Screening Codon 233, 234 and 276 Polymorphisms in Exon 3 of Insulin Receptor Gene in Type 2 Diabetes Mellitus Patients of Kashmir Valley

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**ABSTRACT**: The prevalence of type 2 diabetes mellitus has reached epidemic proportions worldwide. Several single-nucleotide polymorphisms (SNPs) investigated in the genes of insulin signaling pathway have been associated with type 2 diabetes. We investigated three single nucleotide polymorphisms at codon 233, 234, and 276 in exon 3 of insulin receptor gene in type 2 diabetic patients of Kashmir valley. 468 subjects comprising of 198 type 2 diabetic cases and 270 non diabetic controls were included in the study. PCR-RFLP technique was used for genotyping. Amplicons were digested with MspI, Rsal and FokI restriction enzymes. Results were validated by direct sequencing of amplicons. All the subjects were monomorphic as no genotypic or allelic variation was observed in either cases or controls. Our study elucidates that substitutions at codon 233, 234, and 276 in exon 3 of insulin receptor gene do not occur in our population and thereby has no role in conferring any risk or genetic predisposition towards development of type 2 diabetes.

**Keywords**: Insulin receptor, Kashmir valley, Single nucleotide polymorphism, Type 2 diabetes mellitus

I. INTRODUCTION

The insulin receptor is a complex multifunctional protein sub serving various biological effects. The receptor’s organizational structure was firmly established, based largely on the amino acid sequence deduced after cloning of the insulin receptor cDNA\([1,2]\). Subsequently it was shown that the cDNA was derived from a gene composed of 22 exons located on chromosome 19\([3]\). Activation of the insulin receptor on the plasma membrane of cells by binding of insulin is the initial event that triggers the insulin receptor-signaling cascade, leading to the multiple cellular responses induced by insulin\([2]\). Homozygous or compound-heterozygous mutations in the insulin receptor gene (INSR) are found in patients with syndromes of severe insulin resistance\([4]\). Moreover, in patients whose insulin receptor mutations do not lead to a complete loss of insulin receptor function, milder syndromes of insulin resistance are reported, such as the Rabson-Mendenhall syndrome and type A insulin resistance\([4-6]\). Studies have investigated various single nucleotide polymorphisms (SNPs) associated with type 2 diabetes mellitus (T2DM) and found that among heterogeneous populations throughout the world, there are similar polymorphisms that convey genetic risks for T2DM in subgroups of main populations, i.e. Mexican Americans, Pima Indians, gypsies of southern Slovakia, African Americans, etc. This feature has been reported in cases of isolated ethnic groups\([7-11]\) and among underprivileged groups in developed countries\([12]\). Nsil polymorphism at exon 8 of INSR gene has been associated with arterial hypertension\([13]\). Analysis of exon 3 of INSR gene demonstrates four possible regions subject to genetic variation with a potential of five polymorphisms: a non-synonymous transition (T→C) in exon 233\([14]\), a synonymous substitution at exon 234 (C→T)\([15]\), a synonymous change at exon 276 (G→A)\([16]\), and two possible repeats just 5′ of exon 3\([16]\). Each of the three substitutions predicts a restriction site difference: MspI, FokI, and Rsal (3313C allele, 3317T and 3443T), respectively. These polymorphisms are located within exon 3, close to exon 2, which is the region that codes for the insulin binding site on the receptor\([17]\). Very few studies have been carried out on the polymorphic sites in exon 3 of INSR gene and the documented results are conflicting\([14, 15, 17, 18]\).

II. MATERIAL AND METHODS

2.1 Study population

A total of 468 ethnic Kashmiri subjects (198 type 2 diabetes mellitus patients and 270 non diabetic controls) were selected for the study. The study was approved by Institutional Ethics Committee. Selection of cases was based on American Diabetes Association (ADA) and/or World Health Organization (WHO) criteria for type 2 Diabetes Mellitus. The controls comprise of age and gender matched healthy non-diabetic subjects.
Informed consent was taken from each subject. Unwilling subjects and patients not complying with the selection criteria were excluded.

2.2 Anthropometric and systemic examination

Anthropometric data included Body Mass Index (BMI) and Waist to Hip ratio (WHR). Weight and height were measured using standard anthropometric techniques with subjects in light-weight clothing without shoes. Waist circumference was measured midway between the lower rib margin and the iliac crest at the end of gentle expiration using a metal anthropometric tape. BMI was expressed as kg/m^2. Blood pressure (BP) was measured using standard sphygmomanometer in the sitting position after a 10 minute rest. Hypertension was defined as a systolic pressure > 140 mmHg or diastolic pressure > 90 mmHg.

2.3 Sample Collection

2-3 ml of blood was collected from the cubital vein in EDTA vacutainer after 12 hours of fast from each subject. Plasma was separated and immediately sent for biochemical analyses. The samples were stored at -20° C until processed. Laboratory tests included fasting plasma glucose (FPG), total cholesterol (TC), triglycerides (TG), high density, low density lipoprotein (LDL), serum creatinine levels and HbA1c levels.

2.4 Genotyping

DNA was extracted from peripheral leukocytes by Phenol-Chloroform method. A 533 bp segment of exon 3 encompassing codon 233, 234 and 276 SNPs of INSR gene was amplified using a forward 5΄-ACAGGAATTGGACAAAGCCT-3΄ and reverse 5΄-AGCAGAGACCTCAGTAGCCAA-3΄ primer. The PCR products were simultaneously digested with MspI, FokI and RsaI. The wild alleles remain uncut upon digestion with these restriction enzymes whereas the variant allele is digested giving a fragment characteristic of the variant present at the polymorphic site.

