Human Papillomavirus Infection in the Normal Oral Cavity of Young People in Mongolia

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The purpose of the present study was to investigate the frequency of human papillomavirus (HPV) infection in the normal oral cavity of young people in Mongolia using the PCR method. Twenty-five percent of all samples (48/192) were positive for HPV infection. The positive rates of HPV infection for males and females were 22.5% (20/89) and 27.2% (28/103), respectively. Moreover, positive rates for 4 different age groups of 1-5 years old, 6-10 years old, 11-15 years old and 16-20 years old were 4.7% (2/43), 34.3% (24/70), 31.0% (18/58) and 19.0% (4/21), respectively. The frequent types of HPVs in 48 positive samples were HPV-16 (54.0%, 26/48), -4 (29.2%, 14/48), -18 (25.0%, 12/48), -1 (16.7%, 8/48), -37 (16.7%, 8/48), -11 (12.5%, 6/48), -23 (12.5%, 6/48), and -60 (12.5%, 6/48). Multiple infection of HPV was found in 37 of 48 positive samples (77.0%). It was suggested by the results of the present study that the normal oral cavities of young people in Mongolia were already infected with various types of HPVs in childhood and adolescence. Since some types of HPVs are considered related to the development of oral malignant lesions, it is necessary to conduct further studies to ascertain the outcome of HPV infection in the normal oral cavity.

Key words: Mongolia, young people, normal oral cavity, human papillomaviruses

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Introduction

Human papillomavirus (HPV) infection is the most common sexually-transmitted disease worldwide and it plays an important role in the development of many diseases at various body sites, including the respiratory tract, digestive tract and anogenital area, although HPV infection is also seen in various body sites of healthy people. So far, more than 100 types of HPVs have been identified. It has been reported that about 24 types of HPVs are associated with benign lesions such as papilloma, condyloma, focal epithelial hyperplasia, and warts, whereas about 12 types are related to oral malignant lesions such as oral squamous cell carcinoma, malignant melanoma and other lesions (Tables 1, 2) (1-28). On the other hand, many reports have shown that HPV types 1, 6, 11, 16 and 18 are also present in normal tissues (29-42). At present, no studies on HPV infection in the normal oral cavities of Mongolian people have been reported. The purpose of this study, therefore, was to investigate HPV infection in the healthy oral cavities of Mongolian young people using the PCR method.

Materials and methods

Subjects

In this study, oral epithelial samples were collected using cotton swabs from the normal buccal mucosa of 192 volunteer young people (89 males and 103 females), aged from 1 to 20 years old, at the Dental Clinic of Nalaikh Hospital, Nalaikh District, Ulaanbaatar, Mongolia. The 192 samples consisted of 43 samples from 1-5 year olds (23 males and 20 females), 70 samples from 6-10 year olds (37 males and 33 females), 58 samples from 11-15 year olds (25 males and 33 females) and 21 samples from 16-20 year olds (4 males and 17 females). For this study, consent was obtained from both the subjects and their parents. The protocol of this study followed the Guidelines of the Institutional Ethical Review Boards at the School of Dentistry, Aichi Gakuin University.
Extraction of DNA and PCR amplification

DNA was extracted from swabs of the buccal mucosa. Samples were incubated for 24 hours at 37°C in 296 
μl of digestion buffer (50mM KCl; 1.5mM MgCl2; 10 mM Tris-hydrochloric acid, pH 9.0; 1 mM EDTA; 0.5% Tween 20) and 4 μl proteinase K (20mg/ml). DNA was purified using EZNA Tissue DNA Kits (Omega Bio-Tek, Inc., Doraville GA, USA). The 110 base pair 3′-actin gene was detected in all specimens. Consensuses PCR, L1C1/L1C2 (HPV- 1, 2, 3, 5, 6, 8, 10, 11, 13, 16, 18, 31, 33, 34, 36, 38, 42, 52, 58 can detected) and HPV-1003/1004 (HPV-1, 6, 8, 11, 13, 16, 18, 30, 31, 32, 33 can detect) were used. HPV-1, 3, 4, 6, 11, 13, 16, 18, 20, 21, 22, 23, 24, 31, 33, 36, 37, 38, 48, 50, 57, 59, 60, 70, 72, 73, 75, 76, 77, and 80 were typed by PCR using each type-specific L1 region primer. HPV-16 and 18 were typed using E6 region primers. These oligonucleotide primers were synthesized by Sigma-Aldrich Japan K.K. (Tokyo, Japan). In this study, PCR buffer (PE Applied Biosystems, New Jersey, USA), MgCl₂ (Perkin Elmer, New Jersey, USA), Ampli Taq Gold (Perkin Elmer, New Jersey, USA), dNTP (PE Applied Biosystems, New Jersey, USA) and primer for PCR were used. The PCR protocol employed was 40 cycles of dena-
turation (94°C, 1.5 min), annealing (50°C, 1.5 min) and extension (72°C, 2 min) on a Gene Amp 9700 (PE Applied Biosystems, New Jersey, USA). After reaction, one-tenth of the reaction mixture was electrophoresed through 2% NuSieve GTG agarose (Takara Shuzo Co. Ltd., Kyoto, Japan) gel containing 1 μg/ml ethidium bromide, and then visualized under an ultraviolet transilluminator. The gel was subsequently photographed.

