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ASGR1 but not *FOXM1* expression decreases in the peripheral blood mononuclear cells of diabetic atherosclerotic patients

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ABSTRACT

Background: The *ASGR1* was recently shown to play a key role in the development of coronary artery disease (CAD), but its exact mechanism of action in the CAD pathogenesis is not yet known. This study evaluates the possible association between the expression level of *ASGR1* and its downstream transcription factor *FOXM1* in the inflammatory cells of peripheral blood (PBMC) and the pathogenesis of CAD in the Diabetic condition.
Methods: Blood samples were taken from the candidates who had visited the Tehran Heart Center and had underwent diagnostic tests with respect to diabetes and CAD. The peripheral blood cells were harvested, RNA was extracted, and cDNA was synthesized. The qRT-PCR was performed on 79 cDNA samples taken from 49 CAD^+ patients and 30 CAD^- patients.
Results: In this study, we observed a significant decrease of *ASGR1* expression in the PBMC of CAD^+ patients compared to the CAD^- patients. We did not identify any considerable differences in the expression of *FOXM1* in patients' subgroups with respect to the diabetes and CAD.
Conclusion: The results of our study determine the association of *ASGR1* expression and CAD pathogenesis. However, we do not know whether this result is the cause or the effect of CAD.

1. Introduction

Type 2 diabetes is a condition in which the beta cells of the islet of Langerhans lose their ability to secrete insulin in response to varying degree of blood glucose level. This leads to hyperglycemia which disturbs cell function and increases the production of reactive oxygen species (ROS). The excessive generation of ROS leads to increased DNA damage and inflammation.[1] As a consequence, the prevalence of microvascular and macrovascular diseases is higher in diabetic patients. Coronary artery disease (CAD) is a major complication of type 2 diabetes in which the narrowing and subsequent occlusion of the arteries leads to myocardial infarction and death.[2]

CAD is regarded as an inflammatory disease in which both immune and metabolic factors interact and activate lesions in the arterial wall. The accumulation of immune cells in the lesions and the production and secretion of cytokines and chemokines accelerate the progression of lesion and the foam cell formation.[3]

The Extracellular signal-regulated kinase (ERK) signaling pathway is reported in numerous studies to be involved in regulation of inflammation[4,5] and inflammatory associated conditions in cancer,[6,7] Insulin resistance,[8] rheumatoid arthritis,[9,10] vascular inflammation, and atherosclerosis.[11–13] Also, inflammatory cytokines regulate ERK signaling pathway[14].

A recent study revealed that people who are haploinsufficient for the ASGR1 gene are less prone to the CAD development and have lower levels of plasma non-HDL cholesterol and triglycerides. This dramatic reduction in CAD risk is stronger than previously identified genetic variants (e.g. PCSK9 mutation). Interestingly, ASGR1 variant is revealed to have a lesser effect on non-HDL cholesterol level than the mentioned genetic variants.[15] Hence, other protective mechanisms may be involved. One of the proposed mechanisms for the ASGR1's role in the CAD development is the modulation of inflammation.[16]

ASGR1 encodes the major subunit of the Asialoglycoprotein receptor (ASGR), a lectin receptor which has multiple functions; they include the elimination of apoptotic cells, activated lymphocytes, and the immunoglobulin A (IgA) from the circulation.[17] ASGR is mostly expressed on the

Abbreviations: CAD, Coronary artery disease; PBMC, Peripheral blood mononuclear cells; ASGR, Asialoglycoprotein receptor; ROS, Reactive oxygen species; BMI, Body mass index,; HbA1C, Glycated hemoglobin; FBS, Fasting blood sugar; HDL, High density lipoprotein; LDL, Low density lipoprotein; TCH, Total cholesterol; Non-HDL Cholesterol, Total Cholesterol − HDL cholesterol.

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hepatocyte, but its expression has also been observed in the macrophage, monocyte, testes, and intestinal epithelial cells. This receptor is composed of two subunits, the *ASGR1* and the *ASGR2*, both of which are located on chromosome 17p13.1 and in close distance to each other.[18,19]

There are also reports on the immunoregulatory role of these receptors.[17] Moreover, studies in the mouse model show that macrophage lectin receptors could play a role in atherosclerosis development and the foam cell formation by up taking the desialylated LDL cholesterol.[20] Also, based on some studies, the expression, the synthesis, and the function of ASGR are regulated by inflammatory cytokines including interleukin-1 (IL-1), interleukin-6 (IL-6), and tumor necrosis factor (TNF).[21,22]

