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Genetic Polymorphisms of Catalase Enzyme with Hypertension Patients in Babylon Governorate

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Abstract

Oxidative stresses are affected in an inequality among the creation of reactive oxygen species (ROS) and a biological system's capability to voluntarily purify responsive intermediates or simply healing the causing destruction. Catalase was an antioxidant enzyme that acting a most important role in monitoring hydrogen peroxide focus at human cells. H2O2 is dissolved in H2O and O2 by CAT, Protecting the oxidative stress cells. It has proposed which helpful polymorphism moves the enzyme action within the gene coding catalase enzyme, thereby reducing safety against oxidative stress. Between February and October 2018 a total of thirty-five patients and fifteen control subjects were gathered. The genotyping of catalase were achieved consuming polymerase chain reaction (PCR) in addition to restriction fragment length polymorphism (RFLP). we tend to determine no significant difference within the genotype frequencies of catalase among patients with hypertension and controls using the *P*=0.06, OR =7.36 (0.77-69.5)).

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

Introduction

Essential hypertension (HTN) was a popular multifactorial disturbance which includes complex hereditary, vasoconstrictive, environmental and other danger causes [1]. Several family trials have acknowledged the genetic nature of vital hypertension: equivalent to 30% of the changeability of blood pressure has been assessed for genetically specified and the genetic susceptibility of an individual to hypertensive illness sequence of 15 to 35% [2]. The major neurohumoral mechanics included in universal blood pressure ruling and hypertensive barriers have been well known and involve aldosterone renin-angiotensin system, the sympathoadrenal system, the kallikrein-kinin system [3] and others. Moreover, various enzymes like catalase disturbing the metabolism of resident tissue defending or destructive factors [4]. Various enzymes had been designated for participating to blood pressure deregulation. "Responsive classes of oxygen may trigger oxidative hassles that have been shown to play a important role in the pathogenesis of many diseases such as cancer, hyperlipidemia, diabetes mellitus, metabolic disorders, atherosclerosis, cardiovascular diseases (hypertension, ischemic heart disease, chronic heart failure) and neurodegenerative diseases [5]". Typically antioxidant enzyme genes are susceptible to polymorphism and may lead to gene expression modification and reduce enzyme activity [6]. It is shown that various metabolic disorders are associated with the functions of altered antioxidant enzymes [7]. Due to its largest turnover level, catalase (CAT) is one of the strong antioxidant enzymes and exists in almost all aerobic respiratory organisms [8]. Catalase (CAT) is an intracellular antioxidant enzyme that prevents cells against ROS damage by converting hydrogen peroxide into water and oxygen to avoid cell damage [9] People with decreased CAT activity have been highly risky of oxidative stress-related diseases like atherosclerosis and diabetes [10]. Dyslipidaemia18and neurodegenerative disease, Furthermore, the administration of CAT has been shown to avoid bone loss induced by ovariectomy [11]. Recently, CAT gene polymorphisms have been associated with hypertension [12]. However, to our knowledge, Despite CAT's allegedly significant role in bone metabolic and vascular stability and activity, its genetic impacts on hypertension have not yet been researched. This research therefore explored the genetic impact of CAT on the danger of hypertension for the

first time.

Methodology

Sampling

Thirty Five Samples of blood were gathered patient with primary Hypertension whom visit hypertension Center / Hilla/Iraq and fifteen samples as control.

DNA Extraction

Genomic DNA from entire blood cells has been obtained and purified using the Favergen Company (Taiwan) extraction and purification kit.

Genotypical identification by amplification of RFLP-PCR

Specific primers were used to amplify the targeted DNA sites: took of Bioneer, IDTDNA(USA). Primer: Sequence straight on was 5-CTGGGTATCTCCGGTCTTCA -3, Then the opposite pair was 5- CCGCTTTCTAAACGGACCTT-3.

PCR was performed in 20µl response sizes comprising 1 µl of inverse and frontward primers, 12.5 µl of Mix of Green Master , 3 µl of Genomic DNA and a response quantity of up to 20 µl including Nuclease-free water 2.5 µl had been finished. Intensification was completed in a customized thermo-cycler at 94 ° C for two mins; at 94 ° C for 35 cycles Every 5 mints, at 57.8 ° C for one moment and by 72 ° C for one minute; and a last 5 minute extension. Using ethidium bromide, PCR medicines used 1% agarose gel electrophoresis at 75 V for 1 hour. Photographs were taken using the gel documentation structure. The PCR item was sliced using endonuclease restraint of Hinf1, with the Promega Company Protocol the PCR-RFLP technique was achieved steadily.

