ORIGINAL ARTICLE

DETERMINATION OF EXPRESSION OF *SCARB*1 GENE, FASTING BLOOD GLUCOSE AND BODY MASS INDEX IN TYPE 2 DIABETICS PRESENTING WITH AND WITHOUT DYSLIPIDEMIA

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ABSTRACT

Objective: To investigate the expression of *SCARB*1 gene, blood glucose (fasting) and Body Mass Index (BMI) in type- 2 diabetics with and without dyslipidemia.

Design: Cross sectional (comparative)

Place and Duration of Study: Army Medical College, Rawalpindi's Center for Research in Experimental and Applied Medicine (CREAM), in association with the Military Hospital, Rawalpindi, from August 2017 to March 2018.

Patients and Methods: Type 2 Diabetics (n=60), and healthy individuals (n=30) were enrolled randomly between the age of 25 to 75 years. Diabetic patients were further divided into diabetic dyslipidemia (Group I) and diabetic only (Group II). Group III served as control (n=). Ribonucleic acid (RNA) was isolated from the samples of peripheral blood and cDNA was synthesized. Determination of the SCARB1 gene expression was done on Real Time PCR and was presented in cycle threshold. The relative quantification of the gene relied upon 2-ΔΔCT method. Target gene expression, fasting blood glucose and body mass index (BMI) were determined. Comparison of the aforementioned parameters among control, diabetic dyslipidemia and diabetic non-dyslipidemic subjects was done using SPSS 17.

Results: The mean ± SD value of cycle threshold (CT) of *SCARB*1 gene diabetic dyslipidemia (Group I) was 20.499±2.15, it was 21.504±1.10 in Group II (diabetic only) while 20.72 ± 1.44 in Group III (control). In Groups I, II and III mean ± SD CT values of *GAPDH* gene were 22.36 ± 2.27, 22.181 ± 2.23 & 22.548 ± 3.45 respectively. In comparison with the control group, the diabetic dyslipidemia group's expression of the *SCARB*1 gene was altered by 1.0 and 0.4-fold, respectively. Fasting blood glucose levels were most deranged in diabetic dyslipidemia patients ($p < 0.001$) while BMI was significantly different among the three groups ($p < 0.05$).

Conclusion: The *SCARB*1 gene expression was not changed in the diabetic dyslipidemia patients while there was a slight decrease in the expression in the diabetic group. The fasting blood glucose and body mass index were independently altered in diabetic dyslipidemia and diabetic patients.

Key words: *Diabetes, High-density Lipoprotein, Triglyceride*

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INTRODUCTION

Diabetes Mellitus(DM) is ranked fourth among non-communicable diseases (NCD) with 1.6 million deaths yearly[1] and was in eighth position among the diseases that caused deaths in 2019 globally , according to the World Health Organization (WHO)[2]. About 425 million people worldwide have diabetes, according to the International Diabetic Federation (IDF), with 629 million anticipated to have the disease by 2045[3]. It is the leading cause of cardiovascular disease (CVD) therefore WHO recommends managing the levels of glucose and lipids as well as recommends cessation of smoking in diabetics in order to lessen the CVD risk[2]. Type 2 Diabetes Mellitus (T2DM) is distinguished by disturbances of plasma lipoproteins, referred to as diabetic dyslipidemia which is a major contributor to coronary artery disease and atherosclerosis[4]. Pakistan is ranked seventh in terms of the prevalence of DM, with dyslipidemia being the most common complication of T2DM[5]. Diabetic dyslipidemia is distinguished by decreased High-density lipoprotein cholesterol (HDL-C) and increased Triglycerides (TG) levels in the blood. Lipid abnormalities are metabolically linked, and a variety of factors influence normal lipid metabolism[6]. HDL-C is crucial in the reverse cholesterol transport (RCT). High triglyceride with decreased HDL-C observed in type 2 diabetes are linked to an elevated risk of cardiovascular events[7], a high lipid inflow also contributes to β-cell dysfunction[8]. HDL-C participates in the selective uptake of cholesterol esters in order to maintain cholesterol homoeostasis in the human body. Variations in HDL-C levels are influenced by both environmental and genetic factors.

