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CTLA4 gene polymorphisms associated with insulin-dependent diabetes mellitus (IDDM) type I in Iraqi population

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Abstract:

This study was correlated to evaluate polymorphisms in CTLA4 associated with insulin-dependent diabetes mellitus in a Iraqi population. Blood samples were collected from 40 patient (4-25 years) from 1/6/2010- 1/2/2011, isolation of DNA were done and PCR was performed using primer for CTLA4 gene. The result shows that 31 patients have mutation in 49 position A/G polymorphism in exon 1 of CTLA4 by using BstEII enzyme. There is a highly significant correlation between diabetes and incidence of A/G 49 position in exon 1 of CTLA4 gene (X2 = 32.1, P>0.05), the conclusion is that there is enough evidence to support the claim that diabetes is related to A/G 49 transition mutation.

Keyword : *CTLA4 gene , insulin-dependent diabetes mellitus*

Introduction:

Cytotoxic T-lymphocyte antigen 4 (CTLA4) also known as CD152 (Cluster of differentiation 152) is a protein that plays an important regulatory role in the immune system. CTLA4 is a member of the immunoglobulin superfamily, which is expressed on the surface of Helper T cells and transmits an inhibitory signal to T cells. The cytotoxic T lymphocyte-associated antigen 4 gene (CTLA4) encodes the T cell receptor involved in the control of T cell proliferation and mediates T cell apoptosis [1]. In humans, the CTLA4 protein is encoded by the CTLA4 gene [2]. The receptor protein is a specific T lymphocyte surface antigen that is detected on cells only after antigen presentation. Thus, CTLA4 is directly involved in both immune and autoimmune responses and may be involved in the pathogenesis of multiple T cell-mediated autoimmune disorders [3]. The human CTLA4 gene was mapped to chromosome 2q33. It consists of three exons, the first encodes a V-like domain of 116 amino acids. Substitution of A-to-G at nucleotide 49 in exon 1 results in an amino acid substitution (Thr/Ala) in the leader peptide of the protein [4]. Type 1 diabetes mellitus (TIDM) and insulin dependent diabetes mellitus (IDDM) is a common multifactorial autoimmune disease that arises from specific destruction of insulin secreting beta cells of the islets of langerhans by autoreactive T-lymphocyte [5], the pathogenesis of IDDM is complex and multifactorial involving interaction between both genetic and environmental factors [6], mutation and polymorphisms leading to altered activity of CTLA4 are believed to play an important role in the risk of developing autoimmunity [7]. The present study aimed to investigate correlation between the +49 A/G polymorphism in exon 1 of CTLA4 gen and increasing insulin-dependent diabetes mellitus (IDDM) type I in Iraqi population

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Material and Methods:

Sample collection:

Whole blood samples was obtained from 40 Iraqi patients affected by IDDM (20 male and 20 female(, age ranged 4-25 years and also obtained from healthy used as a control group. Whole blood (4ml) was collected into an EDTA- tube; the samples were stored at -20°C until further processing.

DNA extraction

DNA was extracted from the samples by wizard genomic (DNA purification kit, Promega) according to the isolating genomic DNA from whole blood protocol. DNA extracted

from 300 µl whole blood in each case. The volume of the extracted DNA solution was usually 100 µl were stored at -20°C.

PCR Amplification & Genotyping

A fragment 152 bp containing the +49 A/G polymorphism in exon 1 of CTLA4 was amplified using a forward primer (CTLA4–5:5′-AAGGCTCAGCTGAACCTGGT-3′) and a reverse primer(CTLA4–4:5′-CTGCTGAAACAAATGAAACCC-3′) (Primers set supplied by alpha DNA Company, Canada). The forward primer was designed with a single base mismatch for the last nucleotide, which corresponds to the +47 position to introduce a base change in the sequence of the PCR product, according to Marron M.; et al [8]. The PCR amplification was performed in a total volume of 25µl containing 5µl DNA (conc. 20 ng), 12.5 µl Go Taq green master mix 2X (Promega corporation, USA), 1µl of each primer (50 pmol), the volume completed with 25µl with nucleases free water. The thermal cycling was done as follows: Denaturation at 94 °C for 5 min, followed by 30 cycles of 94 °C for 30s, 57°C for 1 min, and 72 °C for 1min, with final incubation at 72 °C for 7 min [9] using a thermal Cycler (Gene Amp, PCR system 9700; Applied Biosystem). The PCR products were separated by 1.5% agarose gel electrophoresis and visualized by exposure to ultraviolet light (302nm) after ethidium bromide staining. The substitution creates a BstEII restriction site in the A allele and this will be confirmed by incubated the PCR Amplified products (12 µL) with 5 U of BstEII (supplied by Promega corporation, USA) at 60 C overnight. Digested products were electrophoresis on 3.0% agarose gel [8].

