

situ ⁽¹⁰⁾. However there has been no report on the association between HLA alleles and brain astrocytomas among Iraqi patents.

In this study, we used polymerase chain reaction with sequence-specific primers (PCR.-SSP) for HLA-DRB alleles typing to investigate the genetic susceptibility of HLA allele polymorphisms in brain astrocytomas of Iraqi patients.

Material and Methods

The brain astrocytoma group (attended Baghdad Neurosurgery Hospital) included thirty unrelated patients (24 men and 6 women), with a mean age of 52.3 ± 4.55 years, who were evaluated radiologically and surgically. And the diagnosis was confirmed by histopathological examination of the tumor mass at Baghdad Neurosurgery Hospital Laboratories.

The control group consisted of 17 unrelated healthy individuals, matched with patients for sex (14 men and 3 women) and age, with a mean of 50.8 ± 3.44 years.

DNA extraction

Genomic DNA was isolated from leukocytes obtained from anticoagulated peripheral blood of patients and controls, using the salting out method ⁽¹¹⁾.

HLA-DRB1 alleles PCR-SSP typing

For HLA-DRB (HLA-DRB1*01-DRB1*16, DRB3, DRB4, AND DRB5) typing by PCR-SSP, 24 separated PCR reactions were performed for each sample (Biotest-ABDR SSP, Germany). It allowed the detection of 353 HLA-DR alleles. Each PCR reaction mixture contained group-specific- DRB primers and the internal positive control primer pair. HLA-DRB alleles PCR-SSP typed system consisted of 50-100 ng genomic DNA, 5U/ul Taq DNA polymerase (Promega ® USA), and PCR tubes contained dried primer / nucleotide mixtures. PCR amplifications were carried out in thermal cycler (Hybaid LTD® England) according to manufacture

instruction. Initial denaturation was made at 94°C for 2 minutes; with 30 cycles each consisting of denaturation at 94° C for 10 seconds, annealing at 65°C for 1 minute. and extension at 72°C for 1 minute. The HLA-DRB alleles typed visualization of amplification was observed using 2% agarose gel electrophoresis. (Promega ® USA), The gels were run for 30 minutes at 8.5 V/ cm in 1X TBE buffer and the bands were visualized using UV illumination and photographed by digital colored camera (Orite, Japan).

Statistical analysis

Odds ratio (OR) was taken to describe the relative risk of a particular HLA-DRB allele. Frequency distribution of the odds ratio for each DRB alleles studied was constructed. Confidence Interval (CI) was also carried out for the normal distribution of the OR. The chi-square (X²) test of significant was used to test the departure of the observed frequency from expected which was built on assumption of normal segregation ⁽¹²⁾.

Results

HLA-DRB1*10011 and DRB1*10012 alleles were significantly present at decreased frequencies in patients with brain astrocytomas, 0.53 vs 0.93, OR = 8.76 (Table 1). CI = 0.643-0.995. The rested HLA-DRB allele's frequencies showed no significant difference in comparison between patients and the controls. Group DR10 might be associated to disease progression due to its low frequency, when compared with control subjects. The same situation could be seen for HLA-DR15 (frequency in patients 0.77 vs controls 0.97, OR= 5.0), thus, HLA-DRB1 alleles that are associated with increased risk of astrocytomas have decreased frequency in patients compared with controls.

The X² test of significance was also conducted to examine the association between the HLA-DRB1 risk group alleles. The results indicated that there was a significant association with DRB*10011 and DRB1*10012 alleles (p<0.05). The x² value (5.88) was higher than that of other alleles (2.34). This was reflected by the significant difference between the observed