ORIGINAL ARTICLE

GENETIC MUTATIONAL AND EXPRESSION ANALYSIS OF SCAVENGER RECEPTOR CLASS B1 *(SR-B1)* GENE IN TYPE 2 DIABETIC DYSLIPIDEMIC PATIENTS

Tayyaba Batool[1], Asifa Majeed[2]

[1]Department of Biochemistry and Molecular Biology, National University of Sciences and Technology, Rawalpindi Pakistan
[2]Department of Biochemistry and Molecular Biology, Army Medical College, Rawalpindi Pakistan

ABSTRACT

Objective: Genetic mutational and expression analysis of Scavenger Receptor class B1 *(SR-B1)* gene in type 2 diabetic dyslipidemic patients.

Study Design: Cross sectional Comparative study.

Place and Duration of Study: The study was caried out at Army Medical Collage Rawalpindi and took one year to complete.

Material and Methods: Sixty subjects were divided into three groups which were comprised of type 2 diabetic dyslipidemia patients, type 2 diabetic patients without dyslipidemia and healthy individuals. DNA was extracted and DNA sequencing of *SR-B1* gene was performed to find genetic mutation in exon-8. RNA was extracted from blood samples and used in quantitative PCR to analyse the expression of the SR-B1 receptor gene. The comparative ΔΔCT method was applied to quantify the expression in diseased groups and control. BioEdit, and SPSS 17 software was applied to find genetic variation, association and statistical significance.

Results: DNA sequencing of *SR-B1* gene revealed presence of rs5888 (GCC) polymorphism with CC genotype in exon-8 in 18 samples of group I, 3 samples of group II and 2 samples of group III. However, rs5888 polymorphism at position c.1050 did not change the amino acid and was synonymous. The expression of SR-B1 receptor gene was found down-regulated in diabetic dyslipidemia patients compared to diabetic and controls. These patients had shown poor glycemic control and deranged lipid profile where HDL-C was most deranged.

Conclusion: The exon-8 of SR-B1 did not contain any genetic mutation and identified polymorphism rs5888 was a normal variant in diabetic dyslipidmia Pakistani subjects. The expression of SR-B1 receptor gene was down-regulated at transcriptional level which may indicate the disturbance in reverse cholesterol transport and resulted in decreased HDL levels.

Keywords: *Dyslipidemia, HDL, rs5888, SR-B1, type 2 diabetes mellitus*

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INTRODUCTION

Diabetes mellitus (DM) is spreading as an epidemic and WHO estimated that currently around 422 million people are diabetic[1] worldwide. Diabetes mellitus leads to the formation of potentially harmful products including "acetylated and glycated LDL, advanced glycation end products, reactive oxygen species as well as chemokines and cytokines"[2]. Plasma high-density lipoprotein plays a role in the determination of risk for cardiovascular diseases through selective uptake of cholesterol ester and maintains the cholesterol homoeostasis in human body. Environmental and genetic factors contribute to the change of HDL-C levels.

Correspondence to: Dr. Tayyaba Batool, Deptt of Biochemistry Quaid -e-Azam Medical Collage, Bahawalpur, Pakistan.
Email: drtayyababatool@yahoo.com

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HDL-C mediates reverse cholesterol uptake (RCT) through transmembrane protein "scavenger receptor SR-B1". Scavenger receptor class I B type (SR-BI) is highly expressed in steroidogenic, intestinal, and hepatic cells. SR-BI has high binding affinity with HDL and regulates the selective HDL cholesterol efflux[3,4]. SR-BI protein is encoded by *SR-B1/SCARB1* gene which is located on chromosome 12 [5]. Expression of SR-B1 in cholesterol uptake was well studied in mice where overexpression of *SCARB1* gene protected against atherosclerosis[6]. Genetic variations in *SCARB1* and *ABCA1* gene have been directly correlated with HDL-C levels[7-9]. Studies have identified association between genetic variants in *SCARB1* gene, body mass index, lipid levels and lipoprotein particle[10-12]. SR-B1 is demonstrated as a key regulator of circulating HDL levels and genetic variations in *SCARB1* gene lead to disturbance in hepatic cholesterol uptake[13-15].

