GENETIC ROLE OF HLA-G14 BASE PAIR INDEL POLYMORPHIC GENE WITH TYPE 1 DIABETIC PATIENTS

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ABSTRACT

The prevalence of Diabetic mellitus in Iraq is high for both males and females, according to the World Health Organization (WHO). The major histocompatibility complex (MHC)/HLA region on chromosome 6p21 has been shown to contain the major genetic component of Type1Diabetic mellitus. A 14-base pair polymorphism inserts and/or removal in exon-8 has a potential role in HLA. This research explores the role of 14-bp HLA-G insertion / deletion polymorphism in Type 1 Diabetes mellitus (T1DM) patients. The polymorphism allele frequency was calculated in patients with T1DM and control. Insertion allele (70.8%) and homozygous deletion genotype are associated with T1D susceptibility (51.6%), while control group (38.3%) and heterozygous genotype of the 14-bp indel are correlated with T1D defense (38.3%) and control group (50%). also a significant differences in the allele frequencies of the HLA-G 14-bp polymorphism were observed. This research shows a sturdy relation among polymorphism HLA-G 14-bp and type 1D.M. (P = 0.009). Our findings describe the combination of the 14-base pair insertion allele and the homozygous genotype deletion to the progress of T1D.

Keywords: 14- HLA-G, type 1 diabetes, polymorphism

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INTRODUCTION

Type 1 diabetes (T1D) is an organ-specific autoimmune condition in which insulin is biologically failure due to the immune system's degradation of the β cells in the pancreas (¹). T1D most commonly occurs in children, including polyuria, polydipsia, and the loss of weight. A minimum of 15 to 67 percent of new T1D patients have ketoacidosis with diabetes, a life-threatening involvement (²). The human leukocyte antigen-G (HLA-G) is a molecule of the main histocompatibility complex (MHC) of non-classic class I (³). The HLA-G
gene is lying within the HLA region (6p21.2-21.3) on the short arm of chromosome 6. This consists of 7 introns and 8 exons coding the HLA-G molecule's heavy chain (4). There is evidence that genetic polymorphisms on the HLA-G gene can affect the tissue expression of HLA-G. Specifically, a HLA-G insertion (ins)/deletion (del) polymorphic gene (14 base pair in exon 8 of the Rs16375 gene) It has been proposed that mRNA stability affect HLA-G protein expression (5, 6). LA-G proteins are capable of binding to Bone marrow inhibitory receptors and thymus cells, NK, and (DCs) dendritic cells (7, 8) specifically found in Safe immunology areas for example thymus and the islets of the pancreas (9, 10). A variety of functional HLA-G gene polymorphisms were specified, involving a 14 base pair deletion / insertion polymorphic gene (Positioned in exon 8 in the three main untranslated region of the gene) related changes the type of alternative isoforms of spliced mRNA and sHLA-G condensation; in addition, the inserted allele affects the stability of mRNA (11). There are some Previous studies have proposed that deletion allele and two of the same allele (homozygous genotype) removal, insertion allele and homozygous genotype addition, and heterozygous genotype are all related with great risk of pathological disorder development (12). Polymorphisms play a major role in controlling HLA-G development in the five prime upstream regulatory region (URR) and in the three prime UTR of the HLA-G gene (13). Mainly, two polymorphic site at the three prime untranslated region: indel of 14 base pairs (14bp) polymorphism (Rs371194629) and a cytosine > Guanine (SNP) at the (+3142base pair) site (Rs1063320) (14). Two types of diabetes (1&2) have immunological disorder that increases insulin resistance due to genetics stable lifestyle, Obesity and other situations like infection or inflammation with chronic state. Higher levels of sHLA-G have been shown to be common in subjects with impaired metabolism of glucose (15). It is interesting to note that hla-G was detected in certain Secretary granules and primary insulin-induced islet cells on the cell surface. Based on these results, It might be presumed that a weaken expression of HLA-G on the Langerhans islets may maintain activation of T cells and diabetes beginning (16).

MATERIAL AND METHODS

Subjects

A total of 60 Iraqi Type 1 diabetes patients (T1D) attending Merjan medical city in Babylon province at the Babylon diabetic center. The control group also included 60 healthy people, balancing their age and gender with patients (30 males and 30 females), 20-45 years of age. Peripheral blood samples (3 ml) were obtained under aseptic technique from each subject, and then transferred to the EDTA tube, preserved at -20 °C for extraction of DNA, then studying HLA-G genotyping.

Genomic DNA extraction and genotyping polymorphism

According to the manufacturer protocol, genomic DNA has been extracted from peripheral blood using the Genomic DNA extraction kit (Promega / USA). The polymerase chain reaction (PCR) used the primers to amplify the HLAG gene: forward primer, GE14hla-G-5’-TGATGGGCTGTATTAAAGTGTACC-3’ and reverse primer RHG-4-5’-GGAAGGATAGCTTCCAGCATGA-3’ (17). The PCR protocol consisted of an initial denaturation stage at 94°C, followed by 35 cycles at 94°C for 20 s, at 64°C for 30 s, and at 72°C for 60 sec., and a final extension at 72°C for 10 min. The segment sizes of the polymerase chain reaction yields...
were calculated (220 and 240 base pair) based on the existence or lack of an exact band on 2% agarose gel discolored with red safe stain then imagined by E-Graph-UV gel.

