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Catalase Gene Polymorphism in Patient with Diabetes Disease Type 1 in Karbala City

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Abstract

Diabetes Mellitus (DM) is a collection of metabolic diseases categorized by hyperglycemia caused by insulin excretion deficiencies, insulin performance, or both DM are categorized into distinct kinds. Type 1 diabetes (T1D) is defined by breakdown of β-cells, generally resulting in complete insulin deficiency. Type 2 diabetes (T2D) is a consequence of peripheral tissue insulin resistance. a complete of fifty patients and fifty control subjects were collected between February to October 2018. The genotyping of catalase were attained consuming polymerase chain reaction (PCR) in addition to restriction fragment length polymorphism (RFLP). We tend to firm vigorous changes within the genotype occurrences of catalase between patients with diabetes and controls using the P=0.038, OR =0.58 (0.48-0.78).

Keywords: *Diabetes, RFLP, catalase, SNPs. Polymorphisms.*

Introduction

Type 1 diabetes mellitus (T1DM) is the greatest public metabolic disturbance in which together hereditary and environmental aspects were complicated [1] .It had been assumed that while kids had a hereditary predisposition to T1DM, there are expected to an environmental aspect that activates progress of T1DM. The initiates have proposed involve viral contagion, vaccines, vit D lack , and cow's milk [2] .

The corrosion of in elevation levels of glucose in diabetic cells products extra electron givers (NADH and FADH2) and rises the electron transmission, thus generating superoxide, spare generation of ROS like superoxide (O2 ●_), hydrogen peroxide (H2O2), and hydroxyl radical (●OH) and responsive nitrogen classes like nitric oxide oxidize aim cellular proteins, nucleic acids, or lipid layers and destruction their cellular arrangement and function [3] .

Overproduction of free radicals i.e., oxidative stress may effect oxidative destruction to lipids, proteins and DNA, finally leading to several chronic diseases like diabetes, myocardial infarction, cardiovascular diseases, atherosclerosis, stroke and other degenerative diseases in humans [4] . Moreover, hyperglycemia-induced generation of reactive oxygen species (ROS) on the mitochondrial level is the original trigger of malicious rotation of oxidative stress in DM [5] .

Catalase (CAT) is present in peroxisomes and occurs as a dumbell-designed tetramer of 4 equal axillary units. CAT quickly catalyzes hydrogen peroxide breakdown into less proactive oxygen and water particles. Insufficiency of catalase has been identified to direct to T2DM improvement [6] .Catalase gene is positioned on chromosome 11p13. Exon 2 and adjoining introns of the catalase gene were supposed to modification heated stains aimed at T2DM vulnerability [7] ..Adjustment of cysteine to cysteic acid to tyrosyl nitration of CAT and reduced activation in cases of oxidative stress [8] .In type 1, 2 and gestational cancer (GD)and disorders such as diabetic retinopathy (DR), diabetic nephropathy (DN), ischemic heart disease (IHD) and CVD [9] , the exon 9-262C / T polymorphism in the catalase gene was screened. In addition, this purposeful polymorphism funded T2DM's progress and its issues [10]. The A / T polymorphism genotype of the CAT gene ' AT ' can elevate the hazard of T2DM in northern Indians [11].

Methodology

Sampling

Fifty blood samples had gathered of patients with

primary diabetes whose visits diabetes Center /Hilla/Iraq and fifty samples as control.

DNA Extraction

Genomic DNA of complete blood cells was derived and filtered by Extraction and filtration Kit of Favergen Company (Taiwan).

Using RFLP- PCR amplification for Genotypic Identification

The directed positions of DNA were intensified using special primers was designed: collected of Bioneer, IDTDNA (USA). Primer: Frontward categorization was 5- CTGGGTATCTCCGGTCTTCA -3, and opposite categorization was 5- CCGCTTTCTAAACGGACCTT-3.

PCR was done in 20µl response measurements covering 1 µl of back and forward primers, 12.5 µl of Green Master Mix, 3 µl of Genomic DNA, and the reaction volume was finished up to 20 ul by containing with 2.5 µl of Nuclease allowed water. Intensification had been completed in a thermo-cycler customized at 94 ° C for 2 min ; 5 minutes at 94 ° C for 35 cycles, 1 at 57.8 ° C and 1 at 72 ° C; and a last five mins extension. PCR products were electrophoresed using 1% agarose gel electrophoresis at 75 V for one hour using ethidium bromide. Photographs had been obtained utilizing the context of gel documentation. Through the Promega Company Protocol, the PCR item became removed utilizing Hinf1 restriction endonuclease, the PCR-RFLP technique achieved stable. The next ingestion of MSPI feedback was electrophoresis utilizing gel electrophoresis (Cleaver Scientific–UK) in 3% agarose gels at 75 V of one hour and 8% polyacrylmide gel electrophoresis control associated with the following: 75 V, 20 Am for 160 minutes. Gels pictured subsequently by ethidium bromide. Utilizing gel documentation system (EBOXCX–UK) photos were obtained.

