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Human Papillomavirus type distribution in Vulval Intraepithelial Neoplasia determined using PapilloCheck DNA microarray

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Short title: HPV type distribution in vulval intraepithelial neoplasia

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Abstract

Vulval intraepithelial neoplasia is a precursor of vulval carcinoma, and is frequently associated with Human Papillomavirus (HPV) infection. Estimates of HPV prevalence in vulval intraepithelial neoplasia vary widely in the UK. The objective of this study was to assess HPV infection in a sample of women with vulval intraepithelial neoplasia, confirmed histologically, and determine the proportion of disease associated with HPV types targeted by prophylactic HPV vaccines. HPV infection was assessed in biopsies from 59 patients using the Greiner Bio-One PapilloCheck® DNA chip assay. Valid results were obtained for 54 cases. HPV infection was present in 43 of the 54 cases (79.6%: 95% CI 67.1-88.2%). The most common HPV types were HPV 16 (33/54: 61.1%), HPV 33 (8/54: 14.8%), HPV 6 (5/54: 9.3 %) and HPV 42 (3/54: 5.6%). The mean age of HPV positive women was significantly less than the mean age of HPV negative women.

This is the largest UK series of vulval intraepithelial neoplasia in which HPV type has been investigated, and 34/54 (63.0 %, 95% CI: 49.6-78.6 %) cases were associated with HPV 16/18, which are targeted by current prophylactic HPV vaccines.

Key words

HPV, Human papillomavirus, VIN, Vulval intraepithelial neoplasia, Papillocheck

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INTRODUCTION

Vulval intraepithelial neoplasia is frequently a painful, distressing condition, and a precursor lesion of invasive vulval carcinoma. In recent decades the incidence of vulval intraepithelial neoplasia has increased, while the mean age of patients has decreased. The incidence of vulval cancer rose by 17% between 1979 and 2001 [MacLean, 2004], with 996 new cases registered in England in 2000 [MacLean, 2006].

Human Papillomavirus (HPV) positivity in vulval intraepithelial neoplasia lesions is reported in international meta-analyses as 84% with most cases attributable to HPV type 16 [De Vuyst et al., 2009]. The most likely explanation for the increased incidence of vulval intraepithelial neoplasia is the substantial rise in HPV infections of the lower genital tract [Peto et al., 2004]; the lifetime risk of HPV infection being estimated at up to 79% [Syrjanen et al., 1990].

The aim of this investigation was to identify HPV types present in vulval intraepithelial neoplasia, confirmed histologically, using the Greiner Bio-One PapilloCheck® DNA chip assay and determine the proportion of vulval intraepithelial neoplasia potentially preventable by HPV prophylactic vaccination.

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MATERIALS AND METHODS

Study population

The study population comprised women attending a specialist vulval intraepithelial neoplasia clinic at the University Hospital of Wales, Cardiff, UK between 2003 and 2009, with vulval intraepithelial neoplasia confirmed histologically, who gave written informed consent for a biopsy to be taken for research purposes. The study was approved by South Wales LREC (SMKW/EL/03/5178). 59 cases were investigated, with an age range of 22-82 years, and a mean age of 45.8 years. There were six cases of vulval intraepithelial neoplasia grade 1, two of vulval intraepithelial neoplasia grade 2, and fifty one cases of vulval intraepithelial neoplasia grade 3.

Sample collection and processing.

Separate biopsies were taken for histology and research use. The research biopsy was immediately placed in liquid based cytology medium (which fixed cells and preserved DNA). DNA was extracted from the research biopsy using the Qiagen QIAamp kit (QIAGEN GmbH, Hilden, Germany) according to the manufacturer's instructions.

Pathology review

A recent review of vulval intraepithelial neoplasia histopathology recommended replacement of the vulval intraepithelial neoplasia 1/2/3 subdivisions with two categories reflecting the putative aetiology of the disease: usual vulval intraepithelial neoplasia (HPV-associated); and differentiated vulval intraepithelial neoplasia (associated with lichen sclerosis) [Sideri et al., 2005]. However, the existence of differentiated vulval intraepithelial neoplasia is disputed, and in practice this diagnosis is rarely made [McCluggage, 2009]. The histopathology of lesions in this study is

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reported using both the vulval intraepithelial neoplasia 1/2/3 and usual/differentiated classification. Pathology review was undertaken blind to HPV status by an experienced Consultant Histopathologist with a special interest in gynaecological pathology (GR).

