Gynecologic Oncology Reports 24 (2018) 83–86

Contents lists available at ScienceDirect

Gynecologic Oncology Reports

journal homepage: www.elsevier.com/locate/gynor

Case series

Human papillomavirus genotypes in Pacific Islander cervical cancer patients

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ARTICLE INFO

Keywords:
Human papillomavirus
Cervical cancer
Pacific Islanders

ABSTRACT

Objective: The role of human papillomavirus (HPV) in the development of invasive cervical cancers is widely known. Few HPV studies have targeted geographically isolated regions. The objective of this study was to determine the HPV genotypes in cervical cancer patients from the Pacific Islands referred to Tripler Army Medical Center (TAMC).

Methods: All cases of invasive cervical cancer treated at TAMC through the Pacific Island Health Care Project between January 2004 and October 2014 were identified through a review of pathology specimens. DNA was extracted from paraffin-embedded tissue blocks. PCR was performed using PLEX-ID plates to isolate and amplify HPV-specific DNA. Mass spectrometry was subsequently performed to identify specific HPV genotypes.

Results: Thirty-five patients had their pathology specimens analyzed. Ten patients had localized disease (Stage 1); 21 had regional disease (Stages 2 and 3); and 4 had distant disease (Stage 4). Thirty-three squamous cell carcinomas and 3 adenocarcinomas were identified. The most common HPV subtypes found were 16 (6, 24%), 45 (6, 24%), and 52 (6, 24%). Other HPV subtypes isolated included 18 (1, 4%), 33 (3, 12%), 39 (2, 8%), 54 (1, 4%), and 67 (1, 4%). In 10 samples, HPV was not isolated.

Conclusion: Pacific Islanders referred to TAMC present with a disproportionally higher rate of regional and advanced disease. Significantly, only 28% of invasive cervical cancers in the Pacific Island population sampled could have been potentially be prevented using the available quadrivalent vaccine targeting HPV 16/18; however, 88% could be covered by the recently licensed nonavalent vaccine.

1. Introduction

Cervical cancer poses a significant worldwide threat to women. In 2012 it was the fourth most common malignancy with an estimated 527,624 incident cases and 265,672 deaths. Developed countries with effective cytology-based screening programs have seen significant declines in cervical cancer rates over the past three decades. Unfortunately, less developed regions continue to shoulder a disproportionately high burden (Ferlay et al., 2013).

While many factors contribute to malignant transformation, human papillomaviruses (HPV) play a central part in the development of cervical cancer. HPV are a group of non-enveloped circular double-stranded deoxyribonucleic acid (DNA) viruses (Barreto et al., 2014). Over 100 distinct subtypes of HPV have been identified, and nearly 40 of these can infect the genital tract (De Villiers et al., 2004). The HPV genome is capable of directing the elaboration of six early proteins of which E6 and E7 are critical to cell transformation. It also directs production of two capsid proteins, L1 and L2 (Schiffman et al., 2007). These late proteins demonstrate subtle differences that determine the viral genotype and also present a target for vaccine development.

Extensive research has identified the distribution of HPV genotypes in invasive cervical cancers for many geographic regions (de Sanjose et al., 2010; Alemany et al., 2014). This data has been instrumental for vaccine development as focus on cervical cancer management has shifted toward primary prevention. Uniformly, a predominance of HPV subtypes 16 and 18 have been detected, accounting for over 70% of cervical cancer cases in the developed world. Two commercially available vaccines in the United States target these genotypes. Despite these efforts, there remain areas where there is limited understanding of HPV genotype contribution to cervical cancer. There are currently few studies which have specifically assessed HPV disease prevalence among cases of invasive cervical cancer from the Marshall Islands and

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https://doi.org/10.1016/j.gore.2018.04.007
Received 12 March 2018; Received in revised form 14 April 2018; Accepted 16 April 2018
Available online 18 April 2018

various other island nations located within the Pacific Rim.

