

RESEARCH ARTICLE

Analysis Polymorphism of DNA Repair Gene (OGG1) in Some Kirkuk Patients with Type 1 Diabetes Mellitus

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ABSTRACT

The current study includes 200 subjects, 120 patients with diabetes mellitus type1 and 80 subjects as control group, the anthropometric characteristics of type1 diabetic (T1D) subjects and non-diabetic controls. The age difference was not significantly different ($p > 0.05$) in diabetic patients when compared with. The distribution of 8-Oxoguanine glycosylase (OGG1) frequency gene polymorphism was significantly difference ($p \geq 0.05$). Frequency of OGG1 C/C (homozygous) genotype showed (32.43%) in patients and (100%) in control. The results of OGG1 heterozygous G/C genotype showed higher significantly in DM type1 (74.76%) and not found in controls (0.00%). The differences were significant. OGG1 G/G genotype frequency was not found, neither in the diabetes mellitus type1 nor controls (0.00%), consequently there was not significantly different ($p \geq 0.01$). there were significant differences in body mass index (BMI) ($p \geq 0.01$) in control group when matching with found in controls (0.00%). The differences were significant. OGG1 G/G genotype frequency was not found, neither in the diabetes mellitus type1 nor controls (0.00%), consequently there was not significantly different ($p \geq 0.01$).

Keywords: Diabetes mellitus, DNA Amplification, Sequencing, Type 1 diabetes.

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INTRODUCTION

Constant hyperglycemia of diabetes relates to long haul harm, brokenness and disappointment of various organs. Diabetes mellitus can be sorted into four clinical gatherings by American Diabetes Association (ADA) (2014) and World Health Organization (WHO).¹ Type1DM beginning happens when 80 to 90% of pancreatic beta cells can presently don't work ordinarily. In type2 diabetes (T2D), the illness advances when insulin emission or insulin activity falls flat. Hyperglycemia and free unsaturated fat utilization are among the foundations for oxidative pressure conditions.² Thus, the age of receptive oxygen species (ROS) is expanded in T1D. The ROS is a free revolutionaries created in cells for engaged with some cell capacities.³ Inordinate ROS have been recorded that it's connected with sickness frequency and advancements. The extensive stretches of extreme free revolutionaries' openness may cause change in DNA fix qualities. Diabetic kidney infection (DKD) is the main source of end-stage kidney illness requiring renal substitution treatment. Hereditary variables seem basic in its pathogenesis. Heightening of glycemic, lipid and pulse control have not significantly affected the commonness of DKD.⁴

The ROS is a free revolutionaries created in cells for engaged with some cell capacities,^{5,6} the inordinate ROS have

been recorded that it's connected with sickness occurrence and improvements. DM observed to be related with oxidative stress unevenness and rise of ROS, and critical job in DM entanglements. The significant stretches of unnecessary free extremists' openness may cause change in DNA fix qualities.^{7,8} The aim of the study research Analysis polymorphism of DNA repair gene (OGG1) in some Kirkuk patients with type1 diabetes mellitus.

METHODS

Patients and Sample Collection

The present work included (200) individual off them (120) suffering from T1D according to the United States Center (US) for diabetes aged between (9–65) years with HbA1c high levels and (80) controls aged between 12 to 67 years non-diabetic. All blood sample collected from privet laboratory in Kirkuk-Iraq for molecular analysis. Anthropometric characteristics of T1D subjects and non-diabetic controls were collected.

DNA Extraction

The DNA become extracted from the whole blood by using wizard genomic (DNA purification package, Intron) according to the isolating genomic DNA from 200 μ L complete blood in

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every case. The volume of the extracted DNA answer become usually 100 µL were saved at -20°C.

Primers Used in the Study

The primers used for amplification of OGG1 gene exon 7, including Ser326Cys, were 5'-ACT GTC ACT AGT CTC ACC AG-3' forward, and 5'-TGA ATT CGG AAG GTG CTT GGG GAA T-3' reverse.¹⁶ Simple polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was used to detect the Ser326Cys variant, because the C to G transversion creates a new Fnu4HI restriction site.