Pakistan is ranked 7th in the world with 7.1million people (7.6%) affected by diabetes. The statistics may get rise to 13.8 million people with diabetes in 2030[16]. Dyslipidemia is a prevailing complication in diabetic patients in Pakistan[17,18,19]. Abnormalities in concentration of plasma lipid and lipoproteins

are commonly occurring in type 2 diabetes mellitus and known as diabetic dyslipidemia[20, 21].

The present study has investigated the cause of dyslipidemia in type 2 diabetic patients at molecular level by targeting the HDL receptor SR-B1. The traditional medicine has been converted into personalized medicine, globally, and it is of utmost need to understand molecular genetics of diabetic dyslipidemia and build genetic pool of our population. The objective of this study was to perform genetic mutational and expression analysis of SR-B1 gene in type 2 diabetic dyslipidemia patients.

MATERIAL AND METHODS

The study was designed in accordance with Declaration of Helsinki (revised version 2013) and approved by the institutional ethical review committee. Written informed consent was obtained from enrolled subjects and divided into three groups. Twenty diagnosed type 2 diabetes patients with dyslipidemia were included in a group I, twenty type 2 diabetes patients without dyslipidemia in group II and twenty healthy individuals in group III. Diagnosed patients of type 2 diabetes mellitus with dyslipidemia were randomly selected as per WHO criteria with the consultation of statistician. The subject age was between 29-70 years. Type 2 diabetes patients and healthy individuals were included in other two groups. Type 2 diabetic patients with hypertension, renal dysfunctions, cancer, chronic liver diseases, cardiovascular diseases and on lipid lowering drugs were excluded. Blood in a fasting state was collected and serum separated. High density lipoprotein, total cholesterol, triglycerides were measured by the enzymatic colorimetric kit method on Selectra E Auto-analyzer. Friedewald's formula was applied to calculate LDL-C[22]. BMI and fasting blood sugar was also investigated.

DNA Sequencing of *SR-B1* gene

Nuclear DNA from blood samples was isolated as described by Sambrook and Russell, 2001. Primers specific to the exon-8 of *SR-B1* gene were designed on Primer3 software. The previous reports associated genetic mutations and polymorphism in exon -8 with muscular dystrophy, premature coronary artery disease, myocardial infarction and serum lipid profile[23,24,25,26]. The gene was amplified through polymerase chain reaction (PCR) followed by cycle 1 at 95°C for 6 min, cycle-2 at 93°C for 35sec, 62°C for 35sec, 72°C for 35sec for 35 times and cycle-3 at 72°C for 8min. DNA sequencing was performed on CEQ8000 genetic Analyzer (Beckman Coulter) using *SR-B1* gene specific primers. BioEdit Sequence Alignment Editor ver 7.0.9.0 was used for mutational analysis and comparison of genetic data.

SR-B1 gene expression

Total RNA extracted from the blood using PureLink RNA Mini Kit (Ambion, Life technologies TM.). cDNA synthesis of the samples and endogenous control was carried out by using RevertAid first strand cDNA synthesis kit (Thermoscientific). cDNA based primers were designed on Primer3 software containing forward primers 5'AGTCCTCGCTGGAGTTCTAC3' and reverse primer 5'CCACAGGCTCAATCTTCC3'. SR-B1 gene expression was analysed on Cepheid Smart cycler II using SYBER® GreenERTM qPCR superMix Universal kit. The conditions were 95°C for 10 min, 40 cycles of 93 °C for 30 secs, 48.5 °C for 35 sec and 72 °C for 1 min. The efficiency of assay was determined by serial dilution of equal quantity of cDNA from each sample by plotting curve slope. The mean Ct (cycle threshold) value was calculated for all samples of three groups. Relative quantification of SR-B1 transcript was calculated by the ΔΔCt method[27]. A housekeeping gene β-actin was used to normalize the qPCR data.

