Genotyping of HLA-class-II by PCR-SSP of Iraqi Breast Cancer Patients

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Abstract

Background: Breast cancer incidence differs among women of different racial/ethnic groups. Several HLA alleles are associated with susceptibility or protection in Breast cancer, the particular allele varies depending on the racial groups.

Objectives: This study was established to shed light on the possible association of HLA class II alleles with BC in Iraqi female patients.

Subjects and Methods: The study included 60 subjects: 30 breast cancer patients, 12 patients with benign breast lesions as first control and 18 apparently healthy subjects as second control. Polymerase chain reaction-specific sequence primers (PCR- SSP) assay was conducted to assess HLA- typing.

Results **:** A survey of the distribution of HLA-DR and HLA-DQ alleles frequencies yielded no evident of positive association between class II alleles and BC as compared with both control groups, but there was appreciable significant decrease in the frequency of DR*010101,0102,0201-0204,04-13 and DQB1*0401,02 alleles in BC patients as compared with healthy control (P=0.031).

Conclusions: These findings demonstrated that HLA– DR*010101, 0102, 0201-0204, 04-13 and DQB1*0401, 02 alleles may confer protective effects against BC.

Keywords: Breast cancer, HLA allele, PCR.

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Introduction

Human based studies have suggested that the host genetics predisposition is important in disease pathogenesis and protection, and considering the importance of immune surveillance during tumorigenesis, some individuals who inherit specific alleles or haplotypes of the highly polymorphic human leukocyte antigen (HLA) system may be exposed or may resist to specific types of cancers [1-5].

Breast cancer incidence differs among women of different racial and/or ethnic groups, and accordingly, several HLA associations have linked HLA system with susceptibility or protection in the disease. However, the studies have been consistent with respect to the influence of these alleles in immune clearance of tumor cells in a way that could affect BC development. Therefore, HLA genotypes have been suggested to be a biologically based risk factor for BC [6, 7]. In the present study, the polymorphism of HLA-DR and -DQ alleles was analyzed in a sample of Iraqi females with BC using the PCR – SSP method.

Subjects and Methods

Subjects:

Thirty breast cancer female patients (invasive ductal carcinoma, invasive lobular carcinoma and in situ ductal carcinoma), with an age range of 28 - 73 years, were eligible for this study. The patients were admitted for surgery at Al-Kadhimiya Teaching Hospital and Nursing Home Hospital (Medical City) in Baghdad, for the period March 2006 - March 2007. Pathological data (histologic tumor type grade, tumor stage and lymph

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node status) were obtained from the medical records of patients and validated by an experienced histopathologist.

Two control groups were included: 12females with benign breast lesions (BBL) (6 cases with fibrocystic disease and 6 with fibroadenoma) and 18 apparently healthy females. The latter subjects had no history or clinical evidence of any breast lesions and matched by age and ethnic backgrounds to BC patients.

Methods:

Blood collection:

Two milliliters of venous blood with EDTA as anticoagulant were collected from each subject.

DNA extraction:

Extraction of DNA from peripheral blood was done according to the modified method of Miller [8], using the EXTRA-GENE-I kit (BAG-Germany), which is the most suitable method for isolation since pure DNA can be obtained from whole blood in a short time without the use of toxic chemicals or solvents.

PCR amplification:

HLA-genotyping was performed by PCR-SSP according to a method presented by Olerup and Zetterquist [9, 10], using low resolution typing kits (HISTO TYPE / DNA-SSP Kits-BAG-Germany). Appropriate amounts of DNA and Taq polymerase (Recombinant Taq polymerase from QIAGEN-company) were added to pre-aliquoted primers and PCR conditions were set according to the manufacturer instructions.

Detection of PCR products:

PCR products were loaded in 2 % agarose gel containing 0.5 mg/ml ethidium bromide, electrophoresed for 25 min at 12 V/cm, and examined under ultraviolet light. The individual alleles were assigned for the specific pattern of appropriately sized bands.

Statistical analysis

The results were presented in terms of percentage frequencies, and alleles showing variations between patients and controls were further presented in terms of odds ratio (OR), etiological fraction (EF) and preventive fraction (PF). The significance of these differences were assessed by fisher's exact probability (P) [11,12].

Results

In this study the mean age of BC patients was 48.1 years with a range of 28 - 73 years, while the mean age of BBL was 35.33 years with a range of 21 - 50 years.

In the PCR-SSP method, 24 HLA-DR and 8 HLA-DQ specific primer mixes were employed as well as, a negative control and ladder mixes. A successful amplification resulted in the generation of a defined length band as a positive internal control in all lanes except the negative control lane, and when there was no amplification, there was no band. In addition, a positive specific amplification resulted in the generation of a specific band in addition to an internal control band (Figure 1).

