What Are the Current HPV Types Contributing to Cervical High-Grade Squamous Intraepithelial Lesions, Adenocarcinoma In Situ, and Early Cervical Cancer?

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ABSTRACT

Objectives: To determine the prevalence of human papillomavirus (HPV) types by genotyping high-grade squamous intraepithelial lesion (HSIL), adenocarcinoma in situ (AIS), and early-stage invasive cervical cancer (ICC) in patients who have been exposed or are naïve to the HPV vaccine.

Methods: This was a cross-sectional study. All patients over the age of 18 years who presented to the colposcopy clinic with HSIL, AIS, or ICC who were expected to undergo a cervical biopsy, loop electrosurgical excisional procedure, or cone biopsy were eligible and approached for informed consent. HPV typing was performed to identify the causative HPV types.

Results: Between November 2016 and May 2023, 113 patients (34 vaccinated with at least 1 dose, and 79 non-vaccinated) consented to this study. The median ages at coitarche and study entry were 18 (range 14–37) and 34 (range 24–66) years, respectively. Only 3 patients were vaccinated prior to coitarche. Histology was as follows: HSIL = 97, AIS = 9, HSIL and AIS = 2, squamous cell carcinoma = 4, and 1 patient with adenocarcinoma. The causative HPV type was 16 or 18 in 59% of the vaccinated group and in 66% of the non-vaccinated group. Most vaccinated patients (74%) reported receiving 2–3 doses of HPV vaccine.

Conclusions: In our cohort, the distribution of causative HPV 16 and 18 in patients presenting with HSIL/AIS/ICC was similar between vaccine-naïve and vaccinated patients. This data suggests cervical screening guidelines should not differentiate between "vaccinated" and "non-vaccinated" women without further details of their vaccination.

Keywords: HPV vaccination; HSIL; early cervical cancer; HPV type

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RÉSUMÉ

Objectifs : Déterminer la prévalence des types de VPH par génotypage des lésions intra-épithéliales malpighiennes de haut grade (HSIL), de l'adénocarcinome in situ (AIS) et des cancers du col invasifs (CCI) de stade précoce chez les patientes exposées ou non au vaccin contre le VPH.

Méthodes : Dans cette étude transversale, toutes les patientes de plus de 18 ans se présentant à la clinique de colposcopie avec une HSIL, un AIS ou un CCI et devant subir une biopsie cervicale, une résection à l'anse diathermique ou une conisation étaient admissibles et ont été invitées à donner leur consentement éclairé. Un typage du VPH a été réalisé pour identifier les types de VPH en cause.

Résultats : Entre novembre 2016 et mai 2023, 113 patientes (34 ayant reçu au moins une dose du vaccin, et 79 non vaccinées) ont accepté de participer à l'étude. Les âges médians au moment du premier rapport sexuel et de l'admission à l'étude étaient respectivement de 18 ans (intervalle : 14-37) et de 34 ans (intervalle : 24-66). Seules 3 patientes ont été vaccinées avant le

premier rapport sexuel.L'histologie des cas se distribue comme suit : 97 cas de HSIL, 9 cas de AIS, 2 cas de HSIL avec AIS, 4 cas de carcinome épidermoïde, et 1 cas d'adénocarcinome. Le type de VPH en cause était le 16 ou le 18 chez 59 % des participantes du groupe vacciné et chez 66 % de celles du groupe non vacciné. La plupart des patientes vaccinées (74 %) ont déclaré avoir reçu 2 ou 3 doses de vaccin contre le VPH.

Conclusion : Dans notre cohorte, la distribution des types de VPH 16 et 18 en cause chez les patientes ayant une HSIL, un AIS ou un CCI est similaire entre les patientes vaccinées ou non vaccinées. Ces données suggèrent que les directives pour le dépistage du cancer du col de l'utérus ne devraient pas faire de différence entre les femmes « vaccinées » et « non vaccinées » sans plus de détails sur leur vaccination.

