Tampereen yliopisto

Laura Kirjavainen

# IMPACT OF DIFFERENT COFACTORS ON NATURALLY ACQUIRED HUMAN PAPILLOMAVIRUS ANTIBODY LEVELS AMONG UNVACCINATED PREGNANT WOMEN

Lääketieteen ja terveysteknologian tiedekunta
Syventävien opintojen opinnäytetyö
Huhtikuu 2024

# TIIVISTELMÄ

Laura Kirjavainen: Riskitekijöiden vaikutus luonnollisesti hankittuihin HPV-vasta-ainetasoihin rokottamattomilla raskaana olevilla naisilla
Syventävien opintojen opinnäytetyö
Tampereen yliopisto
Lääketieteen lisensiaatin tutkinto-ohjelma
Huhtikuu 2024

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Ihmisen papilloomavirukset (HPV, human papillomavirus) ovat yleisiä sekä seksuaali- että ei-seksuaaliteitse tarttuvia virusinfektioita. Kroonisten HPV-infektioiden on todettu lisäävän riskiä erityisesti kohdunkaulan, mutta myös suun ja kaulanalueen syöville. Kohdunkaulan ja suun HPV-infektioiden luonnollista taudinkulkua ja siihen vaikuttavia tekijöitä on tutkittu laajalti, mutta riskitekijöiden vaikutusta luonnollisiin HPV-infektion vasta-ainetasoihin tiedetään vielä vähän. Tämän tutkimuksen tarkoituksena oli tarkastella eri riskitekijöiden vaikutusta HPV:n luonnollisiin vasta-ainevastetasoihin kuuden vuoden seurannan aikana.

Tutkimusaineisto koostui raskaana olevista naisista, jotka osallistuivat suomalaiseen HPV-perhekohorttitutkimukseen vuosien 1998–2007 välillä Turun yliopistollisessa keskussairaalassa ja Turun Yliopistossa. Osallistuvista naisista ei kukaan ollut saanut HPV-rokotetta. Naisilta kerättiin sekä genitaalien että suun alueelta harjanäytteet HPV-genotyypitystä varten; ja verinäytteet HPV-serologiaa varten tutkimuksen alussa sekä 12, 24, 36 ja 72 kuukauden seurantakäynneillä. Vasta-aineet määriteltiin HPV-genotyyppien 6, 11, 16, 18 ja 45 L1-pintaproteiinille multiplex-HPV-serologialla Heidelbergissä, Saksan syöpätutkimuskeskuksessa. Seerumin HPV-vasta-aineet tulkittiin positiivisiksi, kun niiden antigeenispesifiset mediaanifluoresenssi-intensiteettitasot (MFI) olivat yli raja-arvon 200 MFI. Tähän tutkimukseen otettiin mukaan alkuperäisestä 329 naisesta vain 59 naista. Heistä 23:lla oli seurannan aikana aina alhaiset vasta-ainetasot (alle 200 MFI) ja 36:lla oli taas aina korkeat (yli 200 MFI) vasta-aineiden tasot HPV 6, 11, 16, 18 ja 45 genotyypeille. Tutkimuksen aikana naisilta kerättiin tausta- ja riskitekijöitä HPV-infektiolle kattavan kyselylomakkeen kautta.

Lähtötilanteessa tai seurantakäynneillä havaitulla genitaali- tai suun alueen HPV-esiintymisellä ei ollut merkitystä vasta-ainetasoihin. Suuri aikaisempien seksikumppaneiden lukumäärä tai aiemmin diagnosoidut sukupuolielinten syylien määrät liittyivät merkittävästi pysyviin korkeisiin HPV-vasta-ainetasoihin (p-arvot 0,023 ja 0,043). Lisäksi havaittiin, että naisilla, jotka eivät raportoineet lainkaan allergioita, esiintyi useammin (69,4 %) korkeita HPV-vasta-ainetasoja verrattaessa niihin, jotka ilmoittivat allergiasta (p = 0,03).

Seksikumppaneiden lukumäärän sekä sukupuolielinten syylien vaikutukset vasta-ainetasoihin olivat odotettavissa. Yllättävämpää on kuitenkin allergioiden vaikutus HPV-vasta-ainetasoihin. Naisilla, joilla on jatkuvasti alhainen HPV-vasta-ainetaso, todetaan enemmän allergioita kaikissa tutkimissamme allergeenien alaryhmissä (ruoka, luonto, eläin, kemikaalit ja lääkeaineet). Jatkossa voisi olla hyödyllistä tutkia, vaikuttavatko allergiat HPV-vasta-ainevasteeseen yleisesti vai liittyykö tämä vaste vain tiettyihin allergeeneihin.

Avainsanat: vasta-aine, riskitekijä, ihmisen papilloomavirus, allergia, serologia

Tämän julkaisun alkuperäisyys on tarkastettu Turnitin Originality Check -ohjelmalla.

