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MOLECULAR BASIS OF HUMAN LEUKOCYTE ANTIGENS DQB1*0201 ALLELE AND ITS ASSOCIATION WITH TYPE 1 DIABETES MELLITUS OF IRAQI CHILDREN

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ABSTRACT

Background: Type 1 diabetes mellitus (T1DM) is an autoimmune disease arising through a complex interaction of both genetic and immunologic factors. Similar to the majority of autoimmune diseases, T1DM usually has a relapsing remitting disease course with autoantibody and T cellular responses to islet cell autoantigens, which precede the clinical onset of the disease process. The immunological diagnosis of autoimmune diseases relies primarily on the detection of autoantibodies in the serum of T1DM patients. Although their pathogenic significance remains uncertain, they have the practical advantage of serving as surrogate biomarkers for predicting the clinical onset of T1DM. Type 1 diabetes is a polygenic disease association with specific gene such as *HLA* gene with a large number of genes having high effects on T1DM pathogenesis. Risk of T1DM progression is conferred by specific allele of *HLA* gene *(DQB1*0201)*. **Objective:** To investigate the molecular basis of gene encoding human leukocyte antigens *DQB1*0201* allele and its association with T1DM and with various parameters in children of Kerbala province. **Materials and Methods:** The study design was a case-control included 125 T1DM patients, 66 (52.8%) of them were males and 59 (47.2%) females, and another100 subjects of apparently healthy children, 57 (57%) of them were males and 43 (43%) were females randomly recruited from the kerbala province of Iraq as control group. Both T1DM and control groups have the same age ranged between (1-15) years. The data collected during Oct., 2017 to Sep., 2018. Five mL of blood sample from each case was taken to perform various molecular and biochemical investigations. Typing of *HLA* was performed by polymerase chain reaction-sequence-specific priming (PCR-SSP). **Results:** The frequency of *DQB1*0201* in T1DM patients were (72%) and apparently healthy control were (37%). Significant results (P value $\leq$ 0.01) obtained between mean of age and *DQB1*0201* allele. **Conclusion:** The allele of *HLA* gene (*DQB1*0201*) was highly affect T1DM pathogenesis. Highly significant correlation between mean of patients age with allele *HLA* gene (*DQB1*0201*) polymorphism.

KEYWORDS: HLA gene, *DQB1*0201* Allele, T1DM.

INTRODUCTION

Diabetes mellitus is epidemic in Asia characterized by rapid rates of increasing over short period and onset at a relatively young age and low BMI. Abdominal or central adiposity, particularly detrimental to type 2 diabetes and other metabolic diseases, is highly prevalent in Asians. The high rates of gestational diabetes, childhood obesity, and over nutrition in later life, may contribute substantially to the increasing diabetes epidemic in Asia.[1,2] Diabetes mellitus is characterized by the presence of chronic hyperglycemia accompanied by greater or lesser impairment in the metabolism of carbohydrates, lipids and proteins due to various genetic causes.[3]

Type 1 diabetes mellitus (T1DM) is one type of DM and it is a chronic autoimmune disease characterized by increased blood glucose levels (hyperglycemia), which are due to the insulin deficiency that occurs as the consequence of the loss of the pancreatic islet β-cells.[4] T1DM is one of the most common endocrine and metabolic conditions occurring in children and adolescents, Polydipsia, polyphagia, and polyuria is the most symptom of this disease, the loss of β-cells is the consequence T1DM-related autoimmunity. This type has a strong genetic component.[5,6] Pathogenesis of T1DM is an immune-mediated disease. Activated B cells interact with CD4+ and CD8+ T cells, as well as dendritic cells (DCs). Antigen presentation by B cells and DCs drives the activation of β-cell-specific T cells.[7,8] In addition, the exposure of B cells to β-cell autoantigens leads to the production of islet cells - targeting autoantibodies, which serve as biomarkers of asymptomatic disease. Dashed

arrows indicate the potential interactions between B cells and CD8+ T cells and between B cells and DCs.[9]

