Low prevalence of human papillomavirus infection of the cervix in renal transplant recipients

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Abstract

Background. An increased risk of anogenital tract malignancies has been noted among renal transplant recipients. A high prevalence of human papillomavirus (HPV) infection of the cervix in the female renal-transplant population has been assumed based on increasing evidence suggesting that HPV infection is the major risk factor for cervical intraepithelial neoplasia (CIN) and cervical cancer. It has been assumed that immunosuppression leads to either a reactivation of latent HPV or a reduction in the host's ability to contain a primary HPV infection, thereby increasing the risk of CIN and cervical cancer. The objective of this study was to evaluate the prevalence of human papillomavirus (HPV) infection in a population of iatrogenically immunosuppressed renal transplant recipients.

Methods. Twenty-one women were recruited from the renal transplant clinic at Presbyterian Hospital and underwent a gynaecological examination which included colposcopy, a Papanicolaou smear, and a cervicovaginal lavage. Lavage samples were analysed for HPV DNA using L1 consensus primers and the polymerase chain reaction (PCR).

Results. No cases of cervical intraepithelial neoplasia (CIN) were detected in this cohort of 21 immunosuppressed renal transplant recipients. HPV DNA was detected in only a single patient.

Conclusions. Our data suggests that HPV infection is not highly prevalent among older, cytologically normal renal transplant recipients, particularly those who are currently monogamous or not presently sexually active. This study suggests that recent sexual behaviours are more important than past behaviours as a determinant of HPV status in transplant recipients, and also suggests that education concerning the avoidance of high-risk sexual behaviour is an important part of the care of the female renal transplant recipient. Our data is consistent with previous work suggesting that the incidence of CIN is declining in transplant recipients, and it also suggests that the prevalence of HPV infection may be declining as well.

Key words: cervical neoplasia; human papillomavirus; renal transplant

Introduction

Renal transplant recipients require treatment with immunosuppressive medications which appear to increase their susceptibility to opportunistic infections as well as a variety of malignancies. The higher rate of malignancy is due primarily to the development of skin cancers and lymphomas [1–3]. However, several investigators have also noted a high rate of anogenital tract neoplasia in renal allograft recipients [4–7]. In females the anogenital tract lesions that have been reported include intraepithelial neoplasia as well as invasive squamous-cell carcinomas of the vulva, perianal area, and cervix. The intraepithelial neoplasias are frequently multifocal and multicentric [8] and tend to be harder to treat than similar lesions in non-immunosuppressed patients [9].

Cervical cancer appears to be aetiologically related to infection of the cervix with sexually transmitted oncogenic strains of HPV. Indeed, the cytological and histological changes induced by HPV infection form a continuum with the early neoplastic changes of cervical intraepithelial neoplasia (CIN) [10]. CIN lesions span a spectrum of increasing abnormality. Although the clinical significance of low-grade CIN lesions is still debated, high-grade CIN lesions appear to be cancer precursors. Therefore it has been assumed that the increased risk of CIN lesions and cervical cancer in transplant recipients is due to either reactivation of latent HPV or a deficiency in the immunosuppressed host's ability to contain a primary HPV infection.

Although an early estimate of the increased risk of cervical intraepithelial neoplasia among transplant recipients suggested a 14-fold higher incidence [4], a recent investigation suggested that the incidence of abnormal Papanicolaou smears among renal transplant
recipients has been decreasing since 1983. Ter Haar and co-workers [11] attributed this to the introduction of cyclosporin. However, differences in the population of transplant recipients, other changes in the therapeutic regimen, and changes in the prevalence of abnormal cytological smears in the general population are possible explanations for the decreasing incidence of CIN which were not assessed or controlled in this study.

The best available estimates of the risk of invasive cervical cancer come from data collected through transplantation registries and yield standardized incidence ratios of 3.3–8.6 for cervical cancer [7,12].

Despite the existence of reasonably good data on the incidence of cervical cancer, little information is available concerning the prevalence of HPV infection as detected by modern molecular diagnostic methodologies in the transplant population. Alloub and co-workers evaluated renal transplant recipients and hospitalized control patients [13]. Although the prevalence of HPV was quite high in this study (45% of renal allograft recipients versus 38% of controls), they found no significant difference in the prevalence of HPV infection between the groups. A higher prevalence of oncogenic HPV types and a higher prevalence of colposcopically confirmed cervical lesions was noted among the transplant recipients.

Petry et al. noted an HPV prevalence of 17% on cervical DNA samples from transplant recipients [14]. HPV types with high oncogenic potential (HPV 16 and 18) were not detected among the cytologically normal transplant recipients in their population.

More recently, Fairley et al. used a PCR-based HPV detection methodology similar to that used in the present investigation and noted a higher prevalence of HPV infection among transplant recipients (22%) and dialysis patients (20%) as compared to patients with impaired renal function (4.5%) [15]. Nine of the 69 (13%) transplant recipients assessed in this study had cytological findings of atypia or greater. The prevalence of HPV infection among cytologically normal subjects was not reported.

