

Associations Between Quantitative HPV 16 and 18 DNA and RNA Levels in Incident Infections and the Subsequent Risk of Developing Cervical Lesions

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Background

- “High risk” types of genital HPV (e.g. 16 and 18) are causally related to the development of cervical cancer.
- Since most HPV infections are asymptomatic and only a small proportion progress to cervical cancer, it is important to identify factors (e.g. high HPV viral load) that increase the risk of disease progression.
- Previous studies tended to report positive associations between viral load and precancerous cervical lesions (squamous intraepithelial lesions (SIL) or cervical intraepithelial neoplasia (CIN)).
 - Most were cross-sectional or employed semi-quantitative or non-type-specific viral load measurements.

Background (cont.)

- Type-specific associations between high HPV DNA viral load and increasing SIL or CIN grade have been cross-sectionally observed for HPV 16, but not for other high-risk types.
- In nested case-control studies, positive associations between high HPV 16 viral load and risk of developing high-grade CIN (\geq CIN 2) have been observed.

Rationale

- To our knowledge, no study has prospectively investigated the relationship between type-specific viral load and disease progression among women with incident HPV infections.
- In the era of prophylactic HPV vaccines, viral load may provide a useful indicator of the clinical importance of break-through infections.

Specific Aims

- Quantify E7 DNA and mRNA viral loads among women with incident HPV 16 and 18 DNA infections.
- Investigate the associations between type-specific viral loads and the risk of developing HPV-related cervical lesions.
- Hypothesis: E7 mRNA viral load is a potentially more sensitive early indicator of predisposition to high-grade dysplasia than DNA viral load.

Study Population

- 18-22 year old female university students recruited to participate in longitudinal HPV studies between 1990 and 2004
- Clinical visits every 4 months
 - Cervical Dacron swab samples for HPV DNA and RNA testing
 - Cervical cytology specimens collected with cytobrush and plastic spatula
 - Colposcopic inspection of the cervix
 - Women with HSIL or repeated LSIL/ASCUS referred to colposcopy

HPV DNA and RNA Testing

- HPV L1 consensus primer PCR with type-specific probes
 - A subset of samples from women with incident HPV 16 or 18 infections were selected for quantitative testing
- Real-time type-specific PCR (*most accurate and controlled method of estimating viral load*)
 - HPV16 and 18 E7 DNA and mRNA
 - Copy numbers generated from standard curves
 - Log-transformed and normalized according to input of cellular DNA (per 1000 cells) or RNA (per picogram β -actin)
 - Samples with undetectable DNA or RNA assigned a log-transformed copy number of zero

Statistical Methods

- Viral load analyzed as a continuous variable
- Separate models for HPV 16 and HPV 18 DNA and RNA
- Logistic regression for cross-sectional associations between type-specific DNA and RNA viral load and detection of cervical SIL
- Cox proportional hazards models for associations between type-specific DNA and RNA viral load (at time of incident infection) and
 - Risk of developing SIL and CIN 2-3
 - Type-specific HPV “clearance” (defined as the first negative DNA test after an incident positive test)
 - “Clearance” of quantitative HPV RNA

Results

- HPV 16
 - DNA: 191 samples from 34 women
 - RNA: 168 samples from 42 women
 - *Correlation between DNA and RNA viral loads in 52 samples: $r = 0.6$, $p < .01$*
- HPV 18
 - DNA: 81 samples from 13 women
 - RNA: 41 samples from 11 women
 - *Correlation between DNA and RNA viral loads in 13 samples: $r = 0.6$, $p = .04$*

Viral Load at the Time of Incident HPV 16 or HPV 18 Detection

HPV 16			HPV 18		
	% with virus	Mean (SD) log-copy number*		% with virus	Mean (SD) log-copy number*
DNA	88	9.3 (2.4)	DNA	92	8.5 (3.4)
RNA	83	6.8 (1.6)	RNA	91	5.4 (2.4)

*per picogram cellular RNA among samples with detectable E7 mRNA and per 1000 cells among samples with detectable E7 DNA.

Cross-sectional Associations Between Viral Load and Cervical SIL

		Adjusted OR* (95 % CI)
HPV 16	DNA	1.1 (1.01-1.2)
	RNA	1.3 (1.1-1.5)
HPV 18	DNA	1.0 (0.8-1.2)
	RNA	0.9 (0.6-1.2)

*per 1 log increase in viral load, adjusted for concurrent detection of other HPV types

Associations Between Viral Load at Time of Incident HPV and Risk of SIL or CIN 2-3

		Adjusted* HR per 1 log increase in viral load (95% CI)	
		SIL	CIN 2-3
HPV 16	DNA	1.2 (0.95-1.4)	1.2 (0.9-1.6)
	RNA	1.8 (1.3-2.5)	3.9 (2.5-6.0)
HPV 18	DNA	0.9 (0.8-1.1)	1.0 (0.8-1.2)
	RNA	0.9 (0.7-1.2)	1.1 (0.6-2.1)

*Adjusted for HPV type (16 or 18) and concurrent detection of other HPV types (SIL analyses) or other high-risk HPV types (CIN 2-3 analyses)

Associations Between Viral Load in Incident HPV Infections and Time to “Clearance”

- No statistically significant associations were observed between HPV 16 or 18 DNA or RNA viral load and time to “clearance” of HPV 16 or 18.
- No statistically significant associations were observed between HPV 16 or 18 RNA viral load and time to “clearance” of HPV 16 or 18 RNA.

Conclusions

- Increasing HPV 16 viral load (both DNA and RNA) was associated with concurrent detection of SIL.
- Among women with incident HPV 16 infections, higher levels of E7 mRNA (but not E7 DNA) are associated with an increased risk of developing CIN 2-3.
 - *However, the potential clinical utility of E7 mRNA is limited; no clear “cut point” was identified and levels fluctuated over time.*
- No statistically significant associations were observed between HPV 18 viral load and risk of lesions.
 - *Given the small sample, it is unclear whether the results reflect differences in the natural history of HPV 18 vs. 16 infections.*
- No associations between viral load at the time of incident infection and HPV “clearance” were observed.

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