DOI Number: 10.37506/v14/i1/2020/ijfmt/193061

Genotype and haplotype of HLA- class II in Type I and Type II Diabetes Mellitus in Iraqi Patients

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Abstract

Background: Diabetes is one of the common complicated disease associated with multiple influenced factors, immunological serological and molecular factors play a great role in susceptibility and diagnosis of this disease. HLA genotyping were reported to have an influencing in both types of diabetes.

Aim: The current study was conducted to investigate the association of human leukocytes genotypes in Diabetes patients.

Method: Case-control study enrolled 225 blood samples collected from patient attended to the Marjan Teaching Hospital- Hilla and 25 apparently healthy from October 2018 to May 2019. Class II HLA genotyping was performing for 60 patients with diabetes mellitus and 25 healthy unrelated controls by means of the PCR-SSP method. The diagnosis of T1D and T1ID was set up according to American Diabetes Association criteria.

Results: both types of diabetes were significantly associated with HLA-DR3. Associations were also observed with HLA –DQA105:01, the appearance of these two alleles differs in both TID and TIID . Conclusion. Certain HLA class II alleles, haplotypes, and genotypes have related to diabetes mellitus so it can be dependent as a genetic marker for susceptibility of this disease in Iraq.

Keywords: *Type II diabetic, Type I diabetes, HLA classII, genotype, haplotype.*

Introduction

Diabetes mellitus (DM) is a group of metabolic disorders characterized by a chronic hyperglycemic condition resulting from the defects in insulin secretion, insulin action or both.[1] . Type 1 and type 2 diabetes consider the two main types, with type 2 diabetes constituting for the majority (>85%) of the total diabetes mellitus prevalence, According to the latest estimates, there are 425 million people with diabetes in 2017 and this number is expected to rise in 2045 to 629 million.[2] . The genetic loci involved in the rejection of foreign organs knew as the major histocompatibility complex (MHC), and the MHC encodes highly polymorphic cell surface molecules. The human MHC is representing as the HLA (Human Leukocyte Antigen) system because these antigens first identified and characterized using alloantibodies against leukocytes.[3] The encoding of HLA-DQ proteins are belongs to HLA-DQ genes and expressed on α and β chain at cell surface.[4] .The DQ region of HLA include two gene clusters, DQA1 and DQB1.[5] [5]. The polymorphism of HLA had serologically significant impact and the Polymorphism at the HLA-DQB1 locus used to be determined serologically and recognized the specificities DQ1, DQ2, DQ3 and DQ4.[6] . The use of DNA typing techniques has increased the number of alleles. The allelic sequence diversity is also predominantly present in exon 2 and, except for DQB1*0201 and DQB1*0202, all alleles can be discriminated by PCR-SSOP in this exon. A large number of studies have demonstrated that specific alleles at the DRB1, DQA1, and DQB1 loci are strongly associated with diabetes.[7,8] . However, allelic variation at these loci cannot account fully for the pattern of HLA haplotype sharing among affected sib pairs.[9] .

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Material and Method

Patients and controls

Two hundred twenty-five blood samples were collected from clinically diagnosed diabetes patients who regularly admitted by medical committee specialized diabetic center of marjan hospital (Babylon) from October 2018 to May 2019, the age of patients between (2-80) years including both sex male (112) and female (113), in addition to (25) samples were taken from apparently healthy human were taken from Babylon province as control. The study was approved by the Research Ethics Review Boards of the University of Babylon. This work was done by self funding and it is a part of M.Sc project for the first author with the agreement of university .

All participants provided written informed consent. Case Ascertainment. Patients with diabetes were diagnosed by a physician on the basis of the following criteria: a fasting glycemia ≥ 1.26 g/dL, an unexplained weight loss, signs of hyperglycemia (polyuria, polydipsia, polyphagia, and asthenia). These criteria were defined according to the recommendations of the American Diabetes Association.[10].

Blood sampling

For each individual enrolled in the study, 3 ml of venous blood was collected in EDTA-treated tubes for DNA extraction, which performed according to the protocols recommended by the manufacturer (Favorgen / Taiwan).

HLA genotype analysis

HLA –DQA105:01 and HLA-DR3 genotyping was performed with PCR-sequence-specific primers (PCR-SSP) .[11] . In a 20 μl mixture of 2.5 µl DNA, 1.5 µl from each forward and reverse of the primer, 5 µl master mix and 9.5 µl nuclease free water. The DNA amplification for HLA-DQA1 is includes an initial denaturation of 2 min in 94°C, 32 cycles of amplification (every cycle consists of a denaturation of 30 s in 94°C, a hybridization of primers during 30 s in 63°C, and an extension of 30 s in 72°C), and a final extension of 10 min in72°C, [11]. While the process of DNA amplification for HLA-DR3 is same as DQA1, (exception the hybridization temperature was 52°C), then the PCR products were separated in 1.5 % agarose electrophoresis system using ethidium bromide then visualized with the gel documentation, with 100 bp-ladder (Bioneer, Korea) and photographed. The sequences of primers used for the amplification of the genes are presented in table 1.

Table 1: Sequences of the couples of primers used for the amplification of the genes.