According to studies, ASGR1 activates ERK signaling pathway by interacting with Epidermal growth factor receptor (EGFR).[23] The increased expression of EGFR and its ligands has been observed in human CAD plaque, infiltrating monocytes, and macrophage.[24] In addition, a recent study indicates that the therapeutic modulation of this pathway may prevent CAD progression by decreasing inflammation and oxidative stress.[25] ERK acts upstream of Forkhead box M1 (FOXM1) transcription factor, a major downstream effectors of ERK pathway that is involved in controls of the cycle progression and cell proliferation, and activates it by phosphorylation and nuclear translocation which subsequently causes FOXM1 expression by a positive autoregulatory loop.[26,27] The *FOXM1* gene is located on chromosome 12p13.3. Its expression is induced by tissue injury, and it is decreased in senescent cells.[28,29]

FOXM1 is a key regulator of oxidative stress, acts as a sensor for reactive oxygen species (ROS), and its expression increases in the presence of ROS; it downregulates ROS levels by stimulating the expression of ROS scavenger genes.[30] The previous studies demonstrate that *FOXM1* plays a key role in the inflammation and recruitment of inflammatory cells. This is because FOXM1 regulates the expression of various proinflammatory cytokines and chemokines involved in the migration and proliferation of the inflammatory cells including IL-6,[31] INOS (Nitric oxide synthase),[32] CCR2 (C—C chemokine receptor type 2),[33] and CX3CR1 (C-X3-C Motif Chemokine Receptor 1 or fractalkine),[34] all implicated in atherosclerosis pathogenesis.[35]

There has not yet been any report on the association between the *FOXM1* and *ASGR1*'s expression in the PBMC and the pathogenesis of CAD in diabetic patients, as an inflammatory condition. To address this limitation, this study evaluates the possible association between the *ASGR1* and *FOXM1* mRNA expression and the Diabetes-linked CAD development in the Iranian population. This is achieved by performing the expression analysis of the selected genes in the peripheral blood mononuclear cells, the inflammatory cells of the immune system.

2. Subjects, materials and methods

2.1. Peripheral blood sampling

This study is in accordance with ethical standards of the ethical committee of Tarbiat Modares University (Code: 59 d/6920) and with the 1964 Helsinki declaration and its later amendments. Informed consent was obtained from all individual participants included in the study. Samples were selected from the patients who underwent coronary angiography in the outpatient clinic of Tehran heart center. Blood samples were taken from 79 volunteers contain CAD+ patients ($n = 49$) and CAD− patients ($n = 30$) from October 2016 to September 2017. Patients with T2DM were selected based on the standard criteria (FBS > 125 mg/dl (6.9 mmol/l)) and (HbA1C > 6.5% (47 mmol/mol)). The severity of CAD was determined by subgrouping the patients into the two categories of single-vessel (SVD) and multi-vessels disease (MVD).

2.2. Biochemical analysis

The HDL-cholesterol and triglycerides levels were measured using an auto-analyzer (Cobas Integra 400, Roche Diagnostics). To estimates the LDL cholesterol, the Friedewald formula was used. Non-HDL Cholesterol calculated by subtracting the HDL-cholesterol from total Cholesterol. Fasting blood sugar (FBS) was measured by glucose hexokinase method (Cobas Integra 400, Roche Diagnostics). Hemoglobin A1C (HbA1c) was measured by an enzymatic method (Diazyme Laboratories, USA).

2.3. RNA extraction and CDNA synthesize

The PBMC isolation was performed by centrifugation and with the aid of Ficoll-Paque™ Lymphodex (Cedarlane, Netherlands). Total RNA was extracted by RNX-Plus reagent (CinnaGen, Iran). The quality and the quantity of the extracted RNA were examined by agarose gel electrophoresis and spectrophotometry. The extracted RNA was treated with DNase I (Fermentas, Lithuania) at 37 °C for 30 min. The cDNA synthesis was performed using 3 μg of RNA, M-MulV reverse transcriptase (Thermo Scientific, USA), Oligo (dT) and random hexamer primers (MWG, Germany) in a total volume of 20 μl reaction mixture, according to manufacturer's instructions.

2.4. Primer design and Real-time PCR

The primers were designed specifically for each gene by Oligo 7 software (Molecular Biology Insights, Inc., USA). The sequences of primers were as follows: forward: 5′-CAATGGCTCAGAAAGGACCTGC-3′, reverse: 5′-GCCTCCAGTTCTTGAAGCCCGTC-3′ for *ASGR1*, forward: 5′-ATGAGTTCTGATGGACTGGGC-3′, reverse: 5′-TTGTGGCGGATGGAGTTCTTC-3′ for *FOXM1* and forward: 5′-AGCCTTCCTTCCTGGGCATGG-3′, reverse: 5′-AGCACTGTGTTGGCGTACAGGTC-3′ for *ACTB* which used as an internal control for gene expression normalization. The Real-time PCR was performed using ABI StepOne™ (Applied Biosystems, Foster City, CA, USA) and 10 ng of cDNA template, 4 pM of each forward and reverse primers and 5× HOT FIREPol® EvaGreen® qPCR Mix Plus (ROX) (Solis BioDyne, Estonia). The Real-time PCR steps were as follows: pre-denaturation step at 95 °C for 12 min, followed by 40 cycles of denaturation at 95 °C for 15 s, annealing at 60 °C for 20 s and extension at 72 °C for 20 s. All experiments were run at least in duplicate. The specificity of PCR products was confirmed by poly acrylamide gel electrophoresis analysis of Real-time PCR products and melting curve analysis. The expression levels of the target genes were analyzed using $2^{-\Delta CT}$ method and relative fold change of each gene was calculated by Pfaffl method.[36,37]