Genetic polymorphism and T1DM clearly a polygenic disorder, with nearly 40 loci known to affect disease susceptibility.[10] The Human leukocyte antigen (HLA) region on chromosome 6 provides perhaps one-half of the genetic susceptibility that leads to risk of T1DM Of the many *HLA* types,[11] *HLA* class II show the strongest association with type 1 diabetes.[12,13] The another genes associated with T1DM is (*PTPN22, CTLA4, IL2RA* and etc.).[14]

HLA and T1DM in humans is located on the short arm of chromosome 6 (6p21.3).[15,16] The *HLA* consists of three regions which have been designated as class I, class II, and class III based on the structure and function of gene products.[17] The main function of *HLA* on MHC class I gene products (*HLA*-A, -B, and -C) is to present endogenous peptides to responding $CD8^+$ T Cells while the MHC class II coded molecules *HLA-DR, -DP*, and *–DQ* have restricted expression and process exogenous peptides for presentation to $CD4^+$ helper T Cells.[18,19] The class III region, in turn, contains genes which encode for immune regulatory molecules, *HLA* genes are coding for cell surface antigen proteins responsible for a major function of the immune system then bind antigenic peptides and present them to T cells,[20] these to detection the foreign or abnormal antigens. This region contributes to 50% of the inherited risk for T1DM.[13,21] The most alleles that affected on T1DM is *DR3 – DQ2 (DRB1*0301, DQA1*0501 and DQB1*0201)*,[2] there's another alleles affected on T1DM is *DR4–DQ8 (DRB1*0401, DQA1*0301 and DQB1*0302)*.[22]

The aim of the presented work is to investigate the molecular basis of gene encoding human leukocyte antigens *DQB1*0201* allele and its association with type 1 diabetes mellitus and with various parameters in Iraqi children of Kerbala province.

MATERIALS AND METHODS

This study was a case-control study. The number of T1DM patients were 125 with age ranged between (1-15) years, 66 (52.8%) of them were males and 59 (47.2%) females obtained from Al-Hassan Center for Endocrinology in the Al-Hussein Medical City / Kerbala Health Directorates / Kerbala - Iraq, and another100 subjects of apparently healthy children with the same age range, 57 (57%) of them were males and 43 (43%) females and randomly recruited from the kerbala province of Iraq as control group. Blood sample 5 ml was collected from each patient and control subject, 2 ml in EDTA tube for glycated hemoglobin (HbA1c) and molecular studies, the remaining sample 3 ml was used for serum separation at 3000 x g and used for various biochemical investigations including thyroid function tests.

This study was approved by the regional committee of ethics of the Ministry of health Kerbala Health Directorate by issue number (2328 on the 26 September 2017). Written informed consent was obtained from the parents of all the children who participated in this study. All of the authors of this research confirmed that the patients' privacy was protected and the entire process was done with a prior written consent.

Total genomic DNA was extracted from the peripheral blood cells of study participants using the salting-out method. Polymerase Chain Reaction-Sequence-Specific Priming (PCR-SSP) technique (Table 1) by a thermocycler was used for *DQB1*0201* allele, as described.[23] Sequences of primers are shown in (Table 2). The PCR products were analyzed by agarose gel electrophoresis using 1.75 gram of agarose gel, and visualized by staining with ethidium bromide (Promega USA).

Table 1: The program of PCR-SSP for three HLA genes.

Type of Cycle	Temperature °C	Time	No. of Cycles
Initial denaturation	95	5 min.	1 cycle
Denaturation	95	30 sec.	35 cycles
Annealing	61	35 sec.	
Extension	72	1 min.	
Final extension	72	5 min.	1 cycle
Hold	4	10 sec.	
Total time: 2 hours and 10 minutes			

In each PCR reaction a primer pair was included that amplified the third intron of *HLA* gene. These two primers matched non-allelic sequences and thus functioned as an internal positive amplification control. The forward primer is **(5'TGC CAA GTG GAG CAC CCA A3')** (Tm 60°C, complementary to codons 173-179 in the 3' end of exon 3) and the reverse primer is (5'**GCA TCT TGC TCT GTG CAG AT3')** (Tm 60°C, complementary to codons 193-200 in the ***5'*** end of exon 4) that given rise to a **796** base pair **(bp)** fragment.[24]