Statistical Analysis

Continuous variables and relative abudance of *SR-B1* gene were analyzed by one way ANOVA followed by Posthoc tukey test and exppressed as Mean±SD. Data was considered statistical significance with $p \leq 0.05$.

RESULTS

Demographic and Clinical data

The patients with diabetes and dyslipidemia showed significant deranged HDL and TG levels compared to patients with diabetes only (Figure 1). HDL-C levels were markedly decreased in group I as compared to group II. Four patients of this group were presented with normal HDL-C but their TG and LDL levels were found increased. In group I, HDL-C was markedly decrease in 16 (80%) patients and normal in 4 patients. TG levels were raised in 11(55%) patients, normal in 8 (45%) and decreased in 1 (5%) patients. Cholesterol was

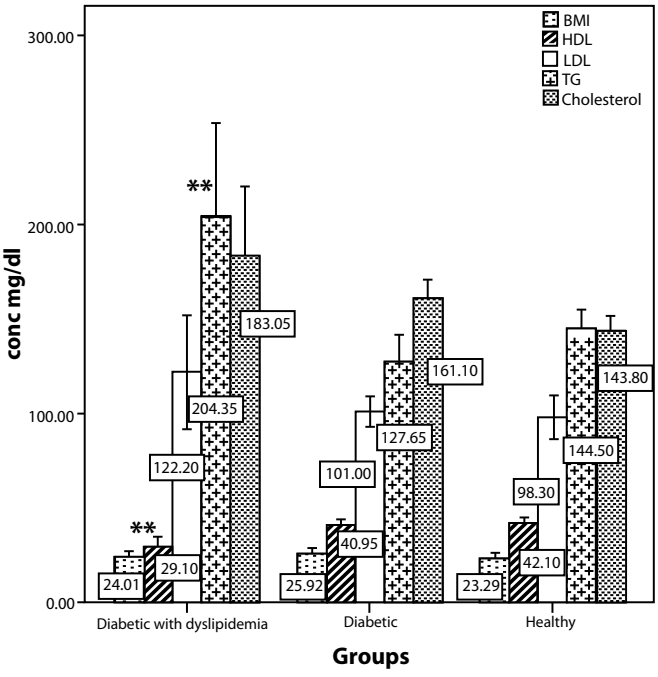

Figure 1: Lipid parameters expressed in mean ±SD.p<0.001 (highly significant).**

observed normal in 11 (55%) patients, increased in 8 (40%) and decreased in 1(5%) patient. LDL levels found normal in 12 (60%) patients, increased in 5(25%) patients and 2 (10%) with decreased levels. Eight subjects were obese. Cholesterol to HDL ratio determined the heart disease risk of these patients. Total 18 out of 20 patients were found at high risk to develop CVD. Different TG levels within the group were detected ranged lowest to the highest value. Group II and III subjects showed normal lipid profile. However, obesity was present in these groups. Therefore, lipid profiles of three groups drawn the conclusion that dyslipidemia in group I was due to diabetes not obesity. The significant difference was ($p<0.05$) observed in HDL-C and TG levels in all three groups.

DNA Sequencing of *SR-B1* gene

Figure 2 shows result of RNA, DNA and PCR. We have screened samples of group I, II and group III for the presence of any genetic mutation in exon-8 of *SR-B1* gene. We did not find genetic mutation in studied exon in disease groups. However, single nucleotide polymorphism rs5888 at position c.1050 (GCC) was detected in 18 patients of group I, 3 patients of group II and 2 control subjects (Figure 3) with major allele "C". This polymorphism was present as CC genotype in all studied subjects.