PapilloCheck® assay

HPV infection was identified using the Greiner Bio-One Papillocheck® assay (Greiner Bio-One GmbH, Frickenhausen, Germany), which enables simultaneous detection and typing of 24 different HPV types in a single reaction (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 70, 73, 82, 6, 11, 40, 42, 43 & 44) . The assay uses multiplex PCR with fluorescent primers to amplify a 350bp fragment of the E1 gene. The PCR product is then hybridised to a PapilloCheck® DNA array, comprising 28 probes, each in 5 replicate spots. To identify false negatives the assay includes amplification of the human ADAT1 gene. Hybridization is assessed using the CheckScanner™ array reader. Amplification, hybridization, detection and interpretation were performed according to the manufacturer's instructions. A valid result was defined as one which passed all of the PapilloCheck® internal controls (including DNA adequacy, sample inhibition, and hybridisation).

Previous studies have confirmed the analytical [Dalstein et al., 2009; Jones et al., 2009] and clinical validity [Halfon et al., 2009; Hesselink et al., 2009] of this assay in large sample sets as compared to GP5+/6+, HC2 and Roche Linear Array. The assay is certified within the European Union as an *in vitro* diagnostic for qualitative detection of HPV in clinical specimens.

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RESULTS

HPV type distribution

HPV type distribution, stratified by histology, is shown in Table I. Valid results were obtained for 54 of the 59 cases. HPV infection was present in 43/54 cases (79.6%: 95% CI 67.1-88.2%). HPV 16 and/or 18 were present in 34/54 cases (63.0 %, 95% CI: 49.6-78.6%). HPV 16 and/or 18 were the only types present in 26/54 cases (48%, 95% CI: 35.4-61.2%).

High Risk (HR) HPV types were present in 38 cases and Low Risk (LR) types in 9 cases; 4 cases contained both HR and LR types. Multiple HPV types were present in 10 cases; in these cases the distribution of types was: one vulval intraepithelial neoplasia grade 1 sample contained HPV 18, 42 and 44/55; all other cases with multiple types were vulval intraepithelial neoplasia 3 and the combinations of types were: 3 cases with HPV 16 and 33; 2 cases with HPV 16 and 6; and one case each of (16,18), (33, 42), (16, 31, 33), (16, 51). In the 5 cases where only LR types were present, 3 were HPV 6 (histology: two cases of vulval intraepithelial neoplasia grade 3, and one vulval intraepithelial neoplasia grade 3 with well-differentiated carcinoma and viral warts), 1 was HPV 40 (vulval intraepithelial neoplasia 1) and 1 contained HPV 42 (vulval intraepithelial neoplasia 1).

HPV and age

HPV positive cases were aged 22-81 with a mean of 43.4 years. HPV negative cases were aged 33-82 years with a mean of 54.9 years. The means of these groups were significantly different (t test p = 0.019).

HPV and histology

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There was no significant correlation between HPV positivity and grade of lesion. Of the HPV positive cases, 40/43 (93.0%) showed vulval intraepithelial neoplasia grade 2 or worse. This was slightly lower in HPV negative lesions, where 9/11 (81.8%) showed vulval intraepithelial neoplasia grade 2 or worse, but this difference was not significant (Fisher's P = 0.266).

There was no significant correlation between HPV type and histological grade; however it was noticeable that all of the HPV 16 positive cases were associated with vulval intraepithelial neoplasia 2 or worse, while a high risk type (HPV 18) was identified in only 1/5 vulval intraepithelial neoplasia grade 1 cases with valid HPV results.

Slides were available for pathology review for 54/59 cases. 52/54 cases were "Usual vulval intraepithelial neoplasia"; 2/54 cases were "Probable Differentiated vulval intraepithelial neoplasia". Both cases of probable Differentiated vulval intraepithelial neoplasia were in HPV negative patients: one an 82 year old woman with vulval intraepithelial neoplasia 3, the other a 61 year old woman with vulval intraepithelial neoplasia 1 (but with a history of vulval cancer). Among the cases with valid HPV results, 42/49 cases of usual vulval intraepithelial neoplasia were HPV positive (85.7% 95%CI: 73.3-92.9%).

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DISCUSSION

This is the largest UK series of vulval intraepithelial neoplasia in which HPV type has been determined. The main limitation of this study was the use of a self selected sample, i.e. women attending a specialist vulval intraepithelial neoplasia clinic. This might bias the sample towards women with persistent or recurrent disease, which might possibly result in some over-representation of more persistent types (HPV 16). A further limitation was the use of separate biopsies for pathology and HPV analysis; in practice both biopsies were immediately adjacent and cases were only included if both biopsies were part of the same macroscopically visible lesion. It is however theoretically possible that the research biopsy may not be representative of the diagnostic biopsy.

