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Investigation of the Gene Expression of APOAI and Gene Variants in ABCAI Gene in Type 2 Diabetic Pakistani Patients with and without Dyslipidemia.

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ABSTRACT

Objective: To determine the *APOAI* gene expression and genetic variations in *ABCAI* gene in type two diabetes mellitus (T2DM) patients with and without dyslipidemia and association with HDL-C profile.

Methodology: A cross sectional analytical study was conducted on ninety subjects; comprised of diabetic dyslipidemics, diabetics without dyslipidemia and healthy subjects, who were randomly selected. RNA and genomic DNA were isolated from human peripheral blood samples. The *APOAI* gene and *ABCAI* gene were amplified through polymerase chain reactions. *APOAI* gene RNA expression analysis on real time PCR and relative quantification via $^{\Delta\Delta}$CT method were carried out. DNA sequencing of *ABCAI* gene was performed and analyzed using BioEdit 7.9 biological software for detection of genetic mutations.

Results: The mRNA abundance of *APOAI* gene was found changed in diseased groups compared to control. The expression of *APOAI* was 5.3fold changed in diabetic dyslipidemia group and 6.9fold changed in diabetic group compared to control group. There was no genetic variation found in Exon-4 of the *ABCAI* gene and DNA sequence was in normal pattern.

Conclusion: The normalized expression of *APOAI* gene in diabetic dyslipidemia and diabetic group was up-regulated compared to the control group and the underlying cause might be diabetes.

KEYWORDS: Apolipoprotein I gene, ATP- binding cassette transporter I (ABCAI) gene, Dyslipidemia, Type 2 Diabetes Mellitus, High Density Lipoprotein (HDL-C)

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INTRODUCTION

The most common type of diabetes, Type 2 diabetes mellitus (T2DM), affected individuals across the globe at an alarming rate [1]. The International Diabetes Federation (IDF) conducted worldwide survey and found 451 million people afflicted with diabetes and this prevalence is estimated to rise up to 693 million by the year 2045 [2]. It has been classified as an epidemic due to the burden it has caused on growing economies of the under-developed countries. The National Diabetes Survey of Pakistan (NDSP 2016-2017) has reported the prevalence of diabetes as 26.3% in Pakistan [3]. Country like Pakistan that has limited resources, is struggling to overcome this clinical burden. Diabetic dyslipidemia, as the most hegemonic complication of T2DM is getting more prevalent in Pakistani population, especially in individuals who aren't following adequate clinical screenings and management trials [4]. Glucose and lipid metabolisms have been linked to each other and diabetic dyslipidemia has been its most important clinical manifestation. In T2DM, derangement in the lipid levels, i.e, decreased high density lipoprotein cholesterol (HDL-C) and increased low density lipoprotein cholesterol (LDL-C) has been caused due to the insulin resistance which releases fatty acids from peripheral tissues into the serum. This increased the risk of development of cardiovascular diseases in the diabetic patients [5].

Apolipoprotein A-I (apoA-I) is a major component of HDL-C. *APOAI* gene encodes apoA-I protein (243 amino acids) and is located on the chromosome 11q23.3 as assigned by Human Genome Database, respectively. *APOAI* gene encodes apolipoprotein A-I receptor protein which is expressed in liver and intestines and it is known to be a highly polymorphic gene [6]. A lot of studies have been done on animal models regarding the role of *APOAI* in Reverse Cholesterol Transport (RCT) mechanisms and mutations in *APOAI* gene that led to HDL-C dysfunctions, exacerbations of atherosclerosis and elicitation of cardiovascular diseases (CVDs) [7]. The positive effect of *APOAI* in reducing HDL-C levels in diabetic obese mice (db/db) was determined in a survey, where over expression of *APOAI* didn't reduce obesity but HDL-C levels were documented to be down-regulated [8]. A high-throughput sequencing data analysis for the genetic expression profiling on T2DM db/db mice showed down-regulation

of *APOAI* gene in the liver of diabetic mice [9]. In humans, the level of serum apoA-I protein and other subclasses have been measured in order to determine its association with the risk for onset of T2DM and diabetic dyslipidemia. And it was imitated by the upshot of a research which revealed that serum levels of apoC3 and apoC3 to apoA-I ratio were linked with the risk of T2DM outcome [10]. A human population study showed direct effect of *APOAI* -75 A allele polymorphism on elevated serum levels of HDL-C and apoA-I proteins with lower risks for CVDs [11].

