Evaluation of urine as a potential alternative sample for the diagnosis of Human Papillomavirus (HPV) related cervical lesions: A pilot study

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INTRODUCTION

The human papillomaviruses (HPV) are non enveloped, double-stranded DNA viruses. More than 100 HPV types have been recognized, of which 30 to 40 types infect the genital tract and are transmitted sexually *(Jacobson et al., 2000).*

HPV types are grouped on the basis of their association with malignant and premalignant lesions, as high risk (16, 18, 31, 45); intermediate risk (33, 35, 39, 51, 52, 56, 58, 59, 68); and low risk (6, 11, 26, 32, 40, 53, 54, 55, 61, 66, 69, 73). The four high risk and the nine intermediate risk types together are designated as cancer associated types *(Jacobson et al., 2000 and Giovannelli et al., 2004)*

In most reports, samples for detection of HPV are obtained from the cervix after vaginal examination, either by endocervical cytobrush *(Forslund et al., 1993)***,** or cervical swab (spatula) *(Ammatuna et al., 2000)*. This requires the expertise of appropriately qualified and suitably equipped personnel and is relatively expensive.

This would be partly obviated if an easily obtainable sample as a source of HPV, not necessitating vaginal examination and biopsy taking, could be identified *(Stanczuk et al., 2003).*

The lower urinary tract lies in close proximity to the cervix, the vagina, and the vulva. Besides, the fact that examination of the cells in urine has been found to be an effective method for diagnosis of other sexually transmitted infections such as Chlamydia and Gonococci *(Gaydos and Quinn, 1998 and Gazzaniga et al., 1998).*

Jacobson et al., (2000) claimed that urine testing for HPV-DNA would identify a majority of women with squamous intraepithelial lesions**.** On the other hand, a negative urine HPV assay for high risk HPVs would strongly indicate normal cervical cytology. They added that HPV assays of the cells in urine either by Polymerase Chain Reaction (PCR) or Hybrid Capture-II (HC-II)**,** may prove to be valuable in certain circumstances, for example in epidemiologic surveys of genital HPV infections in populations where it may be difficult to obtain genital specimens for logistical or cultural reasons and in monitoring of women with cervical dysplasia.

AIM OF THE WORK

The aim of this pilot study was to evaluate the urine as a non-invasive, easily collectable sample for the diagnosis of HPV related cervical lesions.

This will be done by the detection of HPV-DNA in urine samples collected from HPV cervical cases diagnosed by pathological examination of cervical biopsies.

The results would be of great value to evaluate the use of an easy collected (non-invasive) specimen for the rapid diagnosis of large number of cases especially in epidemiologic studies and for screening purposes.

HUMAN PAPILLOMAVIRUS (HPV)

HISTORY

Papillomavirus (PV) was initially isolated from cottontail rabbits by *Shope and Hurst, (1933)***.** The discovery that papillomas induced by papillomavirus had the potential to transform into malignant processes was reported by *Rous, (1935)***.** Squamous cells that developed from papillomavirus infections were noted by *Koss and Durfee, (1956)* who called these cells koilocytic atypia or koilocytosis, meaning "hollow", from the Greek word koilos**.**

Papillomaviruses were detected in a variety of vertebrates. Human papillomaviruses (HPVs) are widespread throughout the population, producing epithelial tumors of the skin and mucous membranes *(Stanley et al., 1997)*.

Despite these early observations, the HPVs have proved difficult to propagate in vitro because these viruses replicate in stratified squamous epithelium, which is not mimicked in monolayer cultures. Most clinical

identifications of HPVs infections, therefore, rely on a technique that identifies the viral DNA, such as polymerase chain reaction (PCR) or a molecular hybridization technique, rather than on virus isolation. These techniques have led to an understanding of the genomic organization of these viruses, the function of different viral genes, and the multiplicity of HPV types *(Bonnez and Reichman, 2005 and Howley and Lowy, 2007).*

CLASSIFICATION OF HPVs

Chan et al., (1997) reported that PVs were classified together with the polyomaviruses as a single family, the **Papovaviridae.** This grouping arose because, although PV genomes and capsids are larger than those of polyomaviruses, both virus groups share many features, including a double-stranded circular DNA genome, an icosahedral capsid composed of 72 pentamers, a nonenveloped virion, and that the site of viral replication and virion assembly is the nucleus.

