Original Article

Mutational Analysis of Sr-B1 Gene in Relation with Dyslipidemia in Diabetic Patients

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ABSTRACT

Objective: To find the mutation in scarb1 gene that may be the cause of dyslipidemia in type 2 diabetes mellitus (T2DM).

Study Design: A cross-sectional comparative study

Place and Duration of Study: This study was conducted at the department of Biochemistry, Quaid e Azam Medical College, Bahawalpur from October 2020 to April 2021.

Materials and Methods: A total 50 individuals (20 having T2DM and dyslipidemia, 20 with T2DM without dyslipidemia and 10 healthy individuals) were enrolled for this study. Informed consent from the study participants was taken. Nuclear DNA was extracted from the blood. Quality and quantity of DNA was checked by 1% agarose gel electrophoresis. Primers of exon 8 were designed by using primer 3 software. Sequencing PCR was performed. On the purified product of sequencing PCR mutational analysis was conducted.

Results: In genotyping analysis no mutation was found but the single nucleotide polymorphism was detected in all groups. The detected polymorphism was rs5888 at c.1050 position. Group I patients were diabetic with the deranged lipid profile.18 patients of this group diagnosed with SNP. Group II individuals were diabetics with normal lipid profile and three patients from this group were diagnosed with SNP. The third group was of healthy individuals and two patients from this group were also detected with SNP. Exon-8 was used for study. This SNP was not lethal. The transition from T to C did not change the amino acid which is coded. In both the cases coded amino acid is Alanine.

Conclusion: The SNP rs5888 was found in all the 3 groups of study. This alteration in nucleotide sequence is non-deleterious as the amino acid which is formed is alanine. This indicates that this polymorphism has no role in causing dyslipidemia in the diabetic individuals.

Key Words: Dyslipidemia, type-2 diabetes mellitus, amino acid.

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INTRODUCTION

Diabetes is a metabolic disease which occurs due to insufficient production of insulin by pancreas or by the inappropriate use of insulin by cell while there was 5% increase in death rate from 2000 to 2016 worldwide among patients having diabetes.[1] In uncontrolled diabetic patients there are episodes of hypoglycemia and hyperglycemia, both of these conditions are related with adverse effects on patient health. These may include myocardial infarction, cerebrovascular accident, retinopathy, nephropathy, neuropathy and death. Hence immediate and vigilant care of diabetic patient is mandatory.[2] Patients of diabetes have 2-4 times greater risk of death due to cardiac issue as well as the cerebrovascular disease like CVA. Dyslipidemia is the major contributor in these diseases.[3] Many consider diabetes as equivalent to the coronary heart disease. More than 65% diabetic patients have high LDL.[4] Insulin resistance leads to increased production of VLDL-C by liver and chylomicron by the intestine. These are rich in triglyceride contents. Moreover, impaired insulin secretion impairs the activity of lipoprotein lipase which decreases the metabolism of VLDL-C and chylomicrons. Hence there is subsequent hypertriglyceridemia which enhances the transfer of triglyceride from VLDL-C and chylomicron to LDL-C and HDL-C respectively.[5] Triglyceride rich HDL-C comparatively has less half-life.[6]

Class B-type 1 of scavenger receptor (SR-B1) is a receptor for multiple ligands and it has high affinity for HDL-C. It is present on hepatocytes and play a pivotal role in reverse cholesterol transport.[7] SR-B1 receptor is encoded by SCARB1 gene. This gene is present on

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12q24.31.polymorphism in SCARB1 gene leads to dyslipidemia.[8] Relationship between the different variants of sr-b1 gene and dyslipidemia was observed among the several communities.[9] A single nucleotide polymorphism rs5888 was found by Acton.[10] At position 1050 of cDNA there was substitution of " C" to" T" in exon 8 whereas rs5888 SNP T allele was associated with the decreased HDL-C and increased LDL-C, TG and apo B level in Guangxi population.[11,12] The present study was aimed to find the mutation in scarb1 gene that may be the cause of dyslipidemia in type 2 diabetes mellitus (T2DM).

MATERIALS AND METHODS

This was a cross-sectional comparative study conducted at the department of Biochemistry, Quaid e Azam Medical College, Bahawalpur from October 2020 to April 2021. A total 50 individuals (20 having T2DM and dyslipidemia, 20 with T2DM without dyslipidemia and 10 healthy individuals) were enrolled for this study. Informed consent from the study participants was taken. Approval from Institutional Ethical Committee was Sough.