ABCAI protein, containing 2261 amino acids is encoded by *ABCAI* gene that is located on the chromosome number 9 at position 31.1 of its long (q) arm and it contains 50 exons, as assigned by Human Genome Database (HGD), respectively. *ABCAI* receptor proteins that are located on the macrophages and liver cells of the body behaves both as a lipid exporter and a signaling receptor. Thus, it plays a vital role in maintaining HDL-C levels. Many studies in animal models and humans have been conducted in order to get a better grip and understanding for the structure and function of *ABCAI* protein. Mice models (C57BL/6j) that lacked adipocytes *ABCAI*, were fed high fat and high cholesterol diet, as a result they showed reduced *ABCAI* efflux activity with higher levels of adipocyte triglycerides and HDL-C, raised obesities, insulin secretion/sensitivity and impaired glucose tolerance [12]. In another study, the association of *ABCAI* variant, which is believed to be the cause of obesity and dyslipidemia in diabetes, was assessed and it was found to be abundant in T2DM subjects [13]. A T2DM systemic review and meta-analysis detected the relation of *ABCAI* mutations *rs9282541* and *rs1800977* with low HDL-C levels. It was also found that these variants were responsible for modulating cholesterol efflux in plasma and mutations caused an increase risk of T2DM outbreak [14]. In this study we have aimed at accessing the relationship of T2DM with dyslipidemia by determining the gene expression of *APOAI* gene and identifying genetic variants of *ABCAI* gene in T2DM patients with and without dyslipidemia in Pakistani population.

METHODOLOGY

Sampling and Study Design

This cross-sectional comparative research study, granted for its ethical approval from the institutional Ethical Review Committee of Army Medical College, National University of Medical Sciences (NUMS), Rawalpindi, Pakistan, was piloted from June 2018 to March 2019. Standardized WHO calculator entailed for calculating the sample size, achieved succeeding statistical assumptions; confidence level as 95%, alpha error as 5%, study power as 80%, anticipated population proportion with *ABCAI* and *APOAI* genes as 20.1% and relative precision as 8.3%. Ninety subjects were targeted for this study, distributing them equivalently in three categories; diabetic dyslipidemic patients (n = 30), diabetic non-dyslipidemic patients (n = 30) and controls, encompassing normal and heavy individuals (n = 30). Written informed consent was sought before enrollment of each subject and they were informed about purpose and procedure of the study. The inclusion and exclusion criterias followed were as described previously [15].

Genomic DNA and Total RNA Extraction:

Total cellular RNA from peripheral blood samples was isolated using GeneJET RNA purification kit. (ThermoFisher scientific). The full length first strand of cDNAs synthesis was generated using RevertAid First Strand cDNA synthesis kit (ThermoFisher scientific). Nuclear genomic DNA was extracted from the whole blood samples by organic method [16]. Quality and quantity of nucleic acids were determined on 0.8% and 1%agarose gel by visualization on Gel Doc System (Bio-Rad). The quantity of nucleic acids was also determined by measuring absorbance at 260nm and 280nm and optical density (OD) at 260/280nm estimated the purity of the DNA and RNA. The ratio ~1.8 for DNA and ~2 for RNA provides good purity of nucleic acids.

Gene Expression Analysis of *APOAI* Gene

The *APOAI* gene was amplified on conventional PCR. Gene sequence of *APOAI* was retrieved from ENSEMBL (https://asia.ensembl.org/index) and primers from coding region were designed using online primer3 software (https://primer3.ut.ee/). The sequences of left and right primers were 5´CACTGTGTACGTGGATGTGC3´ and ´5AGGCCCTCTGTCTCCTTTTC3. PCR program was as follow; hot start at 95°C for 3 minutes, 35 cycles of denaturation at 95°C for 30 seconds, annealing at 58.8°C for 40 seconds and elongation at 72°C for 1 minute. The PCR reaction mix contained following reagents; 1xTaq Buffer, 1.5mM $MgCl_2$, 0.2µM deoxynucleoside triphosphates (dNTPs), 1pmole/µl of left and right primers, 1unit *Taq* DNA polymerase, 1µl template DNA and x volume nuclease free water. Afterward *APOAI* gene was amplified through Real Time PCR on Cepheid Smart Cycler II using Maxima SYBR® Green qPCR SuperMix (ThermoFisher scientific). *Glyceraldehyde-3-phosphate dehydrogenase* (*GAPDH*) gene was used as an endogenous internal control. Both genes were amplified in all samples in duplicate in reaction volume of 25ul containing diethyl pyrocarbonate water, 2x SYBR® Green qPCR SuperMix, 1pmole/µl forward and reverse primers and 2µl cDNA template. Program was set as 40 cycles of denaturation at 96°C for 10 minutes, denaturation at 95°C for 30 seconds and annealing at 58.8°C for 40 seconds. Relative quantification of *APOAI* gene expression in each sample was done using $\Delta\Delta C_T$ method [17].

