EPSTEIN-BARR VIRUS AND ITS ASSOCIATION WITH BREAST CANCER

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Puan,

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Dengan hormatnya perkara di atas adalah dirujuk.

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Kami mengucapkan tahniah kepada puan kerana berjaya mendapatkan peruntukan ScienceFund ini dan semoga berjaya menyiapkan projek penyelidikan ini dengan cemerlang.

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EXTENDED ABSTRACT

EPSTEIN-BARR VIRUS AND ITS ASSOCIATION WITH BREAST CANCER

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Introduction: Epstein-barr virus (EBV) is widely distributed with 90-95% of adults are seropositive. This virus is spread by intimate contact between susceptible persons and asymptomatic shedders. EBV can be the causative agent of some B and T cell lymphomas, Hodgkin disease, nasopharyngeal carcinoma and also as one of the cofactor in the development of many types of carcinomas including breast cancer. The role of Epstein-barr virus (EBV) in breast cancer is not well documented and reports are very controversial. On the other hand, current belief holds that chromosomal deficiency and translocation, deletion, insertion and point mutation in the *p53* tumor suppressor gene (*p53* gene) which plays an important role in the regulation of cell growth, differentiation and DNA repair can play a role. Abnormal cell proliferation is thought to be induced by *p53* gene deficiency. In human carcinogenesis, *p53* mutations are reported in esophageal carcinoma with documented changes in protein expression. There is also evidence that mechanisms other than point mutation may result in p53 protein accumulation and inactivation in a subset of breast cancer. Epidemiologic studies have implicated several lifestyle risk factors: tobacco exposure, alcohol consumption, diet, obesity in esophageal carcinoma. However, the clinical significance and potential applications of these observations in breast cancer remains unclear.

Materials and Methods: A total of 159 archival formalin fixed paraffin-embedded (FFPE) breast tissues were collected from Pathology Department, Hospital Tuanku Ja'afar, Seremban and were screened for (i) EBV, (ii) *p53* gene and (iii) p53 protein. For EBV, Epstein-Barr virus encoded RNA (EBER1) transcripts was screened by *in situ* hybridization (ISH) for nuclear staining and further analyzed by Polymerase Chain Reaction (PCR) for EBER 1 gene. For p53 protein, a commercial kit (Real TM EnVision Detection System ,Dako, Denmark) by immunohistochemistry (IHC) method was used. Samples that were p53 protein positive were further examined by amplification of exons 5 to 8 of *p53* gene using specific primers. For epidemiology studies, the following clinical information were taken from patients' histopathology laboratory report: patients' age, tumor grade status and several other risk factor profiles (Form Perub. J.P. 06). A

modified version of the Bloom and Richardson grading system was used to determine the malignancy grade of tumour samples (based on three tumour features: tubule formation, nuclear pleomorphism and mitotic count). SPSS version 17 was used for statistical analyses. Frequency and chi-square tests were used to explore the association between p53 protein, age, racial differences and tumour grade, the relatedness between EBV and breast cancer, EBV positivity and histological grade.

Results: By ISH, EBV was detected in 83/159 (52.2%) breast cancer samples and 12/159 (7.55%) in normal tissues with an intense blue-black nuclear staining of EBER1 transcripts. EBV was found to have a significant relationship with breast cancer (p=0.000). The presence of EBV genome in these samples were confirmed by PCR. Altogether with both ISH and PCR for EBER 1 gene, 101/159 (63.52%) samples were positive for EBER1 gene. Two PCR positive samples were sent for sequencing, and the sequences were BLAST, aligned and were analyzed phylogenetically. Both samples were found to be 100% similar to the EBV EBER1 gene sequences already deposited in the GenBank (accession numbers AB065135, FN545286, EF187853 and DQ883818) and later were used as in-house positive controls for EBER1 gene.

For p53 protein, by IHC method, a positive reaction was determined when intensely stained nuclei were seen in more than 10% of the total cancer cells with 1+ representing 10-50% staining and 2+ representing >50% staining of the tumor cells present in the sections. Based on these criteria, IHC results were classified into positive and negative. The scoring of p53 protein expressions were performed by two pathologists independently (from Hospital Tuanku Jaafar ,Seremban) and blinded to any clinicopathological information. Nuclear p53 protein correlation with epidemiological risk factors and histological grade found that 39/159 (24.5%) were overexpressed and significantly present in breast tissue samples (p=0.002) and histological grade (p = 0.000). In the Malay and Indian females, there is a significant correlation with histological grade and p53 protein overexpression (p= 0.002) with high frequency of cases between 36-45 years and 46-55 years age group. Among the Chinese women, p53 protein overexpression is in the 46-55 years age group (8.1%) and above 56 years old (18.9%). With regard to p53 protein overexpression positivity and tumor grade, this is highest in tumor grade 3 (42.0%) in all the races.

For *p53* gene analysis, detail analysis of two samples found that exons 5 and 8 exhibited point mutation at nucleotide number 64 (G to C) and at nucleotide number 76 (A to G) in another sample. These mutations resulted in the over-production of a mutated protein that accumulates in the cells.

Conclusions: This study confirmed that EBV can be detected in both breast cancer tissues and normal breast tissues. There is a point mutation of *p53* gene that results in accumulation of p53 proteins. p53 protein overexpression is detectable in breast cancer