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Relationship between NADPH oxidase p22phox C242T, PARP-1 Val762Ala polymorphisms, angiographically verified coronary artery disease and myocardial infarction in South Indian patients with type 2 diabetes mellitus

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ABSTRACT

Introduction: There has been compelling evidence for the role of oxidative stress in the pathogenesis of cardiovascular complications in type 2 diabetes mellitus (T2DM). We analyzed the association of C242T and Val762Ala polymorphisms of NADPH oxidase p22phox and poly (ADP-ribose) polymerase-1 (PARP-1) genes respectively with coronary artery disease (CAD) and its severity, myocardial infarction (MI) and cardiovascular risk factors in T2DM patients.
Materials and methods: We screened 283 T2DM patients, inclusive of 160 with angiographically defined CAD, 73 with and 89 without MI and 121 T2DM individuals with no evidence of CAD for the two gene polymorphisms.
Results: The 242T and 762Ala alleles were significantly more frequent in T2DM subjects without CAD than those with CAD, thereby associating them with a significant protective effect against development of CAD [p = 0.002 (C242T); 0.02 (Val762Ala)]. The association was further characterized by a relatively lower frequency of 242T and 762Ala alleles in T2DM patients with multi (MVD)/triple vessel disease respectively [p = 0.003 (C242T); 0.02 (Val762Ala)]. Conversely, the genotype and allele frequencies of these polymorphisms were not significantly different in T2DM + CAD patients with or without MI. Stratification of risk by putative risk factors for CAD revealed a significant interaction with these polymorphisms. Multiple logistic regression analysis revealed a significant and independent association of C242T and Val762Ala polymorphisms and other putative risk factors with CAD/MVD in T2DM individuals.
Conclusions: Our observations indicate a significant relationship between p22phox C242T and PARP-1 Val762Ala polymorphisms, CAD and its severity, but not with occurrence of MI in T2DM individuals with significant coronary stenoses.

Introduction

Oxidative stress, through a single unifying mechanism of superoxide ($O_2^{\bullet-}$) production has been posited to be a prominent underlying cause of macrovascular complications in type 2 diabetes mellitus (T2DM) [1]. Hyperglycemia induced oxidative stress is inherent to hyperglycemia dependent mechanisms of vascular damage. It has been well documented that T2DM is a powerful and independent risk factor for coronary artery disease (CAD) that accounts for premature mortality in patients with T2DM [2]. Family and epidemiological studies together with those based on animal models have postulated that CAD is a multifactorial disease embodying an important genetic component. Genetic research on CAD centered around identifying disease susceptibility genes has recently focused on the heritable polymorphisms of vascular oxidant enzymes that alter redox balance and thus appear to be associated with an increased risk of atherothrombotic cardiovascular disorders in T2DM individuals.

Studies have averred that cardiovascular tissues release large amounts of reactive oxygen species (ROS), with NADPH oxidase being a major source of $O_2^{\bullet-}$ production in cardiovascular cells and is expressed in atherosclerotic plaque, exacerbating oxidative stress and fomenting plaque ulceration and thrombosis [3]. Hyperglycemia exaggerates $O_2^{\bullet-}$ production from the mitochondrial respiration process with consequential activation of NADPH oxidase and redox sensitive signaling pathways implicated in diabetic macrovascular complications

Abbreviations: T2DM, Type 2 Diabetes Mellitus; CAD, Coronary Artery Disease; ROS, Reactive Oxygen Species; CYBA, Cytochrome b-245; PARP, Poly(ADP-ribose) Polymerase; MI, Myocardial Infarction; SVD, Single Vessel Disease; DVD, Double Vessel Disease; TVD, Triple Vessel Disease; MVD, Multi Vessel Disease; BMI, Body Mass Index; 8-OHdG, 8-hydroxydeoxy guanosine; eNOS, endothelial Nitric Oxide Synthase; OR, Odds Ratio; CI, Confidence Interval; OR_G, Generalized Odds Ratio.

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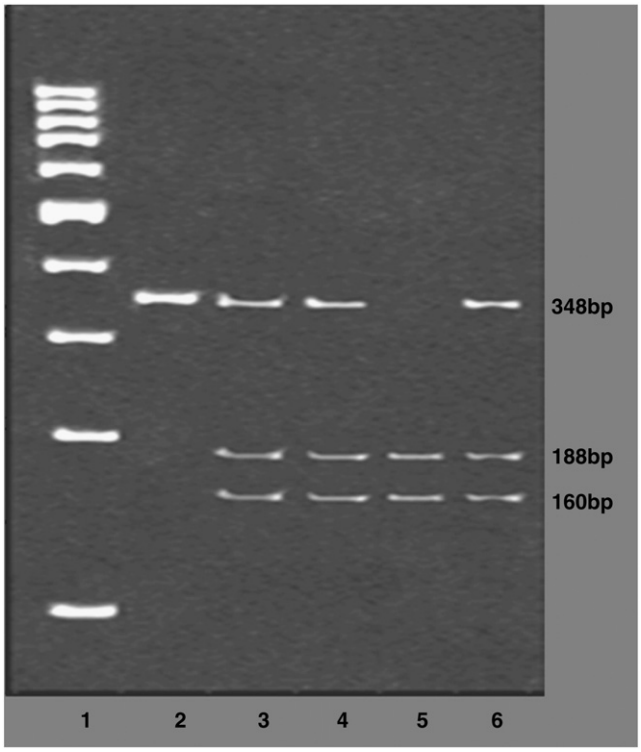

Fig. 1. Representative gel photograph for p22phox C242T polymorphism.