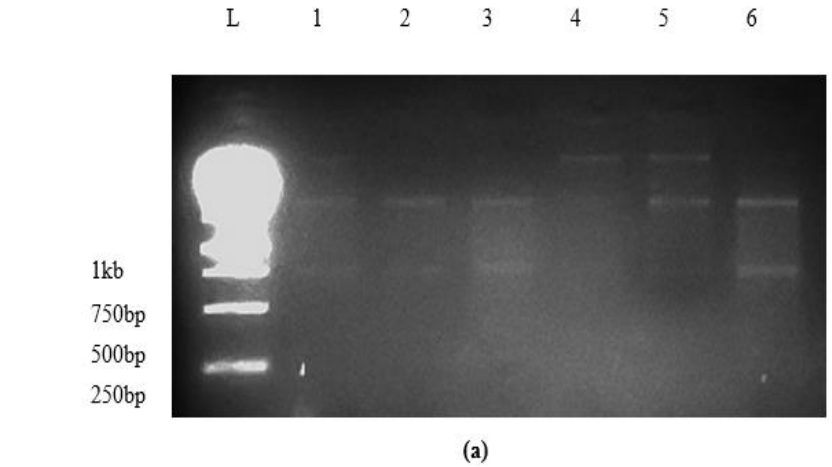
Identification of genetic variants in *ABCAI* Gene

The exon 4 of *ABCAI* gene was selected due to identification of an insertional frameshift mutation (c.219 ins T) previously [18]. *ABCAI* gene sequence was retrieved from ENSEMBL (https://asia.ensembl.org/index) and intron flaking primers of exon 4 were

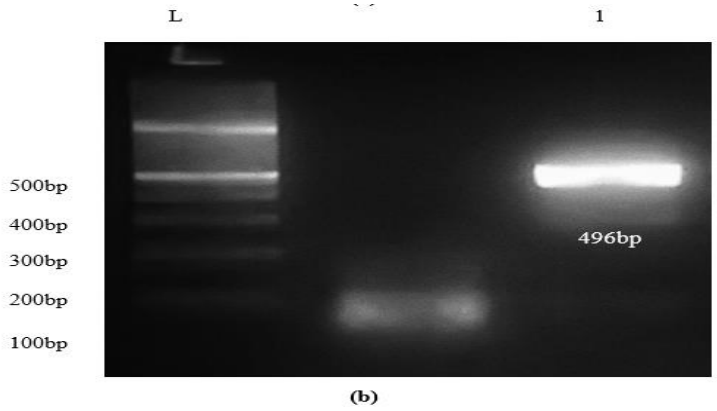
designed on primer3 software (https://primer3.ut.ee/). The sequence for left primer was 5´agcctcaaaatcgcttcagg3´and right primer was 5´CCAGAGGACAAGAAAGGAAGG3´. The amplification was performed on conventional PCR (Corbet In USA) that followed hot start at 95ºC for 3 minutes, 35 cycles of denaturation at 95ºC for 30 seconds, annealing at 58ºC for 30 seconds and extension at 72ºC for 30 seconds, final extension at 72ºC for 8 min. PCR products were purified using GeneJET PCR purification kit (ThermoFisher scientific). A sequencing PCR was done using DTCS kit (Beckman-Coulter GenomeLab™ Dye Terminator Cycle Sequencing). The sequencing reaction (15µl volume) was performed with 50 fmol purified PCR product, 3.2 pmol forward primer, 4µl DTCS mixture. Sequencing PCR was performed at 96ºC for 20 seconds, annealing at 58ºC for 20 seconds and extension at 60ºC for 4 minutes with 30 cycles. The reactions were run on Beckman Coulter CEQ8000 DNA Genetic Analyzer. Sequencing data of group I, II and III was analyzed using BioEdit 7.9.1 biological software for comparison and identification of mutations. The pair-wise alignment of sequencing data was also performed on *Basic Local Alignment Search Tool (BLAST)* against human genome database to detect genetic variations.

