Salla Vuorinen

Natural acquired high-level HPV antibodies do not influence unvaccinated women's genital or oral HPV infection outcomes

Syventävien opintojen kirjallinen työ
Kevätlukukausi 2020

Salla Vuorinen

Natural acquired high-level HPV antibodies do not influence unvaccinated women's genital or oral HPV infection outcomes

Lääketieteellinen tiedekunta, kliininen laitos
Kevätlukukausi 2020
Vastuuhenkilö: Dos. Karolina Louvanto

Turun yliopisto
Lääketieteellinen tiedekunta

Salla Vuorinen
Luonnollisesti saavutetut korkeat HPV-vasta-ainetasot eivät vaikuta rokottamattomien naisten genitaalien tai suun alueen HPV-infektioiden taudinkulkuun

Syventävien opintojen suomenkielinen tiivistelmäosio
Naistentaudit ja synnytykset
Huhtikuu 2020

Krooninen korkean riskin ihmisen papilloomaviruksen (HPV) aiheuttama infektio on suurin tunnettu riskitekijä genitaali- ja suun alueen syöpien kehittymisessä. Lähes kaikki naiset saavat HPV-infektion elämänsä aikana ja tavanomaisesti HPV-infektio paranee spontaanisti kahden vuoden kuluessa aktiivisen immuunipuolustuksen avulla. Genitaali- ja suun alueen HPV-infektioiden luonnollisten vasta-aineiden muodostuminen ja näiden vaikutus genitaali- ja suun alueen HPV-infektion ilmaantuvuuteen, kestoon ja kroonistumiseen rokottamattomilla naisilla ei vielä tarkkaan tunneta.

Tämän tutkimuksen tarkoituksena oli evaluoida eri HPV-vasta-ainetasojen vaikutusta genitaalien ja suun alueen HPV-infektioiden luonnolliseen taudinkulkuun. Tutkimuksen aineistona käytettiin Turun yliopissa kerättyä suomalaista perhetutkimusta (The Finnish Family HPV-study), johon osallistui yhteensä 329 alkutilanteessa raskaana olevaa naista, joita seurattiin yhteensä kuuden vuoden ajan. Tutkimuksen tilastollisissa analyyseissä huomioitiin 267 naisen ensimmäisen kolmen vuoden seuranta ajalta 24:n eri HPV-genotyypin aiheuttamat infektiot suun ja genitaalien alueilla. Naisten seerumin vasta-ainemääritykset tehtiin HPV-genotyyppien 6, 11, 16, 18 ja 45 osalta. Naisten erittäin korkeita sekä pysyvästi koholla olevien HPV 6, 11, 16, 18 ja 45 L1-vasta-ainetasojen vaikutusta tarkasteltiin saman HPV genotyypin ja minkä tahansa HPV-genotyypin aiheuttamien genitaali ja suun alueen HPV-infektioiden kehittymiseen.

Tuloksemme osoittivat, että seurannassa naiset, jotka eivät kehittäneet lainkaan vasta-aineita HPV16-genotyypille koko seurannan aikana ilmaantui herkemmin HPV16-genotyypin aiheuttama genitaalialueen infektio seurannan loppupuolella. HPV 6, 11, 18 ja 45 -genotyyppien osalta pysyvästi koholla olevilla vasta-ainetasoilla tai vasta-aineiden puuttumisella ei pystytty osoittamaan yhteyttä genitaali- tai suun alueen HPV-infektion ilmaantuvuuteen, parantumiseen tai kroonistumiseen. Naisten HPV-infektion kroonistumista yli 24-kuukauden ajan verrattiin HPV-infektion luonnolliseen parantumiseen niiden naisten keskuudessa, joilla oli erittäin korkeat joko HPV 6, 11, 16, 18 tai 45 vasta-ainetasot verrattuna niihin, joille ei vasta-aineita kehittynyt lainkaan seurannan aikana. Edellä mainitussa vertailuissa korkeiden vasta-ainetasojen suhteen emme pystyneet osoittamaan tilastollisesti merkittävää yhteyttä minkään tutkitun HPV genotyypin osalta. Tutkimuksen johtopäätöksenä todettiin, että rokottamattomien nuorten naisten HPV 6, 11, 16, 18 ja 45 -genotyyppien luonnollisesti korkeilla tai pysyvästi koholla olevilla vasta-aineinetasoilla ei näyttäisi olevan merkitystä genitaali- ja suun alueen HPV infektion luonnolliseen taudinkulkuun.