Result and discussion:

The genomic DNA from 40 patient were extracted using wizard genomic DNA promega, CTLA4 gene from genomic DNA were amplified by using specific PCR primers for exon 1, results shown in figure (1) indicated that a yield of single band of the desired product with a molecular weight about 152 bp for exon 1 CTLA4 gene was obtained, in comparison to result Morran, et. al.; HateMohamed, et. al. and Waterhouse et al., [8, 9, 10].

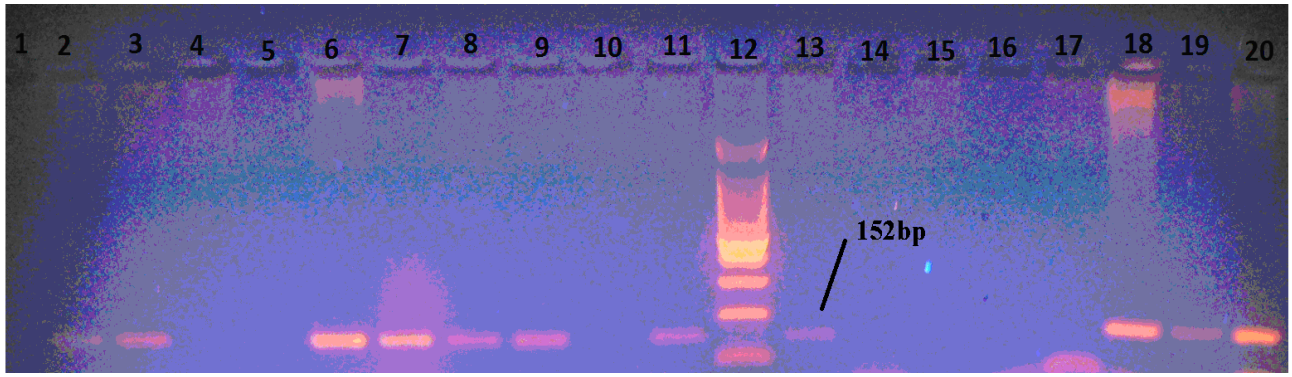

Figure 1: Agarose gel electrophoresis for amplified CTLA4 gene of IDDM patients. Bands were fractionated by electrophoresis on a 1.5 % agarose gel (2 h., 5V/cm, 1X Tris-acetic buffer) and visualized under U.V. light after staining with ethidium bromide staining. Lane: 12 (M:100bp ladder); Lane:1.(negative control); Lane: 2,3,6,7,8,9,11,13,18,19,20 (product band); Lane: 4,5,10,14,15,16,17 (no results).

As shown in figure (2) it appear that 31 samples have transition mutation A to G at nucleotide 47 in exon1 of CTLA4 by incubated PCR products with 5U of BstEII enzyme, the digested A allele yields a fragment of 130 bp (Mutant type) and the G allele yields an intact 152 bp fragment (Wild type), which comparison with other reference that showed similar to this result [8 and 9].

Many studies indicated that the A49G polymorphism is clearly associated with type1 diabetes. CTLA4 49 (A/G) mutation conferred a risk of type 1 diabetes in the Chinese children but not in the West African children, on the other hand, the novel CTLA4 159 (C/G) mutation conferred arisk of type 1 diabetes in the West African children but not in the Chinese type 1 diabetic children [11], and Kavvoura, et al., referred to associated of A49G polymorphism with type 1 diabetes when 29 studies concerning the A49G polymorphism from 33 studies with approximately 12400 type 1 diabetes cases and controls [12]. Mutations and polymorphisms leading to altered activity of CTLA4 are believed to play an important role in the risk of developing autoimmunity [13], The CTLA4 (49+) GG homozygous genotype is associated with T1D in Egyptian children especially with younger age of onset and in younger patients and not associated with grades of diabetic control or diabetic complication [14].