RESULTS

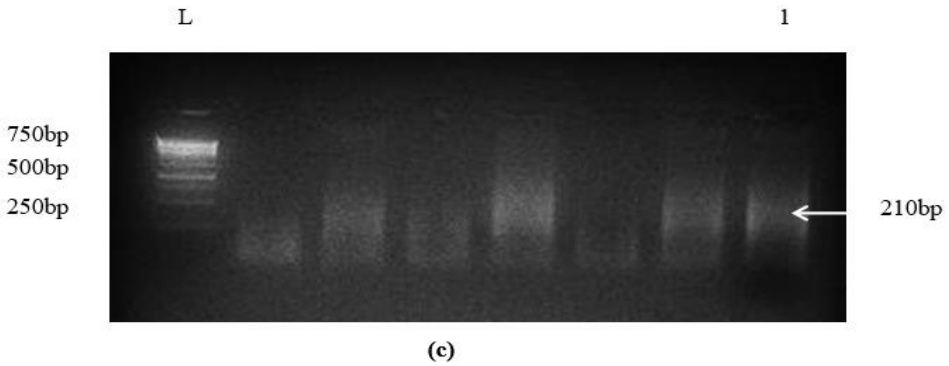
Intact RNA as 28S rRNA and 18S rRNA in good quality were detected. Range of the quantities of RNAs and DNAs was 50ng/µl to 5µg/µl and 12ng/µl to 20ng/µl [Figure-1 (a) & (d)]. The amplicon size of *GAPDH* gene was 496bp and *APOAI* PCR was 210bp [Figure-1 (b) & (c)]. The size of PCR product of exon 4 of *ABCAI* gene was 278kb [Figure-1(e)]. This 278kb fragment amplified spanning on full exon sequence with flanking intronic region.



(a)



(b)



(c)

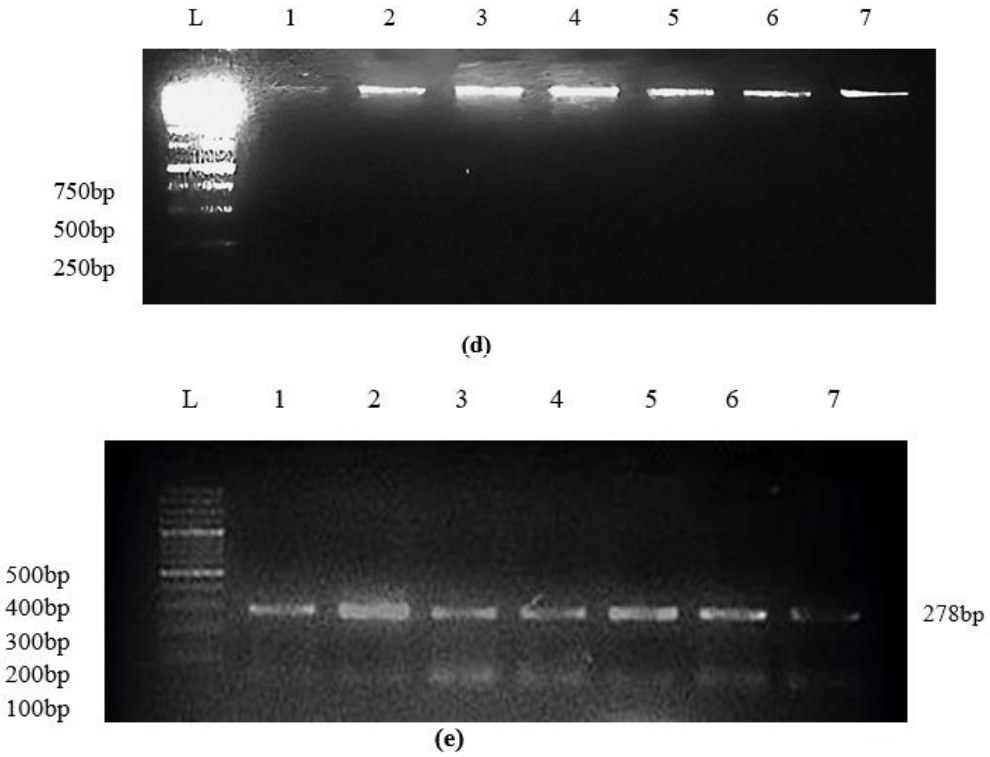
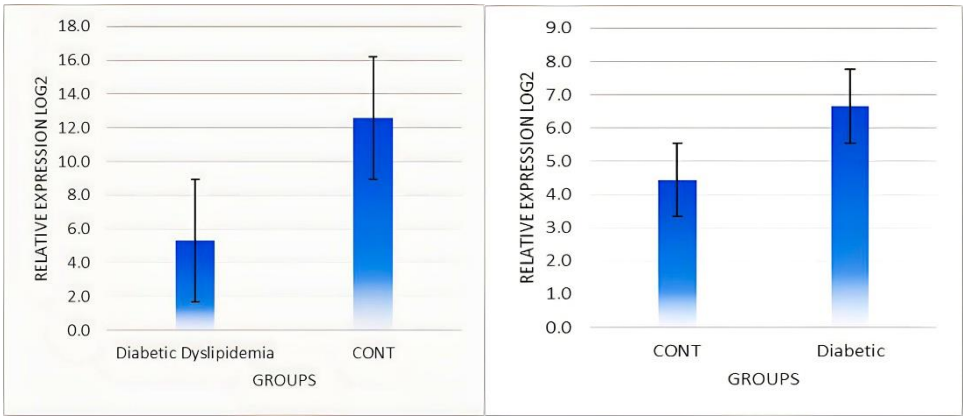
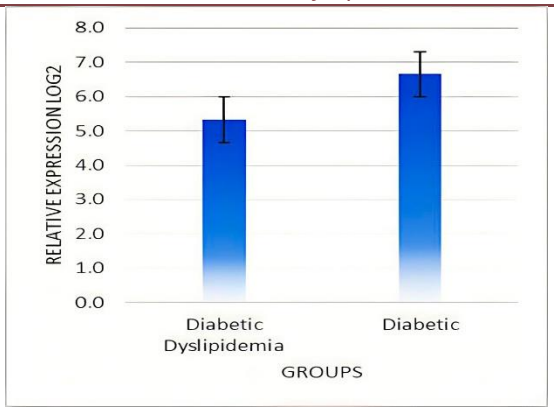


