

Determination of HPV DNA viral load by hybrid capture assay and its association with cytological findings

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Determinação da carga viral de DNA de HPV pelo ensaio de captura híbrida e sua associação com achados citológicos

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key words	abstract
Human papillomavirus	Objective: To compare the relation between HPV viral load by hybrid capture II test (HCII) and cytological findings. Methods: Three hundred sixty-two reagent samples to HPV DNA by HCII had their viral loads classified in four categories and correlated to cytological results. Results: Twenty-two samples (6.1%) were reagent only to low-risk oncogenic types (group A) and 340 (93.9%) were reagent to high-risk oncogenic types (group B). The correlation between viral load for the reagent samples to group A and cytological results showed low-grade squamous intraepithelial lesion (LSIL) predominance (50%). Most of this group samples had viral load between 1 to <10RLU/PCA. Of the patients that were reagent to group B 52.1% had LSIL cytology and 38.2% were negative to intraepithelial lesion and malignancy (NILM) cytology. The patients with LSIL had viral load well distributed with a slight predominance of 100 to < 1,000RLU/PCB category. The samples had viral load between 1 to <10RLU/PCB showed NILM cytology predominance (48.1%). High-grade squamous intraepithelial lesions (3.4%) were present on the samples with viral load between 100 to <1,000RLU/PCB ($p = 0.023$). There was a correlation between the median for group B viral load and LSIL/HSIL results. Conclusions: The quantification of viral load, mainly of high-risk HPV types, may be a useful tool for dealing with patients who have suspicious lesions.
Viral load	
Cytology	

resumo	unitermos
<p><i>Objetivo: Comparar a relação entre a carga viral do HPV por captura híbrida II (HCII) e os achados citológicos. Métodos: Trezentas e sessenta e duas amostras reagentes para DNA de HPV por HCII tiveram suas cargas virais classificadas em quatro categorias e correlacionadas aos resultados citológicos. Resultados: Vinte e duas amostras (6,1%) foram reagentes somente para os tipos de baixo risco oncogênico (grupo A) e 340 (93,9%) foram reagentes para os tipos de alto risco oncogênico (grupo B). A correlação entre carga viral das amostras reagentes para o grupo A e resultados citológicos mostrou predominância (50%) de lesão escamosa intraepitelial de baixo grau (LSIL). A maioria das amostras desse grupo teve carga viral entre 1 e < 10RLU/PCA. Nos pacientes reagentes para o grupo B observamos que 52,1% tiveram citologia LSIL e 38,2% tiveram citologia negativa para lesão intraepitelial e malignidade (NILM). Os pacientes com LSIL tiveram a carga viral bem distribuída, com ligeira predominância da categoria de 100 a < 1.000RLU/PCB. As amostras com carga viral entre 1 e < 10RLU/PCB mostraram predominância de citologia NILM (48,1%). Lesões escamosas de alto grau (3,4%) foram presentes nas amostras com carga viral entre 100 e < 1.000RLU/PCB ($p = 0,023$). Houve correlação entre a mediana da carga viral para o grupo B e os resultados LSIL/HSIL. Conclusões: A quantificação da carga viral, principalmente para os tipos de HPV de alto risco oncogênico, pode ser uma ferramenta útil para o acompanhamento de pacientes com lesões suspeitas.</i></p>	<p>Papilomavírus humano</p> <p>Carga viral</p> <p>Citologia</p>

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Introduction

Cervical cancer is the second most common cancer among women worldwide and the human papillomavirus (HPV) is its main cause. HPV infections are associated with benign and malignant cutaneous and mucosal epithelial lesions⁽⁹⁾. Genital HPV types are subdivided into low-risk oncogenic types, frequently found in genital warts (types 6, 11, 26, 42, 44, 54, 70 and 73), and high-risk oncogenic types, associated with squamous intraepithelial lesions and invasive cancer (types 16, 18, 31, 35, 45, 51, 52, 58 and 59)⁽¹⁾. However, most HPV infections are transient, without clinical lesions or atypical cytological findings.

For some authors the screening of high-risk HPV types should be done to identify lesions with potential for progression to invasive cancer⁽¹²⁾. There is a direct correlation between the number of copies of an oncogenic HPV type and the risk of developing HPV-related diseases⁽⁶⁾.

The hybrid capture II test (HCII) is widely used in routine analysis of cervical scrapes for HPV detection. This test uses probe cocktails that hybridize with specific HPV deoxyribonucleic acid (DNA) permitting the detection of 18 HPV types separated into two groups: low oncogenic risk (group A) and high oncogenic risk (group B). The viral load measure is obtained by comparing the relative light units of the samples to positive controls (RLU/PC).

In the present study, we compared the relation between HPV viral loads and cytological findings.

