Myeloperoxidase-463G/A polymorphism in patients with diabetic nephropathy in Sikkim, India

Diabetic nephropathy (DN) is one of the most common causes of end-stage renal disease (ESRD). Despite major advances made in the area of diagnosis and treatment of DN, death cases related to this ailment are not significantly reduced. Thus it is important to identify the risk factors for this condition.

Hyperglycaemia causes excessive generation of reactive oxygen species (ROS) and oxidative stress, which in turn precedes the development of endothelial cell dysfunction and plays a key part in the pathogenesis of DN. Myeloperoxidase (MPO), a lysosomal enzyme found abundantly in neutrophils, generates ROS. A single-nucleotide polymorphism (SNP), -463G>A at its promoter site leads to loss of SP1 transcription factor binding site and subsequently reduced production of ROS. Thus -463G allele of MPO can be hypothesized to contribute in the development of DN.

Individuals with type-2 diabetes mellitus (DM) visiting Central Referral Hospital, Sikkim Manipal Institute of Medical Sciences, Gangtok, Sikkim, were recruited for the study. DM patients without DN were considered as controls and those with DN were cases. The study was approved by the Institutional Ethics Committee.

DNA was isolated from collected blood samples using QiAmp Blood Mini Kit. Biochemical details were collected from hospital records. Written consents were obtained from all the participants. PCR was carried out with Master Mix (New England Biolab or NEB) using 10 pmol of each forward and reverse primer, 5′-GGTATAGGCACAAATGGTGAG-3′ and 5′-GCAATGTTCAAGCGATTCTTC-3′ respectively. PCR condition included an initial denaturation at 94°C for 2 min followed by 30 cycles of 30 sec denaturation at 94°C, 30 sec annealing at 58°C, 30 sec extension at 72°C, and final extension at 72°C for 5 min. SNP was detected by digesting 6 μl of PCR product with 5U of DdeI restriction enzyme (NEB) at 37°C for 1 h. Digestion product was visualized in 1.5% agarose gel. Following digestion, three bands at 169, 120 and 61 bp were produced for G/G heterozygotes. Following digestion, three bands at 169, 120 and 61 bp were produced for G/G heterozygotes.
homozygotes, two bands at 289 and 61 bp for A/A homozygotes, and heterozygotes (G/A) produced four bands at 289, 169, 120 and 61 bp.

This study included 15 DM patients without nephropathy and four diabetic patients with DN whose urine protein level was more than 25 mg/dl. All the participants were inhabitants of Sikkim. It was observed that percentage of G/G genotype was higher in cases (75%) than the controls (60%), indicating a possible role of oxidative stress inducing genotype in developing nephropathy in diabetic patients. A study including more participants can reveal a statistically significant association.

Although abnormal metabolic state is the major factor influencing the incidence and severity of nephropathy, genetic susceptibility is an important contributing factor for the development of DN. It was found that up to 35% of the patients developed diabetic nephropathy, irrespective of glycaemic control1, which indicates genetic components as important risk factors for DN. Concentration of MPO in type-2 diabetes patients has been reported to be notably elevated, and a positive correlation has been suggested between MPO levels and diabetic nephropathy4.

Sikkim records a high percentage (13.67%) of diabetics among the Indian states5. To the best of our knowledge, there are no previous reports on the putative association of genetic polymorphism with DN from this region. Since -463 promoter polymorphism of MPO is related to oxidative stress level, this may be a potential marker for susceptibility of a diabetic individual to develop DN.


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