2.5 Statistical Analyses

2.6 Statistical Package for Social Sciences (SPSS, version 16.0) and Java Stat software was used for statistical analysis.

III. RESULTS AND OBSERVATIONS

3.1 Anthropometric, systemic and clinical parameters

Mean age of cases and controls was 50.4 ± 11.1 years and 49.2 ± 12.4 years respectively. In both cases and controls, the predominant age group was 51-60 years. Mean BMI of cases and controls was 24.2±5.3 kg/m^2 and 21.2±3.3 kg/m^2, respectively. The cases presented with higher BMI and WHR as compared to controls (p<0.001). This is indicative of the role of central obesity in the etiology of T2DM. The systemic examination showed that 54% of cases were hypertensive. Overall, 85.5% of cases had higher levels of fasting blood sugar (>125 mg/dl) and 75.5% had high post prandial glucose levels (>200 mg/dl). The status of disease was determined by HbA1c levels. Only 22% of cases were in the controlled state (<7%), 29.5% of cases had mild status of diabetes (7-8%) whereas 48.5% of cases suffered from uncontrolled diabetes (>8%). Triglyceride levels (>150 mg/dl), LDL levels (>130 mg/dl) and HDL levels (<40 mg/dl) were observed in 55.5%, 18% and 72.9% of cases respectively. Elevated serum creatinine levels (>1.5 mg/dl) were observed in 20.5% of cases. Based on the type of treatment regimes, patients were categorized into three groups, viz. Insulin, oral anti-diabetic drugs (OADs) and a combination of both. Out of these three groups, 40.5% of the cases were on OADs, 31.5% were using insulin and 28% were undergoing both the treatments (Insulin + OADs). OADs was the most common form of treatment since majority of the patients recruited for the study were newly detected cases of type 2 diabetes mellitus.

3.2 Genotyping Analysis

In INSR gene, three SNPs located in the exon 3: a T↔C at codon 233, a C↔T at codon 234 and G↔A transition at codon 276 were evaluated. Only homozygous wild alleles were present and the frequency of T (233), C (234) and G (276) allele was same in both cases and controls. The 533 bp amplicon of INSR gene generated using polymerase chain reaction was digested individually as simultaneously with MspI, FokI and RsaI. The wild alleles remain uncut upon digestion with these restriction enzymes whereas the variant allele is digested giving a fragment characteristic of the variant present at the polymorphic site.
IV. DISCUSSION

We analyzed a non-synonymous transition Leu233Pro (T→C), and two synonymous substitutions Asp234 (C→T) and Gln276 (G→A) in the exon 3 of INSR gene in patients with type 2 diabetes mellitus for polymorphic alleles. Genotyping analysis and sequencing reports were used to analyze these variants. The results indicate that our population is monomorphic for these three polymorphisms as no variant allele was observed in either case or control. Similar results were observed in Mexican-American population. French and mixed ethnicity samples were also screened in another study that demonstrated monomorphic results for these three SNPs [17]. Based on these explicative results by various studies, the previously documented study has been attributed to sequencing errors and cloning artifacts [19]. In a study on ethnic Iranian population, all 22 exons of the INS gene were screened for variation. Several changes were reported including some novel polymorphisms. However, there was no report of the polymorphisms at codon 233, 234 and 276 [19]. In our study, the patients presented with dyslipidemia and characteristic pattern of lipid abnormalities commonly referred to as “diabetic dyslipidaemia”. Our results are in accordance with the data showing that people with T2DM are overweight or obese primarily with central obesity [20]. Although many studies focus on body mass per se, body fat distribution is also an important independent risk factor for diabetes. Epidemiologic data suggest that for every unit of increase (i.e., between 2.7 and 3.6 kg) in BMI, risk of developing diabetes increases by 12% [21]. HbA1c levels >8% were observed in nearly half (48.5%) of the cases which is suggestive of the extent of uncontrolled diabetes in our population. This discrepancy may be due to several reasons including unawareness, late detection and unresponsiveness and non-compliance to treatment. Identification of the genetic elements of T2DM is one of the most important areas of research because discovery of the risk genes would certainly facilitate understanding of the disease, its complications and its treatment, cure, and prevention. However, even in absence of genetic variants, the preponderance of sedentary habits and obesogenic lifestyle act as precipitating factors for various metabolic disorders. The result is an obesity epidemic paralleling, and contributing to the diabetes epidemic.

V. FIGURES AND TABLES

Figure 1- PCR-RFLP gel picture of INS gene

Lane 1: 100 bp DNA marker
Lane 2 and 6: Undigested samples (533 bp Amplicons)
Lane 3, 4 and 5: Amplicons digested with restriction enzymes MspI, RsaI and FokI respectively
Lane 7 and 8: Amplicons digested simultaneously with restriction enzymes MspI, RsaI and FokI

Figure 2a: Partial sequence electropherogram of codon 233 of INS gene

*Homozygous wild allele (T)
Our study demonstrates that polymorphisms at the codon 233, 234 and 276 in INSR gene do not occur in our population. However screening a large number of genes would facilitate in determining the genetic factors involved in the etiology of type 2 diabetes. The observational epidemiology suggests of the presence of various risk factors like abdominal obesity, deranged lipid profile and hypertension in our population. These risk factors in the setting of a sedentary lifestyle contribute to increasing prevalence of diabetes in Kashmir valley.

VI. CONCLUSION

REFERENCES