After digestion, electrophoresis utilizing gel electrophoresis (Cleaver Scientific–UK) was linked to MSPI reaction in 3 percent agarose gels at 75 V for 1 hour and 8 percent polyacrylmide gel electrophoresis control: 75 V, 20 Am for 160 minutes after the ethidium bromide gels were pictured. The gel documentation system (EBOXCX–U.K.) used to take photographs.

Statistical Method

SPSS applied mathematics software scheme (17; SPSS Inc., Chicago, IL) was finished with all the practical math analysis, P-values < 0.05 statistically significant were regarded.

Results

Genotyping study

deoxyribonucleic acid had removed as of the sample blood Figure (1) showed the electrophoresis agarose gel shot of catalase gene amplification product measuring up to 369 bp portion. The effects of PCR-RFLP's catalase-related quality polymorphisms using Hinf1 containment chemistry revealed that there were three instances of genotype polymorphisms, involving homozygous genotype (2 bands, 200,175 bp), homozygous genotype (1 band, 369 bp) and heterozygous genotype (3 bands, 200,175 and 369 bp) that originated from about three DNA groups.

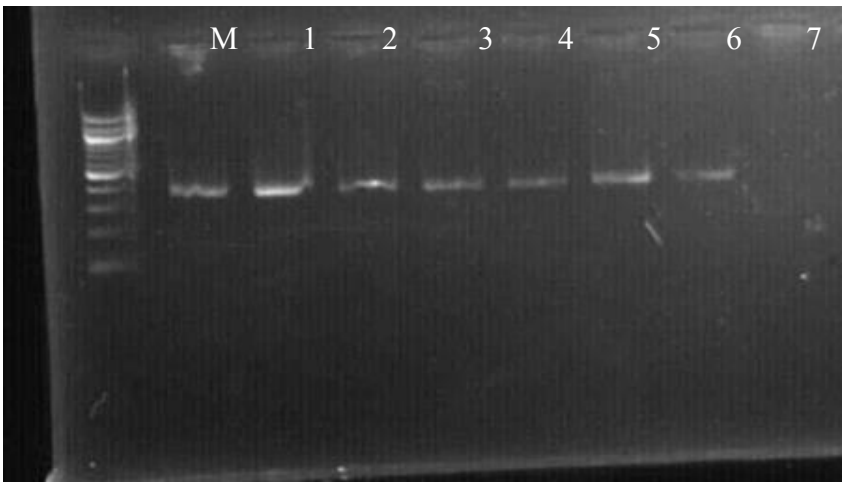

Fig 1. Agarose gel Catalase electrophoresis (Hypertension and control group) amplification products.

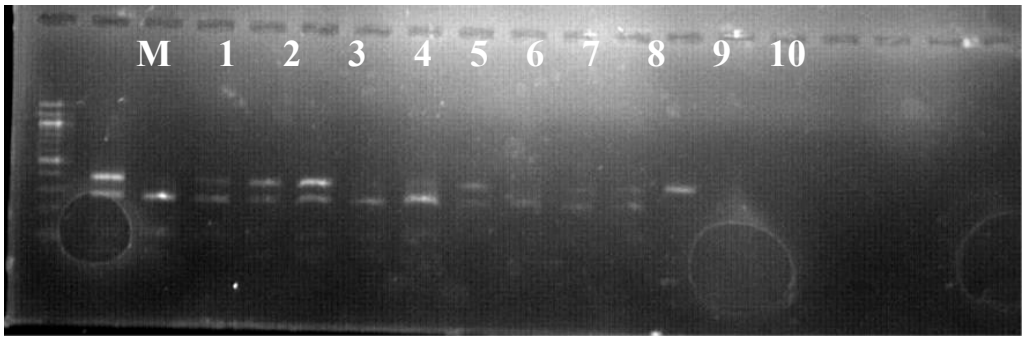

Fig. (2): Agagrose gel electrophoresis of Hypertension and healthy subjects allelotyping of catalase using *Hinf1* enzyme by PCR-RFLP method

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	9 (60%)	11 (31.4%)		
2 band	5 (33.3%)	15 (42.8%)	0.16	2.45 (0.64-9.39)
3 band	1(6.66%)	9 (25.7%)	0.06	7.36 (0.77-69.58)
Total	15	35		

