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Detection of human papillomavirus from archival tissues in cervical cancer patients in Mauritius

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Abstract

Background: Around half a million new cases of cervical cancer are diagnosed worldwide each year, accounting for almost 300,000 deaths. Development of cervical cancer can be multi-factorial, but high-risk human papillomaviruses (HPV) have been associated with the aetiology of cervical cancer. It is believed that HPV DNA integrates into the host DNA causing abnormal cell growth with cells becoming carcinogenic and spreading metastatically. In Mauritius, cervical cancer account for 65% of gynaecological cancers and 3.4% of the cervical cancers are diagnosed at the stage of carcinoma in situ.
Objectives: To determine the prevalence of HPV in histological samples from patients with cervical cancer in Mauritius.
Study design: DNA from archival cervical samples from a cohort of 65 patients suffering from cervical cancer and controls from Mauritius were tested for the presence of HPV using MY09/11 and GP5+/6+ primer sets.
Results: In a cohort of 65 patients from Mauritius, diagnosed with cervical cancer in the year 2000, 19% of cervical histology sections were found to be positive for the presence of high-grade HPV, exclusively HPV18 using MY09 and MY11 primers. Only 15% of the Mauritian population is over 50 years of age, whereas 66% (35) of the diagnosed cases of cervical cancer were seen in patients above 50 years with 50% (5) affected with HPV. These findings suggest that for an infection with HPV to develop into cancer may take years if not decades. Differences were noted using two different primer sets, MY09/11 and GP5+/6+. The latter produce a much smaller amplicon (150 bp) compared to the former (∼450 bp). Seven additional positive cases were detected with the GP5+/6+ primer set, resulting in an apparent prevalence of 32% as compared to the 19% seen with the MY09/11 primer set. This may indicate that some degradation of the target DNA has occurred during processing and storage of histological samples.
Conclusion: Using primer sets MY09/11 and GP5+/6+, only HPV type 18 was found in the Mauritian cohort with a prevalence of 32%.

Keywords: Cervical cancer; Human papillomavirus; Bioinformatics; Mauritius

1. Introduction

Cervical cancer is one of the major causes of morbidity and mortality in women worldwide. There are approximately 450,000 new cases of cervical cancer per year with around 300,000 deaths, (Damasus-Awatai and Freeman-Wang, 2003). Cervical cancer, like any cancer is a state where cell behaviour is compromised. In a normal cell, growth is carefully controlled, while in cancer, the cells divide, infiltrating and proliferating in normal tissues. Malignant cells proliferate to form neoplasia. Cancer is thought to develop through a series of pre-malignant events, in the case of cancer of the cervix, termed cervical intraepithelial neoplasia (CIN) (Hjerpe et al., 1990). Cervical cancer appears to originate from the abnormal cancerous epithelium, classically known as carcinoma in situ.

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Mauritius is a multi-cultural society, consisting of Hindus, Muslims, Tamils, Blacks of African and Madagascan descent, Whites of French and British descent, Mulattos and Chinese. The main religions are Hinduism, Christianity and Islam. Retrospective studies between 1989 and 1993 by Manraj et al. (1998) through the cancer registry of Mauritius showed that the incidence of cervical cancer and ovarian cancer combined was 27% (21% cervical and 6% ovarian). Cancer accounts for 8–9% mortality in Mauritius with an annual cancer-associated death rate of 6 in every 1000. Only 15% of the population is above 50 years of age, whereas 66% (35) of the diagnosed cases of cervical cancer were seen in patients above 50 years. According to Manraj et al. (1998), cervical cancer accounted for 65% of gynaecological cancers and 3.4% of the cervical cancers were diagnosed at the stage of carcinoma in situ. Similar findings have been reported by Mans et al. (2003) who carried a retrospective study in Suriname. The ethnic mix of Mauritius and Suriname is similar. In both studies cancer was, in general, two to six times more common in Creoles, who are of African descent as opposed to those of Indian descent.