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Scavenger Receptor Class B Type 1, SCARB1, receptor is one of the transmembrane proteins with a good affinity for HDL that regulates selective HDL cholesterol efflux. *SCARB*1 gene encodes SCARB1protein, which is located on the 12th chromosome, spanning 75kb & consisting of 13 exons. SCARB1 protein is important in cholesterol homeostasis and reduces thrombotic risk by maintaining HDL-C levels[9]. Genetic variations in the *SCARB*1 gene may reduce the ability of lecithin-cholesterol acyltransferase (LCAT) to bind to HDL, causing cholesterol accumulation by affecting the RCT. The SCARB1 receptor found in macrophages as well as on the endothelial cells protects against atherosclerosis[10]. Insulin resistance has been linked to *SCARB*1 expression and lower HDL levels in mice, according to studies. *SCARB*1 overexpression in mouse hepatic cells has been seen to be linked with lower plasma HDL levels[11]. In one case-control study, *SCARB*1 gene analysis of type 2 diabetics disclosed a link between polymorphism rs9919713 to some lipid parameters[12]. The current study sought to investigate the basis of dyslipidemia of type 2 diabetic patients on the molecular level, by studying *SCARB*1 gene. Need of the hour is to explore molecular anomalies correlated with clinical manifestations of diabetic dyslipidemia. This study's objective was to determine *SCARB*1 gene expression, BMI and the glycemic index in type 2 diabetics with, and without dyslipidemia. This was the first study on Pakistani people to look at diabetic dyslipidemia at the molecular level.

CAPSULE SUMMARY

- Expression of the Scavenger Receptor class B type 1 (*SCARB*1) gene was unchanged in diabetic dyslipidemia patients while it decreased slightly in the diabetic group
- In diabetic dyslipidemia and diabetic patients, fasting blood glucose and body mass index were altered independently

MATERIAL AND METHODS

Patients: The study was done from August 2017 to March 2018 in collaboration with Pak-Emirates Military Hospital, following a formal approval by the Army Medical College's Ethical Review Committee (No. ERC/MS-17 dated 11 August 2017). The research was done according with the Helsinki Declaration's principles (revised version 2013) [13], employing a non-probability purposive sampling technique. The study included ninety subjects. After explaining the purpose of the study, an informed consent, in black and white was taken from all participants. They were well aware of the publications of the result after maintaining confidentiality. The study participants were divided in three groups. Those with Diabetic dyslipidemia were labeled as a Group I, diabetics without dyslipidemia as Group II and healthy subjects as Group III. Medical and family histories of type 2 diabetes of all patients were noted on questionnaire-based proforma in addition to age and sex, BMI, drug history, socioeconomic standing, lifestyle, as well as dietary habits. Lab investigations included fasting blood glucose (FBG), HbA1c, and lipid profiles. The identification codes were assigned to each sample to keep secret patient's identity and were used during the study period. The inclusion criteria were: newly diagnosed type 2 diabetics with and without dyslipidemia, both genders, between the ages of 25 and 75, and age-matched healthy subjects. Individuals with only dyslipidemia were excluded from the study, as were those with any co-morbidity, such as renal dysfunction, hypertension, chronic liver disease, cardiovascular disease, cancer, gestational diabetes, type 1 DM, and type 2 diabetics on hypoglycemic or lipid-lowering medicines.

RNA extraction and Real Time Polymerase Chain Reaction

A 2ml sample of peripheral blood was collected in an ethylenediaminetetraacetic acid (EDTA) vacutainer, and RNA was isolated employing the GeneJet RNA Purification Kit (Thermo Fisher Scientific). RNA was separated on agarose gel (1%), it was visualized using Bio-Rad's (USA) Gel Documentation System. Purified RNA was kept at -80°C. The *SCARB*1 gene's mRNA sequence was got from the National Center for Biotechnology Information . Using downloaded gene sequences, "Primer 3" , an online bioinformatics tool was used to design the required primers, forward being 5'CTGTGGGTGAGATCATGTGG3', while the reverse, 5'GCCAGAAGTCAACCTTGCTC3'. The RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific) was utilized to synthesize cDNA, employing gene specific primers. Using cDNA generated from a control sample, the polymerase chain reaction (PCR) for the *SCARB*1 gene was optimised on a traditional thermal cycler (CGI-96 Corbet). The PCR reaction mixture contained nuclease-free water, 1x PCR buffer, 0.2M dNTPs, 1.7mM MgCl2, 1pmol/l reverse & forward primers, and Taq polymerase (0.3 units). Optimized PCR program was as follows; (1) Denaturation, at a temperature of 94°C for 30 sec, (2) Annealing, at a temperature of 57.2°C for 35 sec, and (3) Extension, at a temperature of 72°C for 8 min, with 35 cycles. On 1% agarose gel, the amplicon was electrophoresed and then seen using the Gel Documentation System ("Bio-Rad, USA").