Table 2: Primer sequence for allele of HLA gene, number of base pair (bp) of primers, number of product size of three genes and the percentage of number of guanine and cytosine (GC).[2]

Allele of HLA gene	Sequences of Primers	Number of bp in primers	Product Size	%GC
*DQB1*0201*	F: 5'GTGCGTCTTGTGAGCAGAAG3'	20 bp	205 bp	55 %
	R: 3'GCAAGGTCGTGCGGAGCT5'	18 bp		67 %

The data were expressed as mean ± SD, student t test and the ANOVA were used for calculating the probability using the PAST version 3.09 which used for calculating probability value (P value), chi- square (X^2), odd ratio (OR) and confidence interval 95% (CI 95%) and where used to express the significance between the studied groups (polymorphisms, biochemical parameters and demographical characteristics). In all statistical analysis the significant value is (0.05) and the highly significant value is (0.01).

RESULTS AND DISCUSSION

Various markers and molecular studies concerning various types of diabetes mellitus have been investigated because genetic variations play important roles in pathogenesis of the disease in various regions including Iraqi patients **(25-28).** Alleles of *HLA* gene *(DQB1*0201)* in T1DM patients (N=125) and control groups (N=100) were study, the results table (3) showed that *DQB1*0201* in positive results were revealed (N=90) (72%) T1DM patients and (N=35) (28%) healthy control, but in negative result showed (N=37) (37%) T1DM patients and (N=63) (63%) healthy control.

The statistical analysis shows results of these allele *(DQB1*0201)* is significant (P value = ≤ 0.01) and (OR = 4.38, 95% CI = 2.493 – 7.69).

Table 3: *HLA* genotypes correlated with T1DM patients and control groups.

Genes		T1DM N (%)	Control N (%)	Odd ratio (OR)	95% CI	P value
DQB1*0201	+	90 (72)	37 (37)	4.38	2.493 – 7.69	≤ 0.01
	-	35 (28)	63 (63)			

The data shown in table (3) indicate the correlation between alleles of *HLA* gene *(DQB1*0201)* with T1DM patients and apparently healthy control.

The significant results obtained concerning *DQB1*0201* (p value ≤ 0.01) and the (OR = 4.38, 95% CI = 2.493 – 7.69) indicate that the T1DM patients frequency higher than healthy control in (4.38) in 95% CI (2.493 – 7.69) which indicate a highly significant association between the current studied alleles and T1DM. Other study indicate that the *DQB1*0201* allele is highly risk that association on T1DM patients compared with healthy control[29] while *DQB1*0201* allele that present in children with T1DM compared with the control group indicate that they have an important role in the development of T1DM.[30] It can be construed that *HLA DR3-DQ2* alleles has a very modest effect with respect to the risk of T1DM.[2]

The amplification of exon 2 of HLA gene of *DQB1*0201* allele was showed in 205 bp and internal control was shown in 796 bp as indicated in (Fig. 1).

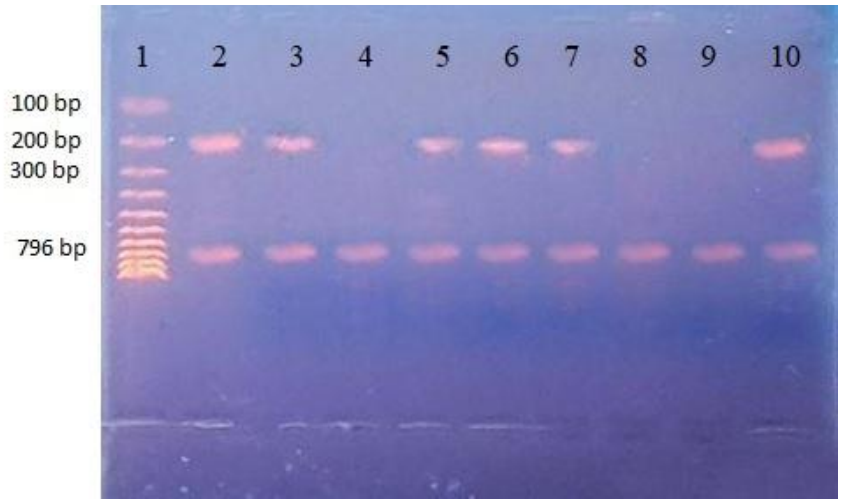

Figure 1: Amplification of *DQB1*0201* allele and control primer, that showed in this figure: Line 1: Represented DNA marker 1000 bp. Line 2, 3, 5, 6, 7 and 10: Represented *DQB1*201* (205 bp) and internal control primer (796 bp). Line 4, 8 and 9: Represented negative case and internal control primer (796 bp).