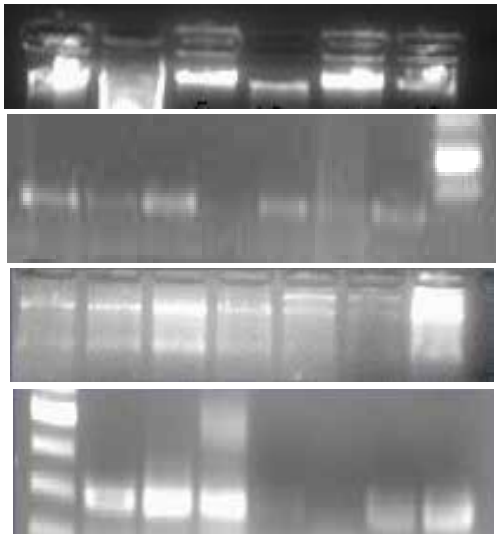

Figure 2a: DNA and Total RNA isolated from leukocyte cells b: PCR of exon-8 of *SR-B1* gene.

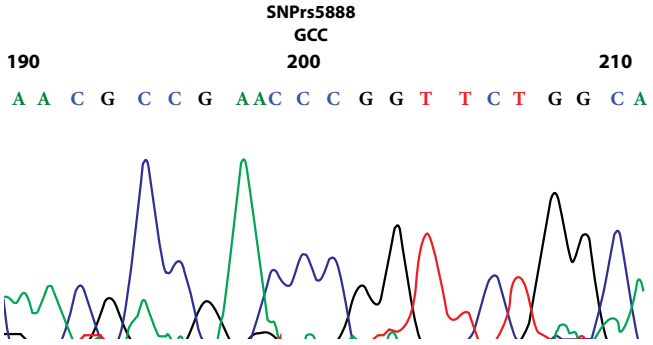

Figure 3: DNA sequencing of *SR-B1* gene. The SNP rs5888 was detected in exon-8 in samples of all groups.

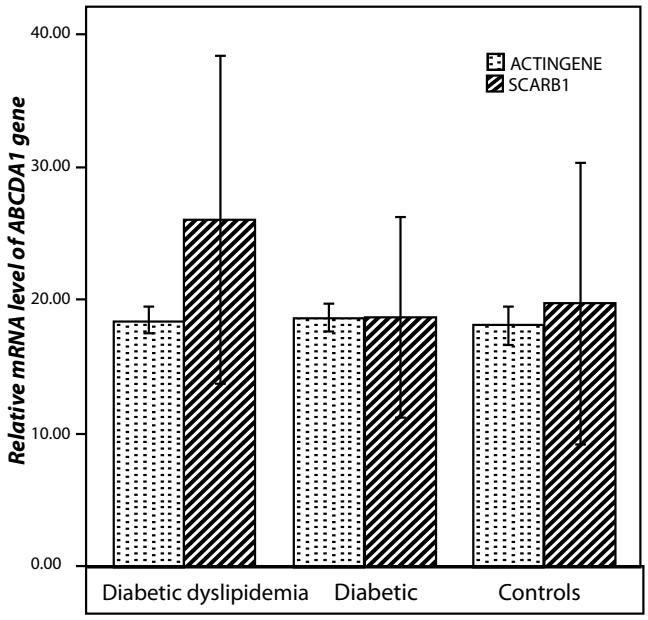

Figure 4. Normalized expression of SR-B1 in human blood cells calculated by 2Δ-ct method. Comparison of CT values showed significant decrease in *SR-B1* gene in diabetic dyslipidemia patients.