Rebrikov et al., (2002) stated that it was later recognized that the two virus groups (PVs and

polyomaviruses) have different genome sizes, completely different genome organizations, and no major nucleotide or amino acid sequence similarities. Besides PV transcription being unidirectional, in contrast to the bidirectional transcription of polyomaviruses, and thus they became officially recognized by the International Committee on the Taxonomy of Viruses (ICTV) as two separate families, **Papillomaviridae** and **Polyomaviridae.**

PVs have been isolated from many mammalian host species and birds, but thus far have not been identified in non-vertebrates *(Antonsson et al., 2003)*.

Based on the analysis of HPV genomes, and those of a number of animal papillomaviruses, *de Villiers et al., (2004)* developed phylogenetic trees that form a stable framework for the placement of additional HPV types as they arise. This proposed taxonomy and nomenclature follows generally accepted criteria. Higher order phylogenetic assemblages that had previously been called ‘‘supergroups’’ or ‘‘major branches’’ are considered to be a genus.

The authors divided **family** Papillomaviridae into 12 genera, each of which is designated by a letter of the Greek alphabet. Within a given **genus**, the major capsid protein "L1" DNA of all members share more than 60% identity; conversely, they have less than 60% identity with members of other genera. A **species** is designated for those PV within a given genus that shares 60% to 70% identity. A viral **type** within a species has 71% to 89% identity with other types within the species. Within a type can exist **subtypes**, which share 90% to 98% identity, and **variants** which have more than 98% identity**.**

The largest group of HPVs comprises the Alpha papillomaviruses, and it is this group that contains the genital/mucosal HPV types and cutaneous viruses such as HPV2. The remaining HPVs come from three genera (Gamma, Mu and Nu) and generally cause cutaneous papillomas and verrucas that do not progress to cancer *(de Villiers et al., 2004)*.

