

HUMAN PAPILLOMA VIRUS AND NASOPHARYNGEAL CARCINOMA: PATHOLOGY, PROGNOSIS, RECURRENCE AND MORTALITY OF THE DISEASE

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Background: One of the malignant tumors among head and neck cancers is nasopharyngeal carcinoma. Many studies consider human papilloma virus (HPV) as a cause for nasopharyngeal carcinoma. **Methods:** 41 paraffin-wax-embedded block samples were examined to detect HPV DNA and its subtype's presence by polymerase chain reaction. The recurrence, prognosis and survival were evaluated for an average of 48 months. **Results:** HPV DNA was positive in 9 patients (22%). The overall recurrence rate was 75% in HPV negative patients and 11% in HPV positive ones. The mortality rate in HPV negative and positive patients was 37.5% and 0%, respectively. **Conclusion:** HPV type 18 and 16 were the most common subtypes. Also, it can be implied that patients which are HPV positive had better prognosis and also less recurrence.

Key Words: human papilloma virus, nasopharyngeal cancer, polymerase chain reaction, prognosis, recurrence.

The overall incidence of nasopharyngeal carcinoma (NPC) is 1/100000 [1]. NPC is a common cancer in Southeast Asia [2] but a rare disease in the United States [3, 4]. Etiologically, NPC is induced by different factors, including infections (e.g., EBV and human papilloma virus — HPV), environmental factors, and genetics. HPV DNA expression has been reported to be between 9% and 51% in NPC [5, 6]. HPV has been also detected in 73% of patients with tonsillar cancers in the United States [4]. In the case of Moroccan patients, HPV prevalence in NPC is 34% [7].

The World Health Organization (WHO) has classified NPC histologically into 3 types: type I (keratinizing squamous cell carcinoma (SCC)), type II (non-keratinizing SCC), and type III (undifferentiated carcinoma) [1, 4]. In this study, we searched for the presence of HPV DNA types (18, 16, 11 and 6) in NPC by PCR in paraffin-wax-embedded blocks to find out if there is any correlation between this presence and pathologic features, prognosis, recurrence and survival of the patients.

All patients underwent biopsy in the Otolaryngology Department of Shahid Sadoughi General Hospital in Yazd between 1995 and 2007. The total number of 41 patients was diagnosed with NPC and paraffin-wax-embedded blocks were collected. The clinical presentation of tumors, including palpable lymph node, nasal obstruction, epistaxis, and hearing loss were collected from admission files. On the basis of hematoxyline and eosin (H + E) staining, these samples were classified according to the WHO classification. Also, by using H + E staining, we were enabled to identify tumor rich

area and core samples were taken from them. To prepare samples for DNA isolation, deparaffinization was done with Xylene and DNA was retrieved by the DNAsy Blood & Tissue QIAGEN kit (Valencia, CA, USA).

HPV kit (GENKAM, Germany) was used for performing PCR. Each sample was subjected to PCR reactions using GP5+/GP6+ primer sets and type-specific primers for HPV6, 11, 16 and 18. The final 50 µl PCR mixture contained 10-µl sample, 25-µl PCR Master Mix Promega, mixed with 3 mM MgCl₂, and 20 pmol of each primer. Cycling profile was done with these amplifications settings: 5 min incubation at 94 °C and 1 min denaturation for 40 cycles at 95 °C, 1 min annealing and elongation at 55 °C and 72 °C, respectively. Final extension of 10 min at 72 °C was performed after the last cycle [8].

To evaluate NPC recurrence, nasopharyngoscopy, CT or MRI was used when the new symptoms of NPC were present at least 6 months after the treatment.

The treatment plan was either radiotherapy or chemoradiotherapy. Regardless of pathology report radiotherapy alone was considered only for stage T1N0 (T1: tumor was confined to nasopharynx). Although none of our patients were classified in this group, all of them were treated with chemoradiotherapy according to the following protocol: 160 mg/m² of cisplatin on day 1, day 22 and day 44 of course of the protocol; and they also underwent a radiotherapy in which 7000 cGy (cumulative dose) was given. These patients have received 80 mg/m² of cisplatin and 1000 mg/m² of 5-fluorouracil per day by continuous infusion days 1–4, after a rest of 28 days. The procedures of the study were approved ethically by Shahid Sadoughi University Ethics committee. The data were analyzed using SPSS statistical software (Chicago, Illinois, USA).

