Human Pathology (2014) **45**, 303–309

ELSEVIER

Human PATHOLOGY

www.elsevier.com/locate/humpath

Original contribution

Prevalence, distribution, and viral burden of all 15 high-risk human papillomavirus types in adenosquamous carcinoma of the uterine cervix: a multiplex real-time polymerase chain reaction–based study☆,☆☆

M. Ruhul Quddus MD[a,*], Pradip Manna PhD[b], C. James Sung MD[a], Spencer Kerley MD[b], Margaret M. Steinhoff MD[a], W. Dwayne Lawrence MD[a]

[a]*Department of Pathology, Women & Infants Hospital, The Warren Alpert Medical School of Brown University, Providence, RI 02905*
[b]*Physicians Reference Laboratory, LLC, Overland Park, KS, 66210*

Received 20 June 2013; revised 3 July 2013; accepted 10 July 2013

Keywords:
Adenosquamous carcinoma;
Cervical cancer;
Human papillomavirus

Summary Human papillomavirus (HPV) 16 and 18 are the types most commonly found in cervical adenosquamous carcinoma. Multiple HPV types have been found in cervical adenocarcinoma but not in the adenosquamous variant. Type-specific detection of high-risk (HR) HPV allows the detection of co-infection by multiple HPV types and assessment of viral load per cell. Our aim was to identify and quantify all HR HPV types in cervical adenosquamous carcinoma and to correlate viral loads with prognosis-related histologic features. All 15 HR HPV types were tested for by multiplex real-time polymerase chain reaction, and standard curves were created for each type. Viral loads were determined retrospectively. Prognosis-related histologic features were correlated with specific HPV types and the viral loads. A total of 80% of the tumors examined expressed HPV. Types 16/18 were detected in 86% of these cases, whereas the remaining 14% of the positive cases were infected by other types. A single type of virus was detected in 67% of cases, 2 in 29%, and 3 in 4%. Poor prognostic features were seen in 84.6% of the tumors infected with HPV 16, 46% of those infected with HPV 18, and 100% of those infected with other types. As expected, HPV 16, HPV 18, or both were the most frequent viral types; HPV 73 was the next most frequent type. Multiple HPV types were detected in 33% of the tumors. Non–HPV 16/18 cases had low viral loads, but all of these had poor prognosis-related histologic features. Two of the three recurrent cases had multiple viral types.
© 2014 Elsevier Inc. All rights reserved.

☆ Financial disclosure: P. Manna and S. Kerley work for Physicians Reference Laboratory, LLC. The other authors declare no conflicts of interest. No funding was received for this work.

☆☆ The results of this study were presented in part at the 98th Annual Scientific Meeting of the United States and Canadian Academy of Pathology, Boston, MA, March 6-13, 2009.

* Corresponding author. Department of Pathology and Laboratory Medicine, Women & Infants Hospital and The Warren Alpert Medical School of Brown University, 101 Dudley Street, Providence, RI 02905.

E-mail address: mquddus@wihri.org (M. R. Quddus).

0046-8177/$ – see front matter © 2014 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.humpath.2013.07.048

1. Introduction

Adenosquamous carcinoma (AdSq) is now classified under "other epithelial tumors" in the most recent World Health Organization system [1] and constitutes from 20% to 50% of all cervical carcinomas that have a glandular component [2]. When a strict criterion was applied, eg, clearly recognizable squamous and glandular component without the need for special stain [3], for the diagnosis of AdSq, the incidence was found to be around 28% [4,5].

The origin of AdSq is debated. Some authors argue in favor of a stem cell origin. Clonality studies (X-chromosome inactivation pattern) and human papillomavirus (HPV) infection analysis support the view that these are mixed tumors of monoclonal origin [5].

Adenosquamous carcinomas are reported to have a poorer prognosis than pure adenocarcinomas of the same stage [4]. However, other studies contradict this claim [6]. Glassy cell tumors, poorly differentiated AdSq variants, are known for their poor prognosis, rapid extrauterine spread, and resistance to radiation therapy [7]. Another variant, clear cell AdSq, is associated with human papillomavirus 18 and also has a poor prognosis [8].

