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Association of Genetic Polymorphism of Catalase in Diabetes Type 2 Susceptibility in Vindhyan Region of Madhya Pradesh India

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ABSTRACT

Type 2 diabetes mellitus (T2DM), is a heterogeneous, multifactorial, polygenic syndrome which is consequences from insulin receptor dysfunction. It is an effect of oxidative stress bring out by interactions of reactive metabolites (RMs) with lipids, proteins and another process of human body. Catalase is a primary antioxidant enzyme inaugurate the primary defense against oxidative stress. In this study, we scrutinize the association of genetic polymorphism of Catalase and the role of catalase activity in diabetes type 2 susceptibility. The blood samples were collected from 450 type 2 diabetic patients attending the outpatient department and admitted to the SSMC. The age of patients ranged from 40-65 years of both sexes. Reactive oxygen species produced by hyperglycemia altered structure and function of lipids, proteins and another molecules. This study was attempted to investigate the association between gene polymorphism of selected antioxidant enzymes. The 450 type 2 diabetes mellitus patients were analyzed for SNP in catalase gene. Restricted fragment length polymorphism studies showed 80% of the patients were TT genotype and 20% were of heterozygous genotype. No AA genotype was observed in our group of study. Observed that patients with T allele had significantly lower fasting plasma glucose, and HbA1c.It is suggestive of feasible association of heterozygocity with poor glycemic control and high catalase activity. It has been concluded that the level of enzyme was found to be significantly high in heterozygous group. High activity may be secondary to high oxidative stress as a result of poor glycemic control. In heterozygous patients the association of high catalase activity with this SNP should be precisely contemplate because early study has described that there is no statistically significant complicated association of CAT alleles and this activity.

Introduction

Diabetes mellitus is a polygenic metabolic disease characterized by hyperglycemia resulting from defects in both insulin secretion and its action [1]. Both the genetic and the environmental factors are involved in the development of type 2 diabetes mellitus [2]. Superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione-S-transferase (GST), nitric oxide synthase (NOS) are antioxidant enzymes associated with in scavenging RMs in normal individuals. Functional polymorphisms of these antioxidant enzymes have been described to be involved in pathogenesis of Type 2 diabetes individuals.

The moderate levels of antioxidant enzymes or their non-functionality effect in extravagant RMs which commence stress associated pathways thereby preeminent to insulin resistance and Type 2 diabetes. Catalase (EC 1.11.1.6) is an omnipresent enzyme present in almost all eukaryotic organisms. It has a very high turnover number, breakdown hydrogen peroxide into oxygen and water at an enormously high rate. Together with superoxide dismutases and glutathione peroxidase, catalase constitutes a primary defense against oxidative stress [1].Catalase is an antioxidant enzyme that plays a major role in controlling hydrogen peroxide concentration in human cells. It decomposes H_2O_2 into H_2O and O_2, thereby preserve the cells from oxidative stress. It has been suggested that functional polymorphism in the gene encoding catalase enzyme affects the

enzyme activity, thereby decreasing the protection against oxidative stress[2]. Oxidative stress is defined as a interruption in the balance between the generation of reactive oxygen species (ROS) and antioxidant defenses, together with enzymatic and non-enzymatic systems [3,4]. ROS is mainly produced within the mitochondria in lens epithelium cells (LECs) and the outermost fiber cells, which are extremely reactive. A precise level of ROS is critical for the proper regulation of cell functions, such as intracellular signal, transcription activation, cell proliferation, inflammation, and apoptosis, but elevated amounts of ROS are harmful to macromolecules [5].

Apart from the decomposition of H_2O_2, catalase pacifies many environmental mutagens. It also obstructs chromosomal abnormality caused by hypoxanthine/xanthine oxidase in Chinese hamster cells [6]. Different studies have reported on the modification in catalase activity in hypertension [6], cancer [2],diabetes, nephropathy [7,4],and other diseases assisted by oxidative stress. Catalase is a homotetramer of220–230kDamasshaving four heme groups in its structure [8, 9]. The CAT gene is localized on chromosome 11p 13.31 consisting of 13 exons and 12 introns. CAT (-21 A/T) variant represents the A to T substitution in the promoter region, which is located inside the promoter region just proximal to the start site [8]. The mutant allele frequency may vary based on the variations in the study population's race and ethnicity and also on the fundamental characters. Other reports accomplish in animals indicate that the over expression of CAT can increase the lifespan, decrease the blood pressure (BP), and delay the onset of atherosclerosis [11, 12, 13]. However, clinical trials disclose contention findings regarding relationship between activity of CAT and pathology due to the divergent consequences [11].

