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Upregulation of Legumain and transforming growth factor beta (TGF-β) in peripheral blood mononuclear cells associated with the severity of coronary stenosis

Arsalan Khaledifar[1], Ameneh Zamani Sedehi[2], Keihan Ghatreh Samani[2], Roohollah Mohseni[2*]

[1]Modeling in Health Research Center, Hajar Hospital, Shahrekord University of Medical Sciences, Shahrekord, Iran
[2]Clinical Biochemistry Research Center, Basic Health Sciences Institute, Shahrekord University of Medical Sciences, Shahrekord, Iran

***Correspondence to**
Roohollah Mohseni, Ph.D.,
Email: Mohseni.r@skums.ac.ir

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Abstract

Introduction: Coronary artery disease (CAD) is one of the most common causes of mortality throughout worldwide. Metabolic deregulation in peripheral blood mononuclear cells (PBMCs) could play important role in the pathogenesis of atherosclerosis.
Objectives: In the present study, we aim to examine gene expression of legumain and transforming growth factor beta (TGF-β) in patients with significant coronary artery stenosis.
Patients and Methods: In this case control study, 56 CAD participants (percentage of coronary artery stenosis ≥50) and 34 non-CAD participants (percentage of coronary artery stenosis ≤30) were incorporated to the study. Blood sample were collected and PBMCs were isolated. Serum factors were analysed and the percentage of coronary artery stenosis was recorded. The gene expression of legumain and TGF-β was evaluated by real time polymerase chain reaction. Finally, correlation of percentage of coronary artery stenosis with Legumain and TGF-β expression was analyzed.
Results: Legumain and TGF-β gene expression levels had a remarkable correlation with the coronary artery stenosis. Furthermore, fasting serum glucose had a significant positive correlation with the expression of legumain, TGF-β and percentage of coronary artery stenosis.
Conclusion: Alteration of expression of legumain and TGF-β maybe participate to pathogenesis of atherosclerosis.

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Introduction

Atherosclerosis is a public health problem globally. The monocyte-derived macrophages have a central role in atherosclerosis (1). Legumain, a proteolytic enzyme from C13 peptidase family, has been involved in several diseases (2). It is worthy to note that legumain expression is increased during the monocytes to macrophages differentiation (3). In addition, current studies have reported that expression and secretion of legumain is augmented in plasma and plaques of patients with carotid stenosis (4). Transforming growth factor beta (TGF-β) cytokine that suppresses the conversion of monocytes to macrophages. Indeed, reduced TGF-β level related to plaque instability (5). Accumulating evidence indicates that the deregulation of TGF-β in patients with atherosclerosis (6). Accordingly, deregulation of TGF-β activity and its related signalling pathway promote development of atherosclerotic.

Key point

Metabolic deregulation in peripheral blood mononuclear cells of participants with coronary artery disease maybe represent severity of coronary artery stenosis.

Objectives

In the present study, the correlation between expression of legumain and TGF-β in PBMCs with percentage of coronary artery stenosis, serum parameters and demographic characteristics were measured.

Patients and Methods

Study subjects

In this case control study. Ninety subjects referred to hospital Hajar in Shahrekord, Iran for angiography from May 2021 to November 2021, 56 participants with percentage of coronary artery stenosis

≥50 and 34 participants with percentage of coronary artery stenosis ≤30 were recruited. Two groups were matched in terms of age and gender. Typical coronary angiography was conducted to diagnosis of percentage of coronary artery stenosis. Questionnaire was completed. Moreover, systolic and diastolic blood pressure was measured. After angiography, blood samples were taken from the participants' arterial sheaths according to standard surgical procedures. Patients with cancer, renal failure, autoimmune diseases, liver disorders and insulin injections history were excluded from the current attempt. Participants with a coronary artery with a percentage of coronary artery stenosis ≥50 in at least one coronary artery was represent as coronary artery disease (CAD) patients. Participants with percentage of coronary artery stenosis≤30 was represented as non-CAD. With a minor modification, the Gensini scoring system was conducted to identify percentage of coronary artery stenosis in the main coronary arteries, including left anterior descending (LAD) coronary artery, left circumflex (LCX) and right coronary artery (RCA). This method awards 1 point for percentage of stenosis coronary artery, 4 points for percentage of coronary artery stenosis of 51 to 75, 8 points for percentage of coronary artery stenosis of 76 to 90, and 16 points for percentage of coronary artery stenosis of more than 90.