Detection of OGG1 Genotype

In order to analyze the genetic diversity of the gene OGG1 in all populace study we optimize the situation of reaction and use the subsequent PCR amplification application:

A 25 µL reactions (Template DNA 1.5 µL, primer forward 0.8 µL F, primer reverses 0.8 µL R, Deionized Water 16.9 µL, PCR Master Mix 5 µL). Thermal cycling conditions for the OGG1 were: an initial denaturation step for 2 minutes at 94°C, 33 cycles of denaturation for 30s at 94°C, primer annealing for 30s at 61°C, and primer extension for 30s at 72°C, followed with the aid of a final extension step for 10 minutes at 72°C.^{11,12}

After PCR, amplification product was digested with 2 devices of Fnu4HI the reaction was performed in 10 µL final volume; at 37°C for 30 minutes, for genotyping of studied samples, the digested fragments have been electrophoresed on 4% agarose gel mixed with crimson stain. The heterozygote C/G incorporates all three bands (100 bp, 107 bp, and 207 bp bands) following restriction digestion. The homozygote C/C isn't always cleaved by FNU4HI, and the unmarried 207 bp band residues. Electrophoresis at 4% agarose gel (2.5 hours/70v).

Sequencing Analysis

After amplification pcr product sent to Humanizing Genomics Macrogen Company– Korea for sequencing.⁹

Statistical Analysis

The To come across wonderful variables in research parameters, the Statistical Analysis System- SAS (2012)¹⁷ changed into utilized. To observe variations in medical parameters between patients and controls, the p-value take a look at became used. OGG1 become observed to be homozygous Ser/Ser in the wild kind and Ser/Cys within the mutant. The *p-values* of less than 0.01, with 0.05 being deemed statistically extensive, were used. In this have a look at, the least significant difference (LSD) test became employed to evaluate approach.¹⁰

RESULTS AND DISCUSSION

The current study includes 200 subjects, 120 patients with T1DM and (80) subjects as control group. Table 1 summarizes the anthropometric characteristics of type 1 diabetic topics and non-diabetic controls. The difference of age changed into no longer extensively one of a kind ($p > 0.05$) in diabetic sufferers whilst in comparison with manipulate organization. Also there was significant differences were observed in the values of BMI ($p = 0.0288$), the BMI for non-diabetic controls was

($p \leq 0.05$) when matching diabetics group but both of them in the normal range of BMI.

The distribution of OGG1 frequency gene polymorphism was significantly different ($p \geq 0.05$) as represented in Table 2. Frequency of OGG1 C/C (homozygous) genotype showed (32.43%) in patients and 100% in control. The results of OGG1 heterozygous G/C genotype showed higher significantly in T1DM (74.76%) and not found in controls (0.00%). The differences were significant. OGG1 G/G genotype frequency was not found, neither in the diabetes mellitus type 1 nor controls (0.00%), consequently there was not significantly different ($p \geq 0.01$). As apparent from Table 1 there were significant differences in BMI ($p \geq 0.01$) in control group when matching with the diabetics group, but both in the normal range of BMI. A decreased BMI in the patient group is due to stop producing insulin hormone, which is required in glucose metabolism, the major type of sugar in the blood, and make the body can't use it correctly, then the calories away From the frame in urine. As a result, kids and adults who broaden kind 1 diabetes can shed pounds despite having an ordinary or improved appetite.^{18,19} The results proves the full-size variations in age on top of things and diabetes organization.

The result in Figure 1 show PCR analysis of OGG1 polymorphism with amplicon amplified DNA in a size 207 bp fragment Figure 2 shows the results of PCR products of the OGG1 gene after FUNHI enzyme digestion represented homozygous (CC-207pb), and heterozygous (CG-100 bp, 107 bp, 207 bp).

The outcomes display desk (3) and Figure 3, Sequence ID: dbj and have range score 345 bits, count on 8e-81, identities 100% and gap 0% and different them (affected person) seemed 99% compatibility with Homo sapiens for OGG1 kind 1 gene, from 376 to 473 number of nucleotide from gene of gene financial institution effects as proven in Table 4 and Figure 3 (037881.1 and feature variety score 147 bits, count on 1e-sixty

Table 1: AGE and BMI for the patients group and control group:

Parameters	Mean ± SD		p-value
	Patients	Control	
Age (year)	28.43 ± 8.10	29.43 ± 7.43	0.3450
BMI (kg/m ²)	24.94 ± 5.23	27.98 ± 2.20	0.632

significant differences at ($p \geq 0.01$)*

Table 2: Distribution of genotype OGG1 gene C/G and C/C polymorphism in T1DM patient and healthy control.