Primers	Sequences 5-3	Size(bp)	Reference
HLA -DR3	5′CACGTTTCTTGGAGTAC3′ 5′CGTAGTTGTGTCTGCAGTAGT3′	237 bp	(Fagbemi *et al*., 2017)
HLA-DQA1∗05:01	5′ACGGTCCCTCTGGCCAGTA3′ 5′AGTTGGAGCGTTTAATCAGAC3′	186 bp	

Statistical Analysis

All data were statistically analyzed according to software program version20 SPSS statistical software (version 17; SPSS, Inc., Chicago, IL, USA). The association between TID, TIID and each identified HLA-DR/DQ alleles, haplotypes, and genotypes was assessed using the odds ratio with its 95% confidence interval (OR, CI 95percentage)

Results

The present study reveals a noticeable variety with HLA haplotype among type I and type II diabetes. HLA alleles discriminated by PCR assay. The distribution of HLA-DQA1 show high appearance in both types, out of 30 samples 26 show positive result 86.66% in type I and out of 33 samples 26 show positive result 78.78% in type II finally, statistical analysis showed no significant differences comparing diabetes patient and control subjects table 2.

Table 2: Human leukocytes antigen (HLA) DQA1 alleles' distribution in type I and II diabetes.

Model	Sample No.	Patients +ve results		patients-ve results		Healthy +ve results		Healthy -ve results		OR (CI)	p-value
		No.	Percent	No.	Percent	No.	Percent	No.	Percent		
Type I	30	26	86.66%	4	13.33%	21	84%	4	16%	1.238 (0.28-5.55)	0.390
Type II	33	26	78.78%	7	21.21%					0.707 (0.18-2.75)	0.308
Total	63	52	82.53%	11	17.46%			25			

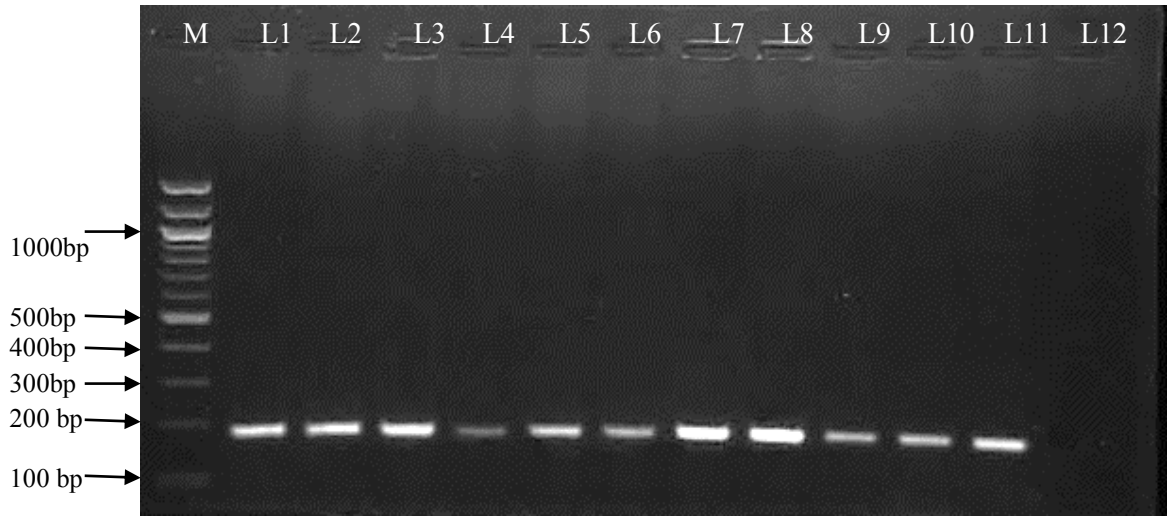

Figure 1: Agarose gel electrophoresis image for HLA –DQA1 haplotype at 100 v for 40 min. and 70 v for 30 min. M, marker 100bp, L1 ,2,3,4,5,6,7,8,9,10,11 give positive results and L12 give negative result.

The distribution of HLA-DR3 in type I and II showed 14 positive result with percentage 46.66% for TID and 11 positive result (36.66%) for TIID whereas the control revealed 8 positive result (32%), finally, statistical analysis showed significant differences comparing diabetes patient and control subjects , table no 3 reveals high appearance of this haplotype HLA DR3 among TID than TIID.

Table 3: Human leukocytes antigen (HLA) DR3 alleles' distribution in type I and II diabetes.