This study which has been determined on the genetic variant -21A/T of the catalase enzyme, was the first kind concerning the comorbidities of catalase gene variant in healthy individuals among vindhyan region population.

The objective was to find out the association of mutant allele frequency of the evaluated catalase (-21 A/T) gene polymorphism in healthy individuals among the vindhyan regions population.

Materials and Methods

Study Design

The study population consisted of 470 controls with no history of cardiovascular disease, diabetes, hypertension, cancer, or any infectious diseases and 450 patients with type 2diabetesmellitus, belonging to vindhyan region population. The patients included were characterized based on the variations in blood sugar level (>126mg/dL). The samples from the subjects were collected into EDTA coated tubes and the informed consent was obtained. Clinical data encompass information on duration of diabetes, presence of any complication, history of other disorders, age, gender, lipid profile, blood sugar level and systolic anddiastolicbloodpressurewerecollectedusingaquestionnaire.Ethicalclearancewasobtainedforthisstudy.

DNA Extraction: Genomic DNA was extracted from the frozen blood by phenol-chloroform method [15]. For DNA extraction, 500μL of blood was used and the isolated DNA dissolvedinTEwasstoredat−20∘C.ThequalityoftheDNA was checked in 1% percent agarose (Hi-Media, Mumbai)gel electrophoresis and quantified using UV spectrophotometry (Hitachi, Japan).

PCR Analysis: PCR analysis was carried out using at thermal cycler (Eppend or f Master Cycler, Germany). Approximately 120 ng of genomic DNA was incubated in a total reaction mixture of 50μL containing both the forward primer 5□AATCAGAAGGCAGTCCTCCC-3□ and the reverse primer 5□-TCGGGGAGCACAGAGTGTAC-3□ (∼70 picomoles) (GenScript Corp., USA), 200μM deoxynucleotide triphosphate, 10X PCR buffer of pH-8.3 containing 15mM MgCl2, and 5 units of Taq DNA polymerase (New England Biolabs, Beverly)[16].DNA was initially denatured at 95∘C for 4min prior to amplification. A250 bp of catalase gene was amplified using the following conditions with 40 cycles consisting of 1min denaturation at 94∘C, 45 sec annealing at 56∘C, and 1 min extension at 72∘C.The final extension included 5 mins at 72∘C and the PCR products were confirmed by ethidium bromide stained 1.2 percent agarose gel and viewed in the UV transilluminator (Figure1).

Restriction Enzyme Analysis: Restriction digestion was performed in a total volume of 10 μL consisting of 5 μL amplicon,1μL NE buffer (50 mM potassium acetate, 20 mM Tris-acetate, 10 mM magnesium acetate, and 1mM dithiothreitol pH-7.9 at 25∘C), and 10U of Hinf1 enzyme (Ferment as Life Sciences ,Germany) [17]. Samples were incubated for 3-5 hrs at 37∘C and the digested PCR products were resolved in 2 percent agarose gel electrophoresis stained with ethidium bromide and separated bands were observed using gel documentation system (Figure 2). The A → T mutation at nucleotide 250bp abolishes a Hinf1 restriction site (Tallele), whichotherwiseforms177and73bpfragments (A allele) when treated with Hinf1. The catalase gene (-21) polymorphism suppress a Hinf1 site and so there will not be any cleavage of the PCR products (250bp) and if there is no mutation (normal genotype-AA) the band pattern in the gel will show the cleavage of the fragment into177bp and73bp. In the case o heterozygous genotype (AT),all the three types of fragments (250bp, 177bp, and 73bp) will be observed in the gel.

Results:

Out of 450 Type 2 diabetes cases, 25.00% (n=114) had AA, 66.25% (n= 303) had AT while only 8.75% (n=33) showed TT genotype. Among 470 healthy control subjects, 45.90% (n=222) ,45.00% (n=229) and 9.1% (n=19) carried the AA, AT and TT genotypes respectively (Table 4.12). Allele 'A' frequency in Type 2 diabetes cases was 58.15% and 'T' was 41.85%. and in healthy controls Allele 'A' frequency was 68.40% and 'T' was 31.60%. 'A highly significant (P≤0.0001) association of CAT-A-21-T gene polymorphism was observed in Type 2 diabetes cases. In addition, carriage rate of T allele was also found significant (P≤0.05) (Table 1). The representative gel picture showing CAT genotypes is shown in Figure 2.

