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Dysregulation of LncRNAs ANRIL, MALAT1, and LINC00305 in Coronary Slow Flow Patients: Implications for Inflammation and Endothelial Dysfunction

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ABSTRACT

Coronary Slow Flow (CSF) is observed in individuals who experience delayed blood supply in the coronary arteries. Inflammation and endothelial dysfunction may play a role in the etiology and development of CSF. The current investigation aimed to compare the expression of specific long noncoding RNAs (lncRNAs) associated with endothelial dysfunction and inflammation in CSF patients. This case−control study enrolled 72 CSF patients and 71 healthy individuals. Blood samples were collected, and serum marker levels were measured. The expression levels of lncRNAs ANRIL, MALAT1, and LINC00305 in peripheral blood mononuclear cells (PBMCs) were assessed using real-time *Polymerase Chain Reaction* (PCR). All statistical analyses were performed using SPSS 22, with the significance level set at $P < 0.05$. The study revealed that the relative expression of MALAT1 and LINC00305 was significantly lower in the CSF group ($p < 0.01$), whereas ANRIL was expressed at higher levels ($p < 0.0001$). The areas under the ROC curves (AUCs) for MALAT1, LINC00305, and ANRIL were 0.64, 0.66, and 0.75, respectively. Notably, the expression level of LINC00305 exhibited an inverse correlation with CSF incidence (OR: 0.83, p: 0.008) in contrast to that of ANRIL (OR: 1.43, $p < 0.0001$). Additionally, compared to those in the control group, the average BMI, WBC, RBC, Hb, LDH, LDL, FBS, and percentage of neutrophils in the CSF group were significantly greater ($p< 0.05$). lncRNA ANRIL is upregulated in CSF patients, whereas MALAT1 and LINC00305 are downregulated. Dysregulation of ANRIL, MALAT1, and LINC00305 may serve as diagnostic and predictive factors for CSF leakage.

Keywords: Coronary vessels, slow flow phenomenon, endothelium, inflammation, LncRNA

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Introduction

The Coronary Slow Flow (CSF) phenomenon is observed in individuals who have delayed blood supply along with a lack of any critical disorder in the coronary arteries on coronary angiography. The clinical manifestations of CSF are similar to those of coronary artery atherosclerotic disease, and the prevalence of this phenomenon has been reported to be between 1% and 7% in patients experiencing angiography due to chest pain (1). CSF patients have a reasonable life expectancy, but frequent angina significantly reduces their quality of life. The etiopathogenesis of CSF is still uncertain. There is a lot of evidence to suggest that inflammation plays a significant role in the initiation, growth, and development of atherosclerosis, suggesting that atherosclerosis is an inflammatory disease. Furthermore, recent evidence has suggested that inflammation may play a role in the progression of CSF (2). In addition, endothelial dysfunction, including CSF dysfunction, is one of the most critical factors for cardiovascular disease (CVD) progression; therefore, it is necessary to scrutinize different mechanisms that lead to endothelial dysfunction, along with other aspects of inflammatory processes and commonalities between them, to better understand the slow-flow phenomenon (2,3). Epigenetics plays an important role in the pathogenesis of CVD. Epigenetic modifications mainly regulate CVD gene function and expression levels through histone modification, DNA methylation, and CVD-related noncoding RNA regulation. Therefore, epigenetic modification may lead to a better understanding of the CSF phenomenon (4).

Long Noncoding RNAs (lncRNAs), which are more than 200 nucleotides long, participate in various mechanisms, including epigenetic changes and posttranscriptional regulation (5). The authors concentrated on lncRNAs and their roles in inflammatory responses and endothelial dysfunction (6). LncRNAs, which are expressed in immune cells, target proteins that play a role in regulating inflammation. They ultimately influence the intensity of the immune response, a factor that can be considered when investigating inflammatory processes (5). Few studies have investigated the role of these factors in CSF occurrence. Despite studies on the relationships of inflammatory factors and various laboratory markers with CSF disease, contradictory results have been reported (7,8). Furthermore, a few studies have investigated the relationships between lncRNAs involved in inflammatory processes and CSF. Further studies have revealed that ANRIL, MALAT1, and LINC00305 play a key role in inflammation and endothelial dysfunction (9).

MALAT1 plays a significant role in inflammatory processes leading to the regulation of endothelial function disorders (10). Moreover, ANRIL, or CDKN2BAS acts on NF-κB signaling to promote inflammation, generating CAD cellular activities (11). LINC00305 is also a new NF-kB activity modulator that is reportedly linked to endothelial dysfunction (12).

Therefore, the current study attempts to compare the expression of these lncRNAs and laboratory findings in patients with CSF phenomenon and healthy individuals to advance our understanding of the etiopathogenic mechanisms underlying CSF.

Materials and methods

Study design and sample collection

This case−control study recruited 72 patients diagnosed with CSF and 71 individuals with a normal coronary function who underwent angiography at Ayatollah Taleghani Hospital in Tehran, Iran. The

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Research Ethics Committee of Shahid Beheshti University of Medical Sciences approved the research, with the code IR.SBMU.MSP.REC.1401.267. The participants included individuals who were hospitalized in the cardiology clinic with chest pain, and CSF was confirmed via TIMI Frame Count (TFC) (>25) during coronary angiography. The control group included individuals with normal coronary flow according to angiography. An attempt was made to select participants who were as similar as possible to those in the case group regarding age and sex. Individuals with arrhythmias other than sinus rhythm, various forms of cardiomyopathy (including restrictive, hypertrophic, or dilated types), renal, pulmonary, or hematological disorders, right or left bundle branch block, congestive heart failure, malignant hypertension (defined as blood pressure >180/110 mm Hg), known connective tissue diseases, moderate to severe valvular heart disease, peripheral artery disease, active infectious or inflammatory diseases, chronic obstructive pulmonary disease, known malignancies, and aortic involvement (such as Marfan syndrome, Ehlers–Danlos, etc.) were excluded from the study. Demographic information, including age, sex, BMI, hypertension history, hyperlipidemia history, smoking history, and diabetes mellitus status, was initially recorded.

Angiographic evaluations

The Judkins technique employed to perform selective Coronary Angiography (CAG) on patients. However, the radial artery was used for patients with high blood pressure or those with a history of femoral artery problems. During CAG, coronary vessels, including the Left Circumflex Artery (LCX), Left Anterior Descending (LAD) artery, and Right Coronary Artery (RCA), were visualized in both the left and right oblique planes, with a caudal tilt in all patients. An average of 6-8 milliliters of contrast medium injected for each image, and coronary vessels were observed at a speed of 30 frames/second. Patients with elevated LV end-diastolic pressure during CAG excluded from the study. Ultimately, following previous methods (13), patients with a Thrombolysis in Myocardial Infarction (TIMI) frame count >25 considered as CSF.

Laboratory testing

After 12 hours of fasting, ten milliliters of venous blood collected from the case and control groups using the standard venipuncture technique. Complete Blood Cell (CBC) counts, including White Blood Cell (WBC) counts, neutrophil percentages, Hemoglobin (Hb) levels, platelet