Model	Sample No.	Patients +ve results		patients-ve results		Healthy +ve results		Healthy -ve results		OR	P-value
		No.	%	No.	%	No.	%	No.	%		
Type I	30	14	46.66%	16	53.33%	8	32%	17	68%	1.86 (0.62-5.61)	0.135
Type II	30	11	36.66%	19	63.33%					1.23 (0.40-3.78)	0.358
Total	60	25	41.66%	35	58.33%			25			

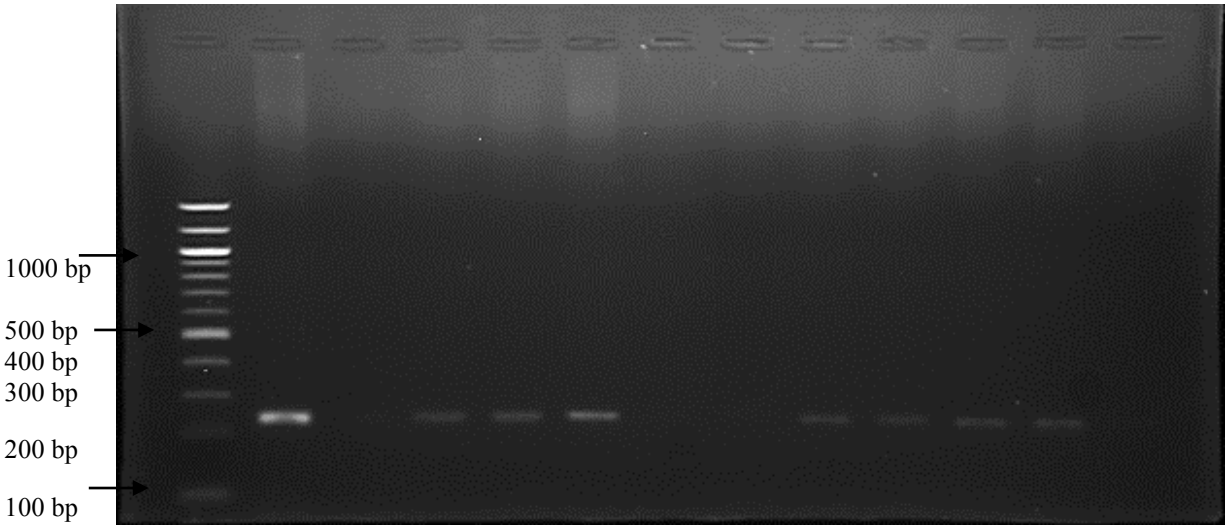

Figure 2: Agarose gel electrophoresis image for HLA –DR3 haplotype at 100 v, 50 min. M, marker 100 bp, L1, 3, 4, 5.8,9,10,11 give positive results. L2, 6,7,12 give negative results.

Discussion

Diabetes mellitus is a combined metabolic disorder that includes several complications and the incidence of diabetes has been increasing worldwide therefore various are relentlessly working out the possible role of a vast number of genes associated with this disease[12] . Human leukocytes antigen represented many proteins that encoded by HLA genes, therefore HLA-DQ genes expressed as heterodimers of alpha and beta chains at the cell surface[13] .

The distribution of HLA-DQA1in the present study show high appearance in both types, out of 30 samples 26 show positive result 86.66% in type I and out of 33 samples 26 show positive result 78.78% in type II finally, statistical analysis showed no significant differences comparing diabetes patient and control subjects table 2.

Although the high appearance of this HLA-DQAI type in both types of diabetes TID and TIID, statistical analysis show no significant differences between patients and control, this led to conclude that this haplotype had no risky effect on this population sample of diabetes patients.

HLA allele and haplotype frequencies vary considerably across ethnic groups[14] .Researchers pointed that HLA-DQA1 ∗ 05:01, HLA-DQA1 ∗ 03:01 play arole in diabetes susceptibility particularly TID[15] .

The analyses of HLA disease associations in different ethnic populations, due to differences in allele frequency distributions and patterns of linkage disequilibrium, can allow important general inferences of disease risk associated with specific alleles and their combinations.

The distribution of HLA-DR3 in type I and II showed that type I diabetes revealed percentage 46.66% and type II revealed a percentage 36.66% whereas the control revealed percentage 32%. Statistical analysis showed significant differences comparing two types of diabetes patient.

On contrary of HLA DQA, HLA DR3 appear in less percentage in diabetes patients particularly in TIID. , this may be due to the protective role of this haplotype in tis population samples of diabetes. The increased risk of DR3/4-DQB1*0302 heterozygotes relative to DR3/3 and DR4/4 genotypes has led to the hypothesis that the *trans*-complementing DQ heterodimers are more effective in presenting diabetogenic epitopes to T-cells[16].

Type I diabetes has the strongest association with HLA-DQA1 and DR3 similar result were also reported by[15] .

Studies on DRB1 and DQB1 allele distributions and the importance of genotype context support the genetic associations observed in previous studies[17,18] .

Variable results on DQA1 and DR3genotypes in this study observed the risky and protective role of these genotypes in TID, TIID and healthy individuals .this is compatible with the of DQ heterodimer encoding by DQA1*0501 and DQB1*0302 explanation[19] .

Conclusion

This study was designed to assess the associations of HLA class II alleles, haplotypes, and genotypes with the risk of developing T1D and T2D in Iraq. Certain HLA class II alleles, haplotypes, and genotypes were related to diabetes and may be used as genetic susceptibility markers to diabetes. Further studies of HLA and diabetes mellitus in Iraq are needed to confirm the present results and to provide data for the development of screening assays and for better management of patients with diabetes at the onset of disease.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the college of Science for women, University of Babylon and all experiments were carried out in accordance with approved guidelines.

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