Analysis of the physical state of human papillomavirus type-16 in early cervical intraepithelial neoplasia.

JEREMY N. KAYE, FARZIN PAKARIAN*, JOHN CASON, RICHARD J. JEWERS, BARBARA KELL, JON BIBLE, KANKIPATI S. RAJU* and JENNIFER M. BEST.

The Richard Dimbleby Laboratory of Cancer Virology, The Rayne Institute and *Department of Obstetrics and Gynaecology, UMDS, St. Thomas’ Hospital, London SE1 7EH.

Cervical cancer is one of the most common malignant diseases in women world-wide. Infection with certain types of Human Papillomavirus (HPV) in particular HPV-16 and other related types (HPV-18, -31 and -33) appears to be an important etiologic factor in the development of cervical intraepithelial neoplasia (CIN) and cervical carcinoma.

HPV infections causing clinically apparent lesions of the lower genital tract seem to occur more frequently during pregnancy and show a high regression rate after delivery. Schneider et al [1] reported the detection of HPV DNA in exfoliated cervical cells in 28% of a pregnant population compared with 12.5% for non-pregnant control patients; a higher detection level occurred in samples obtained later in pregnancy.

Our previous studies [2] have shown a high rate of transmission and persistence of HPV-16 and -18 from mothers to their infants. Concentrating on those mothers that were HPV-16 DNA positive in groups that did and did not transmit viral DNA we investigated the physical state of the virus (integrated or episomal) in order to determine whether this was a significant factor in the vertical transmission of HPV-16 DNA. Mothers were selected on the basis of having current or previous abnormal cervical cytology in order to increase the chance of HPV detection.

It is believed that integration of HPV DNA into cellular DNA usually results in a disruption or a deletion in the E1 or E2 open reading frames (ORFs) (Fig. 1.). For detection of integration two specific primers from the E2 ORF of the HPV-16 genome between nucleotides 2734 and 3872 were used in a polymerase chain reaction [3]. Primer E2-1 (5' AGGACGAGGACAGGAAAA-3') was located between nucleotides 2734-2753 and primer E2-2 (5' GGATGCAGTATCAAGATTTG-3') between nucleotides 3853-3872. A lack of amplification of the 1139 bp product using these E2-specific primers would indicate integration of the viral DNA.

PCR amplification was carried out in a 100ul reaction mix containing 1uM of each primer, 50 mM KCl, 10 mM Tris-HCl pH 8.3, 2.5 mM MgCl2, 200 uM of each dNTP and 2 units of Taq DNA polymerase. Amplification was done for 40 cycles in an Omnigene Thermal Cycler (Hybaid).

Fig. 1. Arrangement of the HPV-16 genome showing early (E) and late (L) genes. Amplification of the E2 sequence was attempted using primers located between nucleotides 2734-2753 and 3853-3872 resulting in a 1139 bp product.

Abbreviations used: HPV, Human Papillomavirus; bp, base pair