MOLECULAR DETECTION OF HIGH RISK HUMAN PAPILLOMA VIRUS SUBTYPES IN NEOPLASTIC CERVICAL TISSUES

NOR RIZAN KAMALUDDIN

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MOLECULAR DETECTION OF HIGH RISK HUMAN PAPILLOMA VIRUS SUBTYPES IN NEOPLASTIC CERVICAL TISSUES

By

NOR RIZAN KAMALUDDIN

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirement for the Degree of Master of Science

June 2007
DEDICATED TO:

My husband, AZAHARI, my children, NUR AMIRA, MUHAMMAD FARHAN and NUR ALYA, my mother, HJH SAMSIAH, my father, HAJI KAMALUDDIN, my brothers and sisters.
Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master of Science

MOLECULAR DETECTION OF HIGH RISK HUMAN PAPILLOMA VIRUS SUBTYPES IN NEOPLASTIC CERVICAL TISSUES

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June 2007

Chairman:  Professor  Abdul Manaf Ali, PhD
Faculty:  Institute of Bioscience

Human papillomavirus (HPV) plays an important role in the pathogenesis of cervical cancer. HPV has been found in 99.7% of cervical cancers worldwide. This common virus is easily transmitted by genital skin-to skin and sexual contact. The HPVs that infect the genital mucosa are classified according to their oncogenic potential and are described as either high or low-risk. The detection of E7 oncogene transcripts of high-risk human papillomavirus type 16, 18, 31, 33, 35, 39, 45, 51, 52 and 56 may be a sensitive indicator of the direct involvement of viral oncogenes in the development of cervical intraepithelial neoplasia and carcinoma. Three methods were used in this study; Type Specific PCR, Dot-blot hybridization to prove no false
positive of the TS-PCR products and SyBr Green Real Time PCR to detect and identify the multiple infection of the HPV subtypes. The objectives of this study are i) to determine the prevalence and the types of human papillomavirus in the neoplastic cervical tissues patients, ii) to detect and identify the multiple infections of HPV subtypes. Paraffin embedded tissues was collected from Hospital Universiti Kebangsaan Malaysia. All 67 specimens from several stages; Cervical intraepithelial neoplasia CIN I, CIN II, CIN III and carcinoma were screened for the presence of high risk HPV types. To determine the presence of HPV in the samples, Type Specific PCR (TS-PCR) and dot blot hybridization were performed. Positive samples from the TS-PCR and dot blot hybridization were analyzed and identified for the presence of the two prevalent HPV genotypes such as HPV 16 and HPV 18 using the SyBr Green Real-Time PCR. The results showed, in 67 samples, CIN I was detected in 37/67 (55%), CIN II was detected in 12/67 (18%), CIN III was detected in 15/67 (22%) and in 3/67 (5%) patients, invasive carcinoma was found. Because of the multiple infections, 67 HPV genomes were found in the 57 positive samples using TS-PCR. HPV 16 genome was detected in 55/67 (82%) cases, HPV 18 in 8/67 (12%) cases, HPV 33 was detected in 1/67 (1.5%), HPV 51 was detected in 1/67 (1.5%) and HPV 56 in 2/67 (3%) and 8 cases had multiple infections. The results showed DNA melting curve for
HPV 16 was having a peak around 80.2º ± 0.2C and threshold C_t value for specific product of HPV-16 was 20 ± 1 cycles whereas DNA melting curve for HPV 18 was having a peak around 79.2 ± 0.2ºC and threshold cycle C_t value for specific product of HPV 18 was 22 ± 1 cycles.

In conclusion, HPV- 16 was the most prevalent followed by HPV-18. This study detected five subtypes of high risk HPV; HPV 16, 18, 33, 51 and 56. HPV types 31, 35, 39, 45 and 52 were not detected. A SyBr Green Real-Time PCR method has the potential for clinical usage in prescreening, detection and identification of HPV infection in the cervical neoplasia at different stages of the disease.
Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PENGESANAN MOLEKUL SUBTIP VIRUS PAPILLOMA MANUSIA RISIKO TINGGI TERHADAP TISU SERVIK NEOPLASIA

Oleh

NOR RIZAN KAMALUDDIN

Jun 2007

Pengerusi: Profesor Abdul Manaf Ali, PhD
Fakulti: Institut Biosains

Human papillomavirus (HPV) memainkan peranan penting dalam patogenesis kanser servik. Pengesanan transkrip onkogen E7 terhadap HPV risiko tinggi jenis 16, 18, 31, 33, 35, 39, 45, 51, 52 dan 56 merupakan petunjuk yang sensitif untuk memperlihatkan penglibatan onkogen viral dalam pembentukan neoplasia intraepithelial servik dan karsinoma. Tiga teknik digunakan dalam kajian ini; TS-PCR, dot-blot hybridization iaitu untuk membuktikan tiada nya positif palsu hasil dari TS-PCR dan SyBr Green Real Time PCR. Objektif kajian adalah untuk menentukan prevalen dan jenis-jenis HPV risiko tinggi dalam pesakit-pesakit kanser servik di Malaysia, dan untuk mengesan dan mengenalpasti jangkitan oleh pelbagai jenis HPV dalam pesakit kanser servik. Spesimen tisu dalam paraffin diperolehi daripada