**Direct DNA sequencing**

PCR products were sequenced with fluorescent dye-labeled dideoxynucleotides and cycle sequencing methods utilizing the Big Dye Terminator Cycle Sequencing Kit (PE Applied Biosystems, New Jersey, USA). Sequencing products were purified of unincorporated dye labeled dideoxynucleotides by processing through Centri-Sep Spin Columns (PE Applied Biosystems, New Jersey, USA). Sequence analysis was performed on an ABIPRISM™ 3100 Genetic Analyzer (PE Applied Biosystems, New Jersey, USA).

In this study, the chi-square test was used for statistical differences in HPV positivity between male and female groups and between the age groups.

**Results**

The numbers given in parentheses in the following paragraphs are the number of positive samples / the total number of samples examined.
Twenty-five percent of all samples (48/192) were positive for HPV DNA. The positive rate of males in all-male samples was 22.5% (20/89) and that of females in all-female samples 27.2% (28/103) (Fig. 1). No statistically significant difference was found for HPV positivity between male and female groups (Fig. 1). The positive rates of the 4 different age groups, that is to say 1-5 years old, 6-10 years old, 11-15 years old and 16-20 years old, were 4.7% (2/43), 34.3% (24/70), 31.0% (18/58) and 19.0% (4/21), respectively (Fig. 2). A statistically significant relation (p<0.05) was found between the age group of 1-5 years old and the rest of the age groups (Fig. 2). The positive rates of the prevalent HPVs types 1, 4, 16, 18 and 37 in 4 different age groups were examined. The positive rates of the 4 different age groups (1-5 years old, 6-10 years old, 11-15 years old and 16-20 years old) were as follows: HPV-16, 50.0% (1/2), 66.7% (16/24), 38.9% (7/18) and 50.0% (2/4); HPV-4, 0% (0/4), 45.8% (11/24), 38.9% (7/18) and 0% (0/4); HPV-18, 50.0% (1/2), 66.7% (16/24), 38.9% (7/18) and 50.0% (2/4); HPV-37, 0% (0/4), 45.8% (11/24), 16.7% (3/18) and 0% (0/4); HPV-1, 0% (0/4), 25.0% (5/20), 25.0% (5/20) and 0% (0/4). The various types of HPVs in 48 positive samples were HPV-16: 54.2% (26/48), -4: 29.2% (14/48), -18: 25% (12/48), -1: 16.7% (8/48), -37: 16.7% (8/48), -59: 6.3% (3/48), -22: 4.2% (2/48), -38: 4.2% (2/48), -57: 4.2% (2/48), -21: 8.3% (4/48), -50: 6.3% (3/48), -3: 2.1% (1/48), -24: 2.1% (1/48) and -20: 4.2% (2/48). The most prevalent type of HPV was HPV-16, followed by HPV types 4, 18, 1 and 37. The positive rates of the prevalent HPVs types of 1, 4, 16, 18 and 37 in 4 different age groups were examined. The positive rates of the 4 different age groups (1-5 years old, 6-10 years old, 11-15 years old and 16-20 years old) were as follows: HPV-16, 50.0% (1/2), 66.7% (16/24), 38.9% (7/18) and 50.0% (2/4); HPV-4, 0% (0/4), 45.8% (11/24), 16.7% (3/18) and 0% (0/4); HPV-18, 50.0% (1/2), 25.0% (5/20), 25.0% (5/20) and 0% (0/4); HPV-37, 0% (0/4), 45.8% (11/24), 16.7% (3/18) and 0% (0/4); HPV-1, 0% (0/4), 25.0% (5/20), 25.0% (5/20) and 0% (0/4).

