Screening for Pathogenic Genetic Variants of APOA1 and ABCG1 Genes Associated with Dyslipidemia in Type 2 Diabetic Patients

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ABSTRACT

Diabetes mellitus is getting an epidemic worldwide and Pakistan stands at position 3rd with the 17% prevalence of diabetic adults. Diabetes develops several clinical complications and dyslipidemia is one among these complications. Dyslipidemia is marked as derangements in lipid metabolism and is one of the risk factors to develop cardiovascular diseases. The genetic predisposition in association with environmental and lifestyle modifications can play a central role in the progression of diabetic dyslipidemia. A cross-sectional study was designed to detect pathogenic genetic variants in APOA1 and ABCG1 genes associated with dyslipidemia in type 2 diabetic patients. A total of ninety subjects of both genders, aged between 30-70 years were randomly selected and further divided into a diabetic dyslipidemia group, a diabetic group and a healthy group. Genomic DNA was extracted from peripheral blood and subjected to the polymerase chain reaction using primers of the APOA1 gene and ABCG1 gene. The amplicons were subjected to Sanger sequencing on Automated DNA Genetic Analyzer. The sequence data was analyzed on BioEdit 7.2 biological software and Basic Local Alignment Search Tool against the human genome database to find any genetic variation. It has been found that ABCG1 gene and APOA1 gene exhibited a pattern of the normal nucleotide sequence in all subjects of three groups with no pathogenic genetic variation. Thus, exon 3 of the ABCG1 gene and exons 2 and 3 of the APOA1 gene do not contribute to the onset of dyslipidemia in type 2 diabetic patients.

Diabetes mellitus (DM) is a heterogeneous class of metabolic syndrome that accounts for disturbed carbohydrate, protein, and lipid metabolism. It is attributed to constantly raising blood glucose in the body which is due to defective insulin secretion, its action or, maybe both. Diabetes mellitus is a globally prevailing chronic disorder (Lau et al., 2019). Numerous factors like elevated levels of body fat percentage, the genetic predisposition, and insulin confrontation are responsible for this metabolic syndrome (Sarfraz et al., 2016). Across Pakistan, the prevalence of type 2 diabetes mellitus (T2DM) is found to be 16.98% and prediabetes is 10.91% (Aamir et al., 2019). In diabetic patients, the reverse cholesterol transport (RCT) and the ability mechanisms of serum cholesterol efflux appeared to impair (Daffu et al., 2015) whereas excess cholesterol needs to be shuttled off to avoid the risk of atherosclerotic plaque formation. Diabetic dyslipidemia is usually described as low high-density lipoprotein cholesterol (HDL-C), elevated triglycerides, and predominance of small-dense LDL particles (Parhofer, 2015). The World Health Organization (WHO) has reported approximately 2.6 million deaths each year because of dyslipidemia (Organization, 2019). There are a number of contributing factors that are considered to be involved in disturbed lipid metabolism or dyslipidemia in patients affected by type 2 diabetes mellitus.

In reverse cholesterol transport, nascent HDL contains apolipoproteins A-I (apoA-I) as a major constituent. ApoA-1 binds with the membrane-associated transporter proteins like ABCA1 and takes on non-esterified free cholesterol and phospholipase from peripheral cells into the cytoplasm. ATP-binding cassette subfamily G member 1 (ABCG1) is a fragment of the ABC transporter family (Sag et al., 2015) and plays a part in RCT. The main
function of ABCG1 protein is to control and regulate cell cholesterol homeostasis in the counter-partner of ABCA1 protein. ABCG1 effluxes the extra lipid from cells by consuming high-density lipoprotein (HDL) particles for reverse cholesterol transport, or, in other words, transports cholesterol to the liver for its disposal from the body (Sag et al., 2015). APOA1 gene is a protein-coding gene that is present on the long arm of chromosome number 11 at location 23.3 and contains 4 exons. ABCG1 gene is located on chr 21q22.3. Genetic alterations in the APOA1 gene resulted in HDL deficiency including Tangier’s disease and systemic non-neuropathic amyloidosis (Mogilenko et al., 2019).

ApoA-1 and ABCG1 proteins are the main components of RCT and mutations in the APOA1 and ABCG1 genes will cause disruption in the transport pathway of cholesterol. A common G/A substitution located at -75bp upstream from the transcription start site and a C/T substitution at +83bp from 1st intron differentially affected the gene expression of the APOA1 gene (Chand et al., 2016). Another study demonstrated an increased risk of atherosclerosis in 22 patients, presented with low levels of HDL due to a mutation in the APOA1 gene (Abdel-Razek et al., 2018). Moreover, rs670 genetic variant in the APOA1 gene was found associated with an enhancement of HDL-C compared to LDL-cholesterol in carriers of A-allele in 360 individuals (De Luis Roman et al., 2019).

APOA1 gene encodes apoA-1 protein, which is an essential component of RCT. There is an immense need to study molecular modifications of the APOA1 gene to better understand diabetic dyslipidemia in our population. The present study was designed with an objective to detect pathogenic genetic variants in APOA1 and ABCG1 genes associated with dyslipidemia in type 2 diabetic patients.