2.5. Statistical analysis

The statistical analysis was performed using GraphPad Prism version 6.0 (Graph Pad Prism Software, Inc., San Diego, CA). The normal distribution of data was analyzed by performing the Shapiro–Wilk test. The correlation analysis was performed using the Pearson and spearman test based on the normality test result. Student's *t*-test and Mann–Whitney *U* test was used to compare parametric and nonparametric variables.

3. Results

3.1. The downregulation of ASGR1 but not FOXM1 in the PBMC of diabetic CAD+ patients

The mRNA expression analysis shows that *ASGR1* expression is downregulated in CAD^+ patients ($n = 49$) in comparison with the CAD^- patients ($n = 30$) (−2.41 Fold, $p = 0.0004$) (Fig. 1A). The expression analysis was also performed in the patient's subgroups based on

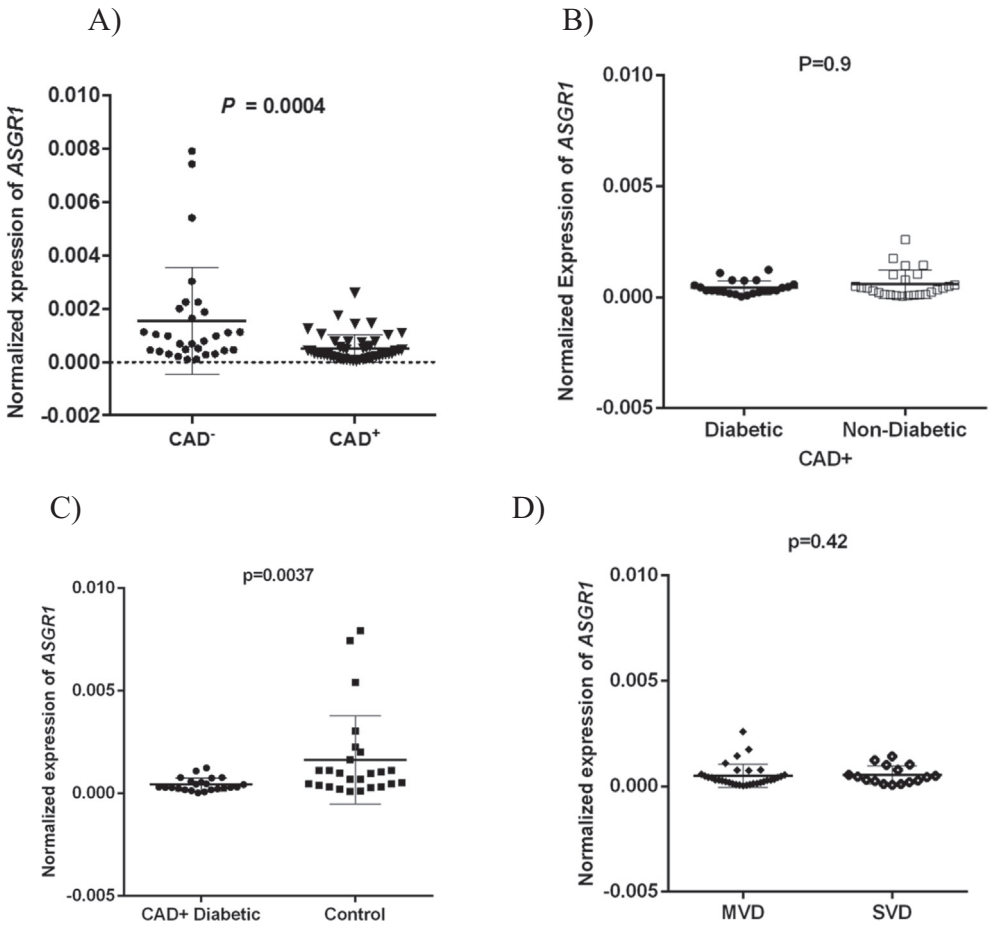

Fig. 1. Expression analysis of *ASGR1*. To normalize the gene expression, ACTB was used as internal control. ASGR1 expression is significantly downregulated in Coronary artery disease (CAD) patients CAD^+ ($n = 49$) in comparison to CAD^- [30] (Mann-Whitney *U* test, $p < 0.05$). There is not any significant difference in the expression of *ASGR1* in CAD^+ patients with ($n = 28$) or without Type 2 Diabetes ($n = 25$) (Mann-Whitney U test, $p > 0.05$). ASGR1 expression is significantly different in CAD^+ Diabetic ($n = 28$) in comparison to CAD^- Non diabetic ($n = 25$) (Mann-Whitney U test, $p < 0.05$). There is not any significant difference in the expression of *ASGR1* in the CAD^+ single vessel disease (SVD) and multivessel disease (MVD) patients (Mann-Whitney U test, $p > 0.05$).Data are presented as mean ± standard deviation.

