Studying of Some Genetics Variables in Patients with Diabetes Mellitus Type I and Type II

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Abstract
Been highlighted through this study to determine the change in the levels of certain standards genetic in people with Diabetes Mellitus both types I and II, contained the current study, 60 people from diabetes patients and by (30) patients of the type I and (30) patients of the type II who underwent some tests, as well as (30) people (healthy apparently) as a group control of the study were collected blood samples from the Hussein Hospital education (blood test unit) and the Hospital of Al-Hindia General (Emergency unit), the Laboratory Razi analyzes of disease in the province of Karbala during the time period from 01.12.2011 until 01.04.2012. DNA was extracted for both types of diabetes patients and healthy people to detect the presence of the mutation in the gene for HLA-DR3 by the technique of the Poly merase chain reaction (PCR). the results of molecular genetic presence of mutations in the gene for HLA-DR3 in both types of diabetes and high incidence of mutations in individuals descended from parents who have a family history positive for the development of injury and also increased the proportion of males higher than in females and also recorded the incidence of high in the young ages of the type I and reconstruction late in the type II.

Keywords: diabetes, HLA-DR3, type 1 diabetes

Introduction
Diabetes based on insulin is an autoimmune disease, and related to kind of attributed to genetic factors and other external, such as food and other diseases and the involvement of these factors occur injury [1], there are 18 district chromosomal linked to injury diabetes based on insulin Each including a set of genes, and these areas named IDDM1-IDDM18, was addressed IDDMI (HLA) because he is responsible for the encryption proteins that the immune response to distinguish between self and Allamat in the human body, and that any defect in this gene leads to non-discrimination between Antginat self Allamat which gives the opportunity for immune T cells to attack the beta cells in the pancreas and destroy them, and called the attack on the cells self autoimmune disease, and as a result a person get diabetes insulin-dependent [2].

There are genes of the HLA on chromosome 6 and encode protein molecules is extremely important for the immune system, these molecules carry small chains of amino acids located on the surfaces of cells help the immune system to analyze these chains and upon discovery of any defect in the order of amino acids will be prepared bearing cells this arrangement is not known to have strange and attack them [3]. There are many genes of the HLA and this leads to a lot of changes on the surfaces of cells and as a result of injury autoimmune diseases, and the alleles of the HLA inherited from the parents responsible for 50% of the risk of developing diabetes based on insulin called these genes responsible for immune to HLA-DP, HLA- DQ, HLA-DR [4].

Can not the immune system without the genes of the HLA differentiate between Antginat self Allamat any of the cells of internal organs and between viruses and bacteria and cancer cells, on the other hand that inheriting copies ( alleles ) of certain genes of the HLA will increase the likelihood of attack the immune system of the body's cells healthy and in the same way immune system cells attack the beta cells, leading to the injury, diabetic insulin-dependent [5].

Like gene HLA-DR, the alleles of specific gene HLA-DQ increase the risk of developing infection diabetic insulin-dependent while alleles other is preventive to reduce this risk, and there is a tendency in people who inherit alleles HLA-DR3, DR4 to inherit alleles gene HLA -DQ Mmaesid of genetic risks of injury, diabetic insulin-dependent, and on the contrary, there are also alleles and protective of the risk to the genes of the HLA-DQ, and can be inherited the genes of the HLA-DR, HLA-DQ together[4].

Inherit 50% of the individuals in the general population allele and one of Jane the HLA either HLA- DR4 or HLA-DR3 and less than 3% of individuals inherit alleles together, and diabetes based on insulin there at least one allele either HLA-DR3 or HLA-DR4 in 95% of Alqoukazeyen that individuals possess both alleles cause a higher likelihood of injury, diabetic insulin-dependent, on the contrary, the Night HLA-DR2 gives protection from the risk of this type of diabetes [6].

Material and Methods
1- Sampling Collection of the specimens
The study sample included 90 people from reviewers to Hussein Hospital educational and al-hindia General Hospital and some outside laboratories in the province of Karbala, during the time period from 12/01/2011 until 04/01/2012, the samples studied were divided form following:
First, people with insulin-dependent diabetes (Type I) (IDDM) - Type I

These included the group 30 people denominations different age, were divided age groups on the five aggregates group included the first age group at least 20 years and that the number of members 2 people, while the second age group (34-20 years) and the number 4 people, and the group they are the third age group (35-49), who are 6 people, while the fourth category (50-64) and the fifth category (65) years and over, who are 16.2 people respectively.

The groups were divided on the weighted totals included five Group One weight less than 50 kg and was the number 2 person, and the second group (59-50) kg and the number 4 people, while the third group (69-60) kg and Group D (79-70) kg and Group E (80) kg and more, as was the 10, 8 and 6 persons respectively.

Secondly, people with diabetes is insulin-dependent (type II):- (NIDDM) Type II

These included the group 30 people infected diabetic non-insulin-dependent Type II, were divided in five age categories and five categories grains as passed in the group infected with the sugar-dependent insulin and by - people in the age group first, and - people in the age group second, and 10 people in the third age group, age group and either the fourth and fifth included 12.8 people respectively. As for the weight categories have been broken down as follows - people in the first group gravimetric, and - people in the second group gravimetric and 14.8 people in the weight aggregates third, fourth and fifth respectively.