Carcinogenesis induced by HPV is under investigation. More than 190 HPV integration loci have been reported [8]. However, a specific DNA target sequence motif has not been described. Epigenetic inactivation through DNA methylation of certain tumor suppressor genes, eg, p16 (*CDKN2A*), adenomatous polyposis coli (*APC*), E-cadherin (*CDH1*), and abnormal fragile histidine triad (*FHIT*) have been reported to be important in carcinogenesis [9]. Similarly, persistent HPV infection is important. A meta-analysis showed that women who had high-risk HPV infections persisting for at least 1 year are at significantly elevated risk of having or developing cervical cancers/precancers in comparison with those whose infection cleared [10]. The HPV 16 and 18 types are regarded as the most common viral genotypes in cervical AdSq [11,12].

Polymerase chain reaction (PCR)–based methods are used to assess the viral load in HPV infections. The sensitivity of PCR is <10 HPV/reaction (<125 copies/ml of Papanicolaou [Pap] smear material). However, the test is not designed to differentiate between free and integrated HPV. The *E2* open reading frame (ORF) is usually deleted from the virus when the viral genome is integrated into the host genome in cervical carcinoma [13]. The detection of both *E2* and *E6* ORFs in equivalent amounts by PCR indicates that the genome remains in its episomal form [5]. However, the detection of *E6* is interpreted as evidence of complete HPV integration into the host genome.

Identification of specific types of HPV appears important in view of reports of better prognosis in certain types of HPV-related carcinomas. For example, Huang and colleagues [12] reported that women with HPV 31–related cervical carcinoma have a better survival rate than those with cancers associated with other types. The presence of multiple HPV viral types did not have any prognostic significance [12]. Zuna and associates [14] reported that the risk of progression to invasive carcinoma in high-grade cervical squamous intraepithelial lesions is related to HPV genotype. In cervical squamous cell carcinomas, multiple viral genotypes may be found. In such cases, the HPV patterns of the primary tumors and metastases are similar in most patients, even when the primary tumors contain double genotypes [15].

Younger women at risk for adenocarcinoma often are not readily identifiable by cytologic examination and may benefit from HPV screening [16], which may be superior to Papanicolaou smear [17]. The index of HPV positivity drops markedly after 30 years of age [18]. A survey of the distribution of HPV done in 2006 found that 27% of smear-negative samples were positive for HPV by PCR-based tests [19]. Hamlin-Douglas et al [20] reported multiple HPV infections in as many as 40% of HPV-positive subjects in a Québec community. High-risk HPV types 18 and 16 have been detected in 62% to 91% of cervical adenocarcinomas [21].

The current study attempted to detect, type, and quantify all 15 high-risk HPV types in AdSq of the uterine cervix from paraffin-embedded tissue samples using a multiplex real-time PCR-based test.

2. Materials and methods

All AdSq of the uterine cervix encountered in the Department of Pathology, from 1 January 2001 to 30 June 2006, were retrieved from institutional archival files after institutional review board approval at the Women and Infant's Hospital, Warren Alpert Medical School of Brown University. Appropriate tissue blocks were selected, and paraffin tissue blocks were retrieved. One hematoxylin and eosin–stained section was examined from each case to confirm the presence of an area of interest. All these cases were diagnosed on the basis of morphologic criteria alone without mucicarmine staining. Prognosis-related histologic features such as depth of stromal invasion, vascular space invasion (VSI), lymph node (LN) metastasis, distant metastasis, and recurrence were noted from patient charts.

The HPV testing was performed on formalin-fixed, paraffin-embedded (FFPE) tissue samples using the COMPLeTe Care HPV test (Physicians Reference Laboratory, LLC, Overland Park, KS), a multiplex real-time PCR test that simultaneously detects, types, and quantifies all 15 high-risk HPV types known to cause anogenital cancer. Five to 10 tissue sections (5 μm each) were used to extract DNA using a QIAmp tissue kit (Qiagen, Valencia, CA). Four multiplex reactions, each targeting four high-risk HPV types, were performed in a LightCycler 480 instrument using 8 μL of extracted DNA per multiplex reaction to detect all 15 high-risk HPV types and an internal control in which HPV 16 was detected in two multiplex reactions. Quantitative standards and controls for each of the high-risk types and internal control β-globin were included in each run.

Download English Version:

https://daneshyari.com/en/article/4133254

Download Persian Version:

https://daneshyari.com/article/4133254

Daneshyari.com