Polymorphism	Patients	Control	P-value
CC	20 (32.43%)	25 (100%)	0.05 **
CG	55 (74.76%)	0 (0.00%)	0.05 **
GG	0 (0.00%)	0 (0.00%)	1.00 NS
P-value	0.05 **	0.05 **	---
<i>Allele freq.</i>			
C	0.75	1.00	---
G	0.94	0.00	---

** ($p > 0.05$), NS: Non-significant.

Table 3: Sequencing ID in gene bank, score, expects and compatibility of DNA sequences obtained.

Organism	Sequence ID	Score	Expect	Identities	No. nucleotide	Type of sample
1	<i>Homo sapiens</i> dbj 037881.1	150	8e-81	100%	376 to 473	Patient and control
2	<i>Homo sapiens</i> dbj 037881.1	147	1e-69	99%	376 to 473	Patient

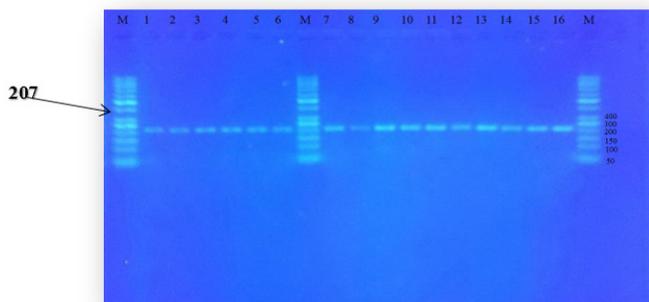


Figure 1: PCR analysis of OGG1 genes in the condition (2%) agarose gel electrophoresis (2 hr/70v). Amplified DNA in a 207 bp fragment, Lane M, DNA marker. Lane (1-6), negative control. Lane (7-16) is samples.

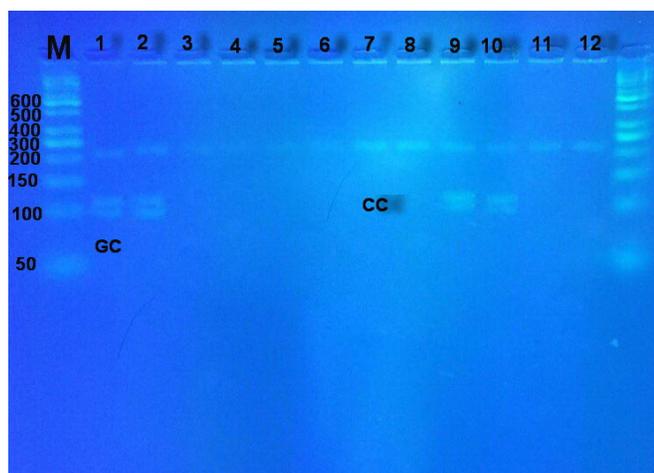


Figure 2: Photograph of the PCR products of the OGG1 gene after FUNH1 enzyme digestion and electrophoresis at 4% agarose gel (2.5 hr/70v). Show lane (3,4,5,6,7,8,11,12) homozygous (CC-207pb), lane (1,2,9,10) heterozygous (CG - 100bp, 107bp, 207bp).

nine, identities 99% and hole 0%. And the Figure 4 shows sample curves that represent each of the two genotypes Figure 5 shows sample Nucleotide sequence chromatogram representing two genotype of OGG1 gene C/C homozygote, and the G/C heterozygote.

There are undoubted experimental and clinical proofs that long-term duration and hyperglycemia would generate ROS, which is increased in both types of diabetes and that the onset of diabetes is closely related to oxidative stress.^{13,14} This study follows other research that demonstrated the correlation of genetic polymorphism of OGG1 with many diseases, T2DM, and cancer. The Ser326Cys polymorphism exon 7 within the OGG1 gene sequences is currently receiving quite a little attention, and researchers are looking to discern if there is a hyperlink between it and diabetes kind 1 susceptibility.¹⁵ Table 3 indicates that the Ser/Ser and Ser/Cys genotype quotes have been 86.67 and 93.33%, respectively, and that the

Homo sapiens mRNA for OGG1 type 1g, partial cds Sequence ID: dbj|037881.1|

Score	Expect	Identities	Gaps	Strand
278 bits(150)	7e-71	150/150(100%)	0/150(0%)	Plus/Plus
Query				21
CAGACTCCACCCTCCTACAGGTGCTGTTTCAGTGCCGACCTGCGCC				
AATCCC GCCATGCTC 80				
Sbjct				322
CAGACTCCACCCTCCTACAGGTGCTGTTTCAGTGCCGACCTGCGCC				
AATCCC GCCATGCTC 381				