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INTRODUCTION

The development of cervical cancer requires persistent infection with oncogenic human papillomavirus (HPV)[1] Previous data from North America have demonstrated that 70% of early cervical cancers are associated with HPV 16 and/or 18.[2] HPV vaccines have demonstrated high efficacy in preventing cervical intraepithelial neoplasia and adenocarcinoma in situ (AIS) in individuals vaccinated prior to exposure to the types in the vaccine.[3] The first HPV vaccine approved by the Food and Drug Administration in June 2006 protected against HPV 16 and 18. Subsequently, a second HPV vaccine (also covering HPV 16 and 18) was approved in October 2009.[4] The latest version of the previous vaccine (covering 9 types) referred to as Gardasil 9 (Merck & Co., Kenilworth, NJ, USA) was approved in 2014.[5] These vaccines have been offered to adolescents through elementary school vaccination programs for girls aged 9–12 years and catch-up programs to girls aged 13–26 years.[6] Expanded indications for HPV vaccination now include women ages 9–45 years.[7]

Early reports of vaccine effects in Australia suggest a decrease in cervical high-grade squamous intraepithelial lesion (HSIL) prevalence in girls who received HPV vaccination at least 3 years prior. While this study was ecological, it was one of the first of its kind.[8] Powell et al. reported that in a population of high-grade cervical intraepithelial neoplasia patients, only 25% had received at least 1 dose of the vaccine, 28% had not been vaccinated, and almost 50% had unknown vaccine status.[9] Another study by Mesher et al. reported that HPV 16/18 prevalence was lowest in the youngest age group at 6.5% compared to pre-vaccinated studies. Although prevalence increased with age, it was expectedly lower than before vaccination.[10]

Currently, no prospective studies have determined HPV types in paraffin-embedded tissue in patients diagnosed with HSIL/AIS/early-stage invasive cervical cancer (ICC). As HPV 16 and 18 have been shown to be the most common types found in HSIL, it is important to determine whether this has changed according to vaccination status. This finding has implications for the importance and anticipated effects of immunization with a nanovalent vaccine that includes other oncogenic types.

The aim of this cross-sectional study was to determine the HPV types in patients diagnosed with HSIL/AIS/ICC and to compare the incidence in HPV-vaccinated patients versus those who were HPV vaccine-naïve. Additionally, we aimed to explore the potential reasons for HPV vaccine failure in women with HSIL/AIS/ICC.

METHODS

This was a single-centre, cross-sectional study. This study was approved by the local institutional review board (study identification #2737). This study was supported by an unrestricted grant from Merck (Canada).

All patients aged >18 years who presented to the colposcopy clinic with a histologic diagnosis of HSIL, AIS, or ICC and were expected to undergo a cervical biopsy, loop electrosurgical excisional procedure (LEEP), or cone biopsy as the standard of care were eligible and approached for informed consent.

Consenting patients underwent cervical biopsy, LEEP, or cone biopsy as part of standard care (or within 2 years of consent, with archived tissue at our institution). Participation did not alter the size of the collected tissue as only standard care requirements were followed.

Patients who were unable to provide consent were excluded. To ensure that specimens were suitable for molecular typing, patients who had a previous LEEP or cone biopsy were also excluded if the tissue was greater than 2 years old or if it was archived at an outside institution.

All patients underwent colposcopy and were treated according to the standard of care. Each patient who agreed to participate was assigned a unique enrolment number. Full medical histories, including current medical conditions, medications, and age, were recorded by the study coordinator. In addition, a self-report questionnaire regarding specific histories related to HPV vaccination type, dosing date, number of doses received, age at coitarche, and history of cervical intraepithelial neoplasia/condyloma was administered to each patient.

Consenting patients underwent cervical biopsy, LEEP, or cone biopsy as part of the standard of care treatment (or within 2 years of consent and whose tissue was archived at our institution). The size or amount of the sample obtained from this baseline procedure was not affected by patient participation; that is, only as much tissue as the standard of care indicated was removed from those who consented.

The tissue obtained at baseline was assessed by the Institutional Anatomic Pathology Department as per the standard of care. Participants whose samples did not reveal high-grade intraepithelial lesions were excluded from the analysis and considered to have screening failure.

This is a descriptive study of the evaluated population. The goal was not to compare the differences between the vaccinated and non-vaccinated groups statistically. Therefore, sample size calculations were not performed. The number of participants in this study was selected based on the feasibility and ability to generate descriptive findings.

We aimed to recruit approximately 100 patients with tissue samples revealing at least HSIL. Of these patients, we planned to have approximately half who were administered at least 1 HPV vaccine dose and the other half were to be HPV vaccine-naïve patients. However, there were no restrictions on eligibility based on HPV vaccination status.