Biochemical analysis

Serum concentration of fasting serum glucose (FSG), cholesterol (Chol), high-density-lipoprotein-cholesterol (HDL-C) and triglycerides (TG) were assessed using kits (Pars Azmoon, Iran) in according with manufacturer's instructors. Serum concentration of low-density-lipoprotein-cholesterol (LDL-C) were calculated using the Friedewald formula.

Peripheral blood mononuclear cells separation

Peripheral blood mononuclear cells (PBMCs) were separated from whole blood by Ficoll-Hypaque solution (7). Briefly, PBMCs were harvested using a Pasteur pipette and cells were washed in two steps with phosphate-buffered saline. Cells were stored frozen at -70°C until RNA isolation.

RNA separation and real time PCR analysis

Total RNA was separated from PBMCs by RNX-Plus solution (Cinnagen, Iran). The quality and quantity of RNA were determined by a NanoDrop spectrophotometer (Thermo Fisher Scientific, USA). Using CDNA preparation kit (Ana Cell, Iran), CDNA was synthesized from total RNA. Real-time polymerase chain reaction (real-time PCR) was conducted by Light Cycler instruments (Roto Gene 6000, Germany). By Pfaffl methods the relative gene expression was analysed. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) housekeeping gene was used as control group. Specific primers were designed with Primer-BLAST software. Primer sequences were as follows:
legumain F: 5′-GGTACATCCTCGACGGCATCT-3′; R: 5′-GTGCCTCTTTGCTGCTTTCAC-3′.
GAPDH F: 5′-CCAGGCAGTCAGATCATCTTC-3′; R: 5′-AGCTGCCCCTCAGCTTGA-3′.
TGF-β F: 5′-CCCAGCATCTGCAAAGCTC-3′;
R: 5′-GTCAATGTACAGCTGCCGCA-3′.

Statistical analysis

The SPSS 18.0 and GraphPad Prism 9.00 software were utilized to data analysis. Kolmogorov-Smirnov was conducted to test normality status. Mann-Whitney U and *t* test were carried out to compare the results between two groups depending on the normality of the data. Moreover, the correlation coefficients between variables were measured by Spearman's and Pearson's correlation coefficient. Meanwhile, Kendall's Tau method was conducted to detect the correlations between quantitative variables and ordinal variables. Results were represented as mean ± standard deviation (SD). Likewise, $P \leq 0.05$ were considered as statistically significant.

Results

Demographic, clinical and biochemical characteristics

As seen in Table 1, FSG levels were greater in patients with significant coronary artery stenosis ≥50 compare to non-CAD group ($P \leq 0.05$). Additionally, serum cholesterol, LDL-C, HDL-C, and TG levels were not different between the two groups (Table 1).

Real time polymerase chain reaction

Gene expression of legumain in PBMCs of participants with coronary artery stenosis ≥50 was considerably greater than participants with coronary artery stenosis ≤30 ($P \leq 0.05$).

Table 1. Demographic characteristics and serum parameters

Characteristic	Non-CAD	CAD	Significancy
Number	34	56	
Age, years	51.62±11	59.70±8	ns
Male (%)	41	45	-
Female (%)	58	54	-
SBP, mm Hg	128.44±20	134.42±16	ns
DBP, mm Hg	78.38±16	83.77±14	ns
BMI, kg/m^2	27.38±3	26.89±3	ns
FSG, mg/dL	107.21±21	173.72±25	s
TG, mg/dL	114.50±65	132.09±95	ns
Chol, mg/dL	123.24±25	135.11±40	ns
HDL-C, mg/dL	38.43±12	40.94±13	ns
LDL-C, mg/dL	61.91±25	67.75±33	ns

BMI; Weight (kg)/ Height^2 (m^2), CAD; Coronary artery stenosis ≥50, Chol; Cholesterol, FSG; Fasting serum glucose, HDL-C; High-density lipoprotein cholesterol, LDL; Low-density lipoprotein cholesterol, Non-CAD; Coronary artery stenosis≤30, TG; Triglyceride, Values are mean± standard deviation. *P* value ≤0.05 is considered significant difference.

