Human Papillomavirus Infection Status of Various Laryngeal Diseases in Japan: A Comprehensive Study

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Summary: Objective. The aim of our study was to clarify the human papillomavirus (HPV) infection status of various laryngeal diseases in Tokyo, Japan.

Study Design. This is a retrospective study.

Methods. A total of 144 patients who underwent surgical resection for various laryngeal lesions were enrolled in this study. These subjects were categorized into four groups based on lesion type: non-neoplastic, 44; precancerous, 29; cancer, 35; and papilloma, 36. To determine the rate of HPV infection, laryngeal secretions and resected tissue from our study participants were examined by liquid-phase hybridization (LPH) and consensus primer-directed polymerase chain reaction (PCR).

Results. The LPH for low-risk HPV was applied to all 144 patients, and that for high-risk HPV was additionally applied to 121 of the 144 patients. The PCR was applied to 94 of the 144 patients. The LPH detected low-risk HPV-DNA in 23 patients (1 cancer and 22 papillomas) and high-risk HPV-DNA in 3 patients (1 cancer and 2 papillomas). The PCR detected HPV-6 and HPV-11 in the papilloma group, whereas it detected HPV-31 in one patient with laryngeal cancer and one patient with precancerous lesion. Both the LPH and the PCR revealed the HPV infection rate in the non-neoplastic group to be 0%.

Conclusions. Although we found no significant difference in the HPV-DNA positive rates of laryngeal cancer and precancerous lesions in the non-neoplastic group, the positive rates were significantly smaller in this group than in the papilloma group. In the Tokyo area, HPV had little or no association with laryngeal cancer, precancerous lesions, and non-neoplastic lesions in the larynx.

Key Words: Larynx–HPV–Cancer–Papilloma–Non-neoplastic lesion.

INTRODUCTION

Human papillomavirus (HPV) infection has been considered a carcinogenic factor of anal[1] and cervical cancer.[2] It has been reported that the development of oropharyngeal cancer is also associated with HPV infection.[3] According to the information from the Japan Health Promotion & Fitness Foundation, the smoking rate has been declining in Japan.[4] However, despite this decline, the incidence of oral and oropharyngeal cancer steadily increased from 1985 to 2007, and the incidence of laryngeal cancer also decreased during the same period.[5] Given these paradoxical findings, the association between HPV infection and laryngeal cancer has remained controversial.

Previous studies have reported an association between HPV infection and individual lesions in the larynx, such as papilloma and cancer.[6,7] However, few have described the infection status of HPV in a wide variety of laryngeal lesions. In particular, there is little information available regarding the HPV infection status of benign laryngeal diseases, except for papilloma. It might therefore be inappropriate to investigate the association between HPV infection and laryngeal cancer if the study design lacks the HPV infection rate of the negative control.

In the present study, we examined the HPV infection rates of various laryngeal diseases in Tokyo, Japan. In addition, the connection between HPV infection and laryngeal cancer and precancerous disease was investigated by comparing the HPV infection rates between these diseases and other diseases, such as non-neoplastic benign disease and laryngeal papilloma.

MATERIALS AND METHODS

Subjects

During the study period from January 2009 to December 2015, 876 patients underwent laryngeal microsurgery in our hospital. Of these 876 patients, 144 agreed to participate in this study. All 144 patients lived in the Tokyo area, with their full details shown in Table 1. Each patient had one lesion (n = 144 lesions). The 144 lesions were categorized into four groups based on type: non-neoplastic (n = 44), precancerous (n = 29), cancer (n = 35), and papilloma (n = 36). The precancerous group consisted of patients with all grades of dysplasia and carcinoma *in situ*.

Determination of HPV infection

To determine the HPV infection status, we assessed the presence of HPV-DNA *via* two different methods: the liquid-phase hybridization method[8–10] and a consensus primer-directed polymerase chain reaction (PCR) system.[11] Using the liquid-phase hybridization method, we examined the HPV high-risk group (HPV-16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68) and the HPV low-risk group (HPV-6, 11, 42, 43, and 44). The sample used for the screening test was tissue from the resected lesion or secretions from the lesion. Liquid-phase hybridization has been widely chosen as a screening test for cervical HPV infection in Japan. Secretions from the cervix are usually obtained as specimens for the examination. As a result, little difference is thus considered to exist in the sensitivity of the test results between secretion specimens and tissue samples. The consensus

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TABLE 1.
Demographic Data of All the Four Groups