whether they were affected by the diabetes or not; the subgroups of CAD^+ diabetic, CAD^- diabetic, CAD^+ non-diabetic, and CAD^- Non-diabetic were examined in this research. The expression level of *ASGR1* was not significantly different in CAD^+ patients who were diabetic ($n = 28$) versus non-diabetic ($n = 25$) ($p > 0.05$) (Fig. 1B).The expression level of *ASGR1* in the diabetic CAD^+ patient's ($n = 28$) vs healthy controls who were not affected by diabetes and CAD ($n = 25$) was also downregulated (−2.38 Fold, $p = 0.0037$) (Fig. 1C). The association of gene expression in the PBMC with the severity of CAD determined by subgrouping the CAD^+ patients into two groups of SVD ($n = 16$) and MVD ($n = 32$). Our results suggest that there is not a significant association between the expression of the *ASGR1* and the severity of coronary artery disease ($p > 0.05$) (Fig. 1D).

FOXM1 expression analysis did not show any significant difference in all the patient categories. Also, we did not observe significant association between the expression of *FOXM1* and the number of narrowed vessels ($p > 0.05$) (Fig. 2 A, B, C).

3.2. The ASGR1 mRNA expression in the PBMC may be a potential biomarker in the discriminating of CAD^+ patients

The receiver operating characteristic (ROC) curve analysis was performed to examine the possible discriminating potential of the ASGR1 mRNA expression in the PBMC of CAD^+ patients. The results show that the ASGR1 gene expression may be a good candidate for identifying the CAD affected patients; the area under the curve is 0.73 (95% Confidence Interval: 0.62–0.84, $p = 0.0005$) in the CAD+ ($n = 49$) versus. CAD− ($n = 30$); also, the (AUC) = 0.74 (95% Confidence interval = 0.6 to 0.88) and p value = 0.004 in the CAD+ Diabetic ($n = 28$) versus. CAD− Non diabetic ($n = 25$) (Fig. 3 A and B).

3.2.1. The correlation analysis between the expression of ASGR1 and FOXM1 expression reveals a small correlation

The correlation analysis was performed to examine the association of the selected genes expression; the correlation results was significant in the CAD^+ patients ($r = 0.31$ $p = 0.03^*$) but not CAD^- patients ($p = 0.15$, $r = 0.27$) (Fig. 4A). The correlation in CAD+ patients was not different with respect to the diabetes condition (Fig. 4 B–D).

3.2.2. The association between the selected genes mRNA expression and lipid profile and the glycemic control was not significant

The demographic characteristics of the participants are presented in (Table 1). The correlation analysis of mRNA expression with the lipid profile of the patients was not significantly different. We also evaluated the association of the *ASGR1* mRNA expression in the PBMC with the non-HDL cholesterol level (a better predictor for the cardiovascular risk assessment compared with LDL cholesterol), but we did not observe any significant association. *ASGR1* expression, however, showed a significant correlation with body mass index (BMI) in the CAD+ patients ($r = 0.3$ $p = 0.04^*$). The association of *ASGR1* and *FOXM1* mRNA expression with the glycemic profile such as HbA1c and FBS was also not significant (Table 2).