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# Impact of Different Cofactors on Naturally Acquired Human Papillomavirus Antibody Levels Among Unvaccinated Pregnant Women

Laura Kirjavainen,[1] Helmi Suominen,[1] Kari Syrjänen,[2] Tim Waterboer,[3] Seija Grenman,[4] Stina Syrjänen,[5] and Karolina Louvanto[1,6]

## Abstract

Human papillomavirus (HPV) infections are common, transmitted by sexual and nonsexual routes. The present case–control setting was designed to examine potential cofactors associated with either persistently low or high HPV-antibody levels. The study subjects were from the Finnish HPV Family cohort of 329 baseline pregnant, non-HPV-vaccinated women, who were sampled for genital and oral HPV-DNA and HPV serology at baseline, and at 12, 24, and 36 months. Antibodies to the L1 major capsid protein of HPV 6, 11, 16, 18, and 45 were analyzed by multiplex HPV serology and HPV genotyping was performed. This study included 59 women, 23 women with persistently low (<200 median fluorescence intensity [MFI]) and 36 women with persistently high and always positive (>200 MFI) levels of these antibodies for all five HPV genotypes. Potential HPV-associated covariates were derived from detailed questionnaires. Only cofactors other than detected HPV genotype significantly impact on the levels of natural HPV antibodies. A higher number of past sexual partners or a history of diagnosed genital warts were significant covariates of high HPV antibody levels ($p = 0.023$ and $p = 0.043$, respectively). Of interest, women with a history of allergies presented with low levels of HPV antibodies ($p = 0.03$), potentially exposing these women to an increased risk of future HPV-related diseases that merit closer surveillance.

**Keywords:** women, antibody, human papillomavirus, cofactors, allergy

## Introduction

Human papillomavirus (HPV) infections are very common, being transmitted by sexual and nonsexual routes (Braaten and Laufer, 2008). More than 30 years ago, it was calculated that >80% of all women acquire genital HPV infection at least once in their lifetime (Chesson et al., 2014; Einstein et al., 2009; Trottier and Franco, 2006). Most HPV infections are transient and clear spontaneously within 1–2 years depending on the HPV genotype (Ho et al., 1998).

[1]Department of Obstetrics and Gynecology, Faculty of Medicine and Health Technology, Tampere University, Tampere, Finland.
[2]SMW Consultants, Ltd, Kaarina, Finland.
[3]Division of Infections and Cancer Epidemiology, German Cancer Research Center (DKFZ), Heidelberg, Germany.
[4]Department of Obstetrics and Gynecology, Turku University Hospital, University of Turku, Turku, Finland.
[5]Department of Oral Pathology, Institute of Dentistry, Faculty of Medicine, University of Turku, Turku, Finland.
[6]Department of Obstetrics and Gynecology, Tampere University Hospital, Tampere, Finland.

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In the January/February 2024 issue of *Viral Immunology* (vol. 37, no. 1; 36–43), the article entitled ''Impact of Different Cofactors on Naturally Acquired Human Papillomavirus Antibody Levels Among Unvaccinated Pregnant Women'' has been updated on March 11, 2024 after first online publication of February 5, 2024 to reflect Open Access, with copyright transferring to the author(s), and a Creative Commons License (CC-BY) added (http://creativecommons.org/licenses/by/4.0).

Persistent HPV infection increases the risk of cervical cancer and its precursors (Braaten and Laufer, 2008).

Cofactors associated with the natural outcomes of cervical and oral HPV infections (incident infections, persistence, and viral clearance) have been extensively studied (Antonsson et al., 2022; Muñoz et al., 2004; Tiiti et al., 2022). However, much less attention has been paid to cofactors influencing the serological response to a natural HPV infection. As compared with HPV vaccination, antibody response to a natural HPV infection is more modest and less protective against incident type-specific HPV infections (Beachler et al., 2016).

Natural HPV antibodies among women have been shown to persist at least for 10 years but individual variation exists (Stanley, 2010). In previous studies, HPV seropositivity has been associated with (1) the lifetime number of sexual partners, shown to increase (2) with the younger age at sexual debut, as well as (3) with the prevalence of genital warts, and (4) history of cervical or oral HPV infection (Ortiz et al., 2018; Pedroza-Gonzalez et al., 2022; Syrjänen et al., 2009). Potential cofactors that impact on the natural HPV antibody response are poorly understood. This study was designed to elucidate these potential cofactors that affect the antibody response to naturally acquired HPV infections during a 6-year prospective follow-up.

## Materials and Methods

### *Subjects*

The Finnish Family HPV (FFHPV) Study is a prospective cohort designed to investigate the dynamics of HPV transmission within regular families (mother, father, index child). The study subjects (mothers, fathers) were enrolled at Turku University Hospital (Finland) during 1998–2001 (Syrjänen, 2018; Syrjänen et al., 2009). Altogether, 329 women who were at least 36 weeks pregnant consented to participate in the cohort. The original study protocol and its subsequent amendments were approved by the Research Ethics Committee of Turku University Hospital (No. 3/1998, No. 2/2006 and 45/180/2010). Informed written consent was obtained from all participants of the study. None of the study participants had received HPV vaccination before or during the follow-up of the study. A detailed questionnaire including information on the participants' demographics, social status, medications, diseases, smoking and alcohol consumption history, sexual behavior, and gynecological history was collected at the baseline of the study (Louvanto et al., 2013; Rintala et al., 2006).