Natural acquired high-level HPV antibodies do not influence unvaccinated women's genital or oral HPV infection outcomes

Salla Vuorinen [1], Kari Syrjänen [2], Seija Grénman[1], Stina Syrjänen [3], Karolina Louvanto [1, 3]

[1] Department of Obstetrics and Gynecology, Turku University Hospital, University of Turku, Turku, Finland.

[2] Department of Clinical Research, Biohit Oyj, Helsinki, Finland.

[3] Department of Oral Pathology, Institute of Dentistry, Faculty of Medicine, University of Turku, Turku, Finland.

Corresponding author: Dr Karolina Louvanto, Department of Obstetrics and Gynecology, Turku University Hospital, University of Turku, Kiinanmyllynkatu 9-11, 20520 Turku, Finland; email: karolina.louvanto@utu.fi; phone: +35823139290

Keywords: HPV, serology, genital, oral, women, antibody, persistence

Abstract

The role of human papillomavirus (HPV) antibodies acquired through natural infection and their role in protection for subsequent cervical or oral HPV remains unclear. A total of 267 women, with a 36-months follow-up, from the Finnish Family HPV (FFHPV) study where evaluated to shed more light on the role of high levels or persistence of HPV specific antibodies to the prevalence, persistence or clearance of genital or oral HPV infection. Women's type-specific seroprevalence for HPV genotypes 6, 11, 16, 18 and 45 were evaluated for the same type-specific and any-HPV oral and genital HPV infections. The following different HPV serological outcomes where included: being always seronegative, seroconversion or persistent seropositivity. Our findings showed that genital HPV16 infections where more prevalent at the end of the follow-up at 24 and 36 months follow-up visit among women who recorded to be always seronegative for HPV16. Otherwise these serological outcomes did not influence the different type-specific or any-HPV genital or oral outcomes. The role of high levels of antibodies for HPV 6,11,16,18 and 45 were further compared to those that were always seronegative to the development of long-term type-specific HPV 6,11,16,18 and 45 persistence (≥ 24 months) or clearance of the genital and oral infections. No significant associations where detected among these evaluations. To conclude, among the unvaccinated young women investigated, we could not detect any significant role for the naturally acquired high or persistent level of HPV antibodies to the genital or oral HPV infections outcomes.

Introduction

Most mucosal human papillomavirus (HPV) -infections are transient and clear spontaneously by active immunological response within a few years (1),(2),(3) . Still approximately 10-20 % of women fail to clear their HPV infection and are at higher risk of progressing pre-cancerous lesions. A persistent genital infection is known to be a key event in cervical carcinogenesis (4). HPV infections are closely linked not only to carcinogenesis of cervical cancer but also to the oropharyngeal cancer.

HPV infection in both oral and genital mucosa can cause an immunological response and process of HPV-type-specific antibodies in the serum (5),(6). However, previous studies have shown that only around 50-70% of women develop in reality these antibodies after a natural infection (7),(8),(9). A meta-analysis concluded HPV antibodies acquired through natural infection to provide modest protection against subsequent cervical HPV infection among unvaccinated women (3). The antibody levels have shown to be also considerably lower after a natural infection compared to those are seen among vaccinated women (10). A mathematical model revealed that there is a large individual variation in the duration of HPV infection and acquired immunity, but suggested that naturally acquired immunity after clearance of an HPV infection may already protect part of the risk population against new HPV infections (11). Although, it is still unclear which is the minimum antibody level necessary for protection or will only higher level of naturally acquired antibody levels only provide protection against future HPV infections.

In this study, we investigated the women's type specific HPV6, 11, 16, 18 and 45 antibody levels, especially persistent seropositivity, high antibody levels and always seronegative during the 36 months follow-up, to the different outcomes of genital and oral HPV infections among young unvaccinated women.

Materials and methods

Subjects. The Finnish Family HPV (FFHPV) study is a prospective cohort study conducted at the Department of Obstetrics and Gynecology, Turku University Hospital, University of Turku and at the Institute of Dentistry, Faculty of Medicine, University of Turku, Turku, Finland. The cohort group included 329 pregnant women who were recruited between 1998 and 2001 at a minimum of 36 weeks of pregnancy and were followed up for six years after the delivery. All women are of Caucasian origin and have the same ethnic background. The HPV status of the women was not relevant at the enrolment. The HPV data of the whole FFHPV study cohort (331 mothers and 131 fathers) has been published earlier (1)(12)(13). The Research Ethics Committee of Turku University and Turku University Hospital has approved the study protocol and its amendment (#2/1998 and #2/2006).