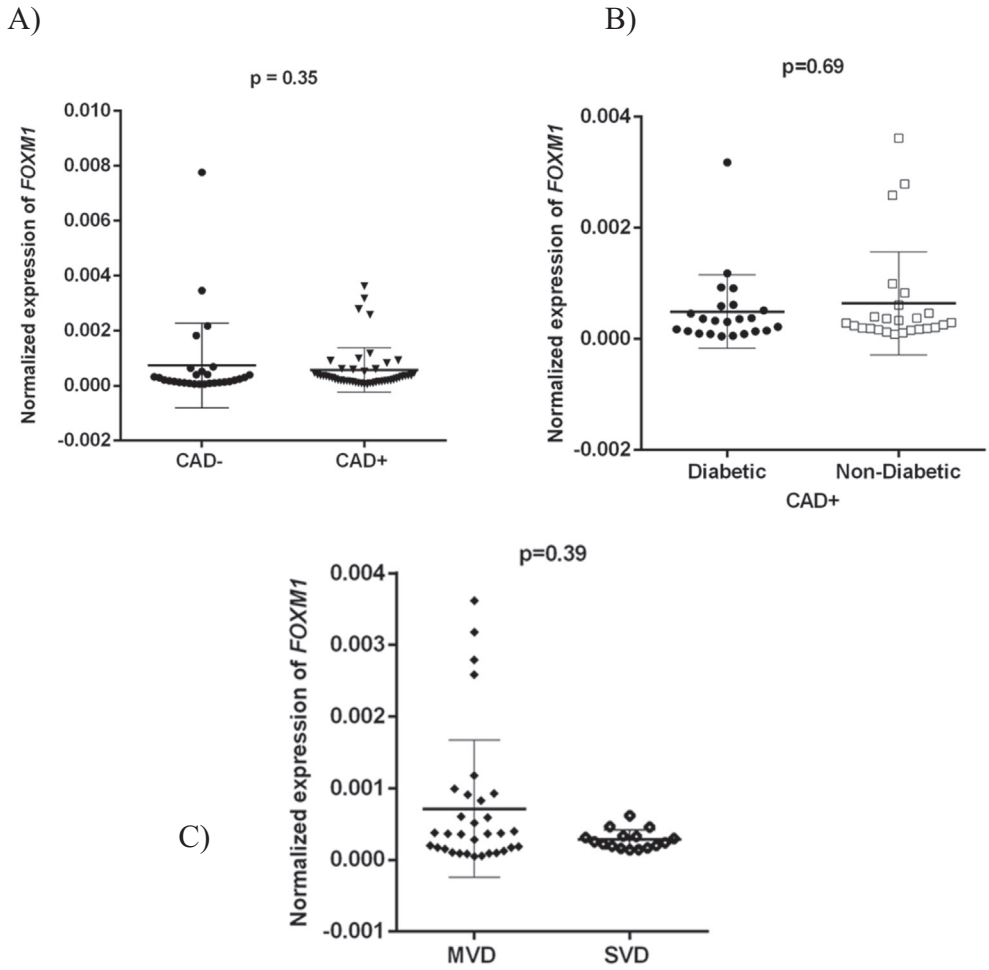

Fig. 2. Expression analysis of *FOXM1*. To normalize the gene expression, ACTB was used as internal control. The expression analysis of *FOXM1* did not show any significant difference in CAD + ($n = 49$) and CAD− patients ($n = 30$); also, the expression of *FOXM1* is not associated with diabetes in the CAD+ patients ($n = 25$ Non-diabetic and $n = 28$ diabetic) (Mann-Whitney U test, $p > 0.05$). There is no significant change in *FOXM1* expression regarding the CAD severity (Mann-Whitney U test, $p > 0.05$). Data are presented as mean ± standard deviation.

3.2.3. The expression analysis of ASGR1 and FOXM1 in the patients affected by hyperlipidemia or hypertension was not significant

The expression analysis of the selected genes in patient's categories did not show any significant association to hypertension (HTN) (HTN+, $n = 44$ and HTN−, $n = 31$). The same result was obtained in hyperlipidemia (HLP) patients (HLP+, $n = 41$ and HLP−, $n = 37$) ($p > 0.05$).

4. Discussion

Coronary artery disease is one of the leading causes of mortality worldwide. Studies show that Type 2 diabetes patients are more prone to the CAD development, and the severity of the disease is higher when compared with the non-diabetic patients[38];

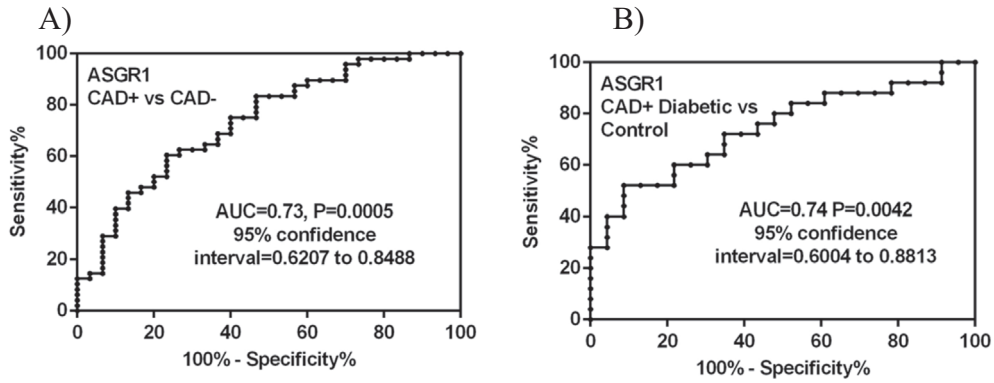

Fig. 3. Evaluation of *ASGR1* biomarker potential for CAD discrimination was performed by ROC curve analysis. This was achieved by plotting (sensitivity versus 1-Specifity) for each value. The results show the Area Under the curve (AUC) = 0.73 (95% Confidence interval = 0.62 to 0.84) and p value = 0.0005*** in CAD^+ versus. CAD^-. (AUC) = 0.74 (95% Confidence interval = 0.6 to 0.88) and p value = 0.004** in CAD^+ diabetic versus. CAD^- Non diabetic (control).