(6/24), 22.2% (4/18) and 25.0% (1/4); HPV-1, 0% (0/4), 12.5% (3/24), 27.8% (5/18) and 0% (0/4); and HPV-37, 0% (0/2), 20.8% (5/24), 16.7% (3/18) and 0% (0/4), respectively (Fig. 5).

Multiple infection of HPVs was observed in 37 of 48 HPV-positive samples (77.0%) (Table 3). In particular, 16.7% of all HPV-positive samples (8/48) were infected simultaneously with 4 or 5 types of HPVs.

**Discussion**

Mongolia is a landlocked country located in the center of the Asian continent between China and Russia. In July 2004, the population of Mongolia reached 2,751,314. Urbanization is increasing with 56.6% of the population now living in cities and the remaining 43.3% living in rural areas. Ulaanbaatar is the capital city where 32% of the population of the country resides. As previously mentioned, so far no studies are available on HPV infection in the normal oral cavities of Mongolian people. Therefore, it is significant with respect to oral health care to investigate HPV infection in this population.

The PCR method is one of the most sensitive and simple techniques for the detection of HPV infection. It has been used in many studies to examine various types of HPV in the oral exfoliative cells of healthy adults and children. In this study, the PCR technique was also used...
to determine HPV infection in the normal oral cavities of young people in Mongolia. The frequency and types of HPV infection in samples from the normal oral cavity were reported in the following studies. Terai et al (36), detecting HPV infection by the PCR method in the swabs collected from the normal oral cavities of 37 adults, reported that HPV infection was detected in 81.1% (30/37). Kojima et al (39), using PCR to investigate the frequency of HPV infection in the oral epithelial cells from swabs in healthy Japanese children, aged 3 and 5 years, reported that 37 of 77 samples (48.1%) were positive for HPV infection. That study also documented that the positive rates of males and females in all samples were 28.3% (22/77) and 19.5% (15/77), respectively, and a statistically-significant association (p<0.01) was found in HPV positivity between males and females. Summersgill et al (38) reported that they investigated the frequency of HPV infection in oral squamous cells from swabs and oral saline solution, using the PCR, dot blot hybridization and DNA sequence methods. In their study, the frequency of HPV infection among young children in Iowa in the USA (under 7 years old) was 8.7% and among adolescents (13-20 years old) it was 5.2%, although HPV infection was not detected in children 7-12 years old. Kurose et al (42), using the PCR method to investigate the presence of HPV infection in oral scrapes from 668 healthy volunteers on Miyako Island in Japan, reported that the presence of HPV was detected in only 4 specimens (0.6%). Bouda et al (37), studying 16 oral scrapes from healthy subjects in Athens, Greece by the PCR method, reported that none were found to be infected with HPV. Kansky et al (40), using the PCR method to investigate HPV infection in tissue specimens of oral squamous cell carcinoma and normal oral mucosa on Ljubljana in Slovenia, reported that HPV infection (HPV-16, -33 and -5, together) was detected in 8.4% (5/59) of oral squamous cell carcinoma and in 6.6% (4/61) of the normal oral mucosa.