Samples. Genital and oral scrapings from the women were collected for HPV-testing with a cytobrush (MedScand, Malmö, Sweden) as described before (12). HPV genotyping was done by Luminex-based Multimetrix kit (Progen Biotechnik GmbH, Heidelberg, Germany), which detects 24 low-risk (LR)- and high-risk (HR)-HPV genotypes (LR-HPV: 6, 11, 42, 43, 44, HR-HPV: 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 70, 73, 82) (14).

Serology. Blood samples were taken at baseline and at 12, 24 – and 36 months of the follow-up and stored as previously described in detail. (1) Antibodies to the major capsid protein L1 of HPV types 6, 11, 16, 18 and 45 were analyzed by multiplex HPV serology based on glutathione S-transferase fusion-protein capture on fluorescent beads, as described previously (15) (16). Sera were scored as positive when the antigen-specific MFI values were greater than the cut-off level of 200 or 400 MFI (stringent) for L1 antigen of individual HPV types (17).

Statistical analyses. The present study focuses on the naturally acquired HPV antibody levels for HPV genotypes 6, 11, 16, 18 and 45 and their association to the genital and oral HPV infection outcomes among 267 unvaccinated women from the FFHPV study. Of the 329 women originally enrolled, 267 women were eligible for the present study, all having at least two serum samples taken during the study period. Frequency tables were analyzed using the $\chi 2$ test or the Fisher's exact test for categorical variables. Differences in the means of continuous variables (i.e. log-transformed HPV antibody titres) were analyzed using ANOVA (analysis of variance) after controlling for their normal distribution. Women's genital and oral HPV 6, 11, 16, 18 and 45 genotype specific and any-HPV prevalence at each follow-up visit was compared with the women's serological status for HPV genotypes 6, 11, 16, 18 and 45. Serological status was categorized into three groups as followed: 1) Always seronegative, MFI value stayed <200 with each visit; 2) Seroconversion, defined by two conditions: (i) an MFI value <200 in the first and >200 in the second sample, and (ii) at least a twofold increase of the previous serum value; and lastly 3) Persistent seropositivity, MFI value stayed > 200 in all follow-up visits. The persistent and clearance of oral and genital HPV 6, 11, 16, 18 and 45 infections where further compared between group of women with high titers of HPV antibody levels to those that where seronegative. High serum antibody levels were defined as consistently MFI >400. Persistent HPV infection was defined as being positive for type-specific 6,11,16,18 or 45 HPV genotype for 24 months or more during the follow-up. The type-specific serology of HPV 6, 11, 16, 18 and 45 status where also further evaluated among the different clinical outcome groups of the oral and genital type-specific and any-HPV infections. Clinical outcome groups for oral and genital infection consisted the following: 1) Always HPV negative, 2) Incidence of HPV-infection, recorded when a woman who was HPV-negative at baseline acquired an incident HPV infection during the follow-up, 3) Clearance, women who had an event at any FU visit when a previously HPV-positive test turned out to be negative and remained HPV-negative until the end of the follow-up, 4) Persistent HPV infection, defined when HPV positivity was

recorded in two or more consequence visits during the follow-up. All statistical analyses were performed using SPSS (IBM, NY, USA, PASW Statistics version 18.0.3) and STATA13.0 (Stata Corp., College Station, TX, USA) software packages. All statistical tests performed were two-sided and declared significant at the P-value <0.05 level.