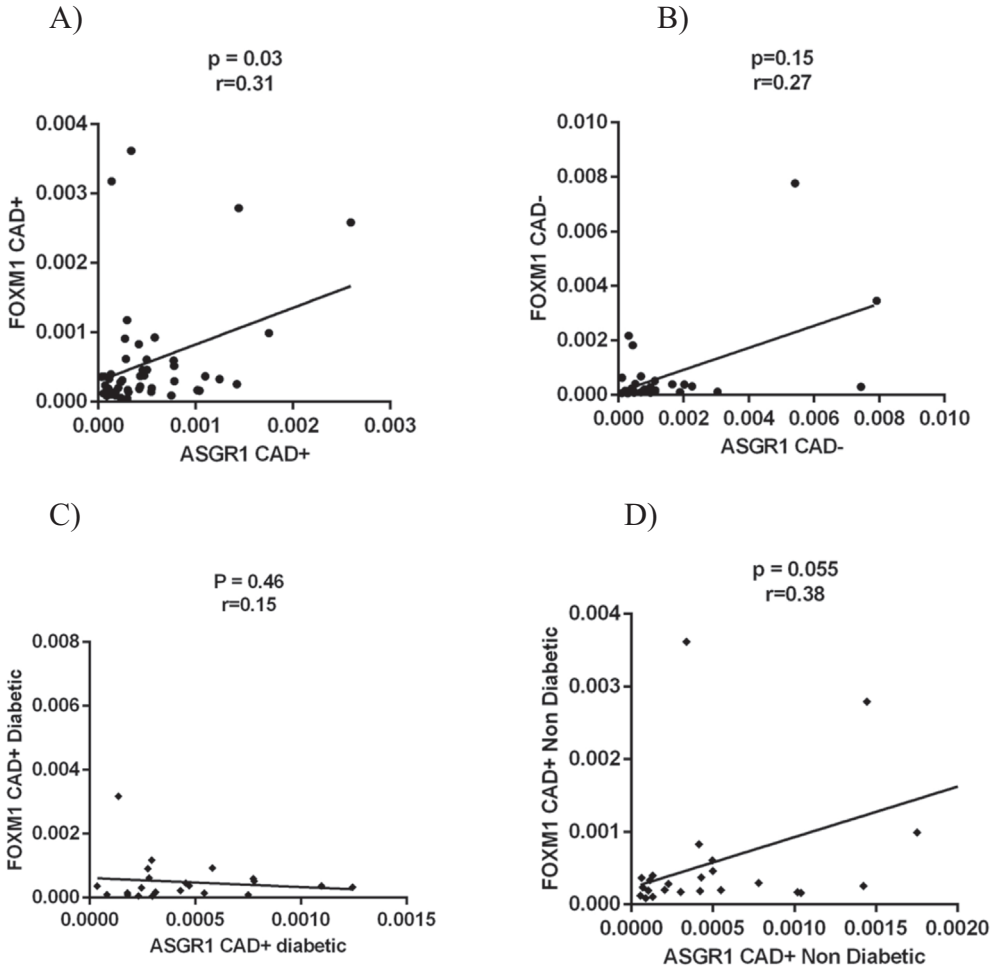

Fig. 4. Correlation analysis between the ASGR1 and FOXM1 mRNA expression in CAD^+ group is significant ($r = 0.31$ $p = 0.03$ Spearman correlation). Conversely, in CAD^- patients there is no significant correlation. ($r = 0.27$ $p = 0.15$ Spearman correlation). There does not exist any significant correlation between the selected genes in the CAD^+ diabetic and Non-diabetic patients. ($p = 0.73$, $r = 0.07$ Spearman correlation for CAD^+ Diabetic and $p = 0.05$, $r = 0.38$ spearman correlation for CAD^+ Non diabetic).

therefore, current studies focus on understanding the molecular mechanisms involved in the pathogenesis of diabetes-related CAD.

The dysregulation in immune system and Inflammatory pathways are the hallmark of Type 2 diabetes and its' associated complications. The change in immunological components is frequently observed in vascular cells, circulating peripheral blood cells, adipose tissue, and liver.[39] According to previous studies, The ERK pathway is involved in inflammatory responses and Insulin resistance associated with Diabetes

Table 1
Clinical characteristic of the patients.