The first Compact of Free Association between the United States and Pacific Island countries such as the Marshall Islands, Federated States of Micronesia, and Palau was negotiated in 1985. Shortly thereafter, Hawaiian senator Daniel Inouye used this compact of free association as inspiration for a bill that allowed Pacific Islanders to receive medical care at Tripler Army Medical Center. Since the inception of the Pacific Island Healthcare Project, many patients with cervical cancer have received care in Hawaii (Person, 2014). The objective of this study was to determine the HPV genotypes in this unique population of cervical cancer patients from the Pacific Islands.

2. Methods

This is a retrospective analysis of all cases of invasive cervical cancer treated at Tripler Army Medical Center through the Pacific Island Health Care Project (PIHCP) between January 2004 and October 2014. The study protocol was approved by the Tripler Army Medical Center Institutional Review Board. Informed consent was waived due to the retrospective nature of the project.

Cases were identified through a review of pathology specimens and the PIHCP database. A chart review was conducted to identify demographic and epidemiologic data, treatment type, response to therapy, and survival data for each patient. Archived paraffin embedded tissues derived from the primary tumors for each patient were located and transferred to the clinical investigation laboratory for analysis. Central review of all pathology was performed by a single pathologist (J Freeman) to ensure accurate and uniform histologic diagnosis.

Total genomic DNA was extracted from each sample using the Qiagen FFPE system (Qiagen Inc., Valencia CA). Briefly, 8–10 μm sections were cut from each block. Paraffin was melted by vigorous agitation of the tissue section with xylene. Residual xylene was removed with an ethanol wash and the “deparaffinized” tissue was digested with proteinase K. High temperature incubation in lysis buffer was utilized as a means of removing any formalin cross-linked to the DNA. The DNA was next bound to a silica membrane, washed with high salt buffers to remove impurities and eluted with nuclease free water.

A polymerase chain reaction (PCR) and mass spectrometry based approach was employed to interrogate any viral DNA sequences in the elutate and to specifically amplify HPV DNA. This was accomplished via the Abbott PLEX-ID BioPharma Viral Assay (Ibis Biosciences, Carlsbad CA). First, a broad range PCR was conducted resulting in the global amplification of all viral sequences. This was followed by a narrowly targeted PCR reaction resulting in the amplification of the HPV L1 gene. Next, each amplicon was analyzed with electrospray ionization time-of-flight mass spectrometry to yield the exact nucleotide base count. Since nucleotide base counts of the L1 amplicon are conserved within HPV genotypes they were utilized to scan a proprietary database and identify the correct genotype by homology.

3. Results

Pathology specimens were analyzed from a total of thirty-five patients. Patients were referred for treatment from clinics located in the Marshall Islands (25), Palau (6), the Federated States of Micronesia (2), and American Samoa (1). Demographic information was not available for one patient; however, specimen analysis was conducted on the basis of the referral criteria of the PIHCP (Fig. 1).

Patient characteristics are summarized in Table 1. The average age at presentation was 49.2 years (range 28–73). Nine patients had localized disease (Stage 1); 22 had regional disease (Stages 2 and 3); and 4 had distant disease (Stage 4). Thirty-three squamous cell carcinomas and three adenocarcinomas were identified, including one patient with a concurrent squamous cell carcinoma and adenocarcinoma.

HPV DNA was detected in 71.4% (25/35) of the study subjects. The most common HPV genotypes found were 16 (6, 24%), 45 (6, 24%), and 52 (6, 24%). Other less common HPV subtypes isolated included 18 (1, 4%), 33 (3, 12%), 39 (2, 8%), 54 (1, 4%), and 67 (1, 4%). In one patient, both HPV 16 and 45 was detected. In the three patients with cervical adenocarcinomas, one patient had HPV 16 isolated and the other two had HPV 52. Overall, combined HPV 16/18 only contributed to 28% of cervical cancer cases (Fig. 2).