Figure-1: (a) RNA from blood sample. (b) GAPDH gene PCR. (c) APOAI gene PCR. (d) L 1kb DNA Ladder, Lanes 1-7, DNA from blood samples. (e) PCR of exon-8 of ABCAI gene.

The endogenous housekeeping *GAPDH* gene showed almost constant expression in samples of group I, II and III with CT values 22. 22.05 and 21.5. *APOAI* gene presented no expression changes in wholly samples. Mean CT values in group I was 19.03, 19.84 of group II and 21.14 of group III. The expression of *APOAI* gene group I was 5.3fold changed and 6.9fold changed in group II compared to control group. These results showed abundance and up-regulation of *APOAI* in samples of group I and group II [Figure-2 (a, b & c)]. There was noticeable increase in expression of *APOAI* gene in diseased groups compared to control but *t test* analysis suggested insignificant difference of gene expression between diseased groups (I & II) and control group (III) ($p=0.06$, $p>0.05$).



(a) **(b)**

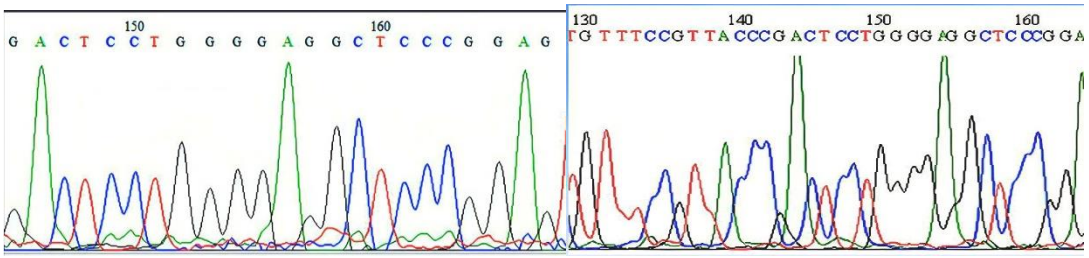


(c)

