Original Article

Expression Analysis of SR-B1 Gene in Type-2 Diabetes Mellitus Patients with and Without Dyslipidemia

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ABSTRACT

Objective: To perform expression analysis of the SCARB1 gene to find out the association of High-density lipoprotein cholesterol (HDL-C) level with SR-B1 receptor expression in patients of type 2 diabetes mellitus

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 January, 2020 to June, 2020.

Materials and Methods: A total of 60 subjects of both genders aged 18 to 70 years were enrolled. Group-A (n=20) comprised of patients who had type 2 diabetes with dyslipidemia, Group-B (n=20) as type 2 diabetes without dyslipidemia while Group-C (n=20) was control (without diabetes and having normal lipid profile). PCR conditions optimized and quantitative real time PCR performed using SYBER Green fluorescence dye kit. Comparative $\Delta\Delta CT$ method applied to quantify the SCARB1 gene expression in control and disease groups.

Results: In a total of 60 subjects, there were 34 (56.7%) male and 26 (43.3%) female. Overall, mean age was recorded to be 48.24±6.77 years. $\Delta\Delta CT$ discovered the reduction in *SCARB1* gene expression in Group-A in comparison to Group-B and Group-C. Difference in expression of target gene was noted to be 1-fold in comparison to other groups. Mean values were found to have significant differences among Ct of Group-A (26.47±6.21), Group-B (18.69±3.76) and Group-C (19.78±5.25). Raised Ct value in Group-A reviewed abundance of target gene transcript with less copy number and found to have alterations in SR-B1 receptor which could be the cause of derangement in lipid profile in type 2 diabetes.

Conclusion: Hyperglycemia inhibits the expression of SCRB1 gene. Hyperglycemia might be responsible for reverse cholesterol transport by controlling SR-BI expression in diabetic patients resulting in the atherosclerotic changes and increasing the incidents of cardiovascular complications.

Key Words: Diabetes mellitus, cholesterol, atherosclerosis

INTRODUCTION

Diabetes mellitus (DM) is known to be a metabolic syndrome occurring because of deficiency in production of insulin and/or inappropriate response of the cells to insulin.[1]

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Comorbidities are common among patients with DM while an important aspect of complications is chronic inflammation related to vessels which could be contributing significantly to atherosclerosis.[2,3]

Type 2 diabetes mellitus (T2DM) is associated with dyslipidemia which includes decrease in high density lipoprotein (HDL) and increase in triglyceride and LDL.[4,5] Hyperglycemia is known to be an important entity contributing to atherosclerosis. "Hepatic scavenger receptor class B type I (SR-BI)" by binding with HDL helps promoting reverse cholesterol transport and minimize the chances of atherosclerosis and plaque creation.[6,7] The protein encoded by SR-B1 is a plasma membrane receptor for HDL. SR-BI is known to localize in the basolateral and canalicular membranes of these cells (Figure 1).[8,9]

Researchers have revealed the linkage of insulin resistance with SR-B1 expression and decline in HDL levels because of hepatic over expression of SR-B1 receptors.[10,11] This research was performed aiming expression analysis of the SCARB1 gene to determine the association of HDL-C level with dyslipidemia in T2DM patients.

MATERIALS AND METHODS

This cross sectional comparative study was done at "The Department of Biochemistry, Quaid e Azam Medical College, Bahawalpur" from January to June 2020. Approval from" Institutional Ethical Committee" was acquired. Informed consent was sought from all study participants.

A total of 60 subjects of both genders aged 18 to 70 years were enrolled. Group-1 (n=20) comprised of patients who had type 2 diabetes with dyslipidemia, Group-2 (n=20) as type 2 diabetes without dyslipidemia while Group-3 (n=20) was control (without diabetes and having normal lipid profile). Patients having comorbidities like hypertension, cardiovascular disease or chronic liver disease were excluded.