Figure (2) showed the correlation between mean of age and allele of *HLA* gene *(DQB1*0201)* and T1DM patients as compared with control groups.

The mean of age compared with *DQB1*0201* allele in positive results (11.2 ± 3.6) of T1DM patients and (8.4 ± 3.6) of healthy control, the results were significant (P value = ≤ 0.01). But negative results appeared (11.18 ± 3.66) in T1DM patients and (8.38 ± 3.5) of healthy control indicate that there is a significant results between mean of age and *DQB1*0201* allele (P value = ≤ 0.01).

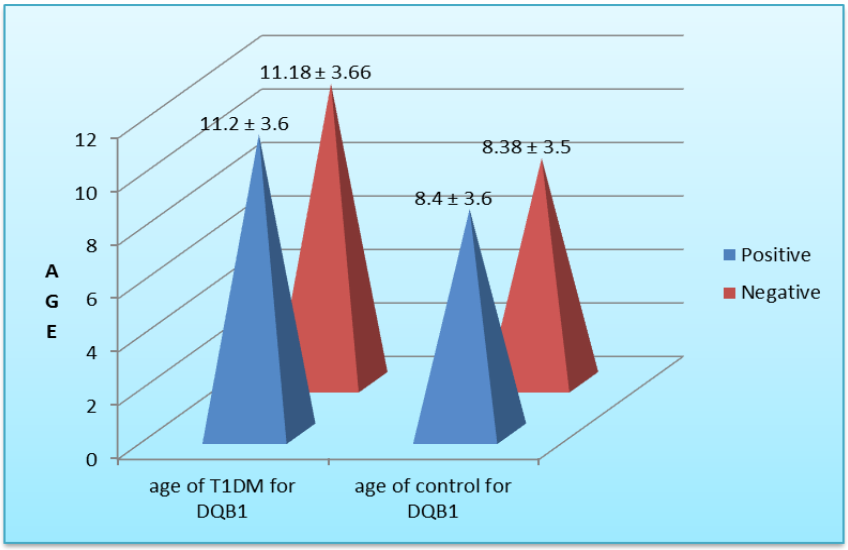

Figure 2: Mean of age correlated with allele of *HLA* gene *DQB1*0201*.

In the current study the comparison between mean of age and *DQB1*0201*, were revealed in (Fig 2) with a significant (p) value (P value = ≤ 0.01), these results was a ligament with other studies[31,32] and insulin delivery method, age of onset and type of insurance each showed an association with change in glycemic control over time in the 8 – 18 years old[33] and the race/ethnicity, income, insulin delivery method, and type of insurance are each associated with mean age-centered.[34]

In increasing period of patients age the symptoms of anxiety and depression are often related to parent stressor on patients as reported,[35] but the obtained results of the presented work has been suggested that the symptoms appear due to the period of increasing in age start with the period of adolescence and sexual puberty.

Other results indicated in table (4) show the correlation between demographical characteristics (gender, history of consanguinity and History of family to T1DM) and allele of *HLA* gene *DQB1*0201* in T1DM patients (N=125) and healthy control (N=100).

The comparison of gender (male and female) with *DR3 – DQ2* alleles *(DQB1*0201* observed as indicated in the following:
The positive results of *DQB1*0201* allele in T1DM patients male (49/125) and female (41/125) compared with healthy control male (22/100) and female (15/100), the statistical analysis in Chi square test of this results was observed non – significant (P value = 0.6, X^2 = 0.27).

The negative results of *DQB1*0201* allele in T1DM patients is male (17/125) and female (18/125) correlated with healthy control male (35/100) and female (28/100), indicated that the results after analysis in Chi square test is non – significant (P value = 0.51, X^2 = 0.44).