Subjects and methods

Female recipients of renal transplants who had functioning renal allografts and were receiving medical care in the renal transplantation programme of Columbia Presbyterian Medical Center were contacted via their primary care provider and recruited into this Institutional Review Board-approved study. Patients who had undergone prior hysterectomy and those who had not commenced sexual activity were excluded. Twenty-one women were enrolled.

The study protocol included an interview using a standard questionnaire regarding sexual, medical, and reproductive history and a gynaecological examination which included colposcopy, a Papanicolaou smear, and lavage and cytobrush samples for HPV testing. Cervical biopsies and endocervical curettage were performed when acetowhite areas were observed at the initial colposcopic examination or at a second colposcopic examination in two patients in whom the original Papanicolaou smear suggested possible abnormalities. Information regarding medical and gynaecological history was verified by review of each subject's medical record.

Cervical cytological slides were stained with the Papanicolaou stain, examined by a cytotechnologist, and reviewed by a single experienced pathologist (TW). Biopsy specimens were fixed, stained by haematoxylin and eosin, and reviewed by the same pathologist. The terminology used for reporting the histological findings was the modified CIN terminology of Richart, in which cervical condylomas, koilocytic atypia, HPV effects, and CIN1 are grouped together into a single category termed low-grade CIN (CIN1) [16].

Cervicovaginal lavage was performed by washing the cervix and vagina with 10 ml of phosphate-buffered saline, pH 7.4, and immediately transferring the fluid to a tube containing 0.5 ml of 0.5 mol/l ethylenediaminetetraacetic acid. Cellular material was pelleted by centrifugation and total genomic DNA was extracted [17]. HPV DNA was amplified using LI consensus sequence primer PCR [18]. Each amplification batch included plasmid DNA as a positive control and reagents without DNA as negative controls. As an additional control for the presence of adequate and amplifiable DNA, all samples classified as HPV DNA negative were subsequently amplified using primers specific for the endogenous, cellular Ki-ras gene.

Amplification of HPV was followed by polyacrylamide gel electrophoresis, ethidium bromide staining, and fluorescence under ultraviolet light. Confirmation that ethidium stained bands of the correct molecular weight were actually HPV amplification products was obtained by transferring the DNA to nylon filters and hybridizing with P32-end-labelled 'generic' HPV probes. HPV typing was performed by examining the restriction endonuclease pattern produced by digestion of the PCR products [17].

Results

Characteristics of the 21 renal transplant patients in this study are shown in Table 1. Patients ranged in age from 25 to 62 years with a mean of 43.9 years. The mean interval after transplantation was 5.3 years with a range of 1 month to 17 years. Eleven Caucasian women were of non-Hispanic origin. Five black women were of non-Hispanic origin. Three Caucasian and two white women were of Hispanic origin. Eighteen women had completed a high-school education and 10 women had additional vocational or college training. Nine women had household incomes in excess of $30,000. Medical problems resulting in end-stage renal disease were varied and included hypertension.

Table 1. Characteristics of transplant patients (n=21)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean</th>
<th>SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>43.9</td>
<td>11.6</td>
<td>25.7–62.7</td>
</tr>
<tr>
<td>Age at first coitus (years)</td>
<td>20.8</td>
<td>4.2</td>
<td>12.0–32.0</td>
</tr>
<tr>
<td>Lifetime sexual partners (n)</td>
<td>4.5</td>
<td>3.2</td>
<td>1.0–20.0</td>
</tr>
<tr>
<td>Sexual partners last year (n)</td>
<td>0.7</td>
<td>0.6</td>
<td>0.0–2.0</td>
</tr>
<tr>
<td>Time on dialysis (years)</td>
<td>3.8</td>
<td>3.1</td>
<td>1.0–12.6</td>
</tr>
<tr>
<td>Time since transplant (years)</td>
<td>5.3</td>
<td>4.2</td>
<td>0.1–17.0</td>
</tr>
<tr>
<td>Pap smears last 10 years (n)</td>
<td>6.6</td>
<td>5.0</td>
<td>1.0–20.0</td>
</tr>
<tr>
<td>Date of last previous Pap</td>
<td></td>
<td></td>
<td>1985–1993</td>
</tr>
</tbody>
</table>
(3 patients), diabetes mellitus (3 patients), primary renal disorders (5 patients), and collagen vascular or autoimmune disorders (7 patients). Patients had received dialysis prior to transplantation for 12–151 months, with a mean of 45 months. Eighteen of the 21 transplant recipients had been maintained on cyclosporin as a major component of their immunosuppressive regimen.

Assessment of sexual risk behaviours revealed that eight women had a single lifetime sexual partner and five women had two lifetime sexual partners. The mean number of lifetime sexual partners was 4.5. This value was heavily skewed from the median value of two partners because three women had greater than 10 lifetime sexual partners. Seven women had not been sexually active within the last year and only one woman had had two sexual partners within the last year. The mean age of first intercourse was 20.8 years with a range of 15–32 years.

Three women had been previously treated for abnormal Papanicolaou smears; in two individuals the abnormal Pap smears occurred while on dialysis prior to transplantation. One of these women and two other women had histories of vulvar condyloma acuminata; these developed prior to transplantation in one woman and after transplantation in the other two individuals.