Blood samples of all subjects were taken as 5ml syringe after ensuring aseptic measures. All samples were transferred to ethylenediaminetetraacetic acid tube. Lipid profile was done. Extraction of RNA was done from the blood within 6 hours adopting "Pure Link RNA Mini Kit ambion" by "life technologies ™. The cDNA synthesis was done through" Revert Aid first strand cDNA synthesis kit", Thermo scientific. The primers of SCARB1 gene were designed employing primer 3 software. Real time analysis was done for the analysis of SCARB1.At 1st, optimization of the gene was performed through conventional polymerase chain reaction (PCR).Optimization program was:

RESULTS

In a total of 60 subjects, there were 34 (56.67%) male and 26 (43.33%) female. Overall, mean age was recorded to be 48.24±6.77 years. The ΔΔCT method found decline in SCARB1 gene expression in Group-Ain compasison to Group-B&C. Difference in expression of traget gene in Group-A was 1-fold in comparison to other two study groups. Our findings revealed that T2DM causes change in SCARB1 gene expression which is leading to dyslipidemia (Table 1).

Table No.1: Relative Expression of SCARB1 genes among study groups

| Groups | Mean CT | | ΔCTSCARB1-ΔCTCONTROL | ΔΔCT DISEASE-CONT | FOLD EXPRESSION |
	SCARB1	CONTROL			
Group-A	2647±6.21	18.62±0.52	7.68±6.2	5.69±6.2	.00028-1.5
Group-B	18.69±3.76	18.62±0.52	0.07±3.7	-1.49±3.7	0.027-0.2
Group-C	19.78±5.25	18.22±0.72	1.56±5.2	0.0±5.2	0.0272-36.75

Significant difference was noted in terms of mean ct values among study groups. Mean ct values were found to 26.47±6.21, 18.69±3.76 and 19.78±5.25 in Group-A, B &C respectively.

Table No.2: Mean CT value of target gene and control gene among study groups

Groups	SCARB1 (Mean±SD)	Control gene (Mean±SD)
Group-I (n=20)	26.47±6.21	18.62±0.52
Group-II (n=20)	18.69±3.76	18.62±0.52
Group-III (n=20)	19.78±5.25	18.22±0.72

95°C............6 min
93°C............30 secs
48.5°C..........35 sec   35 cycles
72°C.............1 min
72°C.............8 min
4°C...............10sec

Following optimization of PCR program, real time PCR was done on "smart cycler (Cephid)". The qPCR was performed utilizing" SYBER® Green ER™ qPCR Super Mix Universal kit" as :

95°C...................10 min
93°C...................30 sec
48.5°C..............35 sec   40 cycles
72°C.................60 sec

Data analysis of qPCR was done through comparative CT method (ΔΔCT method) on 4 steps (Figure 2).[12]
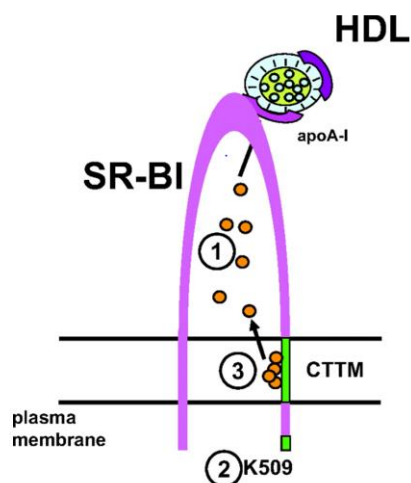

Figure No.1: Structure of SR-B1 Receptor[8]

Step 1: calculation of the standard deviation of ΔCT value by using the formula

$$S= (S1^2 - S2^2)^{1/2}$$

Step 2: calculation of ΔΔCT value by the formula

$$ΔΔCT = ΔCT \text{ test sample} - ΔCT \text{ calibrator sample}$$

Step 3: calculation of the standard deviation of ΔΔCT value

Step 4: incorporation of standard deviation of ΔΔCT value into fold difference

$$\text{Range of target} = 2^{-ΔΔCT}$$

Figure No.2: qPCR by comparative CT method (ΔΔCT method) on 4 steps

The control gene β-actin revealed constant expression in all samples (Figure 3). The efficiency of the target gene assay and control gene assay were estimated as: E=[10(-1/SLOPE)-1.