The observed percentage frequencies of HLA-DR and DQ alleles in the investigated groups are given in tables 1and 2 respectively, while allele showing a significant variation is presented in table 3. A survey of the distribution of HLA-DR and HLA-DQ alleles frequency yielded no evident of positive association between class II alleles and BC, but there was significant decrease in the frequency of DR*010101, 0102, 0201-0204, 04-13 and DQB1*0401, 02 alleles in BC as compared with healthy control (P=0.031) table 3.

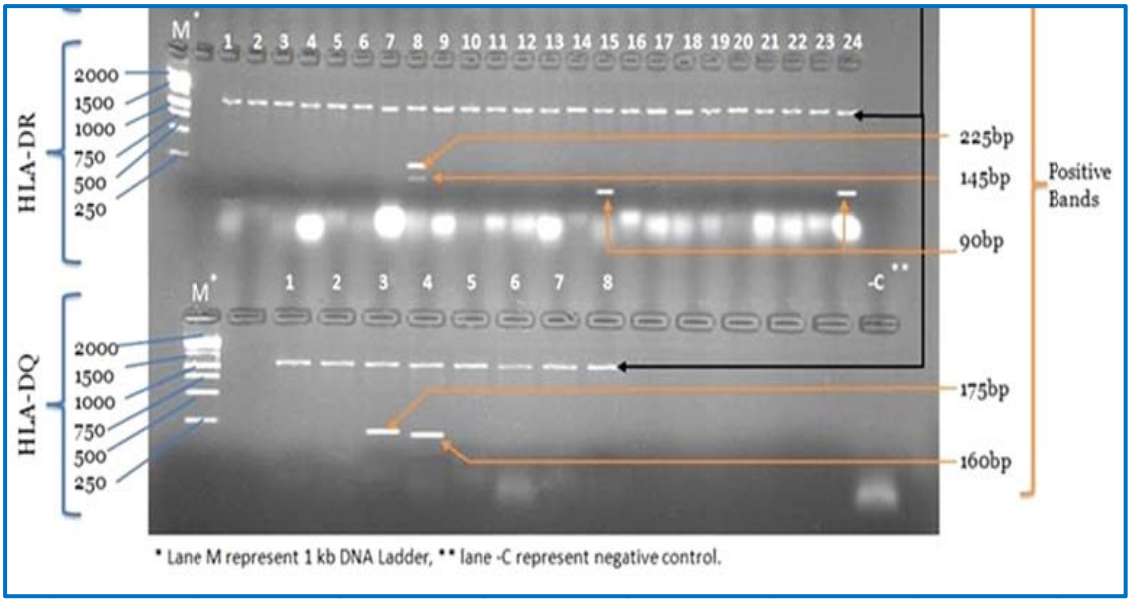

Figure 1: Electrophoresis of HLA-DR and DQ alleles' amplification by PCR-SSP. The first line represent the HLA-DR genotyping was obtained by using primers that detect the following alleles as they were present in the numbered wells, respectively: 1= DR1,-, DR103 ; 2= DR15(2),-, DR2, DR16(2),2,- ; 3= DR17(3),3,- , DR18(3), DR 17(3) ; 4= DR4,- , DR4 ; 5= DR7,- ; 6= DR9,- ; 7= DR8,-, - , DR8 ; 8= DR10 ; 9= DR 11(5),- , DR11(5) ; 10= DR12(5),- ; 11= DR13(6).- ; 12= DR13(6) ; 13= DR,- 14(6) ; 14= DR14(6),- ; 15= DR14(6),- ; 16= DR1403,14(6), -, DR13(6) ; 17= DR 1404,14(6),- ; 18= DR 14(6),- ; 19= DR-6 ; 20= DR 14(6) ; 21= DR8 ; 22= DR6 ; 23= DR14(6),- ; 24= DR14(6),-. The second line represent the HLA-DQ genotyping was obtained by using primers that detect the following alleles as they were present in the numbered wells, respectively:1= DQB5(1) , DQB6(1), DQB6(1) / 1 / - ; 2= DQB2 ; 3= DQB7(3) / - , DQB8(3) / -; 5= DQB9(3) / 3 / - ; 6= DQB7(3) / - ; 7= DQB8(3) ; 8= DQB4.

Table 1: Observed percentage frequencies of HLA-DR alleles in healthy controls, breast cancer patients and benign breast lesion.