4. Discussion

This is one of the few studies to our knowledge to report on the HPV genotype frequency in Pacific Islander cervical cancer patients. In our exploratory cross-sectional study, the most common types isolated include HPV 16, 45, and 52, each accounting for 24% of cases. Some of the subtypes isolated (HPV 54 and 67) have only rarely been associated with malignancy (de Sanjose et al., 2010). Interestingly, combined HPV 16/18 contributed to only 28% of the cervical cancers patients studied. By comparison, in North America these two types provide a relative contribution of 79% (de Sanjose et al., 2010).

In 2013, Hernandez et al. reported a 10% cervical HPV detection rate in a cross sectional study of 211 women undergoing cytologic screening in American Samoa. HPV 6, 16, and 53 were the most common genotypes found (Hernandez et al., 2013). However, this study did not look specifically at patients with known cervical cancer. We detected HPV 39 in the one cervical cancer patient from American Samoa in our study. In another systematic review of cervical cancer incidence and mortality in the Pacific region, Obel et al. reported that HPV 16/18 was found in 77% of ethnic Fijian women with cervical cancer. HPV 31 was the third most common genotype isolated. He also reported that HPV 16/18 was found in 83% of cervical cancer biopsies in women from Papua New Guinea (Obel et al., 2014). While these rates are more comparable to the developed world, none of the patients in this study originated from these islands.

Cervical cancer places a substantial burden on the Pacific Island population. It is the second most common malignancy encountered in women there, accounting for 11% of the total malignancies recorded. The age-adjusted incidence rate averages 16.4 per 100,000 women for the region; however, in this heterogeneous area, some rates exceed that seen in Eastern Africa. For example, in the Marshall Islands, the incidence rate is 65.6 per 100,000 women (Buenconsejo-Lum et al., 2014).

Unfortunately, cervical cancer patients from the Pacific Islands referred to Tripler Army Medical Center present with a disproportionately higher rate of regional spread when compared to the developed world. In our study population of 35 patients, 22 (63%) had regional disease on arrival. While it is unknown what percentage of cervical cancer patients from the Pacific Region are ultimately referred to Tripler Army Medical Center for care, the rate of advanced cervical cancer in our study corresponds with that reported in the Pacific Regional Central Cancer Registry. In 2014, the reported ratio of advanced cancer (Stage 2 or higher) to early disease (Stage 1) ranged from 1.5 to 24.0 in the various island nations (Buenconsejo-Lum et al., 2014). In the United States only 36% are found with similar stages (SEER 18 2006-2012 database) (The website of the National Cancer Institute, n.d.). A lack of an effective screening program directly contributes to the advanced stages seen in Pacific Islanders. Many patients in our study population faced significant logistical challenges seeking medical care and did not have regular cytology screening.

Vaccination for HPV offers an alternative approach to mitigating cervical cancer through primary prevention. In the developed world, initial vaccination efforts targeted HPV 16 and 18 (FUTURE II Study Group, 2007). Six years after introduction of the quadrivalent vaccine in the United States, a 64% reduction in HPV 6, 11, 16, and 18 prevalence has been seen in females aged 14–19 (Markowitz et al., 2016). Further knowledge of prevalence rates has led to the development of a 9-valent vaccine (targeting HPV 6, 11, 16, 18, 31, 33, 45, 52, and 58) in an attempt to increase efficacy (Saraiya et al., 2015). While

