Original Article

Pattern of MICRO-RNA-196A2 Polymorphism among type 2 diabetics

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Objective: To identify and determine the pattern of miRNA-196a2 (rs11614913) polymorphism among type 2 diabetics.

Methodology: This case control study was conducted on 311 individuals comprising 161 type 2 diabetics and 150 healthy controls. Genomic DNA was extracted by using non-enzymatic extraction method. Quality of extracted DNA was evaluated by horizontal gel electrophoresis on 1% agarose. The extracted samples were used to run PCR-RFLP for detecting polymorphism in miR-196a2 gene.

Results: Genotypes found were TT (normal genotype), TC (variant genotype) and CC (variant genotype). The miRNA variant (TC+CC) indicated a significant association with diabetes (69%) as compared to controls (53.3%) (χ 2 = 7.98, P = 0.004).

Conclusion: The miRNA variants (TC+CC) indicated a significant association with type 2 diabetes as compared to controls. The percentage of miRNA-196a2 C allele was greater in patients as compared to controls while the frequency of T allele was less in patients compared to the controls.

Keywords: Type 2 diabetes mellitus, miRNA-196a2, DNA.

INTRODUCTION

Diabetes mellitus (DM) is increasing worldwide and type 2 Diabetes mellitus (T2DM) is a serious health burden.[1] The prevalence of T2DM in Pakistan is 11.77%.[2] It has been indicated that microRNAs play the role of translational repressors and thus control the selected biological processes involved in T2DM.[3,4] Therefore, dysregulation in miRNAs has considerable impact upon the glucose metabolism of various tissues in T2DM such as the pancreas, adipose tissue, and liver.[5] Single nucleotide polymorphisms (SNPs) are important genetic alterations in humans.[6]

SNP belonging to miRNA genes/miRNA binding sites have an ability to alter the affinity of binding of miRNAs to their respective messenger RNA thus affecting the targeted gene expression.[7] Such kinds of SNPs are known part to play a role in susceptibility to a range of diseases including cancer, cardiovascular disease, osteoporosis, and T2DM.[8] Previous studies have shown common polymorphisms in miR-146a (rs2910164), miR-128a (rs11888095) and miR-27a (rs895819) contributing to neuropathy sensitivity in T2DM.[9]

High-risk SNPs and their alleles (TT, TC and CC) have appeared unbalanced genotype score dissemination among the type 2 DM samples and controls.[9] Genetic variation in miRNA genes (regulatory regions, primary miRNA, mature miRNA and precursor miRNA) can contribute to pathogenesis of T2DM by altering the expression and form of target gene and miRNA.[10] Hence, focusing on studies of genetic variations in miRNA or miRNA binding sites will help to understand the pathophysiology of T2DM leading to better health management options.[11,12]

METHODOLOGY

This case-control study was carried out in the department of Pathology, Al Nafees Medical College and Hospital, Islamabad and COMSATS University from Dec, 2017 to Sep, 2018. The sample size of 161 was obtained by purposive sampling technique. Samples were taken from diagnosed cases of T2DM (according to American Diabetes Association criteria). Both males and females were registered in the study with age range 30 to 70 years. Pregnant females and critically ill patients, with diseases having impact on diabetic status (e.g. diabetic ketoacidosis, end-stage kidney disease etc) were excluded from the study. A written and informed consent was taken from all patients.

Data regarding medical illness and demography was collected from each individual. With the help of 5cc sterile syringe venous blood sample was drawn from each individual and transferred to EDTA tubes. To prevent the coagulation of blood gently inverted EDTA tubes to mix EDTA with blood and sample was stored at 4°C. Extraction of genomic DNA was done from the whole blood by using non-enzymatic salting out method. The stored samples were incubated for 15 minutes at room temperature before starting the procedure. DNA was extracted from the blood samples

by stepwise procedure of non-enzymatic extraction method. 1% agarose gel was used for horizontal gel electrophoresis of Genomic DNA. Electrophoresis was done at 500mA and 95 volts for 30minutes in running buffer (1X TAE)[13]. For visualization of product gel was placed under UV light in gel documentation system and gel images were saved (Alpha-Imager Mini Bucher Biotech, Switzerland).

PCR was performed to detect the polymorphisms in rs11614913 gene. Electrophoresis was done at 460mA and 95 volts for 35minutes in running buffer (1XTAE). Gel products were visualized by using gel documentation system under UV light and to confirm the accurate amplification of DNA products compared with DNA ladder. Gel images were saved for record. RFLP technique was performed for SNP genotyping by using Msp1 restriction enzyme (Thermo Fissure-Scientific, USA) (Fig. 1). For selection of enzymes software NEB cutter V2.0 was used. Amplified product was incubated for 24 hours at 37°C. 5% agarose gel was performed for the separation of digested products and visualized by using UV-transiluminator in gel documentation system. The 100bp DNA ladder was used as quality control to confirm the size of digested fragments.[14]

Statistical Analysis: The data were analyzed using SPSS version 20. Pearson's Chi-square test and Fisher's exact test were applied for statistical evaluation. $p \leq 0.05$ was taken as significant.

Table 1: Frequency table of miRNA-196a2 T/C polymorphism in study population.