Characteristics	CAD^+ ($n = 49$)	CAD^- ($n = 30$)	p values
Age (years, mean)	58 ± 9.65	56 ± 9.03	0.50[i]
Sex (male)	35 (71.4%)	15 (50%)	0.09[i]
BMI[a] (kg/m^2)	28.63 ± 4.8	30.31 ± 5.3	0.15[ii]
Triglycerides (mg/dl)	156.6[53–638]	135.8 [53–306]	0.26[ii]
HDL[b] (mg/dl)	39.81 ± 9.6	38.89 ± 12.1	0.34[ii]
LDL[c] (mg/dl)	100.5 ± 37.7	94.25 ± 29.7	0.4[ii]
TCH[d] (mg/dl)	159.54 ± 44.9	149.35 ± 33.71	0.43[ii]
HbA1c[e]	14.49 ± 1.84	15 ± 1.84	0.22[ii]
Hyperlipidemia	32 (65%)	11 (36.6%)	0.02[i]
Hypertension	31 (63.2%)	14 (46.6%)	0.1[i]

Data are presented as mean (SD) for variables with normal distribution and median [interquartile range] for those without normal distribution.

[i] Chi-square or Fisher's exact test is performed to compare variables between Coronary artery disease (CAD+) and CAD− patients.

[ii] Student's *t*-test or Mann–Whitney *U* test is performed to compare variables between CAD+ and CAD− patients.

[a] Body mass index.

[b] High density lipoprotein.

[c] Low density lipoprotein.

[d] Total cholesterol.

[e] Glycated hemoglobin.

Table 2
Correlation analysis between the selected genes mRNA expression and clinical characteristic of patients with and without CAD.

Gene	Correlation with	CAD+		CAD−	
		R	*p*-Value	R	*p*-Value
ASGR1	Triglycerides	0.1	0.44	0.09	0.6
	HDL	−0.08	0.58	0.20	0.28
	LDL	0.2	0.15	−0.008	0.9
	TCH	0.17	0.28	0.022	0.91
	BMI	**0.3**	**0.04***	−0.01	0.63
	Non HDL cholesterol[a]	0.17	0.24	−0.061	0.75
	FBS	−0.02	0.9	−0.08	0.7
	HB1AC	0.18	0.24	0.13	0.5
FOXM1	Triglycerides	0.02	0.8	−0.2	0.3
	HDL	0.003	0.9	0.14	0.47
	LDL	0.07	0.6	0.01	0.9
	TCH	0.13	0.3	0.04	0.8
	BMI	0.02	0.9	−0.06	0.7
	Non HDL cholesterol	0.11	0.45	−0.01	0.9
	FBS	−0.017	0.9	0.23	0.2
	HB1AC	0.08	0.6	0. 1	0.5

$p < 0.05$ was considered statistically significant.

[a] Non-HDL Cholesterol (including cholesterol rich components such as VLDL, LDL, IDL and chylomicrons) = Total Cholesterol − HDL cholesterol.

and atherosclerosis.[12,40] This signaling pathway is controlled by cytokines including interleukin-1β (IL-1β) which is a principal factor activated in Type 2 diabetes.[39] Herein we investigated the differential gene expression analysis of ASGR1 which activates the Erk signaling pathway and one of its major effectors, FOXM1.

The expression analysis was performed on the data obtained from the peripheral blood mononuclear cells. In comparison with the invasive Endarterectomy that is needed to access vascular plaque cells, PBMCs are easy to access. Also, The PBMC gene expression profile is altered in the presence of CAD because of the secretion of the proinflammatory cytokines and chemokines from the vessel walls.[41]

The recent studies in the field of the genetic of complex diseases emphasize the importance of identifying the genes in which the loss of function mutations leads to the protection against the disease. These studies lead to the development of drugs which mimic the observed protective effect by inhibiting the gene function.[42] For example, the loss of function in one or both copy of the *PCSK9* gene is accompanied by the protection against the CAD development and familial hypercholesterolemia. These studies led to the development of the evolocumab and the alirocumab drugs which obtained FDA approval in 2015.[43] A recent whole genome sequencing study in Iceland and subsequently few other countries revealed that the people who are haploinsufficient for ASGR1 have significantly lower chances of developing CAD. *ASGR1* encodes the major subunit of the Asialoglycoprotein receptor; this receptor is reported to be involved in the clearance of glycoproteins or glycolipids that have lost their terminal sialic acid from blood circulation.[15]

Sialic acid biosynthesis occurs through the hexose amine biosynthesis pathway in mammals, which is dysregulated in many kinds of human disorders such as Diabetes, cancer, and cardiovascular diseases.[44] Moreover, serum sialic acid is reported to be higher in patients affected by type 2 diabetes, and this upregulation is an indicator of severe vascular damage.[45] Also, clinical studies reveal that total sialic acid in serum is a risk factor for cardiovascular diseases and a marker of prolonged inflammation.[46] Although our literature review did not reveal any report on the association between this observed dysregulation of sialic acid and ASGR function, some connection may be revealed in future studies.