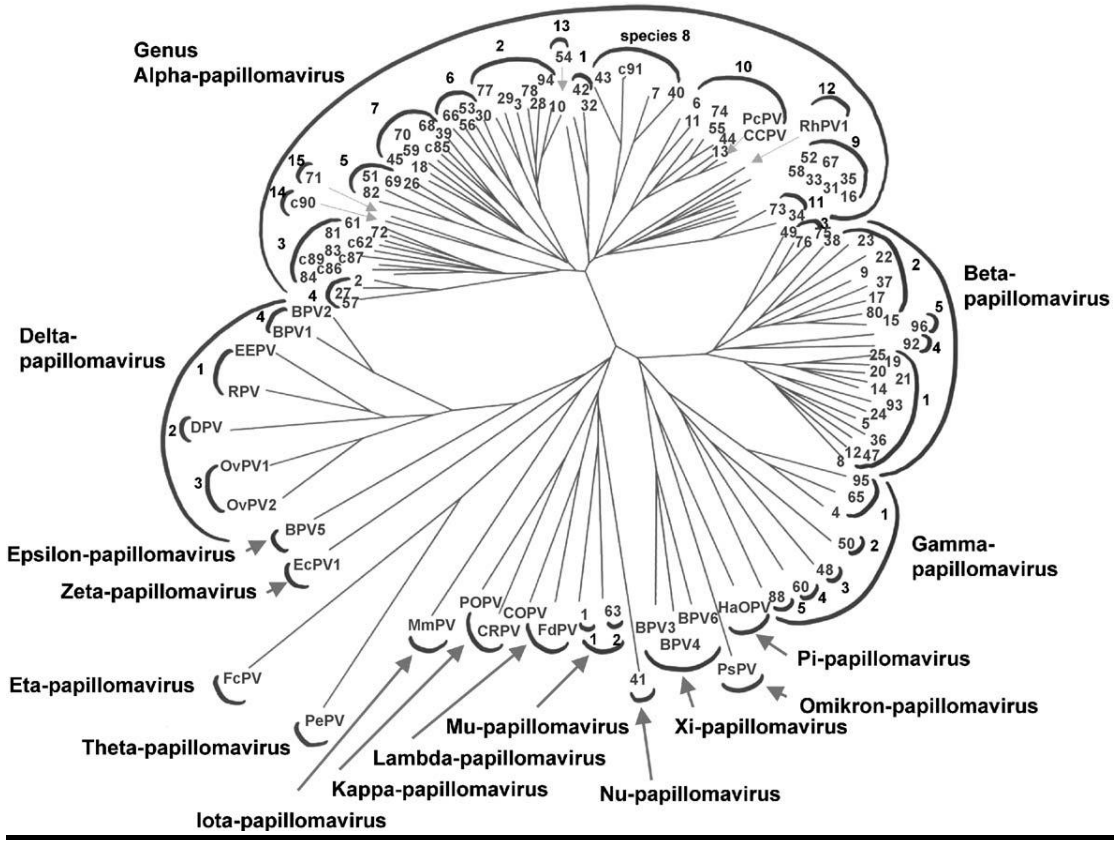

Figure (1): Phylogenetic tree containing the sequences of 118 papillomavirus types (*de Villiers et al., 2004)*.

MORPHOLOGY AND STRUCTURE OF HPVs

HPV is a relatively small, non enveloped virus, 55 nm in diameter**.** The virus harbors a single molecule of double stranded circular DNA contained within icosahedral capsid *(Favre, 1975 and Baker et al., 1991).*

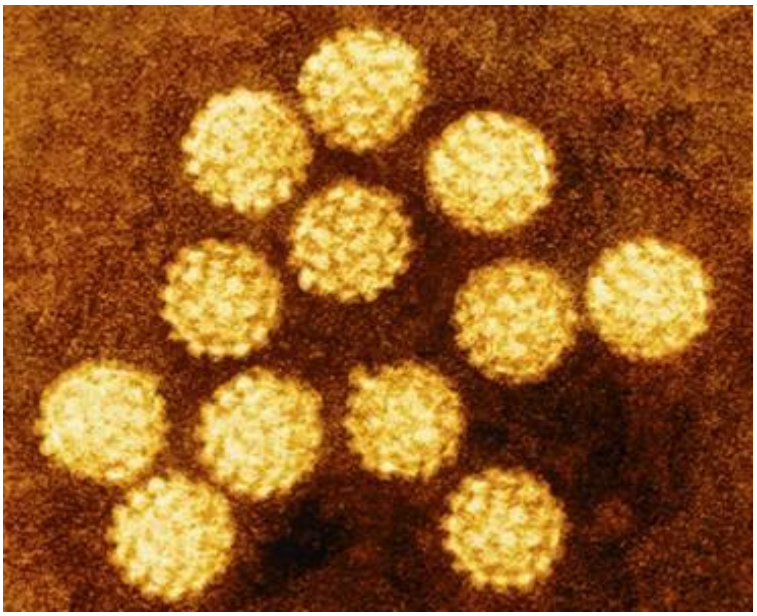

Figure (2): Negatively stained electron micrograph of papillomavirus particles *(Stanley and Pett, 2005).*

HPV genome:

All papillomaviruses contain a double-stranded, circular DNA genome approximately 8 kb in size that can be divided, in general, into three major regions: early, late, and a long control region ([LCR] or noncoding region [NCR]). The three regions in all papillomaviruses are separated by two polyadenylation (pA) sites: early pA (AE) and late pA (AL) sites *(Danos et al., 1982)*.