Third: the control group Control group

This group included 30 people from apparently healthy people as they were divided on the age groups and grains as passed in the previous two groups.

2- blood samples:

(5 ml) of blood take by syringe medical venous blood (Venous blood) for people under study after cleaning the skin with alcohol by 70 % and then divided the samples into two categories (3) ml of blood as it left for a period of 2-1 hours under degree room temperature for the purpose of coagulation full and the occurrence of thrombosis Put (2 ml) of blood in ETDA Tube container on contain anticoagulant was gently to mix the blood with anticoagulant and then transferred cooled to laboratories Faculty of Education, University of Karbala time not to exceed 24 hours to extract the DNA and a molecular tests.

3. dna extraction by promega kit.

4. Polymerase chain reaction (PCR):

Was prepared solutions of the Stock Solution and lotion work Working Solution Company by Alpha DNA and as follows:

Added ℓ 12.5 µ of Premix each tube Bndrof specific to the PCR and added her ℓ 1.5 µ of each F-primer and R-primer of the working solution was added to her ℓ 4.5 µ of each sample DNA, and transferred the tubes to the PCR, which was in the form of 40 cycles start of the first session for 5 minutes at a temperature 94 m and cycle gene HLA-DR3 for 2 minutes degree of 60 m and the final round for a period of one minute and 94 degree m.

Table (1) sequence of primers used in the research processed by Alpha DNA

<table>
<thead>
<tr>
<th>Gene</th>
<th>Sequence</th>
</tr>
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<tbody>
<tr>
<td>HLA-DR3</td>
<td>F-TTG TCC ACC CCC CGC T</td>
</tr>
<tr>
<td></td>
<td>R-CAC GTT TCT TGG AGT ACT CTA CGT CTG TGT</td>
</tr>
</tbody>
</table>

5. Agarose Gel Electrophoresis

Followed the method [7] to deport DNA extracted from the blood on the agarose gel with some modification.

Results and dissection

studying the possibility of having a mutation HLA-DR3 gene for patients with diabetes insulin-dependent and non-insulin-dependent and showed the results of the current study, in form (1) and the presence of this gene in six patients type 1 patient and non-insulin-dependent (type II) and the molecular size of each of these lane is 200 bp and the current study showed that the percentage of the presence of the HLA gene in males is higher than in females.
Form (1) gel electrophoresis of DNA extracted from the blood of patients with diabetes type I and II. 
- field (5-1) a patient’s disease is sugar-insulin-dependent NIDDM.
- field (8-12) disease patients dependent on insulin, sugar IDDM.
- area (6) DNA Ladder.

Genetic factors constitute about a third of cases of predisposition to type I diabetes, since there are more than 20 different areas of the human genome closely with diabetes with insulin-dependent [8].

Most of the attention focused on the region antigens of white blood cells (HLA) within the complexes histocompatibility on the site IDDM1 associated with DR4 or DR3 and two of the patterns of individual’s HLAs with increased susceptibility to infection with the first type of diabetes among Caucasians [9]. And be alleles DR4, DR3 status of the imbalance of my connection Linkage Disequilibrium they tend to break with the alleles of neighboring genes HLA-DQA1 and HLA-DQB1 and the latter are the main determinants of the willingness of the genetic [10].

The antigens of the HLA class II plays a key role in initiating the immune response, that some forms of the gene HLA-DQB1 caused by replacement of a specific amino acid in the beta chains of class II antigens [11].

Although the genetic predisposition can be considered a prerequisite for the development of diabetes based on insulin, the compatibility between the twins unilateral marriages Monozygotic Twins than 40% and that environmental factors with an important role in the induction of expression of the clinical and matched the results of the current study with the study [12], which showed that almost 60% of the risk of genetic diabetes based on insulin be linked area of HLA having found a close relationship between the incidence of type I diabetes and the changes that occur in the region of the HLA, and matched the results of the current study with the study [13], which explained that the occurrence of any defect in the region of the HLA lead to the incidence of diabetes based on insulin. The results showed to calculate the percentage depending on the type of marriage the parents of people with diabetes of both types (Table 1) the high percentage of injury diabetes type I and II to the individuals who have whose parents on kinship (marriage relatives) as the ratio (69.7%), (56%), respectively, compared with outbreeding people who have reached the proportions of injury (30.3%), (44%), respectively.

Table (1) the percentage of the presence of the gene HLA-DR3 in patients with diabetes of both types I and II depending on the different type of marriage.

<table>
<thead>
<tr>
<th>Type I (%)</th>
<th>Type 2 (%)</th>
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<tbody>
<tr>
<td>Outbreeding marriage</td>
<td>marriage relatives</td>
</tr>
<tr>
<td>30.3%</td>
<td>56%</td>
</tr>
<tr>
<td>Outbreeding marriage</td>
<td>marriage relatives</td>
</tr>
<tr>
<td>69.7%</td>
<td>44%</td>
</tr>
</tbody>
</table>

By observing the results of measuring the percentage of the mutation in the gene HLD-DR3 find a high percentage of patients whose parents are unrelated and both types I and II diabetes, which confirms that the record family a big impact in the development of contracting this disease (Table 1).