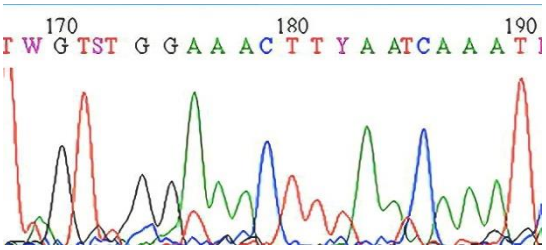
Figure-2: *(a) Relative Expression of APOAI gene in diabetic dyslipidemia group and control group. (b) Relative expression of APOAI gene in diabetic and control group. (c) Relative expression of APOAI gene in diabetic dyslipidemia and diabetic group.*

DNA Sequencing of *ABCAI* gene

ABCAI exon-4 was identified in normal sequence pattern in all subjects of three groups. There was no single base change identified in exon 4 of *ABCAI* gene in studied subjects. These results suggest that other exons need to be studied [Figure-3 (a, b, c)]



(a) **(b)**



(c)

Figure-3 (a, b & c): DNA Sequencing of ABCAI Gene in samples of Group I, II and III.

DISCUSSION

Low levels of HDL-C have been the known risk factor for T2DM, which also triggered the onset of disease. Therefore, when considering the treatment approaches for diabetes, the forefront goal of the different therapies includes the reduction of CVD risks by normalizing the plasma lipid levels [19]. Around the world, CVD due to diabetic dyslipidemia has shown to be the leading cause of death in diabetic patients. Lipid lowering drugs such as statins have shown minimal effect on dyslipidemia but they do reduce the risk of CVDs [5]. About 60-70% of the diabetic patients have shown symptoms of diabetic dyslipidemia. Researches depict that abnormality in the lipid levels in diabetic patients also mingled with the glucose metabolism [20]. *APOAI* and *ABCAI* genes encode proteins that take active part in RCT mechanisms and mature HDL-C formations. Decrease in HDL-C levels has been linked with changes in the expression of *APOAI* gene and genetic mutations of *ABCAI* gene.

Genetic variations in *APOAI* have been linked with HDL-C level disturbances in different populations. Literature review studies illustrated that researches have been conducted on the transcriptional control of apolipoproteins in animal models and cell culture lines related to diabetes. This study was carried out to understand the mechanism of dyslipidemia at molecular level with the association of *APOAI*. *APOAI* gene expression was determined in T2DM subjects linked with and without dyslipidemia by comparing them with normal healthy individuals using *GAPDH* gene as an internal control. The detected mutations might lead

to up-regulation or down-regulation of gene expressions. The results of our study suggested that *APOAI* was transcribed at normal levels and did not contribute for the onset of dyslipidemia in T2DM. However, the results demonstrated slight abundance of gene at transcriptional level in diseased groups compared to control but no remarkable influence of hyperglycemia on *APOAI* gene expression. In the present study, serum HDL-C levels were also noted along with high TAGs, TC and LDL-C.

In many previous studies, *APOAI* gene variants were seen relatable with metabolic syndrome for inducing atherosclerosis, linked with dyslipidemias [21-23]. Though symptomatic metabolic syndrome was detected in our study subjects as well, but no significant changes in *APOAI* gene expression were found. Hence, no linkage with T2DM and dyslipidemia could be concluded. As no down regulation was noted in *APOAI* gene in our subjects, therefore, no alterations in its associated protein structure and function could be recognized as well. In order to observe detailed changes in the gene expression, extensive studies spanning bigger sample size over a prolong period of time should be carried out. It was a cross sectional study entailing freshly diagnosed subjects, so initiation of their *APOAI* gene down regulation might not be evident at that moment.

Little work has been reported on the genetic variations in *ABCAI* with reference to HDL-C levels and dysfunctions in the humans that showed disrupted RCT mechanisms. Genetic variations in *ABCAI* has also showed a key role in the imbalances of plasma HDL-C levels. Previous literatures have focused on the association of reduced expression of *ABCAI* with HDL-C levels in T2DM subjects for triggering CVD risks [24]. In the present study, our target was to find the variations in exon-4 of *ABCAI* that influenced T2DM and diabetic dyslipidemias in Pakistani population. No mutations were found in diseased groups. *ABCAI* consists of 49 exons. However, mutations might be present in the other exons of the gene that might be linked with T2DM or T2DM dyslipidemias. A better understanding of sequential expression of respective off-targets (OTs) and the mutations causing the variation in expression should be studied extensively over the life span so that more effective prevention strategies can be initiated. Our genetic study did not prove combined roles of *APOAI* and *ABCAI* to be the hallmark for aberrant HDL-C values, eliciting RCT modifications in T2DM.