The estimated efficiency of the target and control was noted to be equal to 1.2 showing correctness of qPCR.
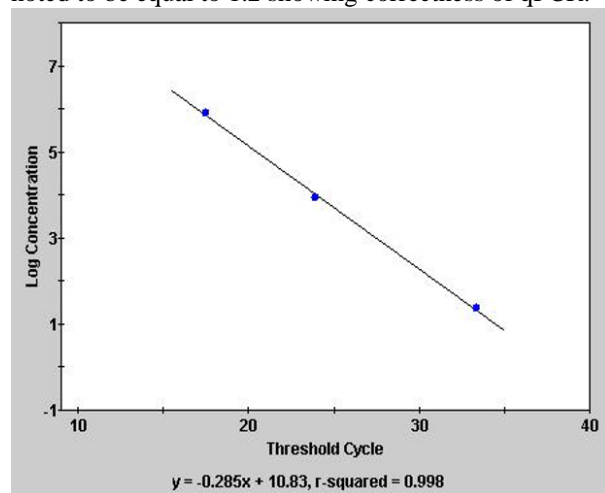

$y = -0.285x + 10.83$, r-squared = 0.998

Figure No.3: Standard Curve of the Internal Standard (Beta actin)

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.[3] 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.[13] Researchers have confirmed the enhanced production of apoBin T2DM which is known to be an important constituent of VLDL and LDL because of up-regulation of intestinal SR-B1 receptor.[14] Low insulinization is also thought to contribute to increased lipolysis in adipocytes resulting into transportation of fatty acid to the liver while this mechanism is thought to be a common abnormality among T2DM patients.[15]

The current study demonstrates that hyperglycemia is directly related to the dyslipidemia. In the present study, aiming expression analysis SR-B1 gene, RT-qPCR was done as it considered to be the best method aiming analysis of change in gene expression.[16] For RNA, only qualitative analysis is not enough. That is why, it is essential performing quantitative analysis through RT-qPCR. A "house-keeping gene" is described as a gene having most stable expression (small coefficient of variation as well as maximum fold changes of less than or equal to 2). Ct is described to be as number of cycles that a reaction requires for reaching the threshold of fluorescence. After collection

of the ct value reaction relative expression level of the samples was estimated [17]

Our results found high abundance of target gene (SCARB1 gene) among controls which was noted following 15-cycles of amplification. The Group-B showed the ct value from 20-26 cycles showing relative low abundance of target transcript in comparison to controls but this low abundance of gene did not alter the SCARB1 gene expression and functioning of SR-B1 receptor. Real time results found that patients having DM have change in expression of SCARB1 gene and might be prone to lipid derangments.[18,19]

The ct value of Group-A showed range of 26-34cycles.The high ct value shows the late amplification of the sample which indicates the low abundance of the target gene and consequently the decrease transcription of the receptor. This ct value was found to have consistency with derangement in lipid profile of this group. Keeping in mind the findings of the present research, we can say that hyperglycemia was directly found to relate with decrease expression of gene. A study done by Murao K et al from Japan concluded that possible glucose suppression by SCARB1 expression could be credited to fractional mediation of p38 MAPK-Sp1 pathway and highlights the probability while all this highlights that changes in SCARB1 expression under influence of hyperglycemia might be providing conditions what accelerate the process of atherosclerosis among patients having diabetes.[20]

CONCLUSION

Hyperglycemia inhibits the expression of SCRB1 gene. Hyperglycemia might be responsible for reverse cholesterol transport by controlling SR-BI expression in diabetic patients resulting in the atherosclerotic changes and increasing the incidents of cardiovascular complications.

Author's Contribution:

Concept & Design of Study:	Tayyaba Batool
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Data Analysis:	Zia Ullah, Saifullah, Saifuddin Nasir
Revisiting Critically:	Tayyaba Batool, Anila Humayun
Final Approval of version:	Tayyaba Batool

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

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