1.1. HPV and cervical cancer

Human papillomavurises (HPV) have been implicated in the aetiology of cervical cancer (Lee et al., 2002; Kailash et al., 2002). HPV, is one of the two genera of papovaviridae; a group of viruses with more than 70 genotypes. HPV consists of a capsid that has icosahedral symmetry with 72 capsomeres of average diameter between 52 and 55 nm. HPV contains double stranded DNA (dsDNA) molecules encoding proteins of estimated weight of 5×10^6 Da and a genome size of approximately 8 Kb. There are about seventy types of HPV that can infect epithelial surfaces and these can be divided into high-risk and low-risk types, dependent on their association with disease (Van Doorn et al., 2001). HPV can either replicate independently as episomes or by integrating into the host DNA. The integration of high-risk type HPV DNA is probably the most crucial event for tumorigenesis (Peitsaro et al., 2002). This heterogeneous group of viruses has the ability to infect and replicate in squamous epithelia of both keratinised and mucosal surfaces. Infection with high-risk types of HPV underlies most cases of high-grade cervical intraepithelial neoplasia (CIN) and practically all cases of invasive cervical cancer. The high-risk HPV types are HPV16, 18, 30, 31, 33 and 45 and there is a significant correlation between HPV16 and 18 and cervical cancer. These two genotypes play a leading role in the process of cervical carcinogenesis as shown in a study conducted by Munoz and Bosch (1997) in which HPV16 and 18 were detected in 50% and 12% of cervical carcinomas, respectively. Several other studies have demonstrated the presence of infectious virions, episomal or integrated HPV DNA. Other high-risk HPV types such as HPV31, 33 and 45 have also been isolated from cancer of the cervix; however, the incidence of these genotypes is low when compared to HPV16 and 18. HPV is host specific and also restricted in tissue tropism.

1.2. HPV genome

The genomes of several HPV types have been cloned and sequenced. There appear to be three distinct regions: the upstream regulatory region, controlling transcription and replication; at least seven early genes encoding proteins required for transcription, DNA replication and cell transformation; two late genes encoding the major and minor capsid proteins.

The molecular organization of the HPV genome is well conserved between viruses of various types. The open reading frame (ORF) is divided into two areas coding for E (early) and L (late) proteins. Because of the overlapping nature of the ORFs, the mRNA transcribed is complex and it is not clear which transcript codes for which protein. The ORFs are situated on, and are transcribed from the same strand and so are read in the same direction from 5′ to 3′.

2. Materials and methods

The samples were obtained after ethical approval from the Ministry of Health, Government of Mauritius. DNA was extracted from wax-embedded tissue from a cohort of 65 Mauritian patients diagnosed with cervical cancer in the year 2000. DNA was extracted from the tissues by an in-house method adapted from several published methods. DNA extraction was based on the method of Crum et al. (1985) who followed a procedure previously described by Ostrow et al. (1982). Most of these research groups had previously worked on either frozen sections or directly on biopsies and not wax-embedded tissue. All described the use of a similar lysis buffer containing Tris, EDTA, sodium chloride and sodium dodecyl sulphate. Crum et al. (1985) and Evander and Wadell (1991) both used proteinase K digestion for 4–6 h, whilst Enomoto et al. (1990) used a pronase digest for 16 h. In our study of wax-embedded formalin-fixed tissue was digested in proteinase K, 10 μl 20 mg/ml solution, in a final volume of 0.75 ml lysis buffer for 16–24 h at 37 °C was used. The DNA extraction method also used phenol chloroform extraction and ethanol precipitation of the DNA. Negative controls were from patients who had undergone abdominal hysterectomy for irregular bleeding, not associated with malignancy. Positive controls were samples previously analysed for the presence of HPV. Additionally, HeLa 229 cells were grown, harvested and lysed as described above. These provided a positive control for HPV18 DNA. Reports suggest that HeLa cells contain between 20 and 50 integrated copies of HPV DNA/cell, and thus, they provide not only a control for the method, but also a means of semi-quantitation.

Wax-embedded tissues cut in serial sections of 5 μm were dewaxed in xylene, transferred to 90% alcohol followed by 70% alcohol each for 10 min. The slides were then brought