Specificities	Healthy controls (n=18)		Cases (breast Ca) (n=30)		Benign breast lesion (n=12)	
HLA-DR-Allele					N	%
DR*010101,0102,0201-0204,04-13	4	22.2	0	0	2	16.7
DR*030101,0102,04-07,09,11-16,18-26,28	4	22.2	3	10	3	25
DR*040101,0102,0301-11,13,16,17,19-20,23,24,26-30,32-35,38-53	4	22.2	9	30	3	25
DR*070101-9,10N	4	22.2	9	30	3	25
DR*080101-07,10-14,16-19,22-24,26-30	2	11.1	3	10	1	8.3

DR*100101,0102	**2**	**11.1**	**3**	**10**	**1**	**8.3**
DR*110201,0202w,1401,1402,21	**2**	**11.1**	**3**	**10**	**1**	**8.3**
DR*131402w	**1**	**5.6**	**3**	**10**	**1**	**8.3**
DR*1317,67	**0**	**0**	**3**	**10**	**0**	**0**
DR*1320,24,29,63	**1**	**5.6**	**3**	**10**	**1**	**8.3**
DR*1346	**1**	**5.6**	**3**	**10**	**1**	**8.3**
DR*140301,0302,12,12,27,40/*1318	**1**	**5.6**	**3**	**10**	**1**	**8.3**
DR*150101-12,14-16	**5**	**27.8**	**9**	**30**	**3**	**25**
DRB5*0205	**1**	**5.6**	**3**	**10**	**1**	**8.3**
Blank	**4**	**22.2**	**3**	**10**	**2**	**16.7**

Table 2: Observed percentage frequencies of HLA-DQ alleles in healthy controls, breast cancer patients and benign breast lesion.

Specificities	**Healthy controls (n=18)**		**Cases (breast Ca) (n=30)**		**Benign breast lesion (n=12)**	
HLA-DQ-Allele	**N**	**%**	**N**	**%**	**N**	**%**
DQB1*030302,030303,06,12,15	**0**	**0**	**3**	**10**	**0**	**0**
DQB1*020101-0102,0202-04	**4**	**22.2**	**3**	**10**	**3**	**25**
DQB1*030101,030102,09,16	**2**	**11.1**	**9**	**30**	**1**	**8.3**
DQB1*030201,030202,030501-0503,07,08,11	**2**	**11.1**	**6**	**20**	**1**	**8.3**
DQB1*0310	**1**	**5.6**	**3**	**10**	**1**	**8.3**
DQB1*0401,02	**4**	**22.2**	**0**	**0**	**2**	**16.7**
DQB1*050101-050302,0504	**3**	**16.7**	**6**	**20**	**2**	**16.7**
DQB1*060101-0103	**1**	**5.6**	**3**	**10**	**1**	**8.3**
DQB1*0602-18,20-22,24-26N	**3**	**16.7**	**6**	**20**	**2**	**16.7**
Blank	**16**	**88.8**	**21**	**70**	**11**	**91.7**

Table 3: HLA- DR*010101, 0102, 0201-0204, 04-13 and DQB1*0401, 02 alleles showing significant variations between breast cancer patients and healthy controls.

HLA-allele	**Patients**		**Healthy controls**		**Statistical evaluations**		
	N	**%**	**N**	**%**	**Odds ratio**	**PF**	**P**
DR*010101, 0102, 0201-0204, 04-13	0	0	4	22.2	0.1	0.22	0.031
DQB1*0401, 02	0	0	4	22.2	0.1	0.22	0.031

PF: Preventive fraction: P: Fisher's exact two-sided probability.

Discussion

The role of genetic factors in the etiology of BC was documented decades ago. As a result, the investigative efforts have focused on the genetic markers of susceptibility to this disease. In particular, HLA system play a pivotal role in cellular immunity and may be an important genetically determined host trait [13, 14]. This study is the first attempt on the association between HLA class II alleles and BC in Iraqi patients, in which the alleles were characterized by PCR-SSP.

Although in the current study, there was high frequency of DR*040101,0102,0301-11,13,16,17,19-20,23,24,26-30,32-35,38-53 and DR*070101-9,10N alleles with patients as compared to control groups, it was statistically not significant. Different results regarding this association was reported, Ghaderi *et al., in* (2001) revealed statistically significant association between the disease and DRN1*12 [15]. On the other hand, present result revealed that DR*010101,0102,0201-0204,04-13 and DQB1*0401,02 alleles showed significant low frequency in BC patients when compared with healthy control, while there was no significant differences as compared with second control group.

Present result was in disagreement with the finding of other studies about the type of protective allele. The HLA DRB1*1101 and DQB1*03032 alleles have been suggested to be protective against breast cancer [16]. This difference may be partly explained by the fact that the patients studied by Chaudhuri *et al.,* all had early-onset breast cancer; they were all younger than 40 years of age. Furthermore, both groups of patients were belonged to very different racial groups [17].

Also, the discrepancies emerged in this study compared with other studies could be, in part, probably due to the highly modified techniques they used for the same purposes, e.g, PCR, however, if the environmental and genetic factors excluded, which yet to be elucidated, the small number of our study groups may be a considerable contributor to these discrepancies, however, further investigations are certainly required to shed further light on this association.

In conclusion, these data suggested that HLA– DR*010101, 0102, 0201-0204, 04-13 and DQB1*0401, 02 alleles may confer protective effects against BC.

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