### *Samples*

Genital and oral scraping samples were collected from all women at the study entry and at 2, 6, 12, 24, 36, and 72 months during the follow-up. The samples were taken by scraping with a cytobrush (MedScand, Malmö, Sweden) from the oral mucosa and the mucus membrane of the cervix as previously described (Louvanto et al., 2013; Rintala et al., 2006). HPV genotyping was performed with a Multimetrix kit (Multimetrix, Progen Biotechnik GmbH, Heidelberg, Germany) as detailed previously (Schmitt et al., 2006). This method detects 24 low-risk (HPV 6, 11, 42, 43, and 44) and high-risk (HPV 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 70, 73, and 82) HPV genotypes (Schmitt et al., 2006).

### *Serological assay*

Blood samples were collected at the baseline visit (study entry) and at 12, 24, 36 and 72 months of follow-up. The blood samples were centrifuged at 2,400 rpm for 10 min and the serum was separated in three 1-mL aliquots (Syrjänen et al., 2009). The samples were first stored at −20°C for 1 week and subsequently at −70°C until analyzed at the German Cancer Research Center (DKFZ) (Syrjänen et al., 2009). Antibodies to the L1 major capsid protein 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 (Waterboer et al., 2006; Waterboer et al., 2005). Sera were interpreted as HPV antibody positive (to any of the tested genotypes) when the antigen-specific median fluorescence intensity (MFI) levels were above the cutoff value of 200 MFI (Michael et al., 2008).

### *HPV outcomes*

In this case–control setting nested within the FFHPV cohort, 59 women were included divided as cases ($n=23$) and controls ($n=36$). The cases comprised women whose sera tested constantly below the 200 MFI cutoff value for all five HPV types, at all four (baseline, 12, 24, and 36 months) visits. The reference group of 36 women included only cases whose sera tested above the 200 MFI cutoff under these circumstances.

The definitions of different viral outcomes (defined by HPV DNA testing) were as follows: (1) incident infection, defined as being HPV negative at baseline and acquiring an HPV infection during the follow-up; (2) viral clearance, defined as HPV positivity shifted to HPV negativity and remaining HPV negative by the end of the follow-up; (3) HPV persistence defined as HPV positivity in two or more consecutive HPV DNA samples (type specific or non–type specific); (4) fluctuation defined as consecutive HPV samples being alternatingly HPV positive and HPV negative (Louvanto et al., 2013; Rintala et al., 2006).

### *Statistical analyses*

Differences between measured variables were analyzed by using the $\chi^2$-test, with Fisher's exact test (or likelihood ratio test) for categorical variables. Statistical analyses were performed using SPSS (IBM, NY; PASW Statistics version 26.0.1) software package. All statistical tests performed were two-sided and declared significant at the $p$-value <0.05 level.

## Results

The mean age (at study entry) of the women with low HPV antibody titers (=cases, $n=23$) was 25.78 years (standard deviation [SD] ±2.6) and that of the women with high antibody titers (controls, $n=36$) the mean age was 26.58 years (SD ±3.752).

Table 1 provides the prevalence of genital and oral HPV genotypes detected in the two groups of women at each follow-up visit when HPV tested. In both genital and oral scrapings, the most frequent HPV genotype was HPV 16 in both low and high HPV antibody titers (cases and controls), and all other HPV genotypes were rarer throughout the

Table 1. Detected of Genital and Oral Human Papillomavirus Genotypes at Each Follow-Up Visit of the Two Groups (Low/High) of Women