Materials and methods

There are 362 samples that were reagent to HPV DNA, analyzed by HCII (Digene Corporation Gaithersburg, Maryland, USA) and included in this study. After the ethics committee approval, these samples were collected from women assisted at Centro de Prevenção ao Câncer, in Campo Grande, Mato Grosso do Sul, Brazil, from 2001 to 2004. The patients came from public health services or medical clinics with clinical suspicion of HPV-associated disease, for control of infection or other cervical disease. Cervical specimens were collected with a cervical brush, put in a tube with specimen transport medium (Digene Brasil, São Paulo, Brazil) and stored at 2°C to 8°C until processing.

Cytological findings

Cytological diagnoses were reported using the 2001 Bethesda System⁽¹⁴⁾.

Hybrid capture II

The protocol was performed according to the manufacturer instructions. After subjected to low and high-risk HPV probes, samples were considered positive when the RLU/PC was ≥ 1 . According to viral load, they were classified into four groups: 1 to < 10 ; 10 to < 100 ; 100 to $< 1,000$ and $\geq 1,000$ RLU/PC⁽⁷⁾ and then correlated to cytological results.

Statistical analyses

Statistical analyses were performed using the Epi Info software, version 3.2, 2004 (CDC, Atlanta, Georgia). The frequency tables were analyzed using χ^2 test, with 95% confidence limits. The correlation between results was calculated by Kruskal Wallis test for two groups.

Results

Patients were 16-64 years old (30.77 mean age). The relation between cytological findings and HCII results is presented on **Table 1** ($\chi^2 = 0.96$). Twenty-two samples (6.1%) were reagent to low-risk oncogenic types (group A) and 340 (93.9%) were reagent to high-risk oncogenic types (reagent to group B or groups A and B). The cytological findings were: atypical squamous cells of undetermined significance (ASCUS) in 9.1% of the smears; high-grade squamous intraepithelial lesion (HSIL) in 0.8%; low-grade squamous intraepithelial lesion (LSIL) in 51.9% and negative to intraepithelial lesion and malignancy (NILM) in 38.1%. HSIL was presented only on the samples that were reagent to group B and groups A and B.

For the reagent samples only to group A, **Table 2** shows that the correlation between cytological results and viral load was not significant ($p = 0.35$). These patients had LSIL cytology predominating (50%) and most of these samples had viral load between 1 to < 10 RLU/PCA.

Table 3 shows the correlation of cytological findings and viral load of the reagent samples to group B or groups A and B ($p = 0.023$). It was observed that 52.1% of these samples had LSIL and 38.2% had NILM. Patients with LSIL had the viral load well distributed, with a slight predominance of 100 to $< 1,000$ RLU/PCB category. Samples with viral load between 1 to < 10 RLU/PCB showed predominance of NILM cytology ($n = 52$; 48.1%). HSIL (3.4%) was present only in samples which had viral load between 100 to $< 1,000$ RLU/PCB.

On **Table 4** mean, median and standard deviation (SD) were observed of the viral load to samples reagent

Table 1 Samples reagent by HCII to high (group B) and/or low (group A) oncogenic risk HPV types in relation to cytological findings

Cytology	HCII			Total
	Group A	Group A and B	Group B	
ASCUS	3 (9.1%)	23 (69.7%)	7 (21.2%)	33 (9.1%)
HSIL	0 (0%)	2 (66.7%)	1 (33.3%)	3 (0.8%)
LSIL	11 (5.9%)	122 (64.9%)	55 (29.3%)	188 (51.9%)
NILM	8 (5.8%)	91 (65.9%)	39 (28.3%)	138 (38.1%)
Total	22 (6.1%)	238 (65.7%)	102 (28.2%)	362 (100%)

ASCUS: atypical squamous cells of undetermined significance; HSIL: high-grade squamous intraepithelial lesion; LSIL: low-grade squamous intraepithelial lesion; NILM: negative to intraepithelial lesion and malignancy.

Table 2 Viral load of the samples reagent only to group A in relation to cytological findings

Cytology	Viral load (group A)				Total
	1 to < 10RLU/PCA	10 to < 100RLU/PCA	100 to < 1,000RLU/PCA	≥ 1,000RLU/PCA	
ASCUS	1 (33.3%)	1 (33.3%)	1 (33.3%)	0 (0%)	3 (13.6%)
LSIL	5 (45.5%)	3 (27.3%)	1 (9.1%)	2 (18.2%)	11 (50%)
NILM	6 (75%)	1 (12.5%)	1 (12.5%)	0 (0%)	8 (36.4%)
Total	12 (54.5%)	5 (22.7%)	3 (13.6%)	2 (9.1%)	22 (100%)

ASCUS: atypical squamous cells of undetermined significance; LSIL: low-grade squamous intraepithelial lesion; NILM: negative to intraepithelial lesion and malignancy.