P ≤ 0.05; OR=(95%CI); [a] reference

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                10        20        30        40        50        60        70        80        90       100
                ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
refrence
CTGGGTATCTCCGGTCTTCAGGCCTCCTTCGGAGAGCCCTGCTCCGAGCCCAT
TGGGCTTCCAATCTTGGCCTGCCTAGCGCCGAGCAGCCAATCAGAAG
190305-040_C03_36_  ..................................................................................................
190305-040_E03_79_  ..................................................G..GA..........A.....C...
190305-040_G03_80_  ..................................................G.............AA.C...C...
190305-040_I03_82_  ..................................................G..GA..........A.....C...
190305-040_K03_83_  ..................................................................A.G..C...
190305-040_M03_10C_ ..................................................................C...C...
190305-040_A03_24C_ ..................................................A.............A.....C...
190305-040_A05_25C  ..................................................................................
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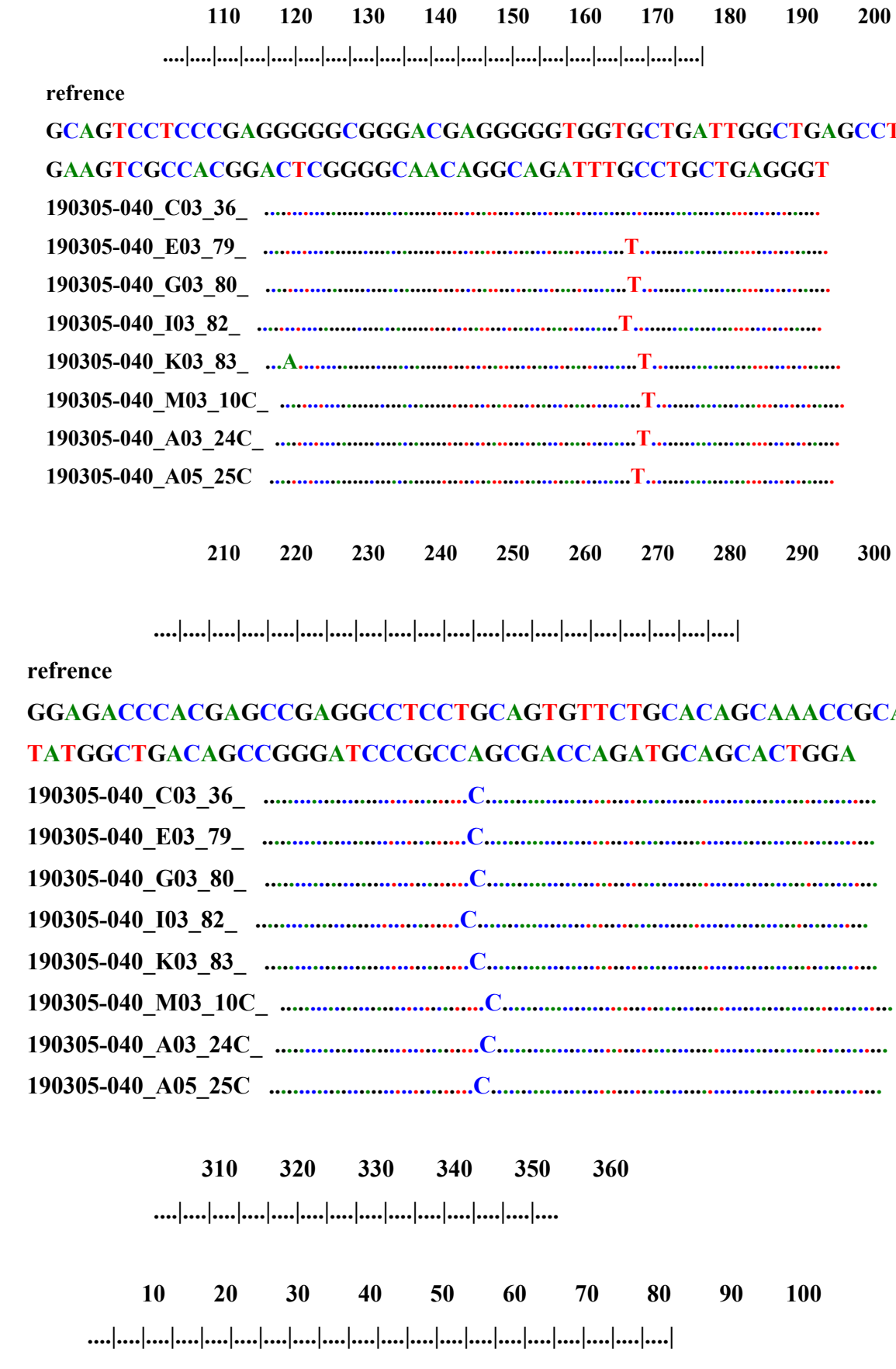
110 120 130 140 150 160 170 180 190 200
refrence
GCAGTCCTCCCGAGGGGGCGGGACGAGGGGGTGGTGCTGATTGGCTGAGCCT
GAAGTCGCCACGGACTCGGGGCAACAGGCAGATTTGCCTGCTGAGGGT
190305-040_C03_36_
190305-040_E03_79_
190305-040_G03_80_
190305-040_I03_82_
190305-040_K03_83_
190305-040_M03_10C_
190305-040_A03_24C_
190305-040_A05_25C
210 220 230 240 250 260 270 280 290 300
refrence
GGAGACCCACGAGCCGAGGCCTCCTGCAGTGTTCTGCACAGCAAACCGCACGC
TATGGCTGACAGCCGGGATCCCGCCAGCGACCAGATGCAGCACTGGA
190305-040_C03_36_
190305-040_E03_79_
190305-040_G03_80_
190305-040_I03_82_
190305-040_K03_83_
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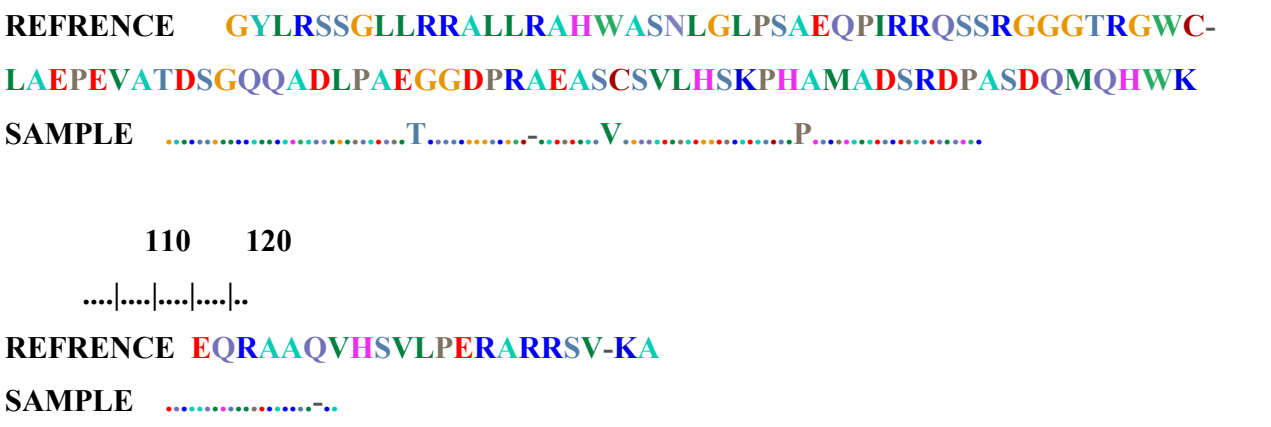