In the present study, 25% of all samples (48/192) from young people were positive for HPV infection. No statistically-significant difference was observed for HPV positivity between male and female groups. As in the above-mentioned studies, as well as in this study, the frequency of HPV positivity of oral samples from healthy individuals ranged from 0% to 81.1%, and HPV positivity between males and female varied from non-significant to significant. These different results in the above-mentioned studies might be due to the differences in samples of age groups, environmental states and race. In this study, positive rates of the 4 different age groups, namely 1-5 years old, 6-10 years old, 11-15 years old and 16-20 years old, were 4.7%, 34.3%, 31.0% and 19.0%, respectively. The positivities of HPV infection in different age groups, except the 1-5 year old group in this study, were higher than those among children (1-12 years old) and among adolescents (13-20 years old) in the study by Summersgill et al (38). These differences in both studies might also be due to differences in environmental conditions and race, although the age groups examined in these two studies were almost the same. Both the frequencies of HPV infection in young children (under 7 years old) in the study of Summersgill et al (38) and in the group of children (1-5 years old) in the present study were similarly low. This indicates that most young children had not been exposed to the HPV infection in the infant stage of life, although environment and race were different for these young children. In the present study, both the positive rates of HPV infection in 4 different male age groups and in 4 different female age groups were similar. These results suggest that, concerning the HPV infection, the male and female groups examined were living in similar environments. Many types of HPVs were considered related to the development of benign and malignant lesions in the oral cavity (Tables 1, 2). Ostwald et al (31), investigating HPV infection in the histological tissues of both oral squamous cell carcinomas and scrapings of the normal mucosa using the PCR method, reported that the presence of HPV was detected in 61.5% (16/26) of oral squamous cell carcinomas and only 1% (1/97) of normal buccal mucosae, respectively. Among the many lesions, HPV-16 was reported to be the most frequently identified HPV in oral squamous cell carcinomas. However, Jalal et al (30), doing a screening of healthy volunteers for HPV infection using scrapes from the buccal mucosa, hard palate and dorsum of the tongue, reported that, among persons even with no clinical disease, the overall prevalence of HPV-16 in normal-appearing oral epithelium was 43% (13/30). Mund et al (35), investigating buccal swabs from healthy children (1-10 years of age) for HPV-16 infection using the PCR method, demonstrated that HPV-16 was found in 35.5% of normal children. Jenison et al (29) reported that they investigated HPV infection in oral mucosal scrapings from healthy adults and preschool children using the PCR method. In adults, HPV-6 and -16 were detected in 17% and 23% of samples, respectively, and, in preschool children, HPV-6 and -16 were found in 24% and 19% of samples, respectively. Kojima et al (39), investigating HPV infection in swabs from the normal oral cavity of children aged 3 and 5 years old using the PCR method, showed that the frequent HPV types were HPV-16 (29.7%), -1 (16.2%), -2 (16.2%) and -16 (16.2%). Terai et al (36), demonstrated that the types of HPV determined by the PCR method in oral swabs from the normal cavity of 30 adults were HPV-18 (26/30; 86.7%), -61 (18/30; 60%), -59 (7/30; 23.3%), -16 (2/30; 6.7%) and -6 (1/30; 3.3%). Lambropoulos et al (34), examining HPV infection in cytobrushes from the oral cavity of healthy people using the PCR method, reported that HPV infection was detected in 9.5% of 169 subjects and that the incidence of types HPV-16, -18, -33, and -11 were 2.4%, 0%, 0%, 4.1% and 0.6%, respectively.
Miller et al (33), examining the presence of HPV infection in cytologic and histologic samples from the normal oral mucosa, reported that HPV was identified with increasing frequency in normal oral mucosa (13.5%), benign leukoplakia (14.8%), intraepithelial neoplasia (18.5%) and squamous cell carcinoma (26.2%). Zhang et al (41), investigating HPV infection (types of 16 and 18) in the histological tissues of oral squamous cell carcinomas and of normal oral mucosa using the PCR method, reported that 74% of oral squamous cell carcinomas (54/73) and 55% of normal tissues (22/44) were positive for HPV-16 and -18. Mao et al (32), examining HPV-16 infection in oral smears from patients with oral cancer and from healthy people using the PCR method, reported that HPV-16 was detected in 30.8% of oral cancer lesions and 15.4% of samples from the normal mucosa.

In this study, the various types of HPVs were identified in 48 positive samples, and the most frequent type of HPV was HPV-16 (54.0%). The frequency of HPV-16 in this study was higher than in the above-mentioned studies (29, 30, 32-36, 39, 41). Probably this difference was related to different environmental states and race. Other types of HPVs were identified in the present study, of types HPV-4 (29.0%), -18 (25.0%), -1 (17.0%), -37 (17.0%) and others. Kojima et al (39) reported that, besides HPV-16, they also identified HPV-1 (16.2%), -2 (16.2%) and -75 (16.2%). In this study, concerning the infection of HPVs, both types of HPV-16 and -18 were identified in all 4 different age groups, but 1, 4 and 37 type HPVs were not found in 2 age groups (1-5 years old and 16-20 years old). The reason for this difference was not clear from the results of the present study.

Multiple HPV infections were observed in 77% of all positive samples in this study. In particular, 16.7% of all HPV positive samples were infected with 4 or 5 types of HPV. The significance of this finding was again not clear from the results of the present study. HPV-16 infection is considered to be a co-factor in oral carcinogenesis. Moreover, an early tendency to HPV-16 infection in childhood may lead to the development of oral cancer in adulthood. Therefore, more studies are needed to clarify the significance of HPV infection in the normal oral cavity.

References