	NN Group (n = 44)	PC Group (n = 29)	CA Group (n = 35)	PA Group (n = 36)	*P*
Age (range), y	53.9 (21–82)	62.1 (38–86)	67.9 (41–90)	55.8 (23–90)	$P < 0$*
Male/male + female (%)	32/46 (70%)	25/29 (87.9%)	29/34 (85.3%)	30/36 (83.3%)	$P = 0.246$

Age is presented as the average value.
Statistical analysis: one-way analysis of variance (age); Fisher exact test (male/male + female).
* $P < 0.05$.
Abbreviations: CA, cancer; NN, non-neoplastic; PA, papilloma; PC, precancerous.

primer-directed PCR, a typing test, can simultaneously detect eight HPV subtypes (HPV-6, 11, 16, 18, 31, 42, 52, and 58). The sample used for the PCR was tissue from the resected lesion. All 144 subjects were examined with the screening test for low-risk types. Because of limitations of our available financial resources to carry out the HPV-DNA tests, 121 of the 144 patients were examined with the screening test for high-risk types. The typing test was applied to 94 of the 144 patients. This selection process was unintentional and depended on our financial resources when the subjects underwent laryngeal microsurgery.

Comparative study

We compared the mean age, the male-to-female ratio, and the positive rates of HPV infection among the four groups. A comparison of the mean age was carried out using a one-way analysis of variance. Fisher exact test was used to compare both the male-to-female ratio and the positive rates of HPV infection. The statistical analyses were performed using EZR (Saitama Medical Center, Jichi Medical University), a graphical user interface for *R* (The R Foundation for Statistical Computing, Vienna, Austria, Ver. 3.1.1), and a modified version of *R commander* (Ver. 2.0–4).[12]

Ethical considerations

This study was reviewed and approved in advance by the institutional review board at Nihon University Hospital. Written informed consent was obtained from all 144 patients.

RESULTS

Comparison in demographic data

A one-way analysis of variance showed a significant difference in the mean age among the four groups ($P = 0.0001$) (Table 1). Thus, *post hoc* pairwise comparisons were conducted using Tukey test, which showed the mean age of the cancer group to be significantly older than those of both the papilloma and the non-neoplastic groups (Figure 1). Fisher exact test showed no significant differences in the male-to-female ratio among the four groups (Table 1).

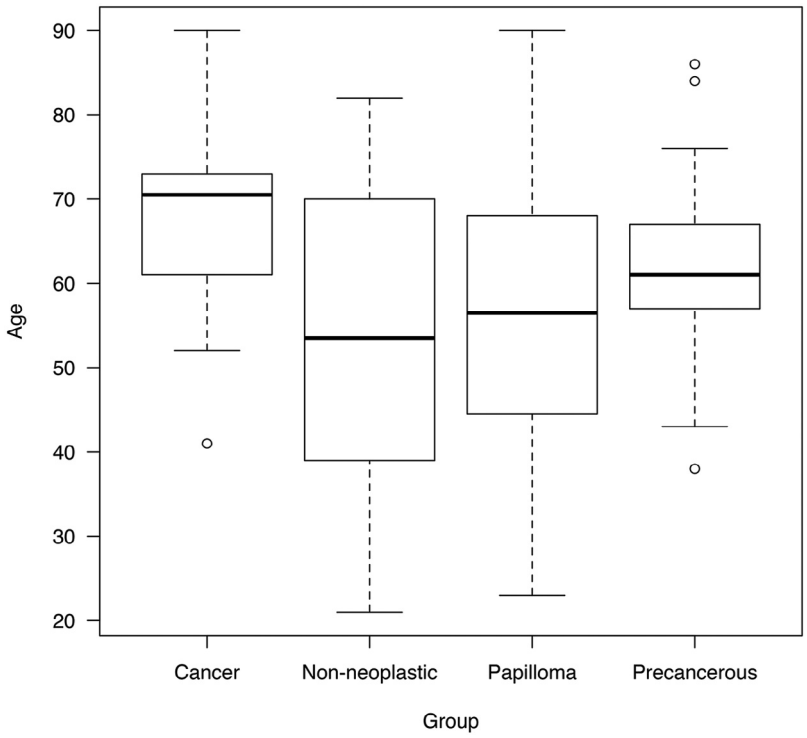

FIGURE 1. There were significant differences in the mean age between the cancer group and the non-neoplastic and papilloma groups. Other parameters showed no significant differences among the groups.