Prevalence and viral load of oncogenic human papillomavirus (HPV) in pterygia in multi-ethnic patients in the Malay Peninsula

Pei Pei Chong,1,2,3 Chee Hong Tung,1 Nurul Asyikin bt Abdul Rahman,1 Misako Yajima,3,4 Fee Wai Chin,1 Crystale Lim Siew Yeng,5 Eng Soon Go,6 Cordelia M. L. Chan,7 Nobuyo Yawata8,9,10 and Naoki Yamamoto3

1Department of Biomedical Science, Faculty of Medicine and Health Sciences, University Putra Malaysia, Serdang, Malaysia, 2Institute of Bioscience, Universiti Putra Malaysia, Serdang, Malaysia, 3Translational Medicine, Department of Microbiology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore, 4Department of Virology, University of Toyama, Toyama, Japan, 5Faculty of Allied Sciences, University College Sedaya International, Kuala Lumpur, Malaysia, 6Ophthalmology Unit, Department of Surgery, Faculty of Medicine and Health Sciences, University Putra Malaysia, Serdang, Malaysia, 7Singapore National Eye Centre, Singapore, 8Infection and Immunity Programme, Singapore Institute for Clinical Sciences, Agency for Science, Research and Technology, Singapore, 9Singapore Eye Research Institute, Singapore, 10Duke-Nus Graduate Medical School, Clinical Sciences, Singapore

ABSTRACT.

Purpose: The aim of the study was to determine the prevalence of human papillomavirus (HPV) in primary and recurrent pterygia samples collected from different ethnic groups in the equatorial Malay Peninsula.

Methods: DNA was extracted from 45 specimens of freshly obtained primary and recurrent pterygia from patients and from 11 normal conjunctival swabs from volunteers with no ocular surface lesion as control. The presence of HPV DNA was detected by nested PCR. PCR-positive samples were subjected to DNA sequencing to determine the HPV genotypes. Real-time PCR with HPV16 and HPV18 type-specific TaqMan probes was employed to determine the viral DNA copy number.

Results: Of 45 pterygia samples with acceptable DNA quality, 29 (64.4%) were positive for HPV DNA, whereas all the normal conjunctiva swabs were HPV negative. Type 18 was the most prevalent (41.4% of positive samples) genotype followed by type 16 (27.6%). There was one case each of the less common HPV58 and HPV59. Seven of the samples harboured mixed infections of both HPV16 and HPV18. All the four known recurrent pterygia samples were HPV-positive, whereas the sole early-stage pterygium sample in the study was HPV-negative. There was no significant association between HPV-positive status with gender or age. A high proportion of patients from the Indian ethnic group (five of six) were HPV-positive, whereas the Malay patients were found to have higher HPV positivity than the Chinese. The viral load of HPV18 samples ranged between $2 \times 10^2$ and $3 \times 10^5$ copies per µg, whereas the viral load of HPV16 specimen was $4 \times 10^4$ to $10^6$ copies per µg.

Conclusion: This report describes for the first time the quantitative measurement of HPV viral DNA for pterygium samples. The high prevalence of oncogenic HPVs in our samples suggests a possible role for HPV in the pathogenesis of pterygia. Moreover, the relatively low HPV viral load is concordant with the premalignant nature of this ocular condition.

Key words: human papillomavirus – multi-ethnic – PCR detection – pterygia

Introduction

Pterygium is a chronic ophthalmic disease characterized by a triangular sheet of superficially growing fibrovascular tissue starting from the conjunctiva. Normally, this tissue is harmless unless it has overgrown into the corneal region, thus threatening the vision of the patients which may then require surgical excision.

To date, the aetiology and pathogenesis of pterygium are still unknown. Factors which have been suggested to contribute towards the development of pterygia include family history of neoplasia, altitude of present residence, age, exposure to ultraviolet light (Coroneo 1993), inflammatory reactions (Wong 1978), a primary degeneration of the cornea followed by fibroblastic proliferation (Elliott 1966), allergic factors (Pinkerton et al. 1984), immunopathological mechanisms (Perra et al. 2002) and oncogenic viruses (Detorakis et al. 2001; Gallagher et al. 2001).

Some studies found that fibroblasts from pterygium behaved like neoplastic cells in vitro (Chen et al. 1994) and histopathological examinations have revealed neoplastic features such as epithelial proliferation, goblet cell hyperplasia, angiogenesis along with concurrence of ocular surface squamous neoplasia and primary acquired melanosis (Degrassi et al. 1993). A case