41 paraffin-wax-embedded blocks were collected from enrolled patients (34 men and 7 women) and assessed

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Abbreviations used: HPV – human papilloma virus; NPC – nasopharyngeal carcinoma; SCC – squamous cell carcinoma.

by PCR methods. The average age at the time of biopsy was 26 years with a range of 14 to 82 years. The average mean follow-up time was 48 months (13–150).

According to the WHO classification, 24 cases (58.6%) were classified as WHO type III, 14 (34.1%) as WHO type II, and 3 cases (7.3%) as WHO type I (Table).

48% of the NPC cases were presented with palpable cervical lymph nodes, 34% with nasal obstructions, 10% with epistaxis, and 8% with hearing loss. HPV was positive in 9 of the 41 cases (22%). Among those samples which were HPV-positive, 5 (55.6%) cases were HPV type 18; 3 (33.3%) HPV type 16; 1 (11.1%) HPV type 6 and none of the samples were positive for HPV DNA type 11 (Table).

Table. WHO histological classifications of NPC and frequency presence of HPV DNA types (18, 16, 11 and 6) in this study

	WHO classifications			HPV negative (78)	HPV positive (22%)			
	Type I (7.3)	Type II (34.1)	Type III (58.6)		Type 18 (55.6)	Type 16 (33.3)	Type 11 (0)	Type 6 (11.1)
Patient (%)	3	14	24	32	5	3	0	1

The overall recurrence rate was 75% (24 of 32) in HPV-negative patients and 11.1% (1 from 9) in HPV-positive ones. The overall mortality rate was 37.5% (12 of 32) in HPV-negative patients and zero in HPV-positive patients (0 of 9).

HPV is a double stranded DNA virus, and several studies have indicated that there is a correlation between HPV infection and head and neck SCC (HN-SCC) [2]. Also, different studies have reported that the expression of HPV DNA among patients with NPC has a range about 9 to 51% [5, 6]. Most studies have been conducted in Southeast Asia [2]. In Southern India, Krishna et al. reported that HPV was positive in 38.8% of 36 patients with NPC [9]. Another study of 30 patients with NPC showed that 7 patients were HPV-positive [1]. Mirzamani et al. [2] performed an in situ hybridization investigation for the detection of HPV DNA in NPC patients inside Iran and found 20% of the samples were HPV-positive. In the current study, we found out the presence of HPV DNA in 22% of NPC tissue blocks. Considering HPV genotypes, HPV 18 (55.6%) and HPV 16 (33.3%) were the most common ones. There was also no significant correlation between HPV infection rate and the median age and gender of patients, which was in agreement with other studies ($p > 0.05$) [7].

It seems that HPV positive tumors have the tendency to spread more rapidly than HPV-negative ones, but the survival of patients which are HPV-positive is better than HPV-negative patients [10]. This is probably because HPV positive tumors are more sensitive to chemotherapy [11]. Fakhry et al. implied that HPV positive cancers of head and neck may have 60 to 80% less mortality rate in comparison with HPV-negative patients [12]. In this study, the overall survival of patients with HPV negative tumors was worse than those with HPV-positive tumors (mortality rate of 37.5 versus 0%) [12]. Ang et al. reported that three year survival

is by 25% higher for HPV positive patients with oropharyngeal cancers rather than ones without HPV in their tumors (82.4 against 57.1%) [13]. In our study, the mortality rates of HPV-negative and HPV-positive patients were 37.5 and 0%, respectively.

In conclusion, the presence of HPV and its subtypes were investigated in NPC in the current study. In addition, it can be implied that patients which are HPV positive had better prognosis and also lesser recurrence. This study has a number of limitations; especially in quantity of cases for determining more precise correlation between the presence of HPV, prognosis and WHO typing. Further investigation may lead to a determination of the effect of HPV on the disease prognosis and could also provide some keys to the prevention and the treatment of NPC.

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