[4]. There has been considerable interest in exploring the possible association of genetic variation in genes encoding NADPH oxidase subunits functioning in electron transport and $O_2^{\bullet-}$ production, particularly the α-subunit of cytochrome b-245 (CYBA) gene encoding p22phox subunit, with disease susceptibility. Accordingly, polymorphisms within the promoter and exonic sequences of the p22phox gene influencing gene expression, NADPH oxidase activation and vascular $O_2^{\bullet-}$ generation effecting a significant inter-individual functional variation in vascular oxidative stress have been identified. Of these,

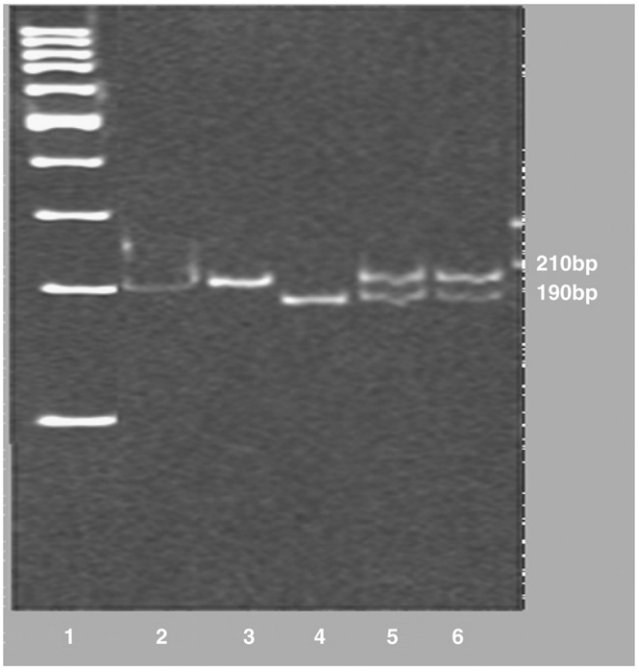

Fig. 2. Representative gel photograph for PARP-1 Val762Ala polymorphism.

the C242T polymorphism in exon 4 of p22phox gene resulting from the substitution of histidine by tyrosine at residue 72 in the putative heme binding site of p22phox subunit has been investigated for its association with coronary risk in different populations generating conflicting results [5–10]. The p22phox A-930G, C549T and A640G polymorphisms have also been reported to be significantly associated with myocardial infarction (MI) and CAD respectively [8,11,12].

Studies have adduced evidence for poly (ADP-ribose) polymerase (PARP) activation constituting a significant step in the pathogenesis of diabetic complications [13,14]. The vicious cycle that could impinge significant damage in the context of macrovascular complications in T2DM involves hyperglycemia induced PARP dependent changes in endothelial ATP content and pyridine nucleotide levels (including NADPH) [15]. By binding to single strand DNA breaks induced by peroxynitrite ($ONOO^-$), a potent oxidant formed by a reaction between nitric oxide (NO) and $O_2^{\bullet-}$, PARP depletes the cellular energetic pools resulting in cellular dysfunction and death [16]. The role of hyperglycemia-induced oxidative stress in producing DNA damage is also substantiated by an experimental observation demonstrating increased amounts of 8-hydroxyguanine and 8-hydroxydeoxy guanosine (8-OHdG) (markers of oxidative damage to DNA) in both the plasma and tissues of streptozotocin induced diabetic rats [17]. Two polymorphic amino acid substitutions located in the coding region of the PARP-1 gene (member of PARP family) have been commonly investigated in relation with susceptibility to various diseases. The most common one being a T to C transition at position 2444 in the mRNA sequence that causes a valine (Val) to alanine (Ala) amino acid substitution at codon 762 of PARP-1 located in the carboxy-terminal catalytic domain. In vitro enzymatic analysis revealed that Ala762 allele is associated with a significantly reduced enzymatic activity [18] thereby suggesting a pivotal role of PARP-1 directed poly(ADP-ribosyl)ation in the pathogenesis of macrovascular diabetic complications like CAD.

Studies investigating the association between p22phox C242T, PARP-1 Val762Ala polymorphisms, MI, CAD and its severity in T2DM patients have been sparse and not conducted in Indian population. Therein, we sought to evaluate the relationship between these polymorphisms, the presence and severity of CAD and MI in T2DM individuals and also the potential interactions between these genetic variants and the putative risk factors for CAD in South Indian T2DM population.

Materials and methods

Study Participants and phenotypic data

A total of 283 consecutive type 2 diabetic patients (defined according to World Health Organization criteria) with duration of T2DM over five years and age ≤65 years visiting Kamineni hospitals, Hyderabad were recruited for the present study. Coronary angiography was performed by standard Judkins technique and images of coronary tree were obtained in routine, standardized projections. Coronary artery stenosis was considered significant in the presence of a luminal diameter narrowing of ≥50% of left anterior descending, right coronary, circumflex artery or their primary branches. The severity of CAD was determined by the number of significantly stenosed coronary arteries (one, two, or three vessels respectively). Myocardial infarction was diagnosed by evaluation of a representative set of electrocardiograms (ECGs), cardiac enzyme values, and typical symptoms. Accordingly, a total of 162 T2DM patients were diagnosed as having CAD, of which 160 had angiographically proven CAD (2 patients did not undergo angiography). These included 42 T2DM patients with single vessel disease (SVD), 50 with double vessel disease (DVD) and 68 with triple vessel disease (TVD) respectively. Among those with CAD, 73 T2DM + CAD subjects suffered from MI and 89 were free of MI. A total of 121 control subjects were T2DM patients who were judged to be free of CAD and peripheral atherosclerotic arterial disease by medical history, clinical examinations, electrocardiography