| | *Baseline,* n *(%)* | | *2 months,* n *(%)* | | *6 months,* n *(%)* | | *12 months,* n *(%)* | | *24 months,* n *(%)* | | *36 months,* n *(%)* | | *72 months,* n *(%)* | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Genital | | | | | | | | | | | | | | |
| | Low ($n=23$) | High ($n=36$) | Low ($n=23$) | High ($n=35$) | Low | High | Low ($n=23$) | High ($n=35$) | Low ($n=23$) | High ($n=34$) | Low ($n=23$) | High ($n=34$) | Low ($n=13$) | High ($n=22$) |
| HPV 6 | 0 (0.0) | 2 (5.6) | 0 (0.0) | 0 (0.0) | … | … | 0 (0.0) | 0 (0.0) | 0 (0.0) | 1 (2.9) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| HPV 11 | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | … | … | 0 (0.0) | 1 (2.9) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| HPV 16 | 1 (4.3) | 1 (2.8) | 1 (4.3) | 4 (11.4) | … | … | 4 (17.4) | 7 (20.0) | 10 (43.5) | 9 (26.5) | 9 (39.1) | 8 (23.5) | 0 (0.0) | 4 (18.2) |
| HPV 18 | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | … | … | 1 (4.3) | 2 (5.7) | 2 (8.7) | 0 (0.0) | 0 (0.0) | 1 (2.9) | 1 (7.7) | 1 (4.5) |
| HPV 31 | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | … | … | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 1 (2.9) | 0 (0.0) | 0 (0.0) |
| HPV 33 | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | … | … | 0 (0.0) | 0 (0.0) | 0 (0.0) | 1 (2.9) | 1 (4.3) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| HPV 43 | 0 (0.0) | 1 (2.8) | 0 (0.0) | 0 (0.0) | … | … | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| HPV 44 | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | … | … | 0 (0.0) | 1 (2.9) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| HPV 45 | 1 (4.3) | 0 (0.0) | 0 (0.0) | 0 (0.0) | … | … | 0 (0.0) | 1 (2.9) | 0 (0.0) | 1 (2.9) | 0 (0.0) | 1 (2.9) | 0 (0.0) | 0 (0.0) |
| HPV 51 | 1 (4.3) | 0 (0.0) | 0 (0.0) | 0 (0.0) | … | … | 0 (0.0) | 0 (0.0) | 0 (0.0) | 1 (2.9) | 0 (0.0) | 1 (2.9) | 0 (0.0) | 0 (0.0) |
| HPV 52 | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | … | … | 0 (0.0) | 0 (0.0) | 0 (0.0) | 1 (2.9) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| HPV 56 | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | … | … | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 1 (7.7) | 0 (0.0) |
| HPV 66 | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | … | … | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 1 (2.9) | 0 (0.0) | 0 (0.0) |
| HPV 70 | 0 (0.0) | 0 (0.0) | 2 (8.7) | 0 (0.0) | … | … | 0 (0.0) | 1 (2.9) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| Any HPV | 3 (13.0) | 8 (22.2) | 3 (13.0) | 7 (20.0) | … | … | 9 (39.1) | 18 (51.4) | 17 (73.9) | 19 (55.9) | 11 (47.8) | 16 (47.1) | 3 (23.1) | 6 (27.3) |
| Multiple types | 0 (0.0) | 4 (11.1) | 0 (0.0) | 3 (8.6) | … | … | 4 (17.4) | 5 (14.3) | 5 (21.7) | 5 (14.7) | 1 (4.3) | 3 (8.8) | 1 (7.7) | 1 (4.5) |
| HPV negative | 20 (87.0) | 28 (77.8) | 20 (87.0) | 28 (80.0) | … | … | 14 (60.9) | 17 (48.6) | 6 (26.1) | 15 (44.1) | 12 (52.2) | 18 (52.9) | 10 (76.9) | 16 (72.7) |
| Oral | | | | | | | | | | | | | | |
| | *Low* (n=*23*) | *High* (n=*36*) | *Low* (n=*23*) | *High* (n=*33*) | *Low* (n=*22*) | *High* (n=*36*) | *Low* (n=*23*) | *High* (n=*35*) | *Low* (n=*22*) | *High* (n=*34*) | *Low* (n=*23*) | *High* (n=*34*) | *Low* (n=*17*) | *High* (n=*20*) |
| HPV 6 | 1 (4.3) | 3 (8.3) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 1 (2.8) | 1 (4.3) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 2 (5.9) | 0 (0.0) | 0 (0.0) |
| HPV 11 | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 1 (4.5) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| HPV 16 | 4 (17.4) | 2 (5.6) | 3 (13.0) | 6 (18.2) | 4 (18.2) | 4 (11.1) | 2 (8.7) | 3 (8.6) | 4 (18.2) | 5 (14.7) | 1 (4.3) | 7 (20.6) | 2 (11.8) | 1 (5.0) |
| HPV 18 | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 1 (4.3) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| HPV 31 | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 1 (2.8) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| HPV 58 | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 1 (4.3) | 1 (2.9) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| HPV 59 | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 1 (2.8) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| Any HPV | 5 (21.7) | 6 (16.7) | 4 (17.4) | 8 (24.2) | 5 (22.7) | 8 (22.2) | 4 (17.4) | 8 (22.9) | 4 (18.2) | 7 (20.6) | 3 (13.0) | 9 (26.5) | 3 (17.6) | 2 (10.0) |
| Multiple types | 0 (0.0) | 1 (2.8) | 1 (4.3) | 2 (6.1) | 0 (0.0) | 1 (2.8) | 0 (0.0) | 4 (11.4) | 0 (0.0) | 2 (5.9) | 1 (4.3) | 0 (0.0) | 1 (5.9) | 1 (5.0) |
| HPV negative | 18 (78.3) | 30 (83.3) | 19 (82.6) | 25 (75.8) | 17 (77.3) | 28 (77.8) | 19 (82.6) | 27 (77.1) | 18 (81.8) | 27 (79.4) | 20 (87.0) | 25 (73.5) | 14 (82.4) | 18 (90.0) |
| Genital and oral combined | | | | | | | | | | | | | | |
| | *Low* (n=*23*) | *High* (n=*36*) | *Low* (n=*23*) | *High* (n=*33*) | *Low* (n=*22*) | *High* (n=*36*) | *Low* (n=*23*) | *High* (n=*35*) | *Low* (n=*22*) | *High* (n=*34*) | *Low* (n=*23*) | *High* (n=*34*) | *Low* (n=*17*) | *High* (n=*20*) |
| HPV 6 | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | … | … | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| HPV 11 | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | … | … | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| HPV 16 | 1 (4.3) | 0 (0.0) | 1 (4.3) | 0 (0.0) | … | … | 0 (0.0) | 1 (2.9) | 1 (4.5) | 1 (2.9) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| HPV 18 | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | … | … | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| HPV 31 | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | … | … | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| Any HPV | 1 (4.3) | 2 (5.6) | 1 (4.3) | 1 (3.0) | … | … | 1 (4.3) | 6 (17.1) | 2 (9.1) | 4 (11.8) | 1 (4.3) | 5 (14.7) | 1 (5.9) | 1 (5.0) |
| Multiple types | 0 (0.0) | 0 (0.0) | 0 (0.0) | 1 (3.0) | … | … | 0 (0.0) | 2 (5.7) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 1 (5.9) | 1 (5.0) |
| HPV negative | 16 (69.6) | 24 (66.7) | 17 (73.9) | 19 (57.6) | … | … | 11 (47.8) | 15 (42.9) | 4 (18.2) | 12 (35.3) | 10 (43.5) | 14 (41.2) | 9 (52.9) | 10 (50.0) |