CONCLUSION

Regarding the expression analysis of *APOAI* gene, our results concluded a slight increase in the expression of *APOAI* in diabetic dyslipidemia and simple diabetic patients, showing the influence of hyperglycemia on gene transcription. However, up-regulation is not linked with dyslipidemia in diabetes because simple diabetic patients also exhibited the same up-regulation.

Authors Contribution

ZKK did conceptual frame work designing, data collection, experimental work and manuscript writing

AR did the overall supervision, conceptual content improvement, reviewed the article and gave the final approval of manuscript. AM designed the project, supervisor of the project, did the analysis and designing along with editing of the manuscript

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B. Conflict of Interest: *None.*

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REFERENCES

1. Shestakova MV, Dedov, II. [Diabetes mellitus in the Russian Federation: Arguments and facts]. Terapevticheskii arkhiv. 2016;88(10):4-8. doi: http://dx.doi.org/10.17116/terarkh201688104-8.
2. Cho N, Shaw J, Karuranga S, Huang Y, da Rocha Fernandes J, Ohlrogge A, et al. IDF Diabetes Atlas: Global estimates of diabetes prevalence for 2017 and projections for 2045. Diabetes research and clinical practice. 2018;138:271-81. doi: http://dx.doi.org/10.1016/j.diabres.2018.02.023.
3. Basit A, Tanveer S, Fawwad A, Naeem N, Members N. Prevalence and contributing risk factors for hypertension in urban and rural areas of Pakistan; a study from second National Diabetes Survey of Pakistan (NDSP) 2016–2017. Clinical and Experimental Hypertension. 2019:1-7. doi: http://dx.doi.org/10.1080/10641963.2019.1619753.
4. Rehan M, Waqar S, Khan H. Prevalence of Dyslipidemia among Diabetic Patients in a Tertiary Care Hospital. Journal of Islamabad Medical & Dental College. 2018;7(2):136-9.
5. Rodriguez V, Newman JD, Schwartzbard AZ. Towards more specific treatment for diabetic dyslipidemia. Current opinion in lipidology. 2018;29(4):307. doi: http://dx.doi.org/10.1097/MOL.0000000000000528.