Results

The role of women's type-specific HPV 6, 11, 16, 18 and 45 antibody levels response to these type-specific oral and genital HPV infections was evaluated. The main aim of this study was to focus on serological persistence or having high level of antibody levels to those women that stayed always seronegative throughout the 36 months follow-up. The mean age of the women at the time of enrolment was 25.5 years (range 18-38) and the characteristics as the dynamics of the seroprevalence, seroconversion and antibody decay of these women in the FFHPV study has been previously described in detail (1)(13). The genotype-specific seroprevalence status of persistently seropositive, seroconversion or always seronegative related to the any-HPV genital infection status is showed in **Table 1**. At baseline HPV16 serology showed a significant comparison between always seronegative women and persistent seropositivity. Among the baseline genital any-HPV-negative women, 134 (69.3%) where recorded always HPV16 seronegative during the follow-up compared to the 47 (24.4%) women recoded to be persistent HPV16 seropositive. None of the other follow-up visits with HPV16 or with other HPV (6, 11, 18 and 45) serology groups recorded any statistically significant comparisons. These same evaluations where further looked among the genotype specific genital infection prevalence (HPV 6, 11, 16, 18 and 45). Interestingly, among women that were genital HPV16 positive at 24 and 36 months visit we could detect a significant outcome difference between women who recorded to be always seronegative (women n range 41-52) compared to those that recorded persistent seropositive (women n range 11-16). With the other

genotype specific analysis with HPV 6, 11, 18 or 45 we could not detect any significant results (data not shown).

These same genotype specific serological groups of persistent seropositive, seroconversion and always seronegative association to oral any-HPV infection status was also evaluated as shown in **Table 2**. The only statistically significant result was among HPV11 serological groups at the second 2-months follow-up visit, 83.0 % (n=39) of any HPV-positive women were HPV11 seronegative compared to 6.4 % (n=3) showed seroconversion. Among HPV negative women 81.6 % (n=146) of were seronegative and 17. 9 % (n=32) where persistent seropositive compared to 0.6% (n=1) who shown seroconversion. The genotype-specific HPV assessment with 6, 11, 16, 18 or 45 oral HPV infection did not show any significant results with their serology (data not shown).

To evaluate more the role of naturally acquired high levels of HPV specific antibody levels in possible protection of the infections, we evaluated women with high levels of antibody compared to those that recorded always HPV seronegative for the same HPV type (**Table 3**). We restricted there evaluations only to those women that recorded a ≥ 24-months type-specific persistence compared to those that cleared their infection (and stayed negative the rest of the follow-up). Evaluations were done between all HPV 6, 11, 16, 18 and 45 genotypes separately, type-specific serology to the same type genital or oral HPV infection outcome. We could not detect any significant results among the role of the antibody levels on these HPV outcomes in the genital or oral mucosa with either of the HPV genotype investigated.

Women's clinical outcomes for genital and oral any-HPV infections where also further also evaluated among the genotype specific HPV 6, 11, 16, 18 and 45 different serological groups that were used in **Table 1 and 2** as with the two comparison groups of having high antibody levels to

those women that where always seronegative as in **Table 3**. None of these serological categories had significant results recorded among either in oral or genital type-specific or any-HPV outcomes groups of incidences, clearance, persistence or always HPV negative (data not shown).

Discussion

The role of naturally acquired high HPV antibody levels compared to those women who never reach seroconversion are conflicting. It has been speculated that naturally acquired immunity after clearing an infection may already protect part of the risk population against new HPV infections (11). Our aim was to shed more light on this by evaluating HPV 6, 11, 16, 18 and 45 genotypes antibody levels and outcomes to the associations to the prevalence, persistence or clearance of genital and oral HPV infections.

Our results showed in the genital site that being always HPV16 seronegative was associated with increased HPV16 genital positivity later in the follow up at 24 and 36-months compared to those women that were recorded to be persistence HPV16 seropositive. This is aligned with a previous study, where also genital HPV16 infected young women were shown to be seronegative (18). This association was however, only seen in the later part of the women's follow-up why one might consider that these women could be later seroconverted with a longer follow-up. One possible explanation for this is that the HPV infection even classified as a persistent one represents a constantly acquired new HPV infection from the partner. A previous study however has reported that there is evidence that some HPV infections do not always cause seroconversion and even women with persistent infection fail to seroconvert (7). Our previous results among these women showed that over half of the women with HPV seroconversion (67 of 134) had seroconverted to at least one HPV type during the follow up (1). With further evaluations in this current study with HPV 6,11,18 or 45 genotype serological categories with the different genital or oral outcomes no significant associations were detected.