The combined section shows the number of women that had simultaneous oral and genital HPV genotypes detected.
HPV, human papillomavirus.

Table 2. Viral Outcomes of Genital and Oral Human Papillomavirus Type-Specific Infections in the Two Groups of Women

| | *Low* (n = *23*) | *High* (n = *36*) |
|---|---|---|
| *Type-specific HPV outcomes* | n *(%)* | n *(%)* |
| Genital | | |
| Always HPV negative | 1 (4.3) | 5 (13.9) |
| Incident HPV (baseline negative) | 8 (34.8) | 4 (11.1) |
| Persistent (HPV type specific) | 10 (43.5) | 14 (38.9) |
| Persistence (non–HPV type specific) | 3 (13.0) | 5 (13.9) |
| Fluctuation | 1 (4.3) | 5 (13.9) |
| Clearance | 0 (0.0) | 3 (8.3) |
| $p = 0.172$ | | |
| Oral | | |
| Always HPV negative | 10 (43.5) | 11 (30.6) |
| Incident HPV (baseline negative) | 2 (8.7) | 3 (8.3) |
| Persistent (HPV type specific) | 0 (0.0) | 7 (19.4) |
| Persistence (non–HPV type specific) | 1 (4.3) | 0 (0.0) |
| Fluctuation | 6 (26.1) | 5 (13.9) |
| Clearance | 4 (17.4) | 10 (27.8) |
| $p = 0.101$ | | |

follow-up. Very few women had a combined oral and genital simultaneous HPV detection. No significant differences in HPV genotype distribution were found between the low (cases) and high antibody titers (controls).

HPV type-specific viral outcomes in low and high antibody titers are given in Table 2. Type-specific HPV persistence was more common in genital samples than in the oral samples, 38.9% versus 19.4% and 43.5% versus 0.0%, respectively, among the high and low antibody titers. Incident HPV was also more common in genital samples than in oral samples. As to the oral samples, 43.5% of the low and 30.6% of the high antibody titers remained always HPV negative. The overall distribution of the outcome profiles of genital and oral HPV infections did not differ between the low and high antibody titers ($p = 0.172$ and $p = 0.101$), respectively.

Table 3 summarizes the frequencies of the demographics and other potential cofactors in the two groups of women. A statistically significant difference between low and high antibody titers was observed in their history of allergies ($p = 0.03$). The women with no history of allergies expressed more (69.4%) often higher HPV antibody levels than those reporting any type of allergy. Indeed, women with persistent low levels of HPV antibodies (cases) reported more allergies in all subgroups of different allergens: food, nature, animal, chemical and medicinal substances.

Potential cofactors associated with gynecological history or sexual behavior in the cases and controls are compared in Table 4. Reporting a higher number of sexual partners was significantly associated with high antibody levels; 33.3% of these control women had >10 past sexual partners, whereas the corresponding percentage among the cases was only 13.6%, ($p = 0.023$). Women in the latter group reported two or less sexual partners more often than did the control women (27.3% vs. 15.2%). The same distinction remained in the category of three to five past sexual partners (45.5% vs. 15.2%). Having a history of genital warts was associated with higher antibody levels in that 46.9% of the control women reported a history of genital warts as compared with only 18.2% of the cases ($p = 0.043$). The other cofactors did not differ between the two groups.