The *SCARB*1 and the reference *GAPDH* genes expression analyses were done on Smart cycler II (Cepheid, USA) by employing SyberGreen Universal Kit (Thermo Fisher Scientific). Real-time PCR was performed in duplicate in order to maintain the data normalization with reference to the housekeeping *GAPDH* gene. In the PCR reaction 1l of cDNA, 12.5l of 2xSyberGreen Mix, 1pmol/l of reverse and forward primers, and nuclease-free water were used. The PCR program was used for 10 minutes of initial denaturation at 96°C, 40 denaturation cycles (Temp 94°C, Time: 30 sec), annealing (Temp: 57.2°C, Time: 35 sec), and extension (Temp:70°C, Time: 35 sec). The *GAPDH* gene was amplified according to the manufacturer's protocol. The equation E= [10(-1/SLOPE)-1] was utilized to examine the control as well as the target gene assays.

Statistical Data Analysis

Mean values of the CT of all samples were calculated in order to determine the gene expression. Relative *SCARB*1 gene

Table 1: Fasting Blood Glucose (FBG) and Body Mass Index (mean ± SD)

Groups	FBG mmol/L	*p value*	BMI Kg/m2	*p value*
I (Diabetic dyslipidemia)	13.40 ± 5.76	<0.001	26.38 ± 3.8	<0.05
II (Type 2 Diabetics)	10.35 ± 4.07		25.97 ± 3.57	
III (Control)	5.05 ± 0.523		23.88 ± 2.56	

Table 2: The cycle threshold of the *SCARB*1 gene expression

Groups	Mean ± SD of *SCARB1* gene (target gene)	Mean ± SD of *GAPDH* gene (ref)
I (Diabetic dyslipidemia)	20.49 ± 2.15	22.36 ± 2.27
II (Type 2 Diabetics)	21.504 ± 1.10	22.18 ± 2.23
III (Control)	20.72 ± 1.44	22.54 ± 3.45

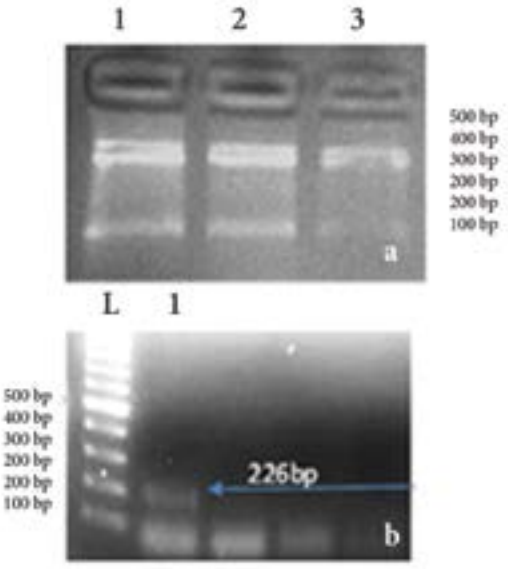

Figure 1: Isolation of Total RNA from peripheral blood & PCR of *SCARB*1 gene:

a: Lane 1-3 showing isolated RNA (Total).

b: L: 100bp DNA ladder, Lane 1: PCR of *SCARB*1 gene (226bp)

expression, with reference to the GAPDH gene, was analyzed by the $2^{-\Delta\Delta CT}$ method[14]. The difference of gene expression of the two groups was analyzed using the independent samples t-test. Analysis of the continuous variables was done by one-way ANOVA using SPSS 17 and was presented as mean±SD. Statistically significance was considered at a p<0.05.

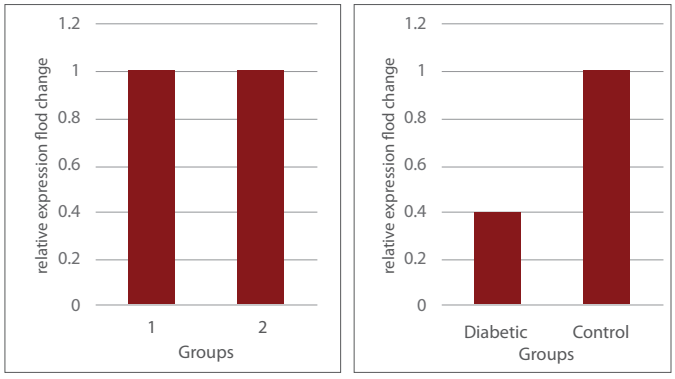

Figure 2: Relative Expression of *SCARB*1 gene in Groups

RESULTS

There were 47 females (51.6%) and 43 males (47.3%) in total. The level of FBS in Diabetic Dyslipidemia patients (Group I) was 13.40 ± 5.76mmol/L. The levels of FBS in diabetic patients (Group II) were 10.35 ± 4.07mmol/L and 5.05 ± 0.523mmol/L in healthy control (Group III) with p<0.05 (Table 1). In the Diabetic Dyslipidemia Group, the mean ± SD of BMI was 26.38±3.8kg/m^2, while it was 25.97 ± 3.578kg/m^2 in the diabetic Group and 23.88 ± 2.568kg/m^2 in control group with p<0.05.