CTLA4 plays a role in limiting T-cell proliferative response, several hypotheses have been proposed to determine the possible mechanism by which this is a achieved, the most known hypothesis states that disruption of the balance between CD28 and CTLA4 interactions with B7 could lead to autoimmune disease by preventing apoptosis or down regulation of activated self reaction T- lymphocyte [15]. Gribben , et al [16] have suggested that this may be through antigen

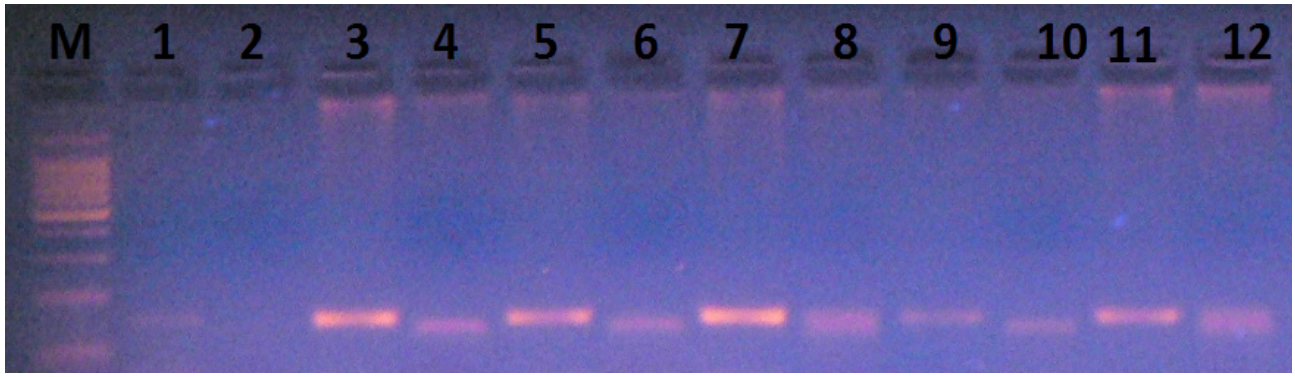

Figure 2 : Agarose gel electrophoresis for amplified CTLA4 gene of IDDM patients. Bands were fractionated by electrophoresis on a 3% agarose gel (2 h., 5V/cm, 1X Tris-acetic buffer) and visualized under U.V. light after staining with ethidium bromide staining. Lane: 1 (M:100bp ladder); Lane: 1, 3, 5, 7, 9, 11, 13, 15, 17 (Intact sample G Allele 152 bp fragment); Lane: 2, 4, 6, 8, 10, 12, 14, 16, 18 (Mutant sample A Allele 130 bp fragment).

specific induction of the apoptotic pathway, the current study investigated the A49G polymorphism in exon 1 of CTLA4 gene in 40 Lebanese and 46 controls from the same ethnic background , an increase in the frequency of the G allele was discovered in patients when compared to control subjects , this difference was statistically significant , despite the small sample size [17]. Wafai, show an association of CTLA4 with type 1 diabetes in Lebanese population which suggests that CTLA4 on chromosome 2q33 (IDDM) is a possible susceptibility locus [17].

There is a highly significant correlate between diabetes and incidence of A/G 49 position in exon 1 of CTLA4 gene (X2 = 32.1, P>0.05), the conclusion is that there is enough evidence to support the claim that diabetes is related to A/G 49 transition mutation. Despite the limited size of our sample, our results together with other population studies show an association of CTLA4 with IDDM in Iraqi population , which suggests that CTLA4 on chromosome 2q33(IDDM) is a possible susceptibility locus .

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الأشكال المتعددة للجين *CTLA4* وعلاقته بداء السكري المعتمد على الأنسولين النوع الأول في عدد السكان العراقية

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الخلاصة:

هدفت هذه الدراسة إلى ألقاء الضوء على التغاير في جين CTLA4 وعلاقته بمرضى السكري النوع الأول عند الأطفال العراق و جمعت 40 عينه دم من مرضى السكري للأعمار من 4- 25 سنه للفترة 1/6/2010 الى 1/2/2011 . تم عزل الدنا وأجريت عملية PCR باستخدام البادئات الخاصة لجين CTLA4 , أظهرت النتائج وجود طفرات في الموقع 49 (A<G) في 31 عينه مصابة بعد استخدام أنزيم القطع BstEII . أعطت نتائج الإحصاء بان هناك علاقة لحد ما بين حدوث المرض وظهور الطفرة الوراثية A/G في الاكسون الأول لجين وبقيمة (X2 = 32.1, P>0.05) , وهي داعمة للادعاء بان هناك علاقة بين المرض والطفرة.