Genotype	Patients (n = 161)	Control (n = 150)	X^2 value	P value
TT	50 (31%)	70 (46.6%)	X^2 = 8.06	**0.017a**
TC	92 (57%)	65 (43.3%)		
CC	19 (125)	15 (10%)		
TT	50 (31%9	70 (46.6%)	X^2 = 7.98	**0.004a**
TC + CC	111 (69%)	80 (53.3%)		
T allele	192 (59%)	205 (68.3%)		
C allele	130 (40.3%)	95 (31.6%)	P = 0.02a OR = 1.4, 95% CI = 1.050-2.031	

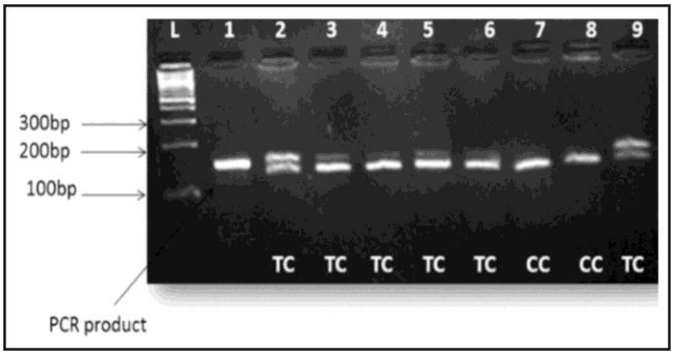

Fig. 1: Identification of the miRNA-196a2 polymorphism. L is 100bp (100 base pair) DNA ladder. Lane 1 is showing PCR product while 2 – 9 are restriction products. Lane 2 – 6 showing TC variant (heterozygous) while lane 7 & 8 showing CC homozygous allele.

RESULTS

Genotypes found were TT (normal genotype), TC (variant genotype) and CC (variant genotype). The miRNA variant (TC + CC) indicated a significant association with diabetes (69%) as compared to controls (53.3%) (χ 2 = 7.98, P = 0.004). The percentage of miRNA-196a2 C allele was 40.3% in patients and 31.6% in controls.

The percentage of T allele was 59.6% in patients compared to 68.3% control. Patients and control percentage showed a significant difference between T and C allele (OR = 1.4, 95%CI = 1.050 – 2.031, P = 0.02) as shown in the Table 1.

DISCUSSION

This is a well-established fact that T2DM has strong genetic susceptibility.[15] After extensive literature review from various search engines, no prior research was found on this topic. However, many studies have highlighted the association of same (microRNA-196a2) polymorphism with various other diseases including cardiovascular diseases, stroke and cancer.[16-18] The present investigation is first of its kind in Pakistan and it aims to find the association of miRNA-196a2 polymorphism at rs11614913 T/C with T2DM as the genetic information of diabetic patients is scarce in Pakistan.

Polymorphism in miRNA-196a2 designated as rs11614913, was the gene observed in the current study. It belongs to the expression of miRNA-196a2. Research shows a long-term workup on this gene to find out its association with various diseases. Ghanbari et al showed that rs11614913: T/C genotype in miR-196a2 was related with waist to hip ratio two target genes were identified.[19] They were experimentally presented as direct targets of miR-196a2. The results suggested that

miRNA-196a2 regulates fat distribution by miRNA-dependent pathway.[19] In another study, Hu et al found that rs11614913: T/C variant genotypes were linked with considerably increased breast cancer risks.[20]

Titan et al suggested that variant homozygote CC of miR-196a2 rs11614913 was linked with about 25% increased susceptibility to lung cancer as compared to wild-type heterozygote TC and homozygote TT and concluded that SNP rs11614913 in miR-196a2 might increase susceptibility of lung cancer.[21] XU et al showed that miR-196a2 rs11614913 plays a part in sporadic congenital heart disease susceptibility.[22] In a study by Kim et al they tried to find out relationship of miRNA-196a2 with silent brain infarction and ischemic stroke but the connection between silent brain infarction susceptibility and microRNA polymorphisms could not be established.[23]

There is one study conducted by Buraczynska et al which concluded that polymorphism in miRNA-196a2 (rs11614913) T/C is associated with an increased risk of cardiovascular disease in patients with type 2 DM. The percentage of T allele was lower in patients with type 2 DM as compared to healthy controls. Frequency of T allele and TT Genotype was high in patients with cardiovascular diseases when compared to those with healthy controls. While frequency of heterozygote TC was same in CVD patients and controls.[13]

However, current study revealed strong evidence on linking role of miRNA-196a2 with type 2 diabetes mellitus. It was found that variant genotypes (TC+CC) showed a significant association with patients of type 2 DM as compared to control group. The percentage of C allele was high in type 2 diabetics while the frequency of T allele was increased in control. This finding may serve in future as basis for screening of type 2 diabetes mellitus in genetically susceptible prediabetes individuals.

CONCLUSION

Our study revealed TT, TC and CC genotypes of microRNA-196a2 are present in type 2 diabetics and healthy individuals. The TC and CC genotypes in microRNA-196a2 (rs11614913) SNP have significant association with type 2 diabetes and hence possible role in screening of susceptible prediabetes. While TT genotype is common among healthy individuals.

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