Materials and methods

The study design was a cross-sectional study and carried out at the Biochemistry and Molecular Biology Department, Army Medical College, National University of Medical Sciences in collaboration with the Chemical Pathology Laboratory of Army Medical College, Pak Emirates Military Hospital Rawalpindi. The Ethical Review Committee, Army Medical College approved the study and study followed the Declaration of Helsinki (World Medical, 2013). The sampling technique was non-probability purposive and ninety subjects between the age of 30 to 70 years were included after informed consent. Subjects were randomly selected, both sexes, and subdivided into three groups on the basis of medical examination. Group I was comprised of thirty diabetic dyslipidemia patients, group II comprised thirty type 2 diabetic patients, and group III comprised of healthy individuals. The diagnosis was based on laboratory investigations of fasting blood sugar, HbA1c, and lipid profile. The inclusion criterion followed was newly diagnosed type 2 diabetic patients with dyslipidemia and newly diagnosed type 2 diabetic patients without dyslipidemia. A newly diagnosis is defined as the first visit of patients to the hospital. Patients with type 1 diabetes, T2DM patients with other chronic complications, patients with malignancies, chronic thyroid, renal or hepatic diseases, pregnant females, and patients on lipid-lowering drugs were excluded.

Genomic DNA was extracted from blood samples as described previously (Sambrook and Russell, 2006). The qualitative DNA analysis was performed on 1% agarose gel electrophoresis. The sequence of exon 3 of the ABCG1 gene and exons 2 and 3 of the APOA1 gene was downloaded from National Centre for Biotechnology Information (https://www.ncbi.nlm.nih.gov) and ENSEMBLE genome databases (https://www.ensembl.org). The primers were designed on primer 3 software (https://primer3.ut.ee/). The sequence of the primer of ABCG1 was 5’-GGTCTTGCTTGCTCCTCTGG-3’ (forward) and 5’-TGAGAAACCTGCTGCCTCTG (reverse), exon-3 was 5’GAGGCAAGGTTTTTCCTCAGT-3’ (forward) and 5’GGCTTTCAACATCCCCACA3’ (reverse). The primers of the APOA1 gene were designed from intronic flanking region and the sequence of exon 2 was 5’CTCTGTGCCCTTCTCCTCAG’ (forward) and TGAGAAACCTGCTGCCTCTG (reverse), exon-3 was 5’GAGGCAAGGTTTTTCCTCAGT-3’ (forward) and 5’GGCTTTCAACATCCCCACA3’ (reverse). DNA samples of three groups were amplified on Thermocycler (Corbett USA). A 25μl PCR reaction mixture was containing 1X Taq buffer, 0.2μM dNTPs, 1.5mM MgCl₂, 1pmol/μl of each primer, 0.5 units Taq DNA polymerase, 20ng/μl template DNA, and 17μl nuclease-free water. The conditions used for amplification of ABCG1 and APOA1 were the same except for annealing temperature. The PCR program was as followed: cycle 1: hot start at 95°C for 4 min, cycle 2: 35 cycles of denaturation at 95°C for 30 sec, annealing for 30 sec at 60.5°C (exon 3 of ABCG1), 61°C (exon 2 of APOA1), 59.6°C (exon 3 of APOA1). DNA sequencing of ABCG1 and APOA1 genes was done on an Automated DNA Sequencer (Beckman Coulter) using DTCS kit (Beckman and Coulter GenomeLab™ Dye Terminator Cycle Sequencing kit). The sequencing PCR followed denaturation at 96°C for 20sec, annealing at optimized temperatures for 20 sec and extension at 60°C for 4 min with 30 cycles. The data was analyzed through BioEdit 7.2 biological software and pairwise alignment was performed on basic local alignment search tool (BLAST) against the human genome database to detect the genetic mutations.
Results and discussion

Type 2 diabetes mellitus is a prevailing health issue. Diabetes leads to several complications that arise mainly due to prolonged hyperglycemia and independent components of metabolic syndrome like insulin resistance (Defronzo et al., 2015). Several genetic and epigenetic factors are considered to be involved in the progression of complications associated with T2DM.

In the present study, genomic DNA was extracted with a concentration of 300ng/μl to 1μg/μl from blood (Fig. 1a). In PCR, 311bp fragment of exon 3 of ABCG1 was obtained and Sanger sequencing revealed no presence of the genetic mutation (Fig. 1b and 1c). The 334bp and 258bp PCR fragments of exons 3 and 2 of the APOA1 gene were obtained (Fig. 2a and 2c). The Sanger sequencing detected the normal pattern of exons 3 and 2 of the APOA1 gene without any genetic variation (Fig. 2b and 2d).

One of the major cardiovascular complications that occur due to prolonged type 2 diabetes is associated with dyslipidemia. Hyperglycemia, advanced glycation end products, and oxidative stress play a significant part in causing diabetic dyslipidemia (Chawla et al., 2016). Several genes contribute to the development of diabetic dyslipidemia. APOA1 gene provides information for the synthesis of apolipoprotein A-1 that is present in the liver and intestines, thus resulting in the translation of apoA-1 receptor proteins. A study reported no association of polymorphism rs670, rs5069, and rs2070665 in the APOA1 gene with dyslipidemia in the Kazakhs of Xinjiang and found equal distribution in cases and control (Feng et al., 2016).

In the population of North-East China, a functional polymorphism in APOA1/C3A4/A5-ZPR1-BUD13 gene cluster was assessed including males and females. It has been found that rs5072 showed a different spectrum in females and males. It provides a new insight to study the molecular mechanisms of dyslipidemia on a gender basis (Bai et al., 2019).

It was hypothesized that mutations in the APOA1 and ABCG1 genes are one of the factors involved in the disruption of cholesterol clearance and diabetic dyslipidemia. These results signify the contribution that studied exons do not involve in diabetic dyslipidemia and open a research gate to study other exons, genes and proteins that take part in the lipid metabolism, especially RCT.

Conclusion

Exon 3 of the ABCG1 and exons 2 and 3 of the APOA1 gene do not contribute to the onset of diabetic dyslipidemia. Thus, genetic mutations can be present in other exons of the APOA1 and ABCG1 genes.

Limitations

Small sample size was due to limited financial resources.

Statement of conflict of interest

The authors have declared no conflict of interest.

References