This study was consistent with the findings of the [14], which Noting there are individuals born of the families have a positive family history of the disease are more susceptible to diabetes and according to the degree of kinship and the rate of infection have more than 20% and that a lot of the families that the evolution of the injury come by inheritance of HLA genes that have a role in the immune response.

[15] noted that the gene HLA-DR determines risk of diabetes type I and that the infection rate in children as a result of a defect in this gene between 0.3% - 30% depending on the model gene to gene HLA class II.

While [16] say that individuals infected with diabetes who do not have a family history of injury,
diabetes rate of infection have more than 5% and between [17] that estimates indicate that individuals Caucasians have a susceptibility to diabetes and 39% of them will catch the disease after the age of twenty years of age. As noted Baisch and others (1990) that the injury diabetes type I in children related to family history of the HLA genes, such as HLA-DR and less than 1% of infections are in children who do not have their ancestral family history of the disease and occurs injury they have as a result of a defect in the genes of HLA Class I (Class I).

Table (2) showed the percentage of the incidence of diabetes quality of both sexes and an increase in the incidence of diabetes of both types I and II in males, as was (56.66%), (57%), respectively, while the percentage of injury among females (43.34%), (43%), respectively. Table (2) the percentage of the presence of the gene HLA-DR3 in patients with diabetes of both types I and II according to sex.

<table>
<thead>
<tr>
<th>Type 2</th>
<th></th>
<th>Type 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>female</td>
<td>male</td>
<td>female</td>
</tr>
<tr>
<td>%43</td>
<td>57%</td>
<td>43.34%</td>
</tr>
</tbody>
</table>

Results showed the table (2) high incidence of diabetes as a result of a mutation in a gene HLD-DR3 among males than females and for both types of diabetes.

results by [18], which pointed to the high susceptibility of injury among males is higher than females as a result of the imbalance in the HLA genes and the genes of the insulin (INS) and attributed the cause of the rise to susceptibility produce antibodies to the pancreatic beta cells and antibodies to insulin and be the ability to configure these antibodies in males compared with females higher, because the males have a habit of decline in the effectiveness of B-cell or an increase in body mass index (BMI) and the evolution of these factors have diabetes type II.

The ability to configure antibodies raise the risk of diabetes as that considered both insulin and beta cell antigens will develop antibodies, as well as stimulate the system complementary since found that there is a close relationship between the cells of the pancreas and the amount of auto-antibodies as a result of the defect in the gene HLA[19].

And [20] that male overweight increases their likelihood of increased insulin resistance by producing antibodies to him at the time did not reach the researchers to find a clear relationship between the increase in body mass index (BMI) and the defect in the gene insulin Insulin gene (INS). Data showed the table (3) to calculate the percentage of injury diabetes of both types depending on different age groups, the high percentage of diabetes type injury in the first category (less than 20 years), (49-40), (59-50), amounting to this percentage (13.33%) (31.33%) (26.66%), and each of them, respectively, while the highest percentage of injury type II diabetes among individuals age group (49-40), (60 and older), as was (20.02%), (50%) and respectively.

<table>
<thead>
<tr>
<th>Type 2</th>
<th></th>
<th>Type 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>(%)</td>
<td>N</td>
<td>Category</td>
</tr>
<tr>
<td>6.66%</td>
<td>1</td>
<td>20-29 من</td>
</tr>
<tr>
<td>6.66%</td>
<td>1</td>
<td>30-39 من</td>
</tr>
<tr>
<td>20.02%</td>
<td>3</td>
<td>40-49 من</td>
</tr>
<tr>
<td>16.66%</td>
<td>2</td>
<td>50-59 من</td>
</tr>
<tr>
<td>50%</td>
<td>7</td>
<td>ضموم فوق 60</td>
</tr>
</tbody>
</table>

The study showed the current that the incidence of mutation in the gene HLA-DR among younger age groups to be high for the rest of the categories of people with type I diabetes, either with respect to categories developed probably due to the large sample taken from this age group, amounting to (9) people. As for people with type II were older age groups the highest proportion than others on the grounds that diabetes type II disease that affects people after middle age [21] and agreed this study with [22], who pointed out that the ability to configure antibodies self in patients with type Althatha of diabetes are higher in the reconstruction adult from young age and be less gradual in the final for the defect in the gene HLA-DR as for the first type, it is a disease in age groups small because of the immaturity the immune system and the lack of competence to recognize self-antigens spoke injury of this type, or perhaps dating back to the lack of efficiency of the immune system to defend the body against attacks by bacteria and viruses such as mumps and measles leads to damage and injuring internal organs such as the liver and pancreas, leading the destruction of beta cells producing insulin or in sometimes formation of antibodies to the insulin itself [23].
Reference