Once pathology confirmed that the participant had at least HSIL, a portion of the total tissue sample from the baseline procedure or archive was obtained for research purposes. This portion of the tissue was subjected to HPV typing at the National Microbiology Laboratory Branch in Winnipeg, Canada. Formalin-fixed paraffin-embedded samples were de-paraffinized using the xylene protocol, and nucleic acids were extracted by enzymatic digestion using the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany). Genotyping was performed using nested polymerase chain reaction amplification with general alpha-papillomavirus primers, followed by specific genotyping using a multiplex microsphere assay with probes for 46 high- and low-risk HPV types, as described previously.[11]

Patients had no further direct involvement in the study after the tissue sample was obtained. Patients interested in the outcome of their HPV genotyping were allowed to call the research coordinator or other members of the research staff to be informed of their results, with the understanding that these results would not affect their care. With patient consent, study personnel were allowed to contact primary care physicians and request patient medical records related to their history of HPV testing, HPV vaccination, abnormal pap smears and/or genital warts, as well as treatments administered as a result.

Descriptive statistics, including the median age at study entry and coitarche, were used to report participants' characteristics using SAS OnDemand. Statistical comparisons were not performed.

RESULTS

Between November 2016 and May 2023, 113 patients (34 vaccinated with at least 1 dose and 79 non-vaccinated) were enrolled. The median ages at coitarche and study entry were 18 (range 14—37) and 34 (range 24—66) years, respectively.

Histological findings were as follows: HSIL = 97, AIS = 9, HSIL and AIS = 2, squamous cell carcinoma = 4; and adenocarcinoma = 1. The distribution of causative HPV types is listed in Table 1 for non-vaccinated patients and in Table 2 for vaccinated patients. There were 5 (15%) in the vaccinated group and 9 (11%) in the non-vaccinated group, and no HPV was identified in the submitted materials. Overall, 5 patients had 2 coexisting HPV types. In the vaccinated group, 35/56, 58/59, and 16/33, and in the non-vaccinated group; 16/31, 16/52, respectively. In AIS/adenocarcinoma patients, the HPV types were HPV 16 = 7, HPV 18 = 3, and HPV-negative = 2.

As shown in Tables 1 and 2, the majority of causative HPV was 16 or 18: 66% (75% if HPV-negative was excluded) in the non-vaccinated group and 59% (69% if HPV-negative was excluded) in the vaccinated group.

Of the vaccinated patients, 25 (74%) reported receiving 2—3 doses. Seven patients could not recall the date of their vaccination, and only 3 patients were vaccinated prior to the coitarche (their HPV status was negative, 16, 16/33). Seven patients were vaccinated after their HSIL diagnosis and were categorized as non-vaccinated for the purposes of this study.

Table 1. HPV types in non-vaccinated patients (n = 79)

HPV	n	%	% (exclude HPV neg)
16	48[a]	61	69
18	4	5	6
31	3[a]	4	4
33	2	3	3
35	2	3	3
42	1	1	1
45	2	3	3
51	2	3	3
52	3[a]	3	4
56	1	1	1
58	2	3	3
59	1	1	1
6	1	1	1
neg	9	11	

[a]Includes 2 patients with dual HPV types (16/31;16/52).

HPV: human papillomavirus; Neg: negative.

DISCUSSION

In this cross-sectional study, the distribution of HPV 16 and 18 in patients presenting with HSIL/AIS/ICC was similar between vaccine-naïve and vaccinated patients.

It is important to note that this was not a study on the effectiveness of HPV vaccination. Multiple studies have demonstrated reduced condyloma, and incidence of abnormal pap smears as a result of broad school-age vaccination programs[12,13] and few would argue the benefits.[12,13] Rather this is a study evaluating the prevalence of HPV types in patients that have developed at least HSIL during a time frame of vaccination.

Table 2. HPV types in vaccinated patients (n = 34)

HPV	n	%	% (exclude HPV neg)
16	20[a]	59	69
18	0		
31	2	6	7
33	3[a]	9	10
35	1[a]	3	3
42	1	3	3
45	2	6	7
51	2	6	7
52	1	3	3
56	1[a]	3	3
58	2[a]	6	7
59	1[a]	3	3
70	1	3	3
neg	5	15	

[a]Includes 3 patients with dual HPV types (16/33;35/56;58/59).

HPV: human papillomavirus; Neg: negative.

The distribution of HPV 16 and 18 in patients presenting with HSIL/AIS/ICC in our data was similar regardless of whether they were vaccine-naïve or not.