We additionally investigated the effect of high L1-antibody levels of HPV 6, 11, 16, 18 and 45, compared to those that were always seronegative, to the development of long-term persistence or clearance of the genital and oral infections. Is has been hypothesized that high levels of natural antibodies would protect from acquiring subsequent HPV infections, although the true role of the natural antibody levels remains still undetermined (3),(19). Due to our long-term follow-up we could evaluate the persistence of oral and genital infection more than 24 months compared to women with virus clearance. Due to our restricted outcome criteria and few women in the groups, we could only estimate the role of high levels antibodies for HPV 16 in the oral and genital site, and in the genital site additionally for HPV6, however we could not detect any significant associations among these evaluations (Table 3). One study concluded that having high antibody levels against HPV16 and HPV18 following natural infection was associated with reduced risk of subsequent HPV16 and HPV18 infections (2). The likelihood for future protection was found stronger with for high antibody levels of HPV18 than with HPV16 (2). We could not confirm this with HPV 18 due to low number of women among this group. Our previous findings among these women showed that those who cleared their cervical HPV16 infection had the highest measures of HPV16 antibody levels, whereas those who acquired incident HPV16 infections had the lowest antibody levels (13). Interesting enough, long-term persistence in the genital or oral site, however, was not predicted in our current analyses by being always seronegative.

Protection through naturally acquired HPV L1-antibodies is likely, given that the strong antibodies responses to prophylactic HPV vaccination are believed to be accountable for the protection of future HPV infections among vaccinated women (9). Natural immunity is, though, considered to be inferior to the immunity acquired by HPV vaccinations in protecting against HPV reinfection (3). Even with smaller effect it is essential to establish whether naturally acquired antibodies protection is observed especially for persistent infections and precancerous lesions (2),(11). Among our

investigations unfortunately we could not shed more light to these questions and true nature of the naturally acquired antibody levels will need further investigations.

Acknowledgments

The authors thank for Tim Waterboer and Michel Pawlita from the German Cancer Research Center (DKFZ), Heidelberg, Germany for the serological assays. We are also very grateful to Mrs Elisa Hovinmäki for collecting the samples and to Mrs Tatjana Peskova and Mariia Henttinen at the MediCity HPV laboratory for technical assistance with the HPV DNA analysis of the samples. The study was funded by Päivikki and Sakari Sohlberg Foundation, the Government Special Foundation for the Turku University Hospital, Sigrid Juselius Foundation and the Finnish Medical Foundation.

References

1. Syrjänen S, Waterboer T, Sarkola M, Michael K, Rintala M, Syrjänen K, et al. Dynamics of human papillomavirus serology in women followed up for 36 months after pregnancy. J Gen Virol. 2009;90(6):1515–26.
2. Safaeian M, Castellsagué X, Hildesheim A, Wacholder S, Schiffman MH, Bozonnat MC, et al. Risk of HPV-16/18 Infections and Associated Cervical Abnormalities in Women Seropositive for Naturally Acquired Antibodies: Pooled Analysis Based on Control Arms of Two Large Clinical Trials. J Infect Dis. 2018;218(1):84–94.
3. Beachler DC, Jenkins G, Safaeian M, Kreimer AR, Wentzensen N. Natural Acquired Immunity Against Subsequent Genital Human Papillomavirus Infection: A Systematic Review and Meta-analysis. J Infect Dis. 2016;213(9):1444–54.
4. Koshiol J, Lindsay L, Pimenta JM, Poole C, Jenkins D, Smith JS. Persistent human papillomavirus infection and cervical neoplasia: A systematic review and meta-analysis. American Journal of Epidemiology. 2008.
5. Stanley M. HPV - Immune response to infection and vaccination. Infect Agent Cancer [Internet]. 2010;5(1):19.
6. Syrjänen S. Oral manifestations of human papillomavirus infections. Eur J Oral Sci. 2018;126:49–66.
7. Carter JJ, Koutsky LA, Hughes JP, Lee SK, Kuypers J, Kiviat N, et al. Comparison of Human Papillomavirus Types 16, 18, and 6 Capsid Antibody Responses Following Incident Infection. J Infect Dis. 2000.
8. Paavonen J, Jenkins D, Bosch FX, Naud P, Salmerón J, Wheeler CM, et al. Efficacy of a prophylactic adjuvanted bivalent L1 virus-like-particle vaccine against infection with human papillomavirus types 16 and 18 in young women: an interim analysis of a phase III double-blind, randomised controlled trial. Lancet. 2007.

9. Wilson L, Pawlita M, Castle PE, Waterboer T, Sahasrabuddhe V, Gravitt PE, et al. Seroprevalence of eight oncogenic human papillomaviruses and acquired immunity against re-infection. JInfectDis. 2014.