In the current study the correlation between history of consanguinity (cousin relation and no relation) with allele of *HLA* gene *DQB1*0201*, indicate a the positive results of *DQB1*0201* allele in T1DM patients cousin relation (60/125) and no relation (30/125) as compared with healthy control cousin relation (25/100) and no relation (11/100), the statistical analysis in Chi square test of this results was observed non – significant (P value = 0.76, X^2 = 0.09).

The negative results of *DQB1*0201* allele in T1DM patients is cousin relation (22/125) and no relation (13/125) correlated with healthy control cousin relation (42/100) and no relation (22/100), the results after analysis in Chi square test is non – significant (P value = 0.78, X^2 = 0.08).

The history of family to T1DM (father and mother) compared with allele of *HLA* gene *DQB1*0201* revealed the following results:
The positive results of *DQB1*0201* allele in T1DM patients father (9/125) and mother (6/125) compared with healthy control father (4/100) and mother (3/100), the statistical analysis in Chi square test of this results was observed non – significant (P value = 0.9, X^2 = 0.016). But the negative results of *DQB1*0201* allele in T1DM patients is father (2/125) and mother (3/125)

correlated with healthy control father (8/100) and mother (7/100), the results after analysis in Chi square test is non – significant (P value = 0.61, X^2 = 0.27).

Table 4: Demographical characteristics compared with allele of HLA gene *DQB1*0201*.

Demographical characteristics		*DQB1*0201*		Statistical analysis
		T1DM N=125	Control N=100	
Gender	Male +	49	22	X^2 = 0.27 P value = 0.6
	Female +	41	15	
	Male -	17	35	X^2 = 0.44 P value = 0.51
	Female -	18	28	
History of consanguinity	Cousin Relation +	60	25	X^2 = 0.09 P value = 0.76
	No Relation +	30	11	
	Cousin Relation -	22	42	X^2 = 0.08 P value = 0.78
	No Relation -	13	22	
History of family to T1DM	Father +	9	4	X^2 = 0.016 P value = 0.9
	Mother +	6	3	
	Father -	2	8	X^2 =0.27 P value = 0.61
	Mother -	3	7	

In table (4) the relationship between demographical characteristics with allele of *HLA* gene *DQB1*0201*, the results observed as in the following:
The correlation between the *DQB1*0201* allele and gender (male and female) was revealed a non–significant value in positive and negative genetics results. Certain reports indicated that *DQB1**0201 allele were more frequent in female with T1DM patients than males[31] and the race and environmental factors vary from patient to patient and between male and female.[32] The results of comparison between gender and *DQB1*0201*allele is not in a linement.[33]

The history of consanguinity (cousin relation and no relation) was compared with *DQB1*0201* allele, the current study was shown a non – significant results, the heritability and genetics effect that transfer from parents to offspring[36] and T1DM is autoimmune disease that affected in consanguinity of parents,[37] this results was disagreed with others.[38]

Relationship between history of family to T1DM (father and mother) with *DQB1*0201* allele is non – significant results, T1DM is autoimmune disease that inherited from parents and other family transfer to the children as indicated by other observations.[22,39] These results of history of family to T1DM compared with *DQB1*0201* was dis-alignment with other study.[40]

Table (4) were showed the relationship between the biochemical parameters (Mean ± SD) and alleles of *HLA* gene *(DQB1*0201, DQA1*0501 and DRB1*0301).*

The correlation between HbA1c and *DQB1*0201* allele, the positive results was observed (9.99 ± 2.50) of HbA1c in T1DM patients and (4.60 ± 1.07) of HbA1c in control groups, the statistical analysis was appeared the significant result (P value = ≤ 0.01).

The negative results of HbA1c in T1DM patients is (10.2 ± 2.3) and (4.6 ± 1.3) of HbA1c in control groups, the results of statistical analysis was significant (P value = ≤ 0.01). In another side the relationship between TSH and *DQB1*0201* allele, the positive results was revealed (2.46 ± 1.26) of TSH in T1DM patients and (2.44 ± 1.11) of TSH in control groups, the results is non – significant (P value = 0.93) but in current study some patients had hypothyroidism.