Contrary to our expectation, *ASGR1* expression was decreased in CAD+ patients by −2.41 fold compared to CAD−. This pattern of gene expression was also observed in patients who were affected by both type 2 diabetes and CAD compared with completely healthy individuals (CAD^-, Non diabetic). However, we did not observe any significant difference in the expression of *ASGR1* in CAD^+ patients who were diabetic versus the non-diabetic ones. The results of studies show that IL-1β in diabetes activates the expression of other cytokines such as IL-6, TNF, IL-8, and monocyte chemoattractant protein 1 (MCP1).[47] As previously mentioned, IL-6 and TNF participate in regulation of ASGR.[22] It should be noted that our result is in agreement with a previous study in which the expression of *ASGR1* was shown to decrease in the PBMC of Heart failure patients.[48] Our result is in contrast to the reported increased expression of the *ASGR1* gene in the PBMC of patients who are affected by multiple sclerosis (an immune disease of central nervous system).[49] This observation may be due to the fact that ASGR1 expression can be affected as a result of cardiovascular disease pathogenesis. The observed lack of association between the ASGR1 expression in the PBMC with the severity of CAD may be in agreement with the previous studies that suggest there is not a correlation between angiography severity and inflammatory markers level.[50] The Recent report by Fowdar et al. showed that there might be a connection between *ASGR1* and hypertension development by an undetermined mechanism.[51] In our study, the expression of *ASGR1* in the patient's subgroup who were affected by hypertension was not significantly different.

We did not observe any significant difference in the expression of *FOXM1*; however, some studies indicate the important function of FOXM1 in inflammatory disease including lung inflammation.[35,52] According to previous reports, FOXM1 activation is linked with improved glucose tolerance and insulin secretion; this is because FOXM1 is required to maintain the normal population of beta cells in the islet of Langerhans.[53] Moreover, a recent research in mouse model have emphasized the role of FOXM1 expression in macrophages through increased proliferation of lesion cells and atherosclerosis aggravation.[54]

The Cellular Senescence, a phenomenon observed in aging diseases such as type 2 diabetes, is triggered by oxidative stress which is also elevated in Diabetic and atherosclerotic patients.[55] FOXM1 expression is increased in response to the oxidative stress[30]; however, its expression is significantly decreased or hardly detectable in the senescent cells.[56] In our study, we did not evaluate cell senescence in the PBMC cell population, and the result of FOXM1 expression analysis may be masked by different conditions in cell populations.

In this study we observed no significant association between the expression of *FOXM1* in the PBMC and serum cholesterol level. Previous studies show that the lack of *FOXM1* expression is accompanied by aneuploidy.[57] Aneuploidy is reported to be a more common incident in the aortic endothelial cells of elderly and atherosclerotic patients, and the cholesterol uptake is reported to be higher in the cells that are affected by aneuploidy.[58]

It was recently shown that the increased expression of O-linked *N*-acetyl glucosamine transferase (O-GlcNAc) is associated with the increased stability of FOXM1 protein; the inhibition of O-GlcNAc leads to degradation of FOXM1 by Sirtuin 1 (SIRT1).[59] Moreover, another recent study revealed that the inhibition of ST3 Beta-Galactoside Alpha-2,3-Sialyltransferase 1 (ST3GAL1) is associated with decreased stability and degradation of FOXM1 protein.[60] More studies are needed to examine the possible role of FOXM1 in the pathogenesis of diabetes-related CAD. This is motivated by a few observations. First, the increased O-GlcNAc and the dysregulation of sialic acid pathway are commonly associated with the diabetic complications.[61] Second, the expression of *SIRT1* is reported to decrease in the PBMC of CAD diabetic patients.[62]

Here, we show that the PBMC mRNA expression of *ASGR1* and *FOXM1* in the CAD patients is slightly associated with each other. The correlation analysis in CAD^- and diabetic subgroups were not significant; however, due to the small sample size of this research and the small correlation, further studies are recommended to reach such a conclusion.

Despite the ASGR1's dramatic role in CAD pathogenesis, there is a lack of studies with respect to ASGR1's mechanism of action in CAD and its risk factors such as diabetes and inflammation. To our knowledge, this is the first time that the expression analysis of the *ASGR1* and *FOXM1* is reported in the PBMC of diabetic CAD patients. The result of our study is somehow in contradiction to what we expected from the genetic studies that suggest that the loss of function of *ASGR1* is accompanied by a lower risk of CAD development. This unexpected result may be due to the personalized medicine and difference in the genomes of different populations. Therefore, further studies with respect to the personalized medicine in different population are recommended.

5. Conclusions

This study evaluated the association between the expression of *ASGR1* and *FOXM1* in the PBMC and CAD pathogenesis. Our results reveal that the *ASGR1* expression in the PBMC is related to the pathogenesis of CAD. But, it is not obvious whether this observation is the cause or the effect of the CAD. This must be addressed in the future studies. Because of the low sample size in our research, further studies with a larger population are recommended. It is also suggested that the expression analysis of the selected genes be performed in the sorted peripheral blood cells.

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