## Discussion

This study is a case–control setting nested within the FFHPV cohort with an active longitudinal follow-up of 6 years. Two distinct groups of women, low responders (called cases) and high responders (called controls) were built up using stringent serological criteria. Cases included all the women whose sera tested seronegative for all five HPV genotypes at all visits, whereas controls included all women whose sera tested invariably seropositive using the same criteria. The two groups were compared regarding the distribution of the HPV genotypes detected in both genital and oral samples during the entire follow-up. In addition, a wide range of variables recorded at the baseline questionnaire were analyzed as potential cofactors of this serological (low/high) response to HPV 6, 11, 16, 18, and 45. Of these potential cofactors, three proved to be of special interest: (1) having an allergy, (2) the number of sexual partners, and (3) the history of genital warts were statistically significantly different among the low and high responders.

According to the date from a recent meta-analysis, naturally acquired HPV antibodies have a moderate protective effect against a subsequent cervical HPV infection (Beachler et al., 2016). However, previous studies assessing potential cofactors that would impact on the different HPV antibody response in longitudinal settings are limited. Most of such studies focus only on HPV seroprevalence and/or HPV genotype detection (Ortiz et al., 2018). In the present setting, the prevalence of any HPV genotypes in both genital and oral samples was generally higher among those women who had high HPV antibody levels, ranging between 10% and 55.9%, respectively. However, we could not establish any significant differences between the HPV genotype prevalence and serological HPV antibody response. Unfortunately, the highly variable techniques and the threshold values for HPV seropositivity used in different studies make direct comparison of these serological studies extremely challenging.

As described before (Louvanto et al., 2013; Rintala et al., 2006) all participants in the FFHPV cohort were interviewed by detailed questionnaires at study entry and at later stage (i.e., 3- and 6-year timepoint). These recorded items include both established and suspected potential HPV-related cofactors that might influence on the natural outcomes of genital and oral HPV infections during the long-term follow-up. In the present analysis, selected items of those were compared between the two study groups to assess their potential impact on the serological HPV antibody response in these women (Tables 3 and 4). Three of these covariates proved to show a statistically significant difference between these two groups and addressed in more detail.

First of these is the number of reported sexual partners. In brief, small number of sexual partners seemed to be typical to women with low levels of HPV antibodies (cases), whereas high levels of HPV antibodies were closely associated with a high number of sexual partners. These results were consistent

Table 3. Demographics and Other Potential Cofactors in the Two Groups of Women

| *Characteristics and background factors* (n/n *low/high)* | *Low* n *(%)* | *High* n *(%)* | p |
|---|---|---|---|
| Mother's age in years (23/36) | | | 0.565 |
| 15–19 | 0 (0.0) | 1 (2.8) | |
| 20–24 | 7 (30.4) | 6 (16.7) | |
| 25–29 | 14 (60.9) | 25 (69.4) | |
| 30–34 | 2 (8.7) | 2 (5.6) | |
| 35–39 | 0 (0.0) | 2 (5.6) | |
| Marital status (22/33) | | | 0.937 |
| Single | 1 (4.5) | 1 (3.0) | |
| Cohabitation | 9 (40.9) | 12 (36.4) | |
| Married | 12 (54.5) | 19 (57.6) | |
| Divorced | 0 (0.0) | 1 (3.0) | |
| Education (22/33) | | | 0.593 |
| Basic school degree | 1 (4.5) | 3 (9.1) | |
| Vocational training | 4 (18.2) | 8 (24.2) | |
| High school graduate | 4 (18.2) | 5 (15.2) | |
| College level degree | 12 (54.5) | 12 (36.4) | |
| University degree | 1 (4.5) | 5 (15.2) | |
| Employment status (22/33) | | | 1.000 |
| Employed | 15 (68.2) | 22 (66.7) | |
| Student | 2 (9.1) | 4 (12.1) | |
| Unemployed | 5 (22.7) | 7 (21.2) | |
| Use of regular medication (20/33) | | | 0.061 |
| No | 16 (80.0) | 32 (97.0) | |
| Yes | 4 (20.0) | 1 (3.0) | |
| Allergy with reported allergen[a] (23/36) | | | **0.03** |
| None | **9 (39.1)** | **25 (69.4)** | |
| Nature | **9 (39.1)** | **4 (11.1)** | |
| Animal | **3 (13.0)** | **3 (8.3)** | |
| Food | **4 (17.4)** | **2 (5.6)** | |
| Chemical | **7 (30.4)** | **2 (5.6)** | |
| Medicinal substance | **4 (17.4)** | **4 (11.1)** | |
| Atopy (22/33) | | | 0.459 |
| No | 17 (77.3) | 29 (87.9) | |
| Yes | 5 (22.7) | 4 (12.1) | |
| Smoking (22/33) | | | 0.946 |
| No | 9 (40.9) | 15 (45.5) | |
| 1–10 cigarettes per day | 7 (31.8) | 8 (24.2) | |
| 11–20 cigarettes per day | 5 (22.7) | 8 (24.2) | |
| >20 cigarettes per day | 1 (4.5) | 2 (6.1) | |
| Pack years of smoking (12/17) | | | 0.621 |
| Lower tertile (<2.5) | 4 (33.3) | 3 (17.6) | |
| Median tertile (<6.0) | 3 (25.0) | 5 (29.4) | |
| Upper tertile (>6.0) | 5 (41.7) | 9 (52.9) | |
| Age of smoking initiation (12/18) | | | 0.321 |
| 10–13 years | 0 (0.0) | 3 (16.7) | |
| 14–17 years | 10 (83.3) | 10 (55.6) | |
| 18–21 years | 1 (8.3) | 4 (22.2) | |
| 22–25 years | 1 (8.3) | 1 (5.6) | |
| Use of snuff (19/30) | | | |
| No | 19 (100.0) | 30 (100.0) | |
| Alcohol consumption (22/33) | | | 0.973 |
| Never | 2 (9.1) | 3 (9.1) | |
| 1 dose 2–3 times a week | 3 (13.6) | 3 (9.1) | |
| 1 dose once a week | 8 (36.4) | 12 (36.4) | |
| 1 dose once a month | 9 (40.9) | 15 (45.5) | |