***SR-B1* gene expression**

The relative quantification was based on ΔΔCT method. Using this method, target gene expression was compared between diabetic dylipidemic (group I), diabetic (group II) and control (group III) samples (Figure 4). The decrease in SR-B1 gene expression was observed in group I compared to samples of group II & III. There was a clear fold change in gene expression of *SR-B1* gene and mean Ct value also showed significant differences between group I, group II & III. The mean Ct of group I was 26.47±6.21 compared to 18.69±3.76 of group II and 19.78±5.25 of group III. We have found clear association between decrease *SR-B1* gene expression and deranged lipid parameters especially in 3 samples. The Ct value of sample 6 was 34 with HDL level 24mg/dl. The fasting blood sugar level was 305mg/dl and the cholesterol and triglyceride levels were also raised (281 and 201 mg/dl). The Ct value of sample no 17 was 27 with a significant decrease in HDL level (17mg/dl). The triglyceride level was 346mg/dl while the fasting blood sugar was 360mg/dl. Sample no. 33 had the Ct value 26. The HDL level of this sample decreased to 32mg/dl with increased serum triglyceride level 464mg/dl. The fasting blood sugar level was 200mg/dl. The higher Ct value shows the low abundance of the target gene. Here, higher Ct value of target gene was with low copy number and clearly showed the changes in *SR-B1* gene expression. This alteration causes deranged lipid profile. These results clearly suggested the decreased expression of the *SR-B1* gene in diabetes. The control β-actin gene showed the constant expression in all samples. The efficiency of the target gene assay and the control gene assay was calculated by equation E= [10(-1/SLOPE)-1. The calculated efficiency of target and control was equal to 1.2. This shows the accuracy of qPCR. In group 1, there were 10 females and 10 males. The patients of a group I

had poor glycemic control and lowest level of HDL. In group II, out of 20 patients, 12 were females and 8 males.

DISCUSSION

Diabetes leads to several complications and dyslipidemia is one of them. Dyslipidemia is characterized as a deranged lipid parameter. In hyperglycemic dyslipidemia, low HDL-C, apoB and high triglyceride, VLDL has been reported. LDL-C level may be normal, but usually it is high[28]. Dyslipidemia is commonly present in type 2 diabetes. We have observed that diabetic patients had significantly low expression of *SR-B1* gene in circulating leucocytes cells compared to controls. The group I (diabetic dyslipidemia) showed poor glycemic control and lipid profile. The patients of group II (diabetic only) had relatively better glycemic control and their co-morbidities were not as bad as the group I. Although, their lipid profile was within the normal range but we have found most values towards the border line. It shows the effect of diabetes on lipoprotein metabolism which later on causes the dyslipidemia.

The down-regulation of the *SR-B1* gene in group I might be associated with the increase risk of atherosclerosis. Vaisman and colleague has reported protective effect of enhanced expression of *SCARB1* gene against atherogenic dyslipidemia and atherosclerosis[29]. Therefore, cure lies in the correction of lipoprotein metabolism and targeting the genes involved in RCT. We have found a clear difference between glucose level and lipid profile in three groups and results were consistent with our expression analysis. Our results suggest that expression of the *SR-B1* gene was reduced at transcriptional level in diabetic dyslipidemia patients. The low HDL-C and high TG & LDL levels showed that these patients could be prone to atherosclerosis. SR-BI is a multiligand receptor having high affinity with HDL-C particles. Diabetes itself causes change in lipoprotein and elevated TG level may impair the beneficial effects of SR-B1. We have seen the normal lipid profile in diabetic patients of group II and normal expression of *SR-B1* gene. Therefore, we can clearly demonstrate that decrease HDL-C levels in a group I was due to the decreased expression of the *SR-B1* gene. Single nucleotide polymorphism rs5888 did not associate with dyslipidemia in diabetes as it was found in control as well.

CONCLUSION

The exon-8 of SR-B1 did not contain any genetic mutation and identified polymorphism rs5888 was a normal variant in diabetic dyslipidmia Pakistani subjects. The expression of SR-B1 receptor gene was down-regulated at transcriptional level which may indicate the disturbance in reverse cholesterol transport and resulted in decreased HDL levels.

LIMITATIONS AND RECOMMENDATIONS

Small sample size was due the lack of interest for participation from patients. The identifications of genetic variants in other exons of *SR-B1* gene in our local population is important to understand dyslipidemia for better management. Large scale studies including whole exome sequencing will give better insight.

AUTHORS' CONTRIBUTION

Tayyaba Batool: Experimental work and drafting of the manuscript.
Asifa Majeed: Conceptual framework design, review of the manuscript and approval of the final draft.

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