While the long-term effectiveness of the nanovalent vaccine has been shown to reduce HPV-related HSIL as compared to the expected incidence in the non-vaccinated population, there is currently no prospective published data on HPV prevalence in those who developed HSIL in vaccinated versus vaccine-naïve patients.[14] An Australian study by Cornall et al. comparing the population in the pre-vaccine to post-vaccine era, and have shown that approximately 70% of HSIL/AIS is due to HPV 16/18 infection, 4–8 years following of implementation of the HPV vaccine program.[15] The majority of participants in the post-vaccine era group were eligible for the catch-up vaccination program and only 0.6% were eligible for the school-based vaccine. These findings are similar to those in our cohort, highlighting the importance of vaccination timing. Nonetheless, this was a retrospective study with different methodologies used for HPV testing in the pre- and post-vaccine-era populations which limited the robustness of the results.

Our results indicate that for many of the patients in our study, the age at coitarche preceded the introduction of school-age vaccination (2012 in Ontario). Most patients presenting with HSIL/AIS/ICC who have previously been vaccinated are likely to have been vaccinated after HPV exposure, although an inadequate number of vaccine doses (dependent on age) could be another explanation for vaccine failure. While HPV vaccination consists predominantly of 3 doses administered over a 6-month period, more recent data and recommendations have reduced the number of doses to 1 or 2, depending on age.[16]

Three patients in our cohort were confirmed to have received HPV vaccination prior to coitarche, and they may fall into the latter group with an inadequate number of doses, although coitarche may not be the definitive measure of HPV exposure, as other routes of transmission can result in HPV infection.[17]

We made interpretative conclusions from the analysis of these data. Patients with no HPV identified in the tissue are likely to have false-negative results because we were

unable to extract good-quality DNA from formalin-fixed paraffin-embedded tissue specimens. In a study by Bogani et al., 15% of patients with high-grade cervical dysplasia were negative for high-risk HPV types.[18] The authors concluded that this could be secondary to false-negative results, low viral load, and infection from low-risk types that were not tested in that study. In a post hoc analysis of the Addressing THE Need for Advanced HPV diagnostics trial,[19] all participants with grade 3 cervical intraepithelial neoplasia and AIS were eventually attributable to HPV infection using different testing methods, although initial testing found them to be HPV-negative.

HPV vaccination uptake is still facing challenges and has not reached the required target for herd immunity.[20] In the province of Ontario, Canada, school-based HPV vaccination uptake was only 62.4% in 2018 (HPV Immunisation for The Prevention of Cancer. Canadian Partnership Against Cancer, March 2021). In addition, HPV vaccination uptake has decreased worldwide during the COVID-19 pandemic[21] and it has not yet recovered to its pre-pandemic state.

As many jurisdictions are considering changing their cervical screening guidelines based on the history of vaccination,[22–24] the above is very important. While early vaccination studies demonstrated very high efficacy in preventing HSIL, these stringent inclusion/exclusion criteria have not been applied to population-based vaccination.[8] Ongoing interventions are required by public health, including the implementation of strategies to increase school-based vaccination uptake before changing screening guidelines based on vaccination history. For those women who are not vaccinated as part of the school-based program (age 13), many are getting vaccinated at varying ages, based upon personal preferences, relationships, and even pap smear abnormalities. The benefits, while reduced, to some of these women are proven, and for others, such as those older than 45 years, unproven.[24–26] National access to screening tests should remain a priority, as delays in the diagnosis and treatment of cervical high-grade lesions are crucial factors in cancer development.

Our study had several strengths. It is a novel study and the first prospective one to explore the differences in HPV types according to HPV vaccination status in patients with HSIL and ICC. Additionally, HPV testing was performed by genotyping in a single laboratory using a uniform protocol for all enrolled patients. However, this study has some limitations. The generalisability of the results is questionable as most participants were likely vaccinated post-HPV exposure, and it is unclear whether these results would be similar for patients receiving their vaccine during school-based programs. Additionally, the study was not powered to detect statistically significant differences in HPV prevalence between the study groups. Another limitation was the small number of patients and unequal distribution of participants in the vaccinated versus non-vaccinated groups, making the subgroup analysis infeasible. It is important to determine whether the number of HPV vaccine doses and the type of vaccine affect the distribution of HPV types in this cohort of patients. Finally, based on previous publications, we assumed that participants with HPV negativity had "false-negative" results, but no additional testing was performed to verify this assumption.

CONCLUSIONS

Our findings suggest that the distribution of HPV 16 and 18 in patients presenting with HSIL/AIS/ICC was similar between previously vaccinated and vaccine-naïve patients. We believe that based on this data until we have higher school-based vaccination uptake and cervical screening based on HPV testing, caution should be exercised before changing screening guidelines that differentiate "vaccinated" versus "non-vaccinated" women without further details of their vaccination.

ETHICS

The study was approved by the Sunnybrook Institutional Review Board (study identification #2737).

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