ABSTRACT

The Epstein-Barr virus (EBV) is closely associated with nasopharyngeal carcinoma (NPC). The role(s) of EBV in NPC remains unclear but antibodies to two EBV proteins namely the viral capsid antigen (VCA) and early antigen (EA), are clinically useful diagnostic markers of NPC.

The NPC scenario in Malaysia has been based on studies done on West Malaysian patients but not much has been described for NPC in Sarawak, East Malaysia. In this study, 164 NPC sera from newly histologically confirmed NPC patients were collected from the Radiotherapy Unit of Kuching General Hospital, Sarawak prior to radiotherapy. One hundred and forty seven non-NPC controls that were sex, age and ethnic groups matched were also collected.

Among the NPC samples, the male to female ratio was 3.4 : 1 with mean age of 47.9 years. The ethnic group distribution among the 164 NPC cases was as follows: 69 cases of Ibans (42.1%), followed by Chinese (22.5%) and the Bidayuhs (14.6%). The Ibans are 29.6% of the population while the Chinese make up 27.4%. The high incidence of Iban NPC patients is of interest since the origin of the Ibans is distinct from the Chinese who are known to have the highest incidence of NPC.

The immunofluorescence assay (IFA) for anti-EBV antibodies was used to titrate the serum IgA and IgG against VCA and EA. The present study found highly significant differences in the titres of all 4 markers (IgA/VCA, IgG/VCA, IgA/EA and IgG/EA) between NPC patients and the non-NPC controls. The geometric mean titres (GMT) for NPC sera were 34.8 for IgA/VCA, 412.4 (IgG/VCA), 14.9 (IgA/EA) and 76.0 (IgG/EA), compared to 2.3, 9.6, 2.0 and 3.1 in the non-NPC control sera. These anti-EBV titres were found independent of sex, ethnic groups and age.

For each serological marker, the sensitivity and specificity of the test determined the cut-off titre for positivity. A positive titre was ≥10, ≥160, ≥5 and ≥40 for IgA/VCA, IgG/VCA, IgA/EA and IgG/EA respectively. The GMT of the serological markers in NPC patients with elevated antibody levels was 55.6 for IgA/VCA, 576.5 for IgG/VCA, 29.0 for IgA/EA and 166.3 for IgG/EA. With a cut-off titre at ≥160, IgG/VCA was the most sensitive marker (89.0%) with a 98.0% specificity. For IgA/EA at a cut-off titre of ≥5, it was the most specific marker (100.0%) but the least sensitive (75.0%). The sensitivity and specificity of IgA/VCA was 83.6% and 97.3% at a cut-off titre at ≥10. IgG/EA had a sensitivity of 76.8% and specificity of 99.3% at a cut-off titre of ≥40.

A significant correlation was found between IgG/VCA (the most sensitive at ≥160) and the titres of the other 3 anti-EBV markers, indicating that if a patient has IgG/VCA antibodies, the other anti-EBV antibodies are likely elevated. One hundred and twelve (68.3%) of the 164 NPC patients were positive for all 4 markers, while 14 patients (8.5%) had no antibodies to any of the 4 EBV antigens. Eighteen (11.0%) NPC patients were positive for 3 markers, which included IgA/VCA, 10 (6.0%) NPC patients were positive for 2 markers, which also included 5 positive IgA/VCA samples and 10 (6.0%) NPC patients were positive for only 1 marker of which 2 were IgA/VCA positive. Based on the comparative sensitivity and specificity of the 4 anti-EBV antigens test, a combined use of IgG/VCA (≥160) and IgA/VCA (≥10) would improve the sensitivity of EBV serology in the diagnosis of NPC to 90.9%.

The role of EBV BHRF1 protein in NPC has been postulated to be through the prevention of apoptosis of EBV-infected cell during the early stages of NPC development, and antibodies to BHRF1 are detected in NPC patients. This protein might be a good marker for NPC diagnosis (Liu *et al.*, 1998). For the determination of the BHRF1 protein linear epitope, 10-mer peptides were synthesised using the Multipin Peptide Synthesis kit. A total of 46 peptide fragments covering the entire 191 amino acids of BHRF1 were synthesised. The synthetic peptides of BHRF1 protein served as target antigens in ELISA and were tested for IgA specific antibodies in 5 NPC sera and 1 control serum. Five of the 46 synthetic peptides showed strong distinguishable IgA binding with NPC sera (peptides 6, 17, 26, 27 and 29). Peptide 17 (FTETWNRFIT) has the highest IgA specific binding and is a potential EBV antigen epitope candidate for NPC monitoring.

ABSTRAK

Virus Epstein-Barr (EBV) telah dikaitkan dengan karsinoma nasofarinks (NPC). Hubungan antara EBV dengan NPC masih tidak jelas tetapi antibodi terhadap dua protein EBV iaitu antigen kapsid virus (VCA) dan antigen awal (EA) memainkan peranan penting sebagai penanda dalam diagnosis NPC.

Senario NPC di Malaysia adalah berdasarkan kajian yang dijalankan di Semenanjung Malaysia tetapi tidak banyak data mengenai NPC di Sarawak, Malaysia Timur dilaporkan. Dalam kajian ini, 164 serum NPC diperolehi daripada pesakit sebelum radiasi dijalankan di Unit Radioterapi, Hospital Besar Kuching, Sarawak. Pesakit-pesakit tersebut telah disahkan menghidap NPC secara histologi. Sebanyak 147 serum kawalan bukan NPC yang jantina, umur dan kaum berpadanan juga diperolehi.