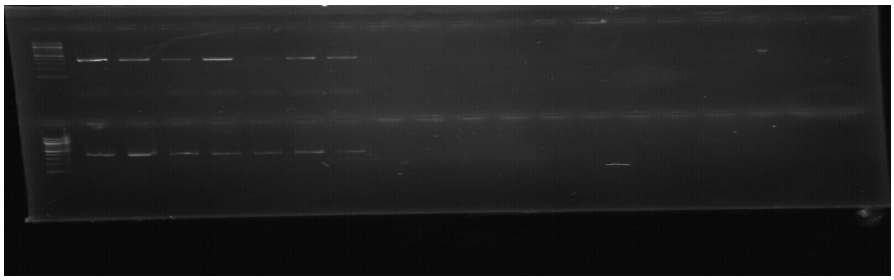
Statistical Method

The SPSS applied mathematics software system (17 ; SPSS Inc., Chicago, IL) was finished with total practical math analysis, and P- values < 0.05 were thought to be statistically important.

Results

study Genotype result

Deoxyribonucleic acid had been extracted from the blood sample Figure (1) revealed the catalase gene amplifying consumer's agarose gel electrophoresis shot, measuring up to 369 bp portion. Issues of PCR-RFLP system catalase-related quality polymorphisms utilizing Hinf1 containment chemistry disclosed that there are three instances of genotype polymorphisms, which involve homozygous genotype (2 band, 200,175 bp), The second type was homozygous genotype (1 band 369 bp) and third example stayed heterozygous genotype (3 bands , 200,175 and 369 bp) which came about three DNA groups



Fig(1) (Agarose gel electrophoresis of Catalase) amplification produces of Diabetes and control groups.

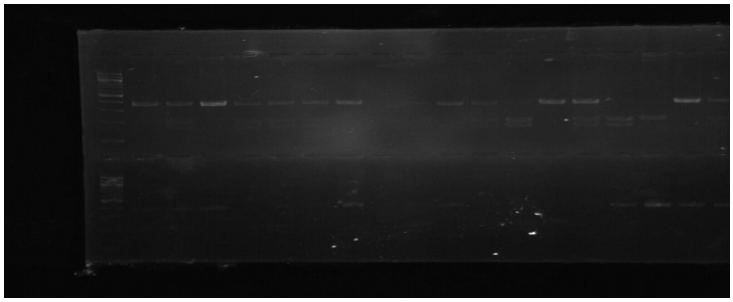


Fig 2: Agarose gel electrophoresis of Diabetes and healthy subjects allelotyping of catalase using Hinf1 enzyme by PCR-RFLP method

Table (1): genotyping distribution of Catalase gene polymorphism and there association in control and patients groups.

Genotype	control No. (%)	patients No. (%)	*P*-value	OR (95%) CKD
1 band [a]	13 (81.25%)	18 (52.29%)		
2 band	3 (19.75%)	9 (26.4%)	0.25	2.16 (0.48-9.60)
3 band	0 (0%)	7 (20.58%)	0.038*	0.58 (0.48-0.78)
Total	16	34		

refrence

CTGGGTATCTCCGGTCTTCAGGCCTCCTTCGGAGAGCCCTGCTCCGAGCCCAT

TGGGCTTCCAATCTTGGCCTGCCTAGCGCCGAGCAGCCAATCAGAAG

190305-040_I01_5_ .....

190305-040_K01_6_ .....

190305-040_M01_8_ .....C.....

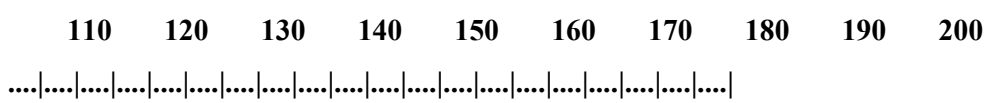
190305-040_O01_16_ .....C.....C.....C...

190305-040_A03_24_ .....A.....A.....C...

190305-040_M03_10C_ .....C....C...

190305-040_A03_24C_ .....A.....A.....C...

190305-040_A05_25C .....



refrence

GCAGTCCTCCCGAGGGGGCGGGACGAGGGGGTGGTGCTGATTGGCTGAGCCT

GAAGTCGCCACGGACTCGGGGCAACAGGCAGATTTGCCTGCTGAGGGT

190305-040_I01_5_ .....T.....

190305-040_K01_6_ .....T.....

190305-040_M01_8_ .....T.....