10. Safaeian M, Porras C, Pan Y, Kreimer A, Schiller JT, Gonzalez P, et al. Durable antibody responses following one dose of the bivalent human papillomavirus L1 virus-like particle vaccine in the Costa Rica vaccine trial. Cancer Prev Res. 2013.

11. Matthijsse SM, Van Rosmalen J, Hontelez JAC, Bakker R, De Kok IMCM, Van Ballegooijen M, et al. The role of acquired immunity in the spread of human papillomavirus (HPV): Explorations with a microsimulation model. PLoS One. 2015;10(2):1–14.

12. Louvanto K, Rautava J, Willberg J, Wideman L, Syrjänen K, Grénman S, et al. Genotype-Specific Incidence and Clearance of Human Papillomavirus in Oral Mucosa of Women: A Six-Year Follow-Up Study. PLoS One. 2013;8(1).

13. Paaso AE, Louvanto K, Syrjänen KJ, Waterboer T, Grénman SE, Pawlita M, et al. Lack of type-specific concordance between human papillomavirus (HPV) serology and HPV DNA detection in the uterine cervix and oral mucosa. J Gen Virol. 2011.

14. Schmitt M, Bravo IG, Snijders PJF, Gissmann L, Pawlita M, Waterboer T. Bead-based multiplex genotyping of human papillomaviruses. J Clin Microbiol. 2006.

15. Waterboer T, Sehr P, Michael KM, Franceschi S, Nieland JD, Joos TO, et al. Multiplex human papillomavirus serology based on in situ-purified glutathione S-transferase fusion proteins. Clin Chem. 2005.

16. Waterboer T, Sehr P, Pawlita M. Suppression of non-specific binding in serological Luminex assays. J Immunol Methods. 2006.

17. Michael KM, Waterboer T, Sehr P, Rother A, Reidel U, Boeing H, et al. Seroprevalence of 34 human papillomavirus types in the German general population. PLoS Pathog. 2008.

18. Coseo S, Porras C, Hildesheim A, Rodriguez AC, Schiffman M, Herrero R, et al.

Seroprevalence and correlates of human papillomavirus 16/18 seropositivity among young women in Costa Rica. Sex Transm Dis. 2010.

19. Castellsagué X, Naud P, Chow SN, Wheeler CM, Germar MJ V., Lehtinen M, et al. Risk of newly detected infections and cervical abnormalities in women seropositive for naturally acquired human papillomavirus type 16/18 antibodies: Analysis of the control arm of PATRICIA. J Infect Dis. 2014.

Table 1. Women's serological HPV 6,11,16,18 and 45 genotype specific outcomes* and any-HPV genital prevalence at baseline, 2-, 12-, 24-, and 36- months follow-up visits among women of the Finnish Family HPV-study. Significant comparisons showed in bold.