The early region of papillomavirus genomes occupies over 50% of the virus genome from its 5′ half and encodes six common open reading frames (E1, E2, E4, E5, E6 and E7) that translate individual non-structural (functional) proteins *(Choe et al., 1989)*.

Two other ORFs, **E3** and **E8**, were also assigned to this region initially, but only the E8 ORF in bovine papilloma virus (BPV-1) and HPV-31 has been proven to encode a protein, a spliced E8^E2C fusion protein, which functions as a negative regulator of viral transcription and replication *(Han et al., 1998)*. Unlike BPV-1 and HPV-31, E8 in several rabbit papillomaviruses have been

characterized as an oncogene, with features similar to those of the **E5** of both BPV-1 and several HPVs **(***Stubenrauch et al., 2001)*.

The late region of all papillomavirus genomes, covering almost 40% of the virus genome, lies downstream of the early region and encodes L1 and L2 ORFs for translation of a major **(L1)** and a minor **(L2)** capsid protein *(Torrisi et al., 2000)*.

The **LCR** region, a segment of about 850 bp (10% of the HPV genome), has no protein-coding function, but bears the origin of replication as well as multiple transcription factor binding sites that are important in regulation of RNA polymerase II-initiated transcription from viral early as well as late promoters *(Figure 3) (Bernard, 2002)*.

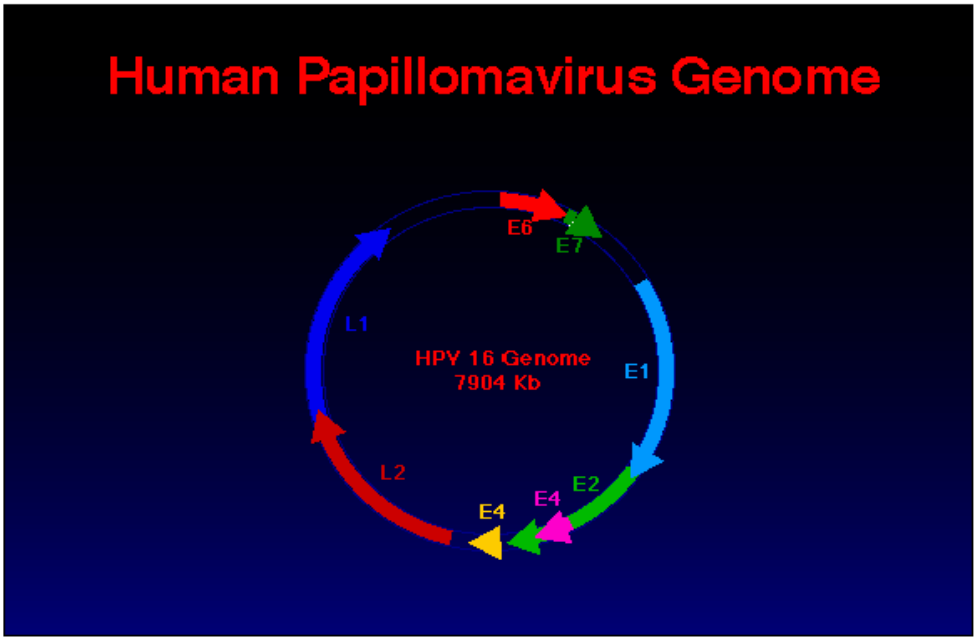

Figure (3): genome structure of HPV *(Doorbar, 2006)*.

HPV capsid:

HPV has an icosahedral capsid composed of 72 pentameric capsomeres arranged on a surface lattice. The capsomeres exist in two states, one capable of making contact with five neighbors in the 12 pentavalent capsomeres and the other capable of making contact with six neighbors in the 60 hexavalent capsomeres *(Figure 4) (Baker et al., 1991).*