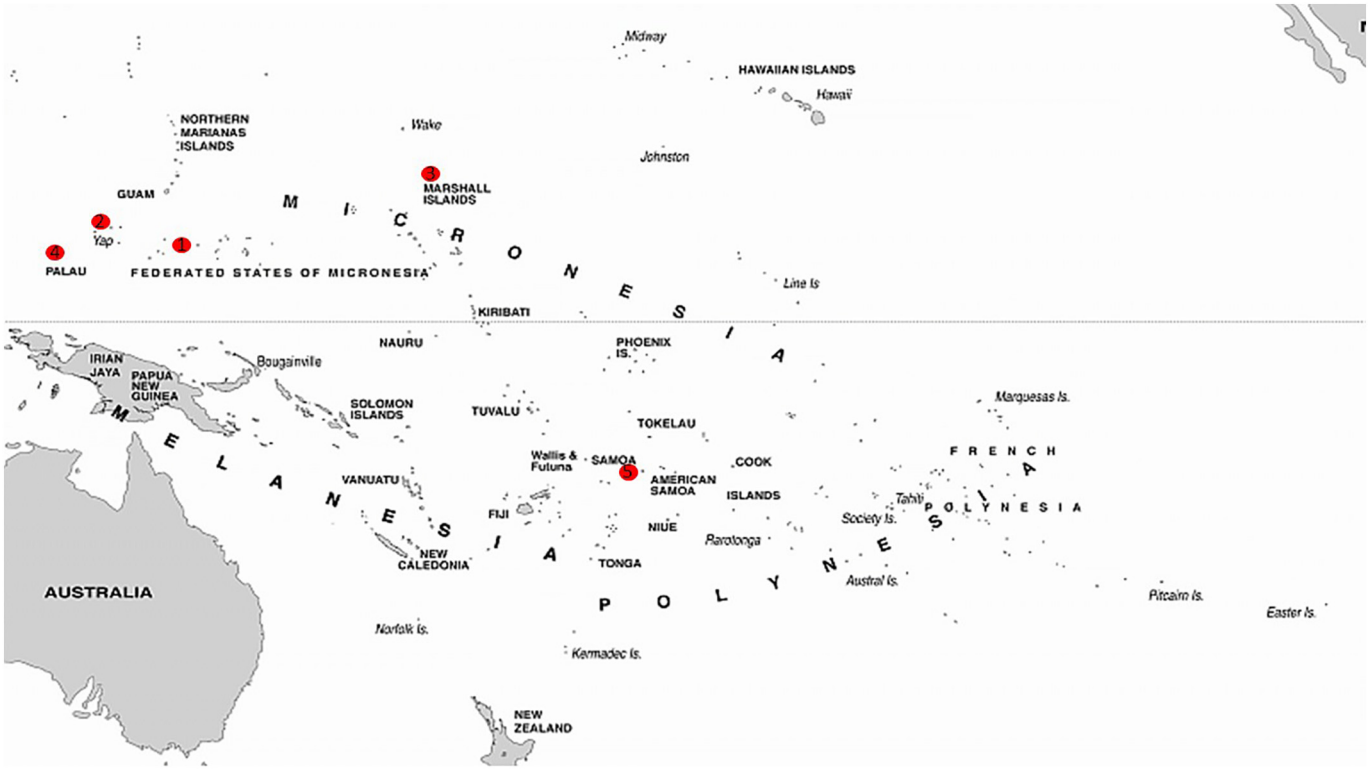

Fig. 1. Map of the Pacific Region showing the approximate zones of origin of the patients evaluated in this study: (1) the island of Chuuk, (2) the island of Yap, (3) the Marshall Islands, (4) Palau, and (5) American Samoa.
(Source: Natural Hazard Planning in the Pacific Island Region. http://trauma.massey.ac.nz/issues/2010-1/campbell.htm. Accessed on April 8, 2018.)

Table 1
Patient characteristics.

Total number of patients	35
Age, y	
20–29	1
30–39	6
40–49	13
50–59	7
60–69	6
70–79	2
Average age, y	49.2
Age range	28–73
Island of origin	
Federated States of Micronesia	2
Chuuk	1
Yap	1
Marshall Islands	25
Ebeye	7
Majuro	8
Unspecified	10
Palau	6
American Samoa	1
Unknown	1
Histology	
Squamous cell carcinoma	33
Adenocarcinoma	3
Stage at presentation	
I	9
II	4
III	18
IV	4