		Baseline		2 months		12 months		24 months		36 months	
HPV type	Serological outcome	HPV + n (%)	HPV - n (%)	HPV + n (%)	HPV - n (%)	HPV + n (%)	HPV - n (%)	HPV + n (%)	HPV - n (%)	HPV + n (%)	HPV - n (%)
HPV 6	Always Seronegative	10 (24.4)	58 (31.0)	15 (38.5)	53 (28.3)	26 (23.2)	41 (36.3)	40 (32.3)	26 (30.6)	33 (29.2)	30 (34.1)
	Seroconversion	2 (4.9)	3 (1.6)	0 (0.0)	5 (2.7)	3 (2.7)	2 (1.8)	4 (3.2)	0 (0.0)	2 (1.8)	1 (1.1)
	Persistent Seropositivity	29 (70.7)	126 (67.4)	24 (58.5)	129 (69.0)	83 (74.1)	70 (61.9)	80 (64.5)	59 (69.4)	78 (69.0)	57 (64.8)
HPV11	Always Seronegative	27 (77.1)	160 (85.1)	28 (87.5)	157 (80.1)	80 (80.0)	103 (81.7)	100 (84.7)	78 (83.0)	98 (84.5)	75 (83.3)
	Seroconversion	0 (0.0)	4 (2.1)	0 (0.0)	4 (2.0)	0 (0.0)	4 (3.2)	1 (0.8)	0 (0.0)	1 (0.9)	1 (1.1)
	Persistent Seropositivity	8 (22.8)	31 (15.9)	4 (12.5)	35 (17.9)	20 (20.0)	19 (15.1)	17 (14.4)	16 (17.0)	17 (14.7)	14 (15.6)
HPV16	Always Seronegative	**15 (44.1)**	**134 (69.4)**	20 (66.7)	128 (65.6)	61 (57.5)	84 (71.8)	76 (63.9)	65 (73.0)	74 (65.5)	63 (70.8)
	Seroconversion	**0 (0.0)**	**12 (6.2)**	0 (0.0)	11 (5.6)	7 (6.6)	5 (4.3)	6 (5.0)	3 (3.4)	8 (7.1)	2 (2.2)
	Persistent Seropositivity	**19 (55.9)**	**47 (24.4)**	10 (33.3)	56 (28.7)	38 (35.8)	28 (23.9)	37 (31.1)	21 (23.6)	31 (27.4)	24 (27.0)
HPV18	Always Seronegative	26 (63.4)	164 (77.7)	27 (69.2)	162 (76.8)	85 (74.6)	101 (75.4)	101 (76.5)	77 (76.2)	100 (79.4)	72 (72.0)
	Seroconversion	2 (4.9)	9 (4.3)	2 (5.1)	9 (4.3)	5 (4.4)	6 (4.5)	8 (6.1)	3 (3.0)	4 (3.2)	7 (7.0)
	Persistent Seropositivity	13 (31.7)	38 (18.0)	10 (25.6)	40 (19.0)	24 (21.1)	27 (20.1)	23 (17.4)	21 (20.8)	22 (17.4)	21 (21.0)
HPV45	Always Seronegative	37 (90.2)	206 (91.2)	34 (89.5)	207 (91.2)	112 (88.2)	127 (93.4)	133 (89.9)	95 (95.0)	127 (92.0)	94 (92.2)
	Seroconversion	0 (0.0)	4 (1.8)	0 (0.0)	4 (1.8)	3 (2.4)	1 (0.7)	1 (0.7)	2 (2.0)	2 (1.4)	1 (1.0)
	Persistent Seropositivity	4 (9.8)	16 (7.1)	4 (10.5)	16 (7.0)	12 (9.4)	8 (5.9)	14 (9.5)	3 (3.0)	9 (6.5)	7 (6.9)

* Serological status was categorized into three groups as followed: 1) Always seronegative, MFI value stayed <200 with each visit; 2) Seroconversion, defined by two conditions: (i) an MFI value <200 in the first and >200 in the second sample, and (ii) at least a twofold increase of the previous serum value; and lastly 3) Persistent seropositivity, MFI value stayed > 200 in all follow-up visits.

Table 2. Women's serological HPV 6,11,16,18 and 45 genotype specific outcomes* and any-HPV oral prevalence at baseline, 2-, 12-, 24-, and 36- months follow-up visits among women of the Finnish Family HPV-study. Significant comparisons showed in bold.