6. de Luis DA, Izaola O, Primo D, Aller R. Implication of the rs670 variant of APOA1 gene with lipid profile, serum adipokine levels and components of metabolic syndrome in adult obese subjects. Clinical Nutrition. 2017. doi: http://dx.doi.org/10.1016/j.clnu.2017.12.007.
7. Sorci-Thomas MG, Zabalawi M, Bharadwaj MS, Wilhelm AJ, Owen JS, Asztalos BF, et al. Dysfunctional HDL containing L159R ApoA-I leads to exacerbation of atherosclerosis in hyperlipidemic mice. Biochimica et biophysica acta. 2012;1821(3):502-12. doi: http://dx.doi.org/10.1016/j.bbalip.2011.08.019.
8. Méndez-Lara KA, Farré N, Santos D, Rivas-Urbina A, Metso J, Sánchez-Quesada JL, et al. Human ApoA-I Overexpression Enhances Macrophage-Specific Reverse Cholesterol Transport but Fails to Prevent Inherited Diabesity in Mice. International journal of molecular sciences. 2019;20(3):655. doi: http://dx.doi.org/10.3390/ijms20030655.
9. Zhang F, Xu X, Zhang Y, Zhou B, He Z, Zhai Q. Gene expression profile analysis of type 2 diabetic mouse liver. PloS one. 2013;8(3):e57766. doi: http://dx.doi.org/10.1371/journal.pone.0057766.
10. Brahimaj A, Ligthart S, Ikram MA, Hofman A, Franco OH, Sijbrands EJ, et al. Serum levels of apolipoproteins and incident type 2 diabetes: a prospective cohort study. Diabetes Care. 2017;40(3):346-51. doi: http://dx.doi.org/10.2337/dc16-1295.
11. Liao B, Cheng K, Dong S, Liu H, Xu Z. Effect of apolipoprotein A1 genetic polymorphisms on lipid profiles and the risk of coronary artery disease. Diagnostic Pathology. 2015;10(1):102. doi: http://dx.doi.org/10.1186/s13000-015-0328-7.
12. O'Reilly M, Dillon E, Guo W, Finucane O, McMorrow A, Murphy A, et al. High density lipoprotein proteomic composition, and not efflux capacity, reflects differential modulation of reverse cholesterol transport by saturated and monounsaturated fat diets. Circulation. 2016:CIRCULATIONAHA. 115.020278. doi: http://dx.doi.org/10.1161/CIRCULATIONAHA.115.020278.
13. Lu C-H, Liao W-L, Wang T-Y, Chen C-C, Chen Y-H, Tse S-S, et al. Association of adenosine triphosphate-binding cassette transporter A1 gene polymorphism with lipid profiles and early-onset type 2 diabetes. Sci Asia. 2014;40:212-18. doi: http://dx.doi.org/10.2306/scienceasia1513-1874.2014.40.212.
14. Haerian BS, Haerian MS, Roohi A, Mehrad-Majd H. ABCA1 genetic polymorphisms and type 2 diabetes mellitus and its complications. Meta Gene. 2017;13:104-14. doi: http://dx.doi.org/10.1016/j.mgene.2017.05.007.
15. Khan Z, Majeed A, Rashid A. THE RISK FACTORS ASSOCAITED WITH DIABETIC DYSLIPIDEMIA AND ANTHROPOMETRIC PARAMETERS LINKED WITH AND WITHOUT DYSLIPIDEMIA IN TYPE 2 DIABETES MELLITUS. PAFMJ [Internet]. 30Apr.2020 [cited 13Apr.2023];70(2):628-3. Available from: https://cern-124-43-210.comsats.edu.pk/index.php/PAFMJ/article/view/4245.
16. Sambrook J, Russell DW. Isolation of High-molecular-weight DNA from Mammalian Cells Using Proteinase K and Phenol. CSH Protoc. 2006;2006(1). doi: http://dx.doi.org/10.1101/pdb.prot4036.
17. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods. 2001;25(4):402-8. doi: http://dx.doi.org/10.1006/meth.2001.1262.
18. Pisciotta L, Bocchi L, Candini C, Sallo R, Zanotti I, Fasano T, et al. Severe HDL deficiency due to novel defects in the ABCA1 transporter. J Intern Med. 2009;265(3):359-72. doi: http://dx.doi.org/10.1111/j.1365-2796.2008.02019.x.
19. Newman JD, Schwartzbard AZ, Weintraub HS, Goldberg IJ, Berger JS. Primary prevention of cardiovascular disease in diabetes mellitus. Journal of the American College of Cardiology. 2017;70(7):883-93. doi: http://dx.doi.org/10.1016/j.jacc.2017.07.001.
20. Parhofer KG. Interaction between glucose and lipid metabolism: more than diabetic dyslipidemia. Diabetes & metabolism journal. 2015;39(5):353-62. doi: http://dx.doi.org/10.4093/dmj.2015.39.5.353.
21. Wu Y, Yu Y, Zhao T, Wang S, Fu Y, Qi Y, et al. Interactions of environmental factors and APOA1-APOC3-APOA4-APOA5 gene cluster gene polymorphisms with metabolic syndrome. PloS one. 2016;11(1):e0147946. doi: http://dx.doi.org/10.1371/journal.pone.0147946.
22. Hosseini-Esfahani F, Mirmiran P, Daneshpour MS, Mehrabi Y, Hedayati M, Soheilian-Khorzoghi M, et al. Dietary patterns interact with APOA1/APOC3 polymorphisms to alter the risk of the metabolic syndrome: the Tehran Lipid and Glucose Study. British Journal of Nutrition. 2015;113(4):644-53. doi: http://dx.doi.org/10.1017/S0007114514003687.
23. Hosseini-Esfahani F, Mirmiran P, Daneshpour MS, Mottaghi A, Azizi F. The effect of interactions of single nucleotide polymorphisms of APOA1/APOC3 with food group intakes on the risk of metabolic syndrome. Avicenna journal of medical biotechnology. 2017;9(2):94.
24. Nam M, Choi M-S, Jung S, Jung Y, Choi J-Y, Ryu DH, et al. Lipidomic Profiling of Liver Tissue from Obesity-Prone and Obesity-Resistant Mice Fed a High Fat Diet. Scientific reports. 2015;5:16984. doi: http://dx.doi.org/10.1038/srep16984,