Significant results ($p$-value <0.05) are shown in bold.
[a]Women who had two or more reported allergens are counted in each group she reported to have.

Table 4. Cofactors Related to Gynecological History and Sexual Behavior in the Two Groups of Women

| *Gynecological and different sexual behavioral background factors* (n/n *low/high*) | *Low* n *(%)* | *High* n *(%)* | p |
|---|---|---|---|
| Recorded infertility problem (22/33) | | | 1.000 |
| No | 21 (95.5) | 32 (97.0) | |
| Yes | 1 (4.5) | 1 (3.0) | |
| Age at first intercourse (22/33) | | | 0.242 |
| <13 years | 0 (0.0) | 1 (3.0) | |
| 14–16 years | 10 (45.5) | 22 (66.7) | |
| 17–19 years | 10 (45.5) | 9 (27.3) | |
| >20 years | 2 (9.1) | 1 (3.0) | |
| Number of sexual partners (22/33) | | | **0.023** |
| 0–2 | **6 (27.3)** | **5 (15.2)** | |
| 3–5 | **10 (45.5)** | **5 (15.2)** | |
| 6–10 | **3 (13.6)** | **12 (36.4)** | |
| >10 | **3 (13.6)** | **11 (33.3)** | |
| Number of sexual partners by the age of 20 (22/33) | | | 0.445 |
| 0–2 | 10 (45.5) | 10 (30.3) | |
| 3–5 | 9 (40.9) | 13 (39.4) | |
| 6–10 | 1 (4.5) | 6 (18.2) | |
| >10 | 2 (9.1) | 4 (12.1) | |
| Number of sexual intercourses per month (22/33) | | | 0.213 |
| 2–4 | 4 (18.2) | 13 (39.4) | |
| 5–10 | 15 (68.2) | 15 (45.5) | |
| >10 | 3 (13.6) | 5 (15.2) | |
| Practise of oral sex (22/33) | | | 0.233 |
| Regularly | 2 (9.1) | 4 (12.1) | |
| Occasionally | 14 (63.6) | 26 (78.8) | |
| Never | 6 (27.3) | 3 (9.1) | |
| Practise of anal sex (22/33) | | | 1.000 |
| Regularly | 0 (0.0) | 1 (3.0) | |
| Occasionally | 2 (9.1) | 4 (12.1) | |
| Never | 20 (90.9) | 28 (84.8) | |
| Use of contraceptive pills (22/33) | | | 0.557 |
| Yes | 20 (90.9) | 32 (97.0) | |
| Never | 2 (9.1) | 1 (3.0) | |
| Intrauterine device (hormonal IUD) (23/33) | | | 0.246 |
| Yes | 5 (21.7) | 13 (39.4) | |
| No | 18 (78.3) | 20 (60.6) | |
| History of sexually transmitted diseases (23/36) | | | 0.200 |
| No | 20 (87.0) | 24 (66.7) | |
| Chlamydia | 2 (8.7) | 9 (25.0) | |
| Genital herpes | 1 (4.3) | 3 (8.3) | |
| Reported genital warts (22/32) | | | **0.043** |
| Yes | **4 (18.2)** | **15 (46.9)** | |
| No | **18 (81.8)** | **17 (53.1)** | |
| Age at the diagnose of genital warts (22/32) | | | 0.086 |
| Never | 18 (81.8) | 17 (53.1) | |
| <20 years | 0 (0.0) | 6 (18.8) | |
| 20–24 years | 3 (13.6) | 6 (18.8) | |
| >25 years | 1 (4.5) | 3 (9.4) | |
| Treatment of condylomas (5/17) | | | 0.935 |
| No treatment | 2 (40.0) | 6 (35.3) | |
| Topical treatment | 2 (40.0) | 5 (29.4) | |
| Electrocautery | 0 (0.0) | 1 (5.9) | |
| Cryotherapy | 0 (0.0) | 1 (5.9) | |
| Laser therapy | 1 (20.0) | 1 (5.9) | |
| Several treatments | 0 (0.0) | 3 (17.6) | |
| Warts in the mouth (22/31) | | | |
| Never | 22 (100.0) | 31 (100.0) | |
| Warts on the skin (11/21) | | | 1.000 |
| Hands | 4 (36.4) | 8 (38.1) | |
| Legs | 5 (45.5) | 8 (38.1) | |
| Several places (hand, legs, head) | 2 (18.2) | 5 (23.8) | |

Significant results ($p$-value <0.05) are shown in bold.

with the data reported in two previous studies. In a study comprising 1.393 Chilean women, higher HPV seroprevalence was typical to participants who had greater number of lifetime sexual partners (Castro et al., 2014). In another study from Puerto Rico, women with HPV types 16/18 and 6/11/16/18 had higher seroprevalence when they reported at least three sexual partners (Ortiz et al., 2018).