Dikalangan sampel NPC, nisbah jumlah lelaki kepada perempuan adalah 3.4:1 dengan purata umur 47.9 tahun. Taburan kaum bagi 164 sampel tersebut adalah seperti berikut: kaum Iban dengan 69 kes (42.1%), diikuti kaum Cina (22.5%) dan Bidayuh (14.6%). Kaum Iban merangkumi 29.6% daripada populasi manakala kaum Cina merangkumi 27.4%. Bilangan kes pesakit NPC yang tinggi dikalangan kaum Iban adalah fenomena yang menarik memandangkan asal-usul kaum Iban berbeza daripada kaum Cina yang mana terkenal dengan bilangan kes NPC yang tinggi.

Teknik imunofluoresens (IFA) untuk antibodi anti-EBV digunakan untuk mengesan IgA dan IgG terhadap VCA dan EA. Kajian ini menunjukkan perbezaan yang bererti dalam titer bagi kesemua 4 penanda (IgA/VCA, IgG/VGA, IgA/EA dan IgG/EA) diantara kumpulan NPC dan kumpulan kawalan. Titer purata geometrik (GMT) bagi kumpulan NPC adalah 34.8 untuk IgA/VCA, 412.4 (IgG/VGA), 14.9 (IgA/EA) dan 76.0 (IgG/EA) berbanding dengan 2.3, 9.6, 2.0 dan 3.1 masing-masing untuk kumpulan kawalan. Titer-titer anti-EBV yang diperolehi tidak bersandar kepada jantina, kaum dan umur pesakit NPC.

Titer 'cut-off' untuk kepositifan bagi setiap penanda ditentukan oleh kesensitifan dan kespesifikan ujian. Titer positif adalah ≥10, ≥160, ≥5 dan ≥40 bagi IgA/VCA, IgG/VGA, IgA/EA dan IgG/EA. GMT penanda serologi kumpulan NPC yang menunjukkan peningkatan takat antibodi adalah 55.6 untuk IgA/VCA, 576.5 untuk IgG/VCA, 29.0 untuk IgA/EA dan 166.3 untuk IgG/EA. Dengan titer 'cut-off' ≥160, IgG/VCA merupakan penanda yang paling sensitif (89.0%) dengan kespesifikan 98.0%. IgA/EA pada titer 'cut-off' ≥5, merupakan penanda yang paling spesifik (100.0%) tetapi kurang sensitif (75.0%). Kesensitifan dan kespesifikan IgA/VCA adalah 83.6% dan 97.3% pada titer 'cut-off' ≥10. IgG/EA mempunyai kesensitifan 76.8% dan kespesifikan 99.3% pada titer 'cut-off' ≥40.

Kolerasi diperolehi diantara IgG/VCA (penanda paling sensitif pada ≥160) dan titer terhadap 3 penanda anti-EBV yang lain. Ini bermakna jika seseorang pesakit mempunyai titer IgG/VCA, antibodi anti-EBV bagi penanda yang lain juga besar kemungkinan adalah tinggi. Seratus dua belas (68.3%) daripada 164 pesakit NPC adalah positif terhadap keempat-empat penanda manakala 14 pesakit (8.5%) tidak mempunyai antibodi terhadap mana-mana 4 penanda tersebut. Lapan belas orang (11.0%) pesakit NPC adalah positif terhadap 3 penanda, termasuk IgA/VCA, 10 orang (6.0%) pesakit adalah positif terhadap 2 penanda, termasuk 5 sampel positif terhadap IgA/VCA dan 10 orang (6.0%) pesakit adalah positif terhadap 1 penanda sahaja,

termasuk 2 sampel positif terhadap IgA/VCA. Berdasarkan perbandingan kesensitifan dan kespesifikan terhadap ujian bagi 4 antigen anti-EBV, penggunaan kedua-dua penanda IgG/VCA (pada ≥160) dan IgA/VCA (pada ≥10) dapat mempertingkatkan kesensitifan serologi EBV terhadap diagnosis NPC kepada 90.9%.

Protein EBV BHRF1 dalam NPC dicadangkan berperanan mencegah apotosis dalam sel yang dijangkiti EBV semasa fasa awal perkembangan NPC. Antibodi terhadap BHRF1 dapat dikesan dalam pesakit NPC. Protein ini mungkin berguna sebagai penanda untuk diagnosis NPC (Liu *et al.*, 1998). Untuk penentuan epitop linear protein BHRF1, peptida 10-mer disintesis dengan menggunakan kit 'Multipin Peptide Synthesis'. Sebanyak 46 set peptida yang merangkumi kesemua 191 asid amino bagi BHRF1 telah disintesis. Peptida sintetik BHRF1 digunakan sebagai antigen dalam ELISA untuk mengesan kehadiran antibodi spesifik IgA dalam 5 serum NPC dan 1 serum kontrol. Lima daripada 46 peptida sintetik (Peptida 6, 17, 26, 27 dan 29) menunjukkan reaktiviti yang tinggi terhadap antibodi IgA. Peptida 17 (FTETWNRFIT) telah mempamerkan ikatan antibodi spesifik IgA tertinggi dan oleh itu berpotensi untuk digunakan sebagai epitop antigen EBV untuk tujuan pemantauan NPC.