2. ☐ Casado/união estável 3. Viúva/outros

1. ☐ Católica 2. ☐ Evangélica 3. ☐ Espiritualista 4. ☐ Outras _____

IV. *CARACTERÍSTICAS REPRODUTIVAS*

Início da primeira relação sexual ☐☐

Número de parceiros sexuais ☐☐

Relação sexual anal 1.☐ Não 2. ☐ Sim 3.☐ com preservativo 4.☐ sem preservativo

Número de partos ☐☐

IST prévias	1.☐ Sim	2.☐ Não
Gonorreia	1.☐ Sim	2.☐ Não
Sífilis	1.☐ Sim	2.☐ Não
Granuloma	1.☐ Sim	2.☐ Não
Herpes Simples	1.☐ Sim	2.☐ Não
Cancróide	1.☐ Sim	2.☐ Não
Hepatite	1.☐ Sim	2.☐ Não

Condiloma 1.☐ Não 2. ☐ Sim 3. ☐ vulva 4. ☐ vagina 5. ☐ colo 6. ☐ Mais de um

Métodos contraceptivos 1.☐ Não 2.☐ Sim 3.Preservativo 4.☐ Pilula 5.☐ DIU

6.☐ Injetável 7. ☐ Outros _____

V. *HÁBITOS*

Etilista 1.☐ Não 2. ☐ Sim 3. ☐Atual 4. ☐Pregresso

Tabagista 1.☐ Não 2. ☐ Sim 3. ☐Atual 4. ☐Pregresso

Usuário de drogas 1.☐ Não 2.☐ Sim 3.☐ Atual 4.☐ Pregresso 5.☐ Cocaína 6.☐ Maconha 7.☐

Outros _____

VI. *CARACTERÍSTICAS CLÍNICAS*

Prurido **1.** ☐ Sim **2.** ☐ Não

Sangramento **1.** ☐ Sim **2.** ☐ Não

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

Título do Projeto: *" Estudo Comparativo e de Eficácia entre Citologia Convencional X Citologia em Meio Líquido e avaliação do diagnóstico e seguimento das DST a nível de Saúde Pública no Estado de Pernambuco "*

Pesquisador Responsável: Micheline Oliveira

Instituição a que pertence o Pesquisador Responsável: IMIP

Telefones: (81) 9972-7008 - (81) 21224100

Voluntária:_____

Idade: _____ anos **RG** _____

Você está sendo convidada como voluntária a participar da pesquisa: **" Estudo Comparativo e de Eficácia entre Citologia Convencional X Citologia em Meio Líquido e avaliação do diagnóstico e seguimento das DST a nível de Saúde Pública no Estado de Pernambuco, "**

A JUSTIFICATIVA, OS OBJETIVOS E OS PROCEDIMENTOS: O motivo que nos leva a estudar o HPV é que o vírus pode causar verrugas nas "partes" (genitais), chamadas condilomas ou "cristas" e também pode causar alterações na pele do colo do útero e do ânus que não tratada pode se transformar em câncer. O objetivo desse projeto é identificar se você tem ou não câncer do colo uterino e alguma doença sexualmente transmissível, como também mostrar a eficácia e justificar o custo-benefício de um novo método de fazer o exame de prevenção.Os procedimentos de coleta de material e dados serão da seguinte forma: Inicialmente farei algumas perguntas para preencher sua ficha, depois você vai fazer exames de sangue para saber se existe alguma infecção sexualmente transmissível já que o vírus do HPV é também uma infecção transmitida através do sexo. Os riscos da coleta de sangue são mínimos podendo ocorrer discreta dor na entrada da agulha. Depois você vai ficar deitada na mesa de exame na posição de fazer exame de prevenção do câncer de colo do útero. Após receber os seus resultados, você irá fazer outro exame, colocando o mesmo aparelho chamado especulo (bico de pato) e vai ser observado através de um aparelho que aumenta o tamanho da imagem, se você tem alguma manchinha no colo do útero. Se durante o exame forem encontradas alterações importantes vou realizar outro exame chamado biópsia que tira um pequeno pedaço do local que estiver comprometido. Seus exames serão arquivados através de fotos. Você deverá voltar para pegar o resultado do exame na data marcada para que possamos fazer o tratamento se necessário.
DESCONFORTOS E RISCOS ASSOCIADOS: Existe um desconforto e risco mínimo para você se submeter à coleta do material. Às vezes, poderá ocorrer certo ardor ou desconforto se for realizado biópsia e pequeno sangramento que é resolvido com medicamento local.

BENEFÍCIOS ESPERADOS: Este exame não faz parte da rotina do serviço, mas você fazendo e se for achada qualquer alteração, você será beneficiada com o diagnóstico precoce e tratamento. FORMA DE ACOMPANHAMENTO E ASSISTÊNCIA: Caso você apresente nos exames de sangue alguma infecção sexualmente transmissível a pesquisadora prescreverá o tratamento e será garantido o fornecimento dos medicamentos, também será realizado tratamento se a biópsia revelar alguma alteração. GARANTIA DE ESCLARECIMENTO, LIBERDADE DE RECUSA E GARANTIA DE SIGILO: Você será esclarecida sobre a pesquisa em qualquer aspecto que desejar. Você é livre para recusar-se a participar, retirar seu consentimento ou interromper a participação a qualquer momento. A sua participação é voluntária e a recusa em participar não irá acarretar qualquer penalidade ou perda de benefícios. Mesmo que você se recuse a participar seu atendimento e encaminhamento para tratamento da alteração do colo do útero no IMIP, prosseguirá de acordo com a rotina. Os resultados dos exames clínicos e laboratoriais da pesquisa serão entregues para você e permanecerão confidenciais. Seu nome ou o material que indique a sua participação não será liberado sem a sua permissão. Você não será identificada em nenhuma publicação que possa resultar deste estudo. Uma cópia deste consentimento informado será arquivada no IMIP e outra será fornecida a você.
CUSTOS DA PARTICIPAÇÃO E RESSARCIMENTO: A participação no estudo não acarretará custos para você e não será disponibilizada nenhuma compensação financeira adicional, porém será garantido caso haja necessidade vale transporte e ticket de refeições pela pesquisadora.

DECLARAÇÃO DA PARTICIPANTE

Eu, _____, RG _____, registro ambulatorial _____, fui informada (o) dos objetivos da pesquisa acima de maneira clara e detalhada e esclareci minhas dúvidas. Sei que em qualquer momento poderei solicitar novas informações e motivar minha decisão se assim o desejar. A pesquisadora Micheline Oliveira certificou-me de que todos os dados desta pesquisa serão confidenciais. Também sei que caso existam gastos adicionais, estes serão absorvidos pelo orçamento da pesquisa. Em caso de dúvidas poderei chamar a pesquisadora através dos telefones (81) 9972-7008 e (81) 3268-1728 ou me dirigir ao Comitê de Ética em Pesquisa no telefone (81) 21224100.

Declaro que concordo em participar desse estudo. Recebi uma cópia deste termo de consentimento livre e esclarecido, tive a oportunidade de ler e esclarecer as minhas dúvidas.

Nome	Assinatura do Participante	Data
Nome	Assinatura do Pesquisador	Data
Nome	Assinatura da Testemunha	Data
Nome	Assinatura da Testemunha	Data