ABSTRACT

This study used 3 recombinant EBV proteins namely p54 and p138 of the EA-D (early antigen - diffuse) and p23 of VCA (viral capsid antigen) as target antigens in ELISA system for NPC diagnosis. The IgA and IgG specific antibodies were analysed in ELISA and compared with EBV serology using indirect immunofluorescence technique (IIF). 85% of IgA-VCA IIF-positive NPC sera had IgA antibodies to p54, 76% had IgA-p138 and 79% had IgA-p23 antibodies. Interestingly, in another group of 46 IgA-VCA IIF- negative NPC sera, IgA-p54 was detected in 33%, IgA-p138 in 35% and IgA-p23 in 28% of the NPC samples. In contrast, only 4% of 163 NHS sera had IgA antibodies to p54, 19% had IgA-p138 and 15% had IgA-p23 antibodies. In control ENT disease serum samples, IgA-p54 was present in 14%, while both IgA-p138 and IgA-p23 showed 12% reactivities. With non-NPC cancer serum samples, IgA-p54 was present in 4%, IgA-p23 in 9% while none of the samples had antibody reactivities to IgA-p138.

Similar tests for serum IgG showed that in 146 NPC, all IgA-VCA IIF-positive sera contained IgG to p54. 73% of these sera were IgG-p138 positive while 88% were IgA-p23 positive. Again, of interest was the finding that in 61 IgA-VCA IIF-negative NPC sera, 74% had IgG-p54, 38% had IgG-p138 and 62% had IgG-p23 antibodies. In contrast, IgG-p54 was present only in 6%, IgG-p138 in 5% and IgG-p23 in 10% of 108 NHS samples. In control non-NPC cancer serum samples, IgG-p54 was detected in 23% and IgG-p23 in 41% of the samples. None of the EBV proteins reacted for serum IgG in control ENT disease samples. Preliminary data

indicate that IgA-p138 appears to have a prognostic potential. p54 was the most sensitive antigen for both IgA (85.3%) and IgG (92.3%) antibodies detection. In addition, the detection of IgG p54 in a substantial portion of IgA-VCA IIF-negative NPC sera is an important finding towards better monitoring of NPC.

In an effort to further simplify the EBV p54-ELISA, we analysed the complete p54 viral protein for linear epitopes that are recognized by IgG in NPC sera. A 10-mer peptide, RFYRSGIIAV, was identified as the immunodominant portion of the p54 EA protein. This peptide was able to identify 97% of NPC sera that were negative for IgA-VCA in IIF.

Hydropathy profiling using Hopp and Woods method (1983) was conducted to compare the predicted hydrophilic with antigenic regions as determined through the epitope PEPSCAN analyses. This was done for p54 as well as two other proteins in which PEPSCAN have been carried out previously, namely the nuclear antigen, EBNA1 and the replication activator, ZEBRA. There was very little correlation seen between the predicted epitope clusters and regions identified through PEPSCAN.

The present study has identified a 10-mer peptide, RFYRSGIIAV of EBV early antigen p54, as a potential valuable complementary test antigen for the diagnosis of NPC.