Statistical analysis
The polymorphic gene frequencies \( \text{hla-G} \) 14-bp Indel was determined by use the specific counting method. The findings were presented in terms of percentage levels and were further presented in terms of odds ratio (OR) alleles indicating differences between patients and controls. Fisher’s exact probability \( (P) \) has assessed the significance of these differences. Statistically significant \( P \) values \( (P<0.05) \) were considered.

RESULTS
In this case-control study, we examined the distribution of HLA-G 14-bp insertion / deletion (INDEL) polymorphism genotypes and allele frequency among patients with type 1 diabetic mellitus and healthy individuals as control. As show in Fig. (1), Insertion or deletion of PCR products size (224 or 210 bp) of the 14-bp sequence in HLA-G, respectively. Three separate genotypes are recognizable by two percent agarose gel electrophoresis \((+14/+14 \text{ bp}), (+14/-14 \text{ bp}) \) and \((-14/-14 \text{ bp})\).

Table (1) presents allele and genotype frequencies of HLA-G (14-bp Ins / Del) polymorphic gene in patients with Diabetes and control group. For 60 T1D patients and 60 healthy people, HLA-G typing was performed. The frequencies of the allelesconformity with Hardy–Weinberg Principle in Diabetes and normal subjects (Hardy–Weinberg Principle \( P \)-value = 0.009). The findings shows that homozygous genotypes frequencies \((+14 \text{ bp}/+14 \text{ bp})\) in diabetes patients and group of controls decrease (Table 1), but the frequency of
homozygous -14 bp/-14 bp was higher in T1D patients. Nevertheless, the frequency of the heterozygous genotype +14 bp−14 bp in the control group was significantly increased relative to T1D patients.

Table 1: The genotype and allele frequency of HLA-G for 14pb indel polymorphism for the T1D patients and a group of controls

<table>
<thead>
<tr>
<th>Genotype</th>
<th>T1D %</th>
<th>Control %</th>
<th>Odds ratio</th>
<th>Confidence interval</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>-14bp/-14bp</td>
<td>31(51.6%)</td>
<td>23(38.3%)</td>
<td>24.500</td>
<td>4.718-127.220</td>
<td>0.009</td>
</tr>
<tr>
<td>+14bp/-14bp</td>
<td>23(38.3%)</td>
<td>30(50%)</td>
<td>24.500</td>
<td>4.718-127.220</td>
<td></td>
</tr>
<tr>
<td>+14bp/+14bp</td>
<td>6(10%)</td>
<td>7(11.7%)</td>
<td>8.050</td>
<td>2.098-30.892</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>60(100%)</td>
<td>60(100%)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Allele</th>
<th></th>
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<tbody>
<tr>
<td>14-bp insertion</td>
<td>85(70.8%)</td>
<td>76(63.4%)</td>
<td>19.240</td>
<td>3.627-124.212</td>
<td>0.01</td>
</tr>
<tr>
<td>14-bp deletion</td>
<td>35(29.1%)</td>
<td>44(36.6%)</td>
<td>14.830</td>
<td>4.787-119.748</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>120</td>
<td>120</td>
<td></td>
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</table>

DISCUSSION
Type 1 diabetes mellitus is a heterogeneous condition which usually develops during childhood and adolescence, previously known as insulin-dependent diabetes. The disorder is characterized by insulin development deficiency due to pancreatic β-cell destruction and requires insulin administration for survival for a lifetime. The genetic determinants of type 1 diabetes susceptibility are better understood than the factors of environmental risk.

The genes of human leucocyte antigen (hla) found on chromosome 6p21 were the first diabetes susceptibility genes identifiable. Subsequent studies showed an association between the disease and the insulin gene region of chromosome 11 (18). In addition to the well-documented association of HLA-DRB1 and DQB1 alleles with type 1 diabetes mellitus (T1D), a gene region similar to the non-classical category I HLA gene has been identified as an independent susceptibility indicator by linkage studies. Since diabetes type one is an autoimmune disease where the tolerance failure is immediately involved in the progress of disease, the hla-G locus could play an main role in this process (19). Previous research in Brazil aimed to investigate the association of the 14-base pairIn/del polymorphic gene (HLA-G) with diabetes type 1 vulnerability for the first time. We also examined the distribution of haplotypes and genotypes of HLA class two-risk in a type one diabetes inhabitant (20). The study was carried out by Baschal et al. (2011) showed that the risk of type one diabetes due to the polymorphism of telomeric MHC locus, an area nearest to classical and non-classical group I alleles of HLA (21). HLA-G has been known as a possible main participant in the pathogenesis and/or disease progression (22). While autoimmune disorders such as multiple sclerosis (23), rheumatoid arthritis (24), systemic lupus erythematosus (25) and psoriasis (26) have been correlated with HLA-G, its role in T1DM is currently being understood. An additional splice site is produced by the presence of the 14-bp insertion allele, where ninety-two (92) bases are deletion from the beginning of exon eight influencing mRNA constancy (27).
The presence of the genotype (del / del) was also nearly threefold greater in the early beginning of T1DM compared with patients who started late. Such results potentially proposed that the genotype of (del / del) is strongly associated with earlier autoimmune diabetes development.

CONCLUSION

Our findings describe the combination of the 14-base pair insertion allele and the homozygous genotype deletion to the progress of T1D.

ETHICAL CLEARANCE

The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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