Table 3 Viral load of the samples reagent to group B, independent of group A result, in relation to cytological findings

Cytology	Viral load (group B)				Total
	1 to < 10RLU/PCB	10 to < 100RLU/PCB	100 to < 1,000RLU/PCB	≥ 1,000RLU/PCB	
ASCUS	10 (33.3%)	7 (23.3%)	9 (30%)	4 (13.3%)	30 (8.8%)
HSIL	0 (0%)	0 (0%)	3 (100%)	0 (0%)	3 (0.9%)
LSIL	46 (26%)	35 (19.8%)	49 (27.7%)	47 (26.6%)	177 (52.1%)
NILM	52 (40%)	28 (21.5%)	26 (20%)	24 (18.5%)	130 (38.2%)
Total	108 (31.8%)	70 (20.6%)	87 (25.6%)	75 (22.1%)	340 (100%)

ASCUS: atypical squamous cells of undetermined significance; HSIL: high-grade squamous intraepithelial lesion; LSIL: low-grade squamous intraepithelial lesion; NILM: negative to intraepithelial lesion and malignancy.

Table 4 Mean, median and standard deviation (SD) of the viral load to samples reagent to group A or B and cytological findings

Cytology	Group A (viral load)			Group B (viral load)		
	Mean	Median	SD	Mean	Median	SD
LSIL	265.9	3.15	723.52	8,895.58	127.41	58,295.37
HSIL	3.27	2.53	3.22	375.2	478.74	182.25
ASCUS	156.7	3.48	355.5	396.58	24.86	851.18
NILM	134.81	3.15	382.51	2,059.54	21.49	16,548.62

ASCUS: atypical squamous cells of undetermined significance; HSIL: high-grade squamous intraepithelial lesion; LSIL: low-grade squamous intraepithelial lesion; NILM: negative to intraepithelial lesion and malignancy.

to group A or B in correlation to cytological findings. A positive correlation was found between median of group B viral load and LSIL/HSIL cytology.

Discussion

Although new alternatives have been proposed, there is a consensus on the HC indication for cases diagnosed as ASCUS^(15, 16). The use of the diagnostic tests for HPV DNA in combination with cytology has been recommended by some authors because it enhances the performance of both tests^(6, 7). HPV DNA detection was considered relevant in screening patients with normal and ASCUS cytology because, if high-risk HPV is found, they may represent people who have high probability of showing abnormal cytology and cervical disease in the future^(4, 8). The results showed that 9.1% of the reagent patients to HPV DNA had cytological diagnosis of ASCUS and 38.1% had NILM (Table 1). In other reports the rate referred to HPV DNA by HCII was 16% in women with ASCUS cytology and 29.6% in women with normal smears^(2, 11).

In this work 51.9% of the reagent patients to HPV DNA, mainly to high-risk types, were found to have LSIL. It is in accordance with other reports that confirm the association of HPV and intraepithelial lesions^(2, 11). Some authors showed that the amount of HPV DNA is related to the grade of intraepithelial lesions and patients with high viral load, of group B HPV types, have greater risk of intraepithelial lesions or cervical cancer^(4, 8). The results are in agreement with other reports, which noted HSIL presence only in samples reagent to high-risk HPV and with high viral load^(2, 5, 11, 13, 17). A positive correlation was observed between group B viral load median and LSIL/HSIL, values above 25RLU/PCB could be of clinical significance. For group A, median values were basically the same to all four cytological classifications.

Hernández-Hernández *et al.* showed that the low viral load of group B HPV was present in patients with negative cytology, and the high viral load, in patients with NIC II/III. They suggested a strong relation between grade of lesion and viral load⁽⁵⁾.

Federschneider *et al.* pointed out that in samples with results of 1 to 3RLU/PCB the agreement between polymerase chain reaction (PCR) and HCII is poor and these results had low reproducibility and predictive value⁽³⁾. Santos *et al.* observed that 10RLU/PCB as cutoff point in HC2 represented the best balance between sensitivity and specificity. They proposed that viral load index values (RLU) should be categorized to estimate the disease progression⁽¹¹⁾. The viral load from 1 to 10RLU is one of low specificity and in these levels may represent false-positive results or HPV with no further clinical implication. These findings are supported by other authors that used PCR to confirm them⁽¹⁰⁾.

Some studies confirmed that the high viral load of group B HPV could be used as a marker of progression to precancerous lesions, although lower viral load does not exclude progressive disease^(10, 11). The quantification of viral load, mainly of high-risk HPV types, may be a useful tool for dealing with patients who bear suspicious lesions and in these cases values > 25RLU/PCB should be considered a key point. Finally, a guideline to the applicability of viral load values for patient management is certainly necessary, but still a not available tool. This study presents a suggestion of clinical cut-off of 25RLU for the high-risk probe, similar to other published data^(13, 17). However, due to the large standard deviations found for both probes and cytological classifications, these guidelines shall be used with caution and in the context of general clinical presentation to guide the attendance of patients with negative cytology or ASCUS and prevent the progression of HPV infection.

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