190305-040_O01_16_ ...C.....A.....C..A.....A.....C.....A.....C.....A.
190305-040_A03_24_ .....T.....
190305-040_M03_10C_ .....T.....
190305-040_A03_24C_ .....T.....
190305-040_A05_25C .....T.....

210 220 230 240 250 260 270 280 290 300
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|

GGAGACCCACGAGCCGAGGCCTCCTGCAGTGTTCTGCACAGCAAACCGCACGC
TATGGCTGACAGCCGGGATCCCGCCAGCGACCAGATGCAGCACTGGA
190305-040_I01_5_ .....C.....
190305-040_K01_6_ .....C.....
190305-040_M01_8_ .....C.....
190305-040_O01_16_ .C.C.....A....T.....C.....T.....
190305-040_A03_24_ .....C.....
190305-040_M03_10C_ .....C.....
190305-040_A03_24C_ .....C.....
190305-040_A05_25C .....C.....

310 320 330 340 350 360
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|

refrence
AGGAGCAGCGGGCCGCGCAGGTACACTCTGTGCTCCCCGAGCGGGCCCGAAG
GTCCGTTTAGAAAGCGG
190305-040_I01_5_ .....
190305-040_K01_6_ .....
190305-040_M01_8_ .....
190305-040_O01_16_ .....
190305-040_A03_24_ .....
190305-040_M03_10C_ .....
190305-040_A03_24C_ .....
190305-040_A05_25C .....

Figure (3): Arrangements alignment ID: catalase gene fragment for *Homo sapiens* by use Bio Edit program

Discussion

Firstly, catalase gene polymorphism was studied in Diabetes conditions and controls. The spreading witnessed in catalase gene polymorphism in control set and conditions clusters are revealed into tab (1) maximum genotype in control group remained homozygote genotype 1 band (81.2%) followed by homozygote genotype 2 band (19.1%) and mutant heterozygote genotype 3 bands (0%) and. In Diabetes disease , peak genotype in Diabetes group was homozygote genotype 1 band (52.2%) followed by homozygote genotype 2 band (26.4%) and mutant heterozygote genotype 3 bands (20.58%). Sequencing findings proven haplotypes detected in our work. Various single nucleotide polymorphism (SNP), were taken between DNA polymorphisms (1, 2 and 3 bands) and Catalase NCBI Primer3 ^{plus} reference**.** Worldwide, DM is one of the maximum non-communicable diseases in the public. As per the International Diabetes Federation (IDF), they had 366 million persons among 20-79 years of age who had diabetes [12]. IDF also reported that DM in 2011 culminated in as many as 4.6 million fatalities [13]. Among kids, T1D has the greatest prevalence of various kinds of DM, with the largest occurrence recorded in Finland and Sardinia and the lowest in China and Venezuela [14]. Among the youngest kids, particularly among the European population, the rise is the highest. "The number of children developing this form of diabetes every year has increased rapidly all over the world, except in Central America and West Indies where the trend is decreasing (14). However, latest research regarding incidence of T1D for children in Finland highlighted two significant changes in the trends between 1980 and 2011". The study revealed up to 2005 an annual rise, accompanied by a plateau until the later part of 2011 [15] . A comparable plateau incidence from 2005-2007 [16] and Norway from 2004-2012 [17] has also been recorded by Sweden. "Catalase is one of the main enzyme components of cell defense counter to oxidative stress and it had been hypothesized that the polymorphism CAT decreases the antioxidant capacity and can serve as a risk element for oxidative stress associated diseases. The association among SOD1-251 A/G, CAT-21 A/T, and GPX1-198C/T antioxidant gene polymorphisms in the risk of cataract was informed between Chinese population" [18]. Our results showed significant differences between patients and controls, Other results showed that CAT polymorphism was linked to enhanced hazard and began playing an essential role in diabetes pathogenesis [19]. On the other side, little reviews had revealed the polymorphism of the catalase gene not related to the risk of cardiovascular diseases among type 2 diabetes mellitus in Finnish population [20] and also among type 1 diabetes mellitus patients in Czech population [21]. Other study exhibited No association between the catalase polymorphism and DM was detected in diabetic patients of type 2 Caucasian-Brazilian. That's the earliest research planned for our data to investigate the relation between a polymorphism of the catalase gene and diabetic problems of patients with type 2 diabetes. Though catalase has mostly been researched, little studies have been specially examined relations of catalase polymorphisms through diseases [22] To conclude, our findings provision the hypothesis which the polymorphism in the catalase enzyme connected to both the progress for diabetic retinopathy, diabetic nephropathy or ischemic heart disease popular Iraqis with type 2 diabetes.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the College of Nursing, University of Warith Al-anbiya'a and all experiments were carried out in accordance with approved guidelines.

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