		Baseline		2 months		6 months		12 months		24 months		36 months	
HPV type	Serological outcome	HPV + n (%)	HPV - n (%)	HPV + n (%)	HPV - n (%)	HPV + n (%)	HPV - n (%)	HPV + n (%)	HPV - n (%)	HPV + n (%)	HPV - n (%)	HPV + n (%)	HPV - n (%)
HPV6	Always Seronegative	12(32.4)	56 (29.6)	17(34.7)	51 (29.5)	16(30.2)	51 (30.2)	12(27.3)	56 (30.6)	14(27.5)	51 (32.7)	10(31.3)	53 (31.0)
	Seroconversion	1 (2.7)	4 (2.1)	0 (0.0)	5 (2.9)	1 (1.9)	4 (2.4)	1 (2.3)	4 (2.2)	1 (2.0)	3 (1.9)	0 (0.0)	3 (1.8)
	Persistent Seropositivity	24(64.9)	129 (68.3)	32 (65.3)	117 (67.6)	36 (67.9)	114 (67.5)	31 (70.5)	123 (67.2)	36 (70.6)	102 (65.4)	22 (68.8)	115 (67.3)
HPV11	Always Seronegative	28 (80.0)	158 (81.4)	**39(83.0)**	**146 (81.6)**	46 (85.2)	137 (80.1)	33 (80.5)	153 (81.4)	42 (89.4)	137 (83.0)	26 (81.3)	148 (84.1)
	Seroconversion	2 (5.7)	2 (1.0)	**3 (6.4)**	**1 (0.6)**	1 (1.9)	3 (1.8)	1 (2.4)	3 (1.6)	1 (2.1)	0 (0.0)	0 (0.0)	2 (1.1)
	Persistent Seropositivity	5 (14.3)	34 (17.5)	**5 (10.6)**	**32 (17.9)**	7 (13.0)	31 (18.1)	7 (17.1)	32 (17.0)	4 (8.5)	28 (17.0)	6 (18.8)	26 (14.8)
HPV16	Always Seronegative	26 (70.3)	122 (64.6)	30 (62.5)	116 (66.7)	28 (62.2)	117 (66.1)	24 (58.5)	124 (68.9)	26 (59.1)	115 (70.1)	19 (59.4)	118 (69.0)
	Seroconversion	1 (2.7)	11 (5.8)	2 (4.2)	9 (5.2)	2 (4.4)	10 (5.6)	2 (4.9)	10 (5.6)	3 (6.8)	6 (3.7)	1 (3.1)	9 (5.3)
	Persistent Seropositivity	10 (27.0)	56 (29.6)	16 (33.3)	49 (28.2)	15 (33.3)	50 (28.2)	15 (36.6)	51 (28.3)	15 (34.1)	43 (26.2)	12 (37.5)	44 (25.7)
HPV18	Always Seronegative	33 (78.6)	155 (74.5)	42 (79.2)	144 (75.0)	46 (79.3)	140 (74.5)	33 (80.5)	156 (74.3)	41 (75.9)	137 (77.0)	29 (80.6)	144 (75.0)
	Seroconversion	3 (7.1)	8 (3.8)	2 (3.8)	8 (4.2)	1 (1.7)	10 (5.3)	0 (0.0)	11 (5.2)	3 (5.6)	8 (4.5)	0 (0.0)	11 (5.7)
	Persistent Seropositivity	6 (14.3)	45 (21.6)	9 (17.0)	4 (20.8)	11 (19.0)	38 (20.2)	8 (19.5)	43 (20.5)	10 (18.5)	33 (18.5)	7 (19.4)	37 (19.3)
HPV45	Always Seronegative	42 (93.3)	199 (90.5)	53 (93.0)	184 (90.6)	58 (89.2)	181 (91.9)	47 (92.2)	195 (90.7)	55 (93.2)	173 (92.0)	35 (89.7)	187 (92.1)
	Seroconversion	1 (2.2)	3 (1.4)	1 (1.8)	3 (1.5)	2 (3.1)	2 (1.0)	2 (3.9)	2 (0.9)	2 (3.4)	1 (0.5)	1 (2.6)	2 (1.0)
	Persistent Seropositivity	2 (4.4)	18 (8.2)	3 (5.3)	16 (7.9)	5 (7.7)	14 (7.1)	2 (3.9)	18 (8.4)	2 (3.4)	14 (7.4)	3 (7.7)	14 (6.9)

* Serological status was categorized into three groups as followed: 1) Always seronegative, MFI value stayed <200 with each visit; 2) Seroconversion, defined by two conditions: (i) an MFI value <200 in the first and >200 in the second sample, and (ii) at least a twofold increase of the previous serum value; and lastly 3) Persistent seropositivity, MFI value stayed > 200 in all follow-up visits.

Table 3. HPV genotype 6,11,16,18,45 -specific serology as predictor of 24-month persistence compared to those women who clearance their infection. Association was compared only between women with genotype specific high-level serology titers (MFI >400) and those that were always seronegative. No significant results were detected.

HPV genotype	Persistence ≥ 24 months (n)		Clearance (n)		OR* 95%CI
Genital mucosa	High-level serology	Always seronegative	High-level serology	Always seronegative	
6	1	1	4	1	0.25 (0.002-39.09)
11	0	0	0	1	NC
16	8	42	4	35	1.66 (0.40-8.16)
18	0	3	1	4	NC
45	0	3	0	5	NC
Oral mucosa					
6	0	0	1	1	NC
11	0	0	0	1	NC
16	8	26	6	26	1.33 (0.34-5.35)
18	1	1	0	3	NC
45	0	0	0	0	NC

*OR calculated as likelihood of a positive high-level serology titers to predict HPV persistence; NC= OR non-calculable