Several studies have investigated HPV prevalence in vulval intraepithelial neoplasia in the UK and estimate HPV positivity at 37.5 to 100%, with the proportion of HPV positive cases containing HPV 16 varying from 37.5 to 93.3% [Abdel-Hady et al., 2001; Baldwin et al., 2003; Daayana et al., 2010; Fiander et al., 2006; Gasco et al., 2002; Todd et al., 2002; Tristram and Fiander, 2005; Woo et al., 2007]. The sample size in these studies ranged from 8-29 cases. The sample size for the current study is larger, and the findings are more consistent with the overall results of a recent international meta-analysis, in which 84.0% of vulval intraepithelial neoplasia cases tested HPV positive (67.5% were HPV 16, 7.7% were HPV 33, and 4.6% were HPV 18) [De Vuyst et al., 2009]. The corresponding proportions in the current study were overall HPV positivity of 79.6% (61.1 % were positive for HPV 16, 14.8% for HPV 33, and 3.7% for HPV 18). These are also similar to a separate meta-analysis which

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indicated an overall HPV positivity of 80.4% for vulval intraepithelial neoplasia 2/3 (HPV 16 present in 71.2%, HPV33 in 7.7% and HPV 18 in 5.5% [Smith et al., 2009]). Patients who tested positive for HPV infection were significantly younger (by over 11 years on average) than those testing negative. This is consistent with HPV associated disease being more common in younger women and vulval intraepithelial neoplasia associated with lichen sclerosis being more common in older patients [Bonvicini et al., 2005].

The finding that 85.7% of usual vulval intraepithelial neoplasia were HPV positive is in accord with data previously reviewed [van de Nieuwenhof et al., 2008]. The 7 HPV negative usual vulval intraepithelial neoplasia patients represent an interesting group; it is not clear whether these patients are accounted for by mis-diagnosis of differentiated vulval intraepithelial neoplasia (possibly due to sampling error), or whether there is a subset of usual vulval intraepithelial neoplasia that is truly HPV negative.

Three cases showing vulval intraepithelial neoplasia grade 2 or worse were associated with a low risk HPV type alone (HPV6). One of these cases showed vulval intraepithelial neoplasia grade 3 with well differentiated squamous cell carcinoma and viral warts; it is possible that the warts were associated with the HPV 6 but the vulval intraepithelial neoplasia and carcinoma were not. This apparent association between high grade vulval intraepithelial neoplasia and LR HPV types runs contrary to the suggestion that LR types cause only warts and low grade lesions. However an association between LR HPV types and high grade disease was observed in the De Vuyst meta analysis [De Vuyst et al., 2009] which reported approximately 5% of vulval intraepithelial neoplasia 2/3 as linked to HPV 6.

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HPV associated vulval intraepithelial neoplasia is most common in women in their 30's and 40's [Hart, 2001]. The incidence of VIN is approximately 5 per 100,000 women per year and is increasing [Joura, 2002]. HPV infection is endemic in the UK with almost 30% of women in the 20-25 year age range now infected with a high risk anogenital HPV type [Hibbitts et al., 2008]. Hence, in the short term, the number of women affected by vulval intraepithelial neoplasia is likely to continue increasing. HPV vaccination has been shown to be 100% effective in prevention of vulval epithelial neoplasia 2/3 associated with HPV 16/18 in a per protocol population [Joura et al., 2007]. In the UK vaccination of 12-13 year old girls against HPV 16/18 infection began in 2008. Hence it is likely to be 20 to 30 years before the effects of this intervention become apparent in reduced incidence of vulval intraepithelial neoplasia. Ultimately however, as HPV 16/18 were present in 63.0% of vulval intraepithelial neoplasia, our data suggest that greater than half of vulval intraepithelial neoplasia cases could be potentially prevented by vaccination.

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Competing interests: None declared.

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Histology	HPV Type								
	16	18	31	33	51	6	40	42	44/55*
VIN 1	0	1	0	0	0	0	1	2	1
VIN 2	1	0	0	0	0	1	0	0	0
VIN 3	32	1	1	8	1	4	0	1	0
Total	33	2	1	8	1	5	1	3	1
%	61.1	3.7	1.9	14.8	1.9	9.3	1.9	5.6	1.9

Table I. HPV type distribution within histological grade.
There were 10 cases showing multiple infection, hence the number of types identified is greater that the number of cases. Percentages indicate the proportion of cases that tested positive for a given type.
*the HPV 44 probe cross-hybridizes to HPV 55, hence these types are reported together.

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