The second of these significant covariates was the history of genital warts. In the present analysis women with genital warts were more likely to have persistent higher HPV antibody titers. In a previous report from the whole FFHPV cohort, predictors for HPV seropositivity were (1) age at the onset for sexual activity, (2) number of sexual partners by the age of 20, (3) lifetime number of sexual partners, and (4) history of genital warts (Syrjänen et al., 2009). Genital warts are usually considered as manifestations (surrogates) of sexual activity, and together with a high number of sexual partners, the high HPV antibody response can be feasibly explained by this sexual behavior. An increased (often promiscuous) sexual activity increases the likelihood of contracting multiple HPV infections during the lifetime, resulting in a more prolific antibody response to HPV. Recent data confirm that antibody response to naturally acquired HPV infection is not as strong as achieved by HPV vaccination, but the natural response is still strong enough to confer some protection against type-specific HPV infections in the future (Beachler et al., 2016).

The third of the covariates shown to be significant in this analysis is the history of allergies. Of interest, women who did not report any history of allergies were more likely to belong to the controls, that is, presented with high HPV antibody titers. A previous study reported an association between a positive history of allergies and decreased risk of cervical squamous cell cancer (Johnson et al., 2011). In the same study analyzing all allergies, pollen allergy was found to have the most marked impact in reducing the risk of cervical cancer risk (Johnson et al., 2011). Without going into the discussion whether HPV antibody responses are in any way related to the development of cervical cancer (protective or enhancing), one can speculate that in the present series, women with no allergies are more prone to elicit a high antibody response to HPV simply because their humoral immune system is not overloaded by the continuous exposure to allergens.

There are also some interesting similarities in the link between immune response and allergy in other viral diseases. People with food allergies were found to have at least 50% lower risk to get severe acute respiratory syndrome coronavirus 2-infection compared with the people without allergies (Seibold et al., 2022).

There were some limitations in our study. The sample size was relatively small; however, the selection was very stringent, which is a vast strength, as we had HPV antibody levels low or high for all five different HPV genotypes for a longitudinal setting of 36 months. In evaluation questionnaires there is always the recall bias that must be considered. Our strength however is that all women in this study responded to the questionnaires. In the allergy selection, a larger sample size would be needed to investigate each different allergen separately more clearly.

In future, it might be useful to investigate whether allergies influence HPV antibody response in general or only some specific allergens. When comparing the antibody levels after natural infection and HPV vaccinations, antibody levels achieved by the bivalent anti-HPV 16/18 vaccine were far above the levels observed after natural HPV infection (Artemchuk et al., 2019). Therefore, in a vaccinated person, the HPV antibody levels persist longer and are more stable (Artemchuk et al., 2019). However, even after vaccination, these high antibody levels do not persist at this level forever, but the titers slowly decrease and there is also some notable individual variation (Mo et al., 2022). One cannot exclude the possibility that in people with allergies, the effectiveness and the duration of the effect of HPV vaccines may also be different, because of these differences related to antibody response to natural HPV infections.

Taken together, of all cofactors affecting the antibody response to natural HPV exposure analyzed in this study, the number of sexual partners and history of genital warts were not unexpected. On the contrary, disclosing the impact of allergies on the HPV antibody response was unexpected. In future studies, increasing attention might be warranted to allergens as potential cofactors of the antibody response to natural HPV infections, and possibly also of the antibody response to HPV vaccines.

## Authors' Contributions

Conceptualization, S.S., S.G. and K.L.; methodology, T.W., S.S., S.G. and K.L.; software, T.W. and K.S.; validation, T.W., S.S. and K.L.; formal analysis, L.K., H.S., K.S. and K.L.; investigation, S.S, S.G. and K.L.; resources, T.W., S.S. and K.L.; data curation, S.S, L.K., H.S. and K.L.; writing—original draft preparation, L.K,.; writing—review and editing, H.S, K.S., T.W., S.G., S.S, and K.L.; visualization, L.K, H.S. and K.L.; supervision, K.L.; project administration, S.S and K.L.; funding acquisition, S.S. and K.L. All authors have read and agreed to the published version of the article.

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Address correspondence to:
*Prof. Karolina Louvanto*
*Department of Obstetrics and Gynecology*
*Faculty of Medicine and Health Technology*
*Tampere University Hospital and Tampere University*
*Arvo Ylpön katu 34, PO Box 100*
*Tampere 33014*
*Finland*

*E-mail:* karolina.louvanto@tuni.fi