But the negative results, (2.38 ± 0.7) of TSH in T1DM patients and (2.45 ± 0.9) of TSH in control groups, there's non – significant results between *DQB1*0201* allele and TSH (P value = 0.68) but in present study some patients had hypothyroidism.

In the T3 parameters the compared with *DQB1*0201* allele, the positive results was observed (2.09 ± 0.64) of T3 in T1DM patients and (2.13 ± 0.65) of T3 in control groups, the statistical analysis was revealed non – significant results (P value = 0.75) but some patients had hypothyroidism were seen in present study.

While in the negative results was revealed (2.29 ± 0.56) of T3 in T1DM patients and (2.18 ± 0.73) of T3 in control groups, the results was non – significant between *DQB1*0201* allele and T3 (P value = 0.40) but some patients had hypothyroidism were seen in current study.

Relationship between T4 and *DQB1*0201* allele, the positive results was appeared (116.76 ± 29.58) of T4 in T1DM patients and (121.19 ± 24.87) of T4 in control groups, the results is non – significant (P value = 0.39) but in these study some patients had hypothyroidism.

The negative results of T4 in T1DM patients (120.09 ± 29.3) and T4 in control groups (115.4 ± 22.39), the results of present study was observed non – significant (P value = 0.41) but in these study some patients had hypothyroidism.

Table 4: Biochemical parameters compared with HLA genotypes.

Parameters		*DQB1*0201*, No. (225), Mean ± SD		P value
		T1DM	Control	
HbA1c	+	9.99 ± 2.50	4.60 ± 1.07	≤ 0.01
	-	10.2 ± 2.3	4.6 ± 1.3	≤ 0.01
TSH	+	2.46 ± 1.26	2.44 ± 1.11	0.93
	-	2.38 ± 0.7	2.45 ± 0.9	0.68
T3	+	2.09 ± 0.64	2.13 ± 0.65	0.75
	-	2.29 ± 0.56	2.18 ± 0.73	0.40
T4	+	116.76 ± 29.58	121.19 ± 24.87	0.39
	-	120.09 ± 29.3	115.4 ± 22.39	0.41

Table (4) showed a significant value of HbA1c as compared with allele of *HLA* gene *(DQB1*0201)* these due to elevated of HbA1c in diabetic patients, Environmental risk factors are believed to interact with susceptibility genes and thereby contribute to the disease process[37] and The average HbA1c value of (9.3) among the Somali children with T1DM is high in value, poor glycemic control among ethnic minorities has been reported.[38] The current study was non competence with others.[2] In the other side, when TSH compared with *DQB1*0201*the results is non – significant but some patients indicate that they have hypothyroidism. Hypothyroidism in HT is most likely due to thyrocyte destruction, including apoptosis, mediated by several immunological mechanisms such as Th1 cells and cytokines[39] and In the 3.5% the hypothyroidism coexisted with type 1 diabetes mellitus, consistent with the known increased prevalence of other autoimmune conditions in this disorder.[40] These data is disagreed with others.[41]

The results of comparison between T3 with *DQB1*0201*is non – significant but observed some patient had hypothyroidism, susceptibility genes have been acknowledged to confer a risk for development autoantibodies and T1DM, lead to thyroid disorder that result hypothyroidism[42] and Hypothyroidism in children with T1DM is frequently associated with hypoglycemia resulting from increased insulin sensitivity. Growth disorders diagnosed in these children are associated with chronic hypoglycemia and thyroid hormone deficiency.[43] Dis-alignment of current data was observed with others.[44]

T4 that compared with *DQB1*0201*, non – significant results that observed but some patient had hypothyroidism, susceptibility genes have been acknowledged to confer a risk for development autoantibodies and T1DM, lead to thyroid disorder that result hypothyroidism,[42] they inhibit enzyme activity and stimulate cytotoxicity by natural killer. Anti-thyroglobulin antibodies (TgA) are detectable in a small percentage of patients, while high levels of thyrotropin receptor-blocking antibodies are often present, particularly in patients who develop autoimmune hypothyroidism which was not in a liniments with others.[44,45]

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