Keputusan ujikaji menunjukkan daripada 67 sampel yang dikaji, CIN I telah dikesan sebanyak 37/67 (55%) kes, CIN II sebanyak 12/67 (18%) kes, CIN III sebanyak 15/67 (22%) kes dan 3/67 (5%) kes bagi pesakit invasif karsinoma. Oleh kerana adanya jangkitan oleh pelbagai jenis HPV, keputusan keseluruhan menunjukkan 67 jenis HPV genom telah ditemui dalam 57 sampel positif daripada teknik Tindakbalas Berantai Polymerase-Jenis Spesifik. Genom HPV 16 telah dikesan sebanyak 55/67 (82%) kes, genom HPV 18 sebanyak 8/67 (12%) kes, genom HPV
33 sebanyak 1/67 (1.5%), genom HPV 51 sebanyak 1/67 (1.5%) dan genom HPV 56 sebanyak 2/67 (3%).

Fluorescen SyBrGreen telah diukur bagi setiap amplifikasi dan terbitan pertama fluorescen telah diplotkan sebagai fungsi suhu. Untuk membezakan hasil spesifik HPV 16 dan HPV 18, graf pencairan DNA telah dilakukan selepas tindakbalas berantai polymerase. Keputusan menunjukkan graf pencairan DNA bagi HPV 16 adalah pada suhu puncak 80.4°C dan nilai C_t bagi produk spesifik terhadap HPV 16 adalah pada pusingan ke 20. Manakala graf pencairan DNA bagi HPV 18 adalah pada suhu puncak 79.4°C dan nilai C_t bagi produk spesifik terhadap HPV 18 adalah pada pusingan ke 22.

Sebagai kesimpulan, HPV 16 adalah lebih prevalen, diikuti oleh HPV 18. Kajian ini hanya mengesan lima jenis HPV risiko tinggi iaitu HPV 16, HPV 18, HPV 33, HPV 51 dan HPV 56. HPV risiko tinggi yang lain seperti HPV 31, HPV 35, HPV 39, HPV 45 dan HPV 52, tidak dapat dikesan. Kaedah Tindakbalas Berantai Polimerase-Masa Sebenar SyBrGreen mempunyai potensi klinikal dalam penyaringan awal, pengesanan dan pengenalpastian jangkitan oleh pelbagai -jenis HPV bagi peringkat-peringkat yang berbeza dalam pesakit-pesakit kanser servik.
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I certify that an Examination Committee has met on 27th June 2007 to conduct the final examination of Nor Rizan Kamaluddin on her Master of Science thesis entitled “Molecular detection of high risk human papilloma virus subtypes in neoplastic cervical tissues” in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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Date: 15 November 2007
DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

____________________________________
NOR RIZAN BT KAMALUDDIN

Date: 2 June 2007
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<td>bp</td>
<td>Base pair</td>
</tr>
<tr>
<td>CIN</td>
<td>Cervical intraepithelial neoplasia</td>
</tr>
<tr>
<td>dATP</td>
<td>Deoxyadenosine triphosphate</td>
</tr>
<tr>
<td>dCTP</td>
<td>Deoxycytidine triphosphate</td>
</tr>
<tr>
<td>dGTP</td>
<td>Deoxyguanosine triphosphate</td>
</tr>
<tr>
<td>DMSO</td>
<td>Dimethylsulfoxide</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>dNTPs</td>
<td>Deoxynucleotide triphosphate</td>
</tr>
<tr>
<td>dTTP</td>
<td>Deoxythymidine triphosphate</td>
</tr>
<tr>
<td>ECL</td>
<td>Enhanced Chemilluminescent Labelling</td>
</tr>
<tr>
<td>HCl</td>
<td>Hydrogen chloride</td>
</tr>
<tr>
<td>H &amp; E</td>
<td>Hemotoxylin and Eosin</td>
</tr>
<tr>
<td>HPV</td>
<td>Human papillomavirus</td>
</tr>
<tr>
<td>ISCN</td>
<td>International System for Human Cytogenetic Nomenclature.</td>
</tr>
<tr>
<td>Kbp</td>
<td>Kilo base pair</td>
</tr>
<tr>
<td>mM</td>
<td>Mili molar</td>
</tr>
<tr>
<td>M</td>
<td>Molarity</td>
</tr>
<tr>
<td>Min</td>
<td>Minute</td>
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