Fig (3) Protein Sequences alignment: results for *Homo sapiens* catalase gene fragment version 7.2.5 of the Bio Edit program

Discussion

The catalse gene polymorphism was studied of hypertension and checks. The distribution found in catalase gene polymorphism in control group and group of instances is shown in Table (1): homozygote genotype was the largest genotype in control group 1 band (60%) followed by homozygote genotype 2 band (33%) and mutant heterozygote genotype 3 bands (6.66%) and, in hypertension disease Homozygous genotype 2 band (42.8%) and homozygous genotype 1 band (31.4%) false genotype 3 bands (20.7%) were the largest genotype. Followed by the results of sequencing, confirmation of the haplotypes found in our job. A lot of single nucleotide polymorphism (SNP) has been taken among DNA polymorphisms (1-, 2- and 3-band) and Catalase NCBI Primer3 and more reference.

Our group's genetic studies have effectively resulted in no important connection between patients and control, while other trials have effectively resulted in the identification and correlation of particular polymorphisms connected with regulation of blood pressure [13]. There have been numerous genetic markers to be acknowledged on the basis of volunteers cardiovascular system dysfunctions and hypertensive patient kidneys, as well as the already recognized HTN physiology pathway. There are several significant constraints in the attempt to detect genes connected with vital HTN: the big amount of genes that can control blood pressure, the combined impact of their expression, the potential genetic polymorphisms of each gene, the patient's phenotypic heterogeneity, as well as various environmental variables that influence blood pressure [14].

"An association between CAT and hypertension is compatible with a research in China demonstrating the association of homozygous people with CAT-844 AA and high blood pressure [15]". "However, in this Chinese population, the CAT-844 G allele is more common (68.4%) than the two groups recorded here (41.1% for African Americans and 36.2% for Caucasians). This can be result of variations in population or selection bias in the Chinese sample that involved only hypertensive topics. The CAT-262 SNP has not earlier been examined for blood pressure values, although the CAT-262 T allele is linked with the catalase gene's greater expression level [16]".

The CAT enzyme is capable of controlling oxidative stress by hydrogen peroxide degradation [17]. In the promoter region of the CAT gene, polymorphism may decrease gene expression, eventually decreasing enzymatic activity and increasing oxidative stress [18].

To sum up, these findings indicate that CAT gene polymorphisms are unlikely to related with hypertension vulnerability. This research is, to our understanding, the first report showing that CAT polymorphisms and haplotypes are not correlated with hypertension.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Adult Nursing Department, College of Nursing, University of Babylon, Iraq and all experiments were carried out in accordance with approved guidelines.

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