The results of the colposcopic and cytological examinations are shown in Table 2. No condyloma acuminata of the vulva or vagina were observed. Colposcopic examinations revealed minor abnormalities which were not deemed to be significant in two patients. These two patients underwent repeat colposcopy with cervical biopsy and endocervical curettage because atypical squamous cells of undetermined significance were diagnosed on the Papanicolaou smears obtained at the initial examination. Biopsy specimens were diagnosed as within normal limits in both cases.

A single woman who had a prior history of CIN in 1989 and vulvar condyloma acuminata in 1991 had a novel HPV type detected by PCR of her cervicovaginal lavage. Notably this woman was one of three women who were not receiving cyclosporin. The remaining 20 patients tested HPV negative.

Compliance with current screening recommendations was low among the 18 women who had not had a previous abnormal smear. Four women had undergone Papanicolaou smear screening only once in the previous 10 years. Only five women had been screened annually.

Table 2. Results of colposcopy, cytology, and HPV DNA testing in 21 renal transplant recipients

<table>
<thead>
<tr>
<th>Variable</th>
<th>Positive (n)</th>
<th>Positive (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild colposcopic abnormality</td>
<td>2</td>
<td>10%</td>
</tr>
<tr>
<td>Atypical Pap smear</td>
<td>2</td>
<td>10%</td>
</tr>
<tr>
<td>CIN</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>HPV DNA Positive*</td>
<td>1</td>
<td>5%</td>
</tr>
</tbody>
</table>

*Novel HPV type by RFLP analysis of L1 consensus amplimers

Discussion

In the current study we screened 21 women for HPV and CIN using optimal diagnostic modalities and found only one patient with an HPV infection and no patients with CIN. However, even though none of the 21 women in this study had CIN detected at the time of their examination, three women in our study had previously been diagnosed with CIN. When women with a previous diagnosis of CIN are taken into consideration, the rate of CIN in this cohort appears to be comparable to that reported by other investigators [5,6,8].

The prevalence of HPV infection noted in this investigation is lower than previous reports in which the diagnosis was based upon cytological and colposcopic criteria [19–20] and is lower than that reported to date in studies which used molecular diagnostic modalities [13–15]. However, the HPV prevalence in this study is not markedly lower than that observed by Petry et al. [14] and Fairley et al. [15], studies conducted in cohorts receiving modern immunosuppressive regimens and using similar diagnostic techniques. Although neither of those reports gave data on HPV prevalence among the cytologically normal women which were studied, it appears that the majority of HPV infections were associated with abnormal cytologies and that the HPV prevalence among cytologically normal transplant recipients was quite low in their subjects as well.

The low prevalence of CIN and HPV in this cohort may be due to an overall decline in incidence of these problems since the addition of cyclosporin to the immunosuppressive regimen [11]. Although the woman who tested positive for HPV was among the three women who were not receiving cyclosporin, this subject may have been at increased risk for HPV by virtue of her longer duration of immunosuppressive therapy (11 years).

Characteristics of our population were similar to those reported by Fairley et al. [15]. However, our patients were older than those reported by Petry et al. [14]. HPV infection has been noted to be more highly prevalent in younger women and has been uncommon in cytologically normal, immunocompetent women over the age of 35 years [21].

The low prevalence of HPV in our population may also be related to conservative sexual behaviours. Harpert et al. compared the characteristics of renal transplant recipients with normal cytologies to those with evidence of HPV or CIN on Papanicolaou smear [8]. Women with abnormal cytological findings differed only in the percentage who reported more than two sexual partners (16.7% of cytologically normal versus 57.1% of those with HPV/CIN, P < 0.01).

Eight women in our cohort reported a single lifetime sexual partner and five reported two partners. Seven women had not been sexually active within the last year and only one woman reported more than one sexual partner within the last year. The observation...
that five of these women had histories of HPV-induced lesions, yet none presently had CIN and only one had detectable HPV DNA, suggests that recent sexual behaviour is an even more important determinant of HPV status in immunosuppressed women than is past sexual behaviour. This is consistent with the hypothesis that persistence of a newly acquired HPV infection and progression of the infection to a CIN lesion is more problematic than reactivation of latent virus in this population. If this finding is confirmed in additional studies, it suggests that avoidance of high-risk sexual contacts may impact directly on the cervical cancer risk of transplant recipients and suggests an important role for education concerning avoidance of high-risk sexual behaviours.

In conclusion, our data demonstrates that HPV infection of the cervix is not highly prevalent among older, cytologically normal, female renal transplant recipients, particularly those who are currently monogamous or not presently sexually active. This study suggests that recent sexual activity is a more important determinant of HPV status in this population than past sexual behaviours and suggests that education concerning the avoidance of high-risk sexual behaviour is an important part of the care of the female renal transplant recipient. Although the conclusions of this study are limited by the small size of the population studied, our data is consistent with previous work suggesting that the incidence of CIN is declining in transplant recipients and suggests that the prevalence of HPV infection may be declining as well. Additional studies are needed to verify these findings.

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References


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