Exctraction of genomic DNA from blood was done as described by Sambrook and Russell 2001.[13] The quality and quantity of extracted DNA was checked on 0.8% agarose gel through horizontal electrophoresis. Primer 3 software was used to design the primers of *SR-B1* gene and also of exon 8 (Table 1).

Primer 3 software calculated the annealing(Tm) and reconfirmed by the formula:

Tm= 4(G+C) + 2(A+T)

The following are the steps of PCR. These stages were optimized by parameters through chain of reaction;

1. Denaturation
2. Annealing
3. Extension
4. Final Elongation

By PCR all the samples were amplified. The 1% agarose gel was used to check the results and these were visualized on Gel documentation (Bio-Rad). PCR purification kit (Genomed GmbH Inc) was used to purify the PCR product by following the instructions. The quality and quantity of product was checked on 1% agarose gel. On both strands of DNA with forward and reverse primer the sequencing PCR was performed as it had 30 repeats. The mutational analysis was carried out by CEQ8000 automated sequencer genetic analyzer.

RESULTS

In genotyping analysis no mutation was found but the single nucleotide polymorphism was detected in all groups. The detected polymorphism is rs5888 at c.1050 position. Group I patients were diabetic with the deranged lipid profile.18 patients of this group diagnosed with SNP. Group II individuals were diabetics with normal lipid profile and three patients from this group were diagnosed with SNP. The third group was of healthy individuals and two patients from this group were also detected with SNP. Exon-8 was used for study. This SNP was not lethal. The transition from T to C did not change the amino acid which is coded. In both the cases coded amino acid is Alanine. Figure 1 is showing DNA sequencing of SR-B1 gene as SNP rs5888 was detected in exon8.

Table No.1: SR-B1 gene and exon 8 primer sequence

Exon	Upstream primer	Exon	Downstrem primer	Size
Sr-8-f	Ggttatcttg tcatcgccac	Sr-8-r	Gtgctccaaccaggaatc	291

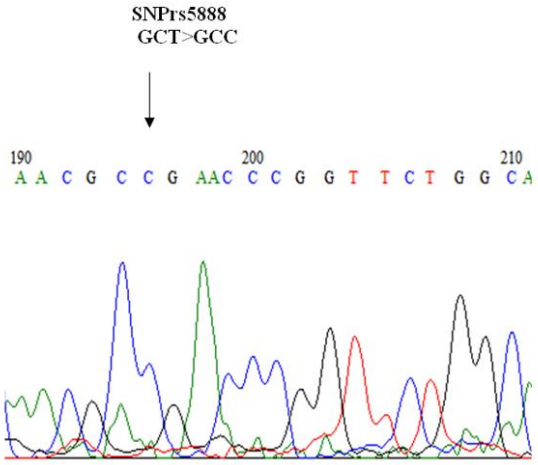

Figure No.1: DNA sequencing of *SR-B1* gene as SNP rs5888 was detected in exon8

DISCUSSION

"Diabetic dyslipidemia" is known to be a mixture of plasma lipid as well as lipoprotein disorder which are metabolically interconnected to each other. Dyslipidemia is known to be linked with insulin resistance, visceral obesity and liver fat contents.[14] Researchers are putting efforts in discovery of definite regulation steps that can help controlling the complications related to dyslipidemia among patients with DM. Genetic contribution to natural course of DM is well established.[15] Researchers have confirmed the enhanced production of apoB in T2DM which is known to be an important constituent of VLDL and LDL because of up-regulation of intestinal SR-B1 receptor.[16] Variant of HDL receptor gene SR-B1 may be a key influential factor of dyslipidemia in females which leads to the coronary artery disease.[17] In a study done by Acton S et al, five genetic variants of SR-B1 gene were found while two were found in the introns 3 and 5 while three were found in exons 1, 8 and 11 whereas exon 8 variant was associated with low LDL-C.[10] SR-B1 polymorphism is related to the coronary artery disease and atherosclerosis. Age and gender plays an important role in this regard.[18] In our study SNP rs5888 was found at the exon 8. This SNP is found in all the individuals including diseased one and the healthy one. As the sr-b1 gene has 13 exons there is the possibility

that there may be any other mutation or polymorphism which may cause the dyslipidemia in the diabetic patients but it needs further investigation.[19] The inconsistent results of variants of sr-b1 regarding the metabolism of lipids and coronary artery disease indicates that there may be an involvement of another pathway in this regard.[20].

CONCLUSION

The SNP rs5888 was found in all the 3 groups of study. This alteration in nucleotide sequence is non-deleterious as the amino acid which is formed is alanine. This indicates that this polymorphism has no role in causing dyslipidemia in the diabetic individuals.

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Conflict of Interest: The study has no conflict of interest to declare by any author.

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