Query	81
AGGAGCCACCAGCAAAGCGCAGAAAGGGTCCAAAGGGCCGGA	
AGGCTAGATGGGGCACC 140	
Sbjct	382
AGGAGCCACCAGCAAAGCGCAGAAAGGGTCCAAAGGGCCGGA	
AGGCTAGATGGGGCACC 441	

Query 141 CTGGACAAAGAAATCCCCAAGCACCTTCC 170	
Sbjct 442 CTGGACAAAGAAATCCCCAAGCACCTTCC 471	

Figure 3: OGG1 gene Sequencing for population study obtained from Gene Bank. Query represents of sample; Subject represent of database of National Center Biotechnology Information (NCBI).

Homo sapiens mRNA for OGG1 type 1g, partial cds Sequence ID: dbj|AB037881.1|

Score	Expect	Identities	Gaps	Strand
272 bits(147)	1e-69	149/150(99%)	0/150(0%)	Plus/Plus
Query				20
CAGACTCCACCCTCCTACAGGTGCTGTTTCAGTGCCGACCTGCGCC				
AATG CCGCC ATGCTC 79				
Sbjct				322
CAGACTCCACCCTCCTACAGGTGCTGTTTCAGTGCCGACCTGCGCC				
AATCCC GCC ATGCTC 381				

Query	80
AGGAGCCACCAGCAAAGCGCAGAAAGGGTCCAAAGGGCCGGA	
AGGCTAGATGGGGCACC 139	
Sbjct	382
AGGAGCCACCAGCAAAGCGCAGAAAGGGTCCAAAGGGCCGGA	
AGGCTAGATGGGGCACC 441	

Figure 4: OGG1 gene Sequencing of for cases of (patient), obtained from Gene Bank. Query represents of sample; Subject represent of database of National Center Biotechnology Information (NCBI).

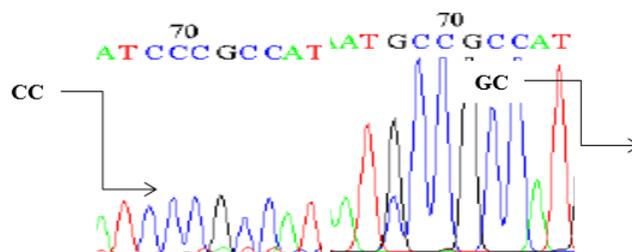


Figure (5): chromatogram representing Nucleotide sequence two genotype of OGG1 gene. C/C homozygote, and the G/C heterozygote.

Cys/Cys genotype frequency become no longer recognized, neither in diabetes mellitus kind 1 nor in controls (0.00%).¹⁶ The allele frequencies for serine (wild kind) and cysteine (version) have been 0.63 and 0.37, respectively. With the OGG1 Ser326Cys polymorphism, the genotype and allele frequencies said from diabetes patients have been not extensively different from those observed on top of things individuals. In Table 3, the sequencing of amplified made from OGG1 gene from (healthful and patient) out of them regarded a 100% compatibility and different them (patient) appeared 99% compatibility with Homo sapiens for OGG1 type 1 gene. In current years, much research confirmed the critical role of OGG1 gene polymorphism in various illnesses consisting of cancer risk. This has a look at complying with every other research demonstrated the correlation of genetic polymorphism of OGG1 with many ailment diabetes mellitus type2, most cancers.^{17,18}

This present study exhibits huge differences in OGG1 polymorphism in diabetes mellitus type1 affected person with admire the two special types of polymorphism takes place in patients (CC, GC). There are undoubted experimental and medical proofs that long time of duration and hyperglycemia would generate ROS, which is increased in both forms of diabetes and that the onset of diabetes is carefully associated with oxidative stress. Our consequences in DM1 observed the role of hyperglycemia in the production of ROS. It has been proposed that the long duration of DM1 and hyperglycemia can produce continual oxidative strain by the glucose oxidation pathway main to an extra in mitochondrial superoxide production,¹⁹ that may inhibit expression of the 8-oxoG-DNA glycosylase1 (OGG1), one of the key restore enzymes for DNA oxidative harm. ROS cause strand breaks and base adjustments in DNA, including the oxidation of quinine residues to 8-hydroxy-2-deoxyguanine (8-OHdG). This lesion is powerful mutagen and might generate transversion (substitution) of C/G serine with cytosine in codon 326 which lower the interest of the DNA restore enzyme.²⁰

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