HLA- DRB Genotyping of Brain Astrocytomas among Iraqi Patients

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Abstract

Background: The major histocompatiblity complex (MHC) refers to as human leukocyte antigen (HLA). The loss of HLA antigens by neoplastic cells is considerably important for tumor growth and metastasis and expression of certain certain HLA allels may predispose to have certain types of tumors.

Objective: To investigate the genetic susceptibility of HLA-DRB1, DRB3, DRB4 and. DRB5 alleles to brain astrocytomas in Iraqi patients.

Methods: HLA-DRB1, DRB3, DRB4 and, DRB5 allele polymorphisms were typed by polymerase chain- reaction with sequence-specific primers (PCR-SSP) in 30 unrelated patients astrocytomas and 17 unrelated normal control subjects. The association was measured by appropriate statistical tests.

Results: Allele frequency (AF) of HLA-DRB1*10011 and DRB1*10012 was significantly decreased in brain astrocytomas patients than that in normal controls (0.53 vs 0.93) the odds ratio 8.76). There was no association between patients and controls in the rested HLA-DRB1 alleles.

Conclusion: HLA-DRB1*10011 and DRB1*10012 alleles were less common in the patients with brain astrocytomas than in the healthy controls. Individuals carrying HLA-DRB1* 10011 and DRB1*10012 alleles might be considered as protective markers. These protective alleles; might have a role in the degree of malignancy of the tumors and its histological type.

Keywords: PCR-SSP, brain astrocytomas, HLA-DRB

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Introduction

Astrocytic tumors comprise a wide range of neoplasms that differ in their location within the central nervous system (CNS). The majority of tumors had either heterogeneous or positive expression of HLA class I heavy chain (HLA-HC), and β2 microglobulin[1]. The loss of HLA antigens by neoplastic cells is considered important for tumor growth and metastasis [2-4]. Since tumor neoantigens on the surface of aberrant cells are recognized by T-cells only in the context of the HLA "self" antigens, loss of the HLA antigens may allow the tumor to escape immunosurveillance [5]. Defects in the expression and/or function of the human leukocyte antigen (HLA) class I antigen-processing machinery (APM) components are found in many tumor types.

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These abnormalities may have a negative impact on the interactions of tumor cells with host's immune system and on the outcome of T cell–based immunotherapy [6]. The alleles of the HLA system controls a variety of immune functions and influence the susceptibility to more than 40 diseases, many of which have an autoimmune components [7,8] Association of a particular HLA allele with a disease implies that the frequency of the allele is different in the patient population as compared with that of matched control population . A study done by (Angelica etal. 2005) [9] showed that HLA class I antigens were lost in 50% of glioblastoma multiforme (GBM) lesions and in 20% of grade 2 astrocytoma lesions. Selective HLA-A2 antigen loss was observed in 80% of the GBM lesions and in 50% of grade 2 astrocytoma lesions stained. HLA class I antigen loss was correlated with tumor grade. HLA class II antigen expression was detected in 30% of the 44 lesions analyzed. HLA-Dr expressed by brain tumor cells selectively inhibit CD8 subset which participates in immunoreaction against brain tumors in

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 (Cl) was also carried out for the normal distribution of the OR. The chi-square (X^2) 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). Cl: =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

and expected values. The reverse was true for the control group. In addition, if we take this comparison into consideration when checking for the observed and expected values for the alleles that showed no significant differences, it was also evident that the observed was higher than the expected for the patients group. In this case, either the non-significant differences might be attributed to the small number of observations or that the contribution of such alleles was too little to be associated with the occurrence of the disease. This would demonstrate a clear picture about the association of these two alleles under the specificity of DR10 with astrocytoma.

Table 1: Frequency and odds ratios for HLA-DRB1, DRB3, DRB4, DRB5 alleles in patients with astrocytoma in comparison with normal control subjects

HLA-DRB1, DRB3. DRB4, DRB5		FREQUENCY (%)		ODDS VALUE		ODDS RATIO
Group	*Allele*	*Patient*	*Control*	*Patient*	*Control*	*Patient*
DR10(DRB1	10011	53	93	0.87	0.13	8.76
DR10(DRB1	10012	53	93	0.87	0.13	8.76
DR15(DRB1	15011	77	97	0.30	0.06	5.00
DR15(DRB1	15012	77	97	0.30	0.06	5.00
DR15(DRB1	15022	77	97	0.30	0.06	5.00
DR15(DRB1	15023	77	97	0.30	0.06	5.00
DR15(DRB	1503	77	97	0.30	0.06	5.00
DR15(DRB1	1504	77	97	0.30	0.06	5.00
DR15(DRB1	1505	77	97	0.30	0.06	5.00
DR15(DRB1	1506	77	97	0.30	0.06	5.00
DR15(DRB1	1507	77	97	0.30	0.06	5.00
DR52(DRB3)	0207	90	97	0.11	0.06	1.83
DR52(DRB3)	0208	90	97	0.11	0.06	1.83
DR53(DRB4)	01011	90	97	0.11	0.06	0.00
DR53(DRB4)	010111	90	100	0.11	0.00	0.11
DR51(DRB5)	01011	90	100	0.11	0.00	0.11
DR51(DRB5)	01012	90	93	0.11	0.13	0.84

Discussion

Lack of human leukocyte antigens and costimulatory molecules have been suggested as mechanisms by which human malignant gliomas avoid immune recognition and elimination [(12)]. The major finding in this study was that the frequency decreased incidence of HL.A-DRB1*10011 and DRB1*10012 in the Iraqi patients with brain astrocytomas compared with that in healthy controls. HL.A SSP DNA typing on 30 patients revealed a significant decreased of DR10 alleles (DRB1*10011 and DRB1*10012) 0.53 vs 0.93, OR = 8.76, Cl= 0.643-0.995 ($p< 0.05$), X2 = 5.88. In addition to the absence of DRB1*15 alleles in patients compared to controls. None of the tested HLA-DRB alleles occurred at markedly altered frequency between the patients and normal individuals. It is may be the alleles that is associated with genetic susceptibility of this tumors but why? It was entirely unclear up to now; the pathogenesis of genetic association may be linkage disequilibrium (nonrandom association) and/ or changing in the recognized procession of the specific antigen. It is controversial whether or not HLA antigens expression in astrocytoma cells correlates with the development of disease and progression [(14-16)]. As reported in some studies, the reduced expression of HLA antigens in malignant tissues has been proposed as a mechanism thereby tumor-associated proteins cannot be

presented in the T cells[13], therefore the tumor cell proliferates are unperturbed by the immune system and tumor cells protect themselves from host' immunosurveillance. There is possibility that HLA allele genetic association and expression on tumor cells may provide a clue to the understanding of the therapeutic mechanisms of biological response modifiers or immunotheraphy which may cut through the induction of HLA antigens on malignant cells [17-22]. The cells of a given individual may express HLA alleles, which altered binding to tumor peptides, thereby leading to a modified immune response to the tumor. Identification of the mechanism associating HLA-DRB1*10011 and DRB1*10012 with brain astrocytoma could ultimately help target individuals most likely to benefit from cancer screening and prevention strategies and could facilitate novel therapeutic programs for cancer immunoprevention. Further studies with large number of patients with use of nucleotide sequence of targeted alleles may show more clear correlation [23]. The presence of HLA antigen defects in malignant brain tumors may provide an explanation for the relatively poor clinical response rates observed in the majority of the T cell–based immunotherapy clinical trials conducted to date in patients with malignant brain tumors [24].

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