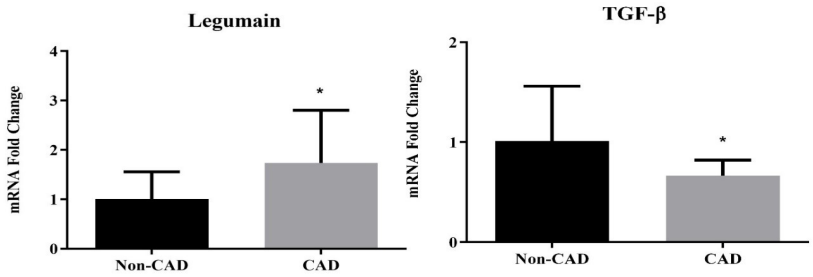

Figure 1. Relative gene expression of legumain and TGF-β in PBMCs in subjects with coronary artery stenosis≥ 50 and, ≤30. CAD; patients with coronary artery stenosis ≥50, non-CAD: patients with coronary artery stenosis≤30. * Compared between two groups ($P \leq 0.05$). Data were expressed as mean±standard deviation.

Moreover, real time PCR analysis shown that TGF-β expression in PBMC of patients with coronary artery stenosis ≥50 significantly exceeded those participants with coronary artery stenosis ≤30 (Figure 1).

Correlation between severity of coronary artery stenosis, serum parameters, and legumain and transforming growth factor-beta expression

Among serum biochemical parameters, increased serum FSG declared strong relation with biochemical parameters and severity of LAD, LCX, and RCA coronary arteries. Among lipid profile parameters, serum cholesterol was more positive correlated to severity of coronary artery stenosis. The overexpression of legumain and TGF-β was related to stenosis of LAD artery. Upregulation of legumain and TGF-β has significantly correlated with serum level of FSG (Figure 2).

Discussion

Main findings of the present pilot research were raised baseline expression of legumain and TGF-β in PBMCs of patients with percentage of coronary artery stenosis ≥50. Indeed, upregulation of legumain and TGF-β in PBMCs was significantly to do with coronary artery stenosis.

Lipid profile parameters had not augmented appreciably in CAD. While earlier studies have explained the correlation between serum lipid profile abnormalities and the beginning of coronary artery stenosis. According to previous studies, lipid profile changes, such as oxidized low-density-lipoprotein (Ox-LDL), are associated with foam cell formation and atherosclerotic events (9). It is worthy to note that, foam cells formation is affected by Ox-LDL more than LDL-C.

Data in this study indicates that legumain is increased in PBMCs of patients with coronary artery stenosis. Likewise,

