Occurrence of Amino Acid Mutation (Ala98Val) Of HNF1α in Association with Type II Diabetes

Shakya P,1 Aryal S,1 Aryal R,1 MazgaeenL,1 ShahA,1 JoshiB2
1SANN International College, Kathmandu, Nepal, 2Annapurna Neurological Institute and Allied Sciences, Kathmandu, Nepal.

ABSTRACT

Background: Maturity onset diabetes of the young type 3 is a monogenic form of diabetes. Gene defects in the Hepatocyte Nuclear Factor -1 alpha (HNF1α) causes MODY3. HNF1α gene located in the chromosome (12q24.2) codes for a transcription factor which helps in signalling of insulin exocytosis in pancreatic Beta cells. A prevalent amino acid polymorphism at codon 98-Ala98Val (exon 1) of the HNF1α was shown to be associated with diabetes in the South Indian population. Since Nepal shares the ancestral origin with India and people have been sharing similar lifestyles for a long period of life it was relevant to check the occurrence of same mutation in diabetic population of Nepal as well. The study was carried out to identify the occurrence of amino acid mutation (Ala98Val) of HNF 1 alpha in association with type 2 diabetes in diabetic population of Kathmandu.

Methods: DNA samples were randomly collected from 12 non-diabetic and 56 diabetic patients. The DNA samples were amplified using Polymerase Chain Reaction (PCR). Restriction Fragment Length Polymorphism (RFLP) was carried out to identify the occurrence of the mutation.

Results: During the study, out of 12 non-diabetic samples, nine were normal while three samples showed heterozygous Ala98Val mutation. Whereas, eight diabetic patients were found to have Ala98Val mutation and rest 48 had normal genotype. The study thus showed 16.17% occurrence of Ala98Val mutation among 68 samples.

Conclusions: The study showed the occurrence of Ala98Val amino acid mutation in diabetic samples that were taken under study.

Keywords: Ala98Val; diabetes; hepatocyte nuclear factor 1α; restriction fragment length polymorphism; single nucleotide polymorphism; type II diabetes.

INTRODUCTION

Hepatocyte Nuclear Factor-1alpha (HNF1α), a transcription factor, found in pancreatic-beta-cells1 and hepatocytes,2 helps in differentiation of pancreatic beta cells3 and in transcription of genes required for insulin secretion.2 HNF1α directly regulates the transcription of insulin-1 gene, in rat.3 Mutation in HNF1α causes a type of monogenic diabetes called MODY3.4

Mutation in HNF1α gene, located on the chromosome 12 (12q24.2), is associated with both late onset type II diabetes7 and type-I diabetes.8-10 Variation in this gene is reported in Finnish,7 Danish Caucasians,11 Chinese and Japanese subjects12 in association with type-II diabetes and MODY, and also in association type-I diabetes in Japan.8-10

Mutational hotspot in exon-4 of HNF1α is demonstrated in German,13 Finnish and North American populations.14 Moreover, ala98val (exon-1) mutation in HNF1α is associated with diabetes in Danish Caucasians, Finnish8,12 and the South Indian population.15 This study has thus
been designed to detect the occurrence of ala98val polymorphism in Nepal.

METHODS

Sixty eight blood samples were collected; 12 from non-diabetic subjects from SAN International College and 56 from Annapurna Neurological Hospital. Informed consent, made under proper format of Nepal Health Research Council (NHRC), was obtained from all the participants.

DNA was extracted from the blood samples by phenol chloroform DNA extraction method. HNF1α gene segment (251bp including region of mutation) was PCR amplified using primers described earlier. The sequences of the sense and antisense primers (Promega corporation) : 5-GAAGGCCCTGGACAAGG-3 and 5-CCCTCTAGGCTCTCCTGGGA-3 respectively.

The PCR (long-gene PCR machine) was carried out in a volume of 50ul containing: 1ul DNA, 1.5mmol/l MgCl2, 1mmol/l dNTPs, 5pmol of each primer, and 1unit of TaqDNA polymerase (Promega corporation). The PCR conditions set were: denaturation (95°C for 30 s), annealing (65°C for 30 s), extension (72°C for 30 s) followed by 35 cycles, and a final extension (72°C for 9 min). 3 units of the enzyme Hae III was used for 3hrs to carry out Restriction digestion. The cleaved products were run in 3% agarose gel containing ethidiumbromide. The variation in digested fragments was visualized under UV gel-documentation system. The study was conducted in molecular biology laboratory of Department of Biotechnology, SAN International College.

RESULTS

Sixty-eight samples were considered during the study, out of which 12 were non-diabetic and 56 were diabetic. Out of 12 non-diabetic subjects, three showed heterozygous ala/val polymorphism while none showed homozygous val/val polymorphism. Similarly, out of 56 diabetic subjects, 8 showed heterozygous ala/val polymorphism and 3 showed val/val homozygous polymorphism. Rest of the subjects did not show any polymorphism. In sum, 11 out of 68 subjects showed ala98val polymorphism which is 16.17%.

<table>
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<th>SN</th>
<th>Type</th>
<th>Number of samples</th>
<th>Non-mutant (ala/ala)</th>
<th>Mutant</th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>Non-diabetic</td>
<td>12</td>
<td>9</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Diabetic</td>
<td>56</td>
<td>48</td>
<td>5</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>68</td>
<td>57</td>
<td>8</td>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>

DISCUSSION

The study carried out by Anuradha et al. showed the prevalence of ala98val polymorphism in association with MODY3 and early onset diabetes in South Indian population. This investigation on diabetic population of Kathmandu further reports the occurrence of ala98val polymorphism in relation to type II diabetic patients.

Similarly, the study showed presence of mutation in the youths of SANN College who showed no symptoms of diabetes. The presence of mutation in those samples shows the possibility of diabetes in near future and also indicates the need for genetic testing of their family members. Out of the 11 volunteers who had the mutation 5 of them have reported diabetes in their near relatives. This finding further supports the presence of mutation in the family. Since genetic testing of disease is a new practice in Nepal we suppose those volunteers might have running mutations in their family which they are unaware of.

The practice of testing plasma glucose for testing diabetes dates back to no more than roughly 20 years in Nepal. Therefore, the age of onset of the patients of diabetes can only be known from the last 20 years. Since MODY3 presents a mild form of diabetes people might be unaware of their exact age of higher plasma glucose. Furthermore due to practice of going for a check-up only after visible symptoms the exact age of onset is difficult to be known.

The presence of mutation in youths shows that there may be presence of slightly higher plasma glucose in them. It would be a good suggestion for them to go for routine check-up and correctly diagnose MODY3 and be sure it is not type I diabetes. The result may alter their medication process.

CONCLUSIONS

The observation of 11 Ala98Val mutation samples, out of 68 samples under study showed that there is occurrence of Ala98Val mutation in diabetic population of Kathmandu, as well. The occurrence of the ala98val
polymorphism could have biological significance, which remains to be established. The study on larger sample size with samples from different parts of Nepal would help to establish definite relation between Ala98Val polymorphism and diabetes in Nepalese population.

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REFERENCES


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