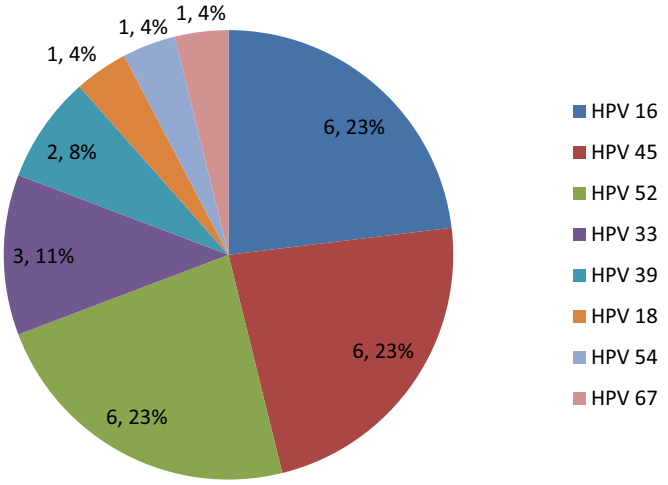

Fig. 2. Distribution of HPV Genotypes in Pacific Island Cervical Cancer Patients (Percentages based on total detections (26) in 25 patients).

implementing a vaccination program in developing regions poses significant challenges, in the long run it may be a more effective strategy in eradicating cervical cancer in regions with limited resources to dedicate toward cancer screening or treatment. In 2013, ten Pacific Island countries had incorporated HPV vaccination in their national immunization schedule, but only half of these had reached national coverage goals. Another nine territories either had no vaccination strategy or only had small pilot programs (Obel et al., 2015). All of the patients referred to Tripler Army Medical Center originated from territories where vaccination programs are still being developed. Complete understanding of the pre-vaccine type-specific HPV prevalence in a region is critical for successful implementation of any vaccination program. Based on the HPV genotypes isolated in this study population, the 9-valent vaccine would be a better selection for Pacific Islanders to maximize coverage.

The PIHCP was a major strength of this study. Under this program, patients from a wide range of remote islands in the Pacific are afforded

care in a central location, making them a convenient population to study when looking at this ethnic group. Other strengths included a centralized pathology review, a regimented laboratory protocol, and the length of the study period.

The primary weakness of our study is the relatively small number of cervical cancer patients available for analysis. Our low numbers prevented any meaningful subgroup analysis of HPV contribution to specific location, age groups, histology, or cancer stage at presentation. Although our study was retrospective in nature, it would be difficult to conduct this type of analysis in any other fashion. It is possible that selection bias is present, but this risk is minimized since consecutive patients over a 10 year period were included in the analysis.

In our study, the HPV genotype isolation rate was 71%. This rate is lower than that published in other recent studies (Park et al., 2013). This may be due to the quality of the tissue available for analysis in older blocks. As most patients presented with more advanced disease, smaller biopsies were collected for diagnosis. It is possible that the sections cut from the paraffin blocks for analysis may have contained relatively normal histology lacking HPV DNA. Furthermore, polymerase inhibitors can occasionally contaminate digested tissue samples. These may interfere with PCR quality leading to false negative results (Park et al., 2013). Finally, our methodology utilizing time-of-flight mass spectrometry to yield a L1 nucleotide base count for HPV genotype identification was novel and differed from that described in other studies (Schiffman et al., 2007; de Sanjose et al., 2010; Alemany et al., 2014; Park et al., 2013).

In conclusion, HPV 16, 45, and 52 are the most common genotypes contributing to cervical cancer in the population of Pacific Islanders we studied. Only 28% of these cervical cancer cases could have been prevented with the currently available bivalent or quadrivalent vaccines targeting HPV 16 and 18. Fortunately, a recently approved 9-valent vaccine could protect up to 88% of women in this population. This study highlights the need to continue to explore HPV's contribution to cervical cancer in geographically isolated and under-developed regions.

Conflict of interest statement

The authors have no conflicts of interest to disclose.

The views expressed in this manuscript are those of the authors and do not reflect the official policy or position of the Department of the Army, Department of Defense, or the US Government.

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