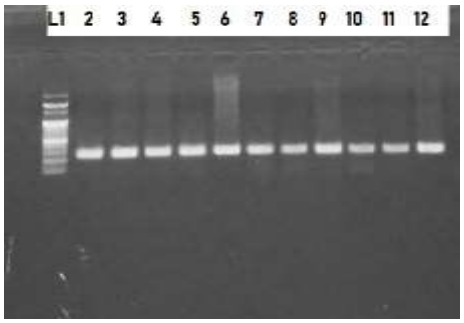
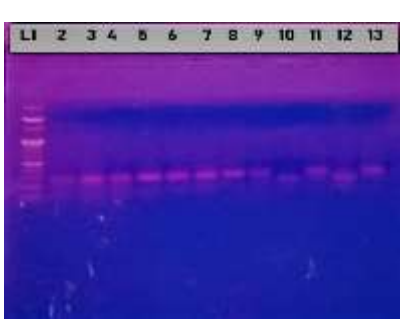


Fig 1: PCR analysis of CAT A-21-T gene. Lane 1: 100bp DNA ladder. Lanes 2–12: 249 bp PCR amplicon



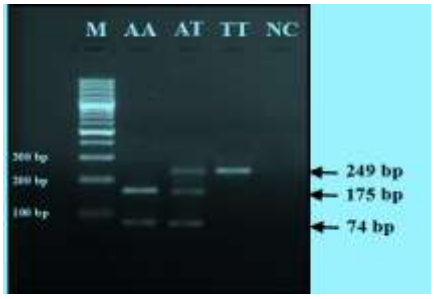


Fig 2 : Representative gel showing CAT-21A/T genotypes. Lane 1 100bp DNA ladder (Marker, M), Lane 2 AA (175 & 74 bp), Lanes 3 AT (249, 175 & 74 bp), Lane 4 TT UD Undigested (249bp).

Table 1 : Genotype distribution and allele frequencies as well as carriage rate of *CAT* A-21-T in controls and Type 2 diabetes cases.

Genotype frequencies						
	Count (n)	**Frequency (%)**	**Count (n)**	**Frequency (%)**	**P-value Chi ^{2} value**	**Reciprocal Odd's Ratio (Fisher Exact) with 95% CI (Lower-Upper)**
	Controls (n=470)		**Cases (n=450)**			
AA	222	45.90	114	25.00	--	1 (Ref.)
AT	229	45	303	66.25	**< 0.0001**	2.726 (2.066-3.608)
TT	19	9.1	33	8.75		0.4610 (0.3528-0.6024)
Allele frequencies						
	Count (n)	**Frequency (%)**	**Count (n)**	**Frequency (%)**	**P-value Chi ^{2} value**	**Reciprocal Odd's Ratio (Fisher Exact) with95% CI (Lower-Upper)**
	Controls (n=470)			**Cases (n=450)**		
A	673	68.40	541	58.15		1 (Ref.)
T	267	31.60	369	41.85	< 0.0001	1.719 (1.416-2.087)
Carriage rate						
	Count (n)	**Frequency (%)**	**Count (n)**	**Frequency (%)**	**P-value Chi ^{2} value**	**Reciprocal Odd's Ratio (Fisher Exact) with 95% CI (Lower-Upper)**
	Controls (n=470)			**Cases (n=450)**		
A (+)	451	90.0	417	91.25	<0.0004	1 (Ref.)
T (+)	248	54.1	336	75.0		1.465 (1.186-1.810)