At 260nm absorbance, no genomic DNA, proteins, or salts were seen. On a 1% agarose gel, total RNA was separated and found to be intact as 28S rRNA and 18S rRNA (Figure 1a). On the thermocycler, control Group's cDNA was used to optimize the PCR reaction for both genes *(SCARB1* and *GAPDH*). At an annealing temp of 57.4°C, a band size of 226bp was amplified (Figure1b).

The mean ± SD CT value for *SCARB*1 in Group I turned out to be 20.499±2.15 in comparison with a value of 21.504±1.10 in Group II, and 20.72±1.44 in Group III. In Groups I, II, and III, the mean ± SD CT values of the *GAPDH* gene were 22.36 ± 2.27, 22.181 ± 2.23, and 22.548 ± 3.45, respectively (Table 2). The mean CT values for the *SCARB*1 gene differed slightly between Groups. The relative gene expression analysis revealed a 1.0-fold increase in *SCARB*1 gene expression in Group I, which was the same as in the control Group. This data showed a statistically non-significant difference ((p≥0.05)) in the expression of *SCARB*1 gene between the two Groups. Type 2 diabetics (Group II) had a 0.4-fold increase in expression, which was less than the control Group (Group III) (Figure 2).

DISCUSSION

Lipoprotein receptors are involved in the signaling pathways that regulate cell functions. Scavenger receptor class B, type 1, plays a role in the recognition of oxidized LDL to prevent the process of atherosclerosis[15] and it's an HDL receptor. The majority of *SCARB*1 studies have mainly focused on mutational analyses, this is the first study on Pakistani diabetics.

The lower C_T value shows a higher abundance of gene and the high C_T value shows a low abundance of gene. However, results could not establish a direct link between *SCARB*1 gene

expression and diabetic dyslipidemia. The threshold cycles did not achieve any significant difference among the three Groups ($p \geq 0.05$). In a Chinese study, it was observed that HDL levels became dysfunctional in hyperglycemic conditions, which was linked to the downregulation of the *SCARB*1 gene mRNA[16]. Downregulation of this gene reduces HDL's cholesterol efflux capacity. Diabetes alters lipoproteins, and high TG levels may reduce the beneficial effects of SR-B1. These findings suggest that diabetes may influence *SCARB*1 expression at the transcriptional level. It was revealed that abnormal lipid levels resulted in induction of overexpression of miRNA-24 on the SR-B1 receptor protein, that resulted in the suppression of *SCARB*1 expression by targeting its 3 UTR[17]. Diabetes is caused by a decrease or insufficiency in insulin release. VLDL has been linked to hyperglycemic dyslipidemia, low HDL-C, apoB, and high triglycerides[18]. The HDL-C levels in Group I patients were found to be lower than normal, whereas Group II was within normal limits. Hyperglycemia may alter the metabolism of lipoproteins in type 2 diabetic patients. The glycemic index of Group I (patients with diabetic dyslipidemia) and Group II (diabetic) patients differed from the control. The majority of patients in Groups I and II were overweight compared to the control Group but Group I patients had higher BMI values compared to Group II. Though Group I and Group II subjects were newly diagnosed with diabetes Group I patients developed dyslipidemia. These findings also described the need to formulate awareness and monitoring programs for timely diagnosis of diabetes to control future complications[19]. The present study hypothesized that altered expression of *SCARB*1 gene affects the reverse cholesterol transport and HDL-C levels. The expression of *SCARB*1 gene was not changed in Group I (Diabetic dyslipidemia) while the lipid profile was remarkably deranged in the diabetic dyslipidemia Group. Moreover, fold-change showed a slight change in the expression of SCARB1 gene Group II (Diabetic) while lipoprotein levels were normal. However, data were obtained from 60 subjects and cannot apply to the whole population of diabetic dyslipidemia. Some patients of Group I and II were presented with higher CT values above 25 cycles compared to control Group. These results suggest that diabetes mellitus might change the expression of the *SCARB*1 gene.

CONCLUSION

Expression of *SCARB*1 gene was normal under diabetic dyslipidemia while slightly decreased in diabetic patients. The fasting blood glucose and BMI were independently altered in diabetic dyslipidemia.

Acknowledgment: Authors are thankful to the participants for voluntary participation.

Recommendation and limitations: Majority of patients was not willing to participate. The patients who presented with dyslipidemia without diabetes were not included due to limited funds. Large-scale studies will present better outcomes to understand diabetic dyslipidemia. Other genes can also be included.

AUTHORS' CONTRIBUTION

Khadeeja Siddique	Acquisition of data, Analysis and interpretation of data, Drafting the Article
Asifa Majeed	Conception and design, Analysis and interpretation of data, Critical revision

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