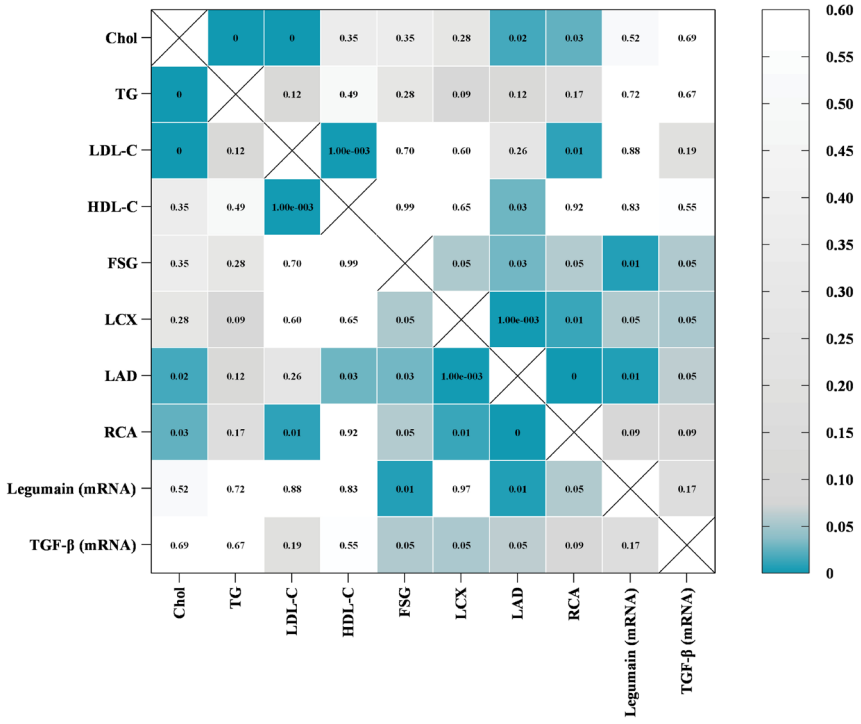

Figure 2. Correlation coefficient matrix among serum parameters, legumain and TGF-β expression, as well as coronary artery stenosis. Chol; Total cholesterol; FSG; Fasting serum glucose; HDL-C: High-density lipoprotein cholesterol; LDL-C: Low-density lipoprotein cholesterol; TG: Triglyceride; LCX: Left circumflex artery; LAD: Left anterior descending; RCA: Right coronary artery. $P \leq 0.05$ were contemplated statistically significant correlation by Pearson, spearman, and Kendall's tau correlation. The change in colour from white to blue-green is go along with by a significant augmented.

analysis of gene expression change of legumain alongside other involving genes in metabolism of PBMCs may be a valuable factor in the occurrence of atherosclerotic. Our results are agreement with previous finding that showed legumain cause the differentiation and activation of macrophages by activating several proteases (9-12). Moreover, legumain overexpression is accompanied by plaque instability. Moreover, Umei et al indicated that in complex coronary lesion, serum legumin was significantly increased (13). In addition, recent research has shown that legumain play role in atherosclerotic vascular remodeling (14).

In the present research, we found that in patient with significant coronary stenosis, TGF-β mRNA level in PBMCs was augmented. Our results are consistent with primary findings. For example, past studies show TGF-β, a major driver of vascular inflammation and atherosclerosis, is overexpressed in asymptomatic plaques (15).

On the other hand, the study conducted by Grainger et al showed serum TGF-β level decreases in steps advanced atherosclerosis. Indeed, TGF-β is considered as the main inhibitor of atherosclerosis (16).

Recent studies showed that TGF-β through recruiting and activating of monocytes as a proinflammatory agent cause development atherosclerosis and following suppressing differentiation monocytes to macrophages exerts its protective effect. However, according to prior studies no study has examined the exact role of TGF-β through the progression of atherosclerosis (17-20).

The strong correlation between coronary artery stenosis especially LAD, serum FSG levels and expression of Legumain and TGF-β in PBMC in patients with coronary artery stenosis was observed in the present study. Our result with earlier studies that said constant hyperglycemia plays an important role in the pathogenesis of atherosclerosis was aligns (21).

Correlation analysis in the current attempt illustrate that LAD coronary artery stenosis was more common than RCA and LCX arteries among participants. It seems LAD, the most important coronary artery in connection with blood supply to the myocardium, is more susceptible than other coronary arteries to recruit and cumulate of PBMCs and inflammatory agents.

In the current study, we report that a positively correlation between expression levels of Legumain and, TGF-β in PBMCs with coronary artery stenosis. Indeed, identifying molecular markers in PBMCs can not only improve our consciousness of its pathogenesis but also propose new strategies prevent and/or treat involving disease.

Conclusion

Taken together, the present study gives new insights into measurement of legumain and, TGF-β expression in PBMCs may be considered as a fruitful biomarker for estimation of coronary artery stenosis. Further attempts, for example serum measurement of Legumain and, TGF-β can provide extra information about the prognostic value Legumain more precisely.

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Author's contribution

Conceptualization: RM.
Data curation: AK, AZS.
Formal analysis: KGS.
Funding acquisition: RM.
Investigation: AK, AZS.
Methodology: RM, AZS.
Project administration: AK, AZS, AK.
Supervision: AK.
Validation: AK.
Visualization: AZS.
Writing–original draft: AZS.
Writing–review & editing: RM.

Conflicts of interest

The authors declare that they have no competing interests.

Ethical issues

The research followed the tenets of the Declaration of Helsinki. The Ethics Committee of Shahrekord University of Medical Sciences approved this study (Ethical code#IR.SKUMS.REC.1400.036). Accordingly, written informed consent was taken from all participants before any intervention. Ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the authors.

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