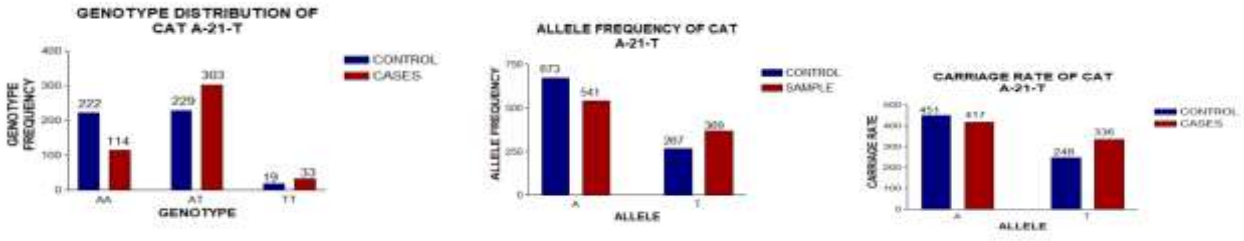


Fig 3: Status of CAT A-21-T Polymorphism. (A) Genotype, (B) Allele frequency and (C) Carriage pattern. Sample from Control and Cases (T2DM) were analyzed and were subjected to statistical analysis. All values are shown in percentage.* P<0.05, ** P<0.01, *** P<0.001, **** P<0.0001

Discussion

The-21A/T(rs7943316) polymorphism in CAT gene achieve its importance mostly by its position being close to transcription start site, or at sites where the transcription factors bind[18]. In the present study, 470 healthy volunteers were analysed for SNP in catalase-21A/T gene polymorphism. It has been observed that only 7% of the individuals have AA genotype and 70% of individuals have TT genotype and the remaining 23% of individuals have AT heterozygous mutant genotype (Table 3). The highest TT genotype (70%) of 21 A/T catalase gene polymorphism was observed among south Indians and the lowest TT genotype (8%) was observed among Chinese population [2]. A study accompany among Indian population with diabetes mellitus has reported that 80% of the patients were of TT genotype and 20% were of AT genotype. AA genotype was not observed among patients and healthy individuals were not included in their study. The study also found lower activity of scavenger enzymes in patients with diabetes mellitus [22]. However, diminished catalase activity can be determined by the disease itself and not by gene polymorphism. Catalase is one of the significant enzyme components of cell defense against oxidative stress and it has been hypothesized that the polymorphism of -21 A/T CAT decreased the antioxidant capacity and may distribute as a risk factor for oxidative stress associated diseases. The association between SOD1251A/G, CAT-21 A/T, and GPX1-198C/T antioxidant gene polymorphisms in the risk of cataract was reported among Chinese population and was found to have no significant associationinCAT-21A/TandGPX1-198C/T polymorphisms between the controls and patients. However, SOD1-251 A/G polymorphism was found to be associated with an increased risk of cataract [2]. Experimental and clinical studies conducted among essential hypertension and cerebral stroke patients suggested that the polymorphism -21 A/T CAT was associated with increased risk and was found to play an important role in pathogenesis [23]. Similar study conducted among Russian population reported that there was an association between polymorphism -21 A/T of the catalase gene and bronchial asthma. The study also reported that cigarette smoking and fruits and vegetables intake have potentially inverse modifying influences on the risk of asthma [24]. In contrast few studies reported that the catalase gene polymorphism was not related to the risk of cardiovascular diseases among type 2 diabetes mellitus in Finnish population [11] and also among type1diabetes mellitus patients in Czech population [13]. Even though, catalase was not essential for some cell types under normal conditions, it was reported to play an important role in the adaptive response to oxidative stress [17,18].Earlier studies reported that the catalase activity was found to be higher among cancer patients. However it has been reported that an adaptive increase of catalase activity may not be sufficient for inactivation of reactive oxygen species [19]. Decreased catalase gene expression due to the presence of mutant allele may further decrease the activity of catalase enzyme. The presence of insufficient antioxidant enzyme system may increase the susceptibility to oxidative stress-associated diseases among south Indian population. Apart from -21 A/T polymorphism of the catalase gene, another polymorphism (-262C/T) in the promoter region has been shown to influence transcription factor binding and correlates well with blood catalase levels [24]. There has been a well-established association between the type 1 diabetic neuropathy patients and the latter polymorphism in the Russian population [24]. In conclusion, the present study demonstrates that CAT (-21 A/T) SNP is more prevalent among the south Indian population. Further study should be conducted to find out whether there is alteration in catalase activity in the individuals with mutant genotype for CAT(-21A/T)polymorphism among healthy individuals and also need to be correlated with any disease associated with oxidative stress such as cancer, diabetes, and coronary artery diseases. The other polymorphisms within the same gene should also be studied for better understanding of the role of catalase gene in the mediation f oxidative stress response.

Conclusion

Significant association of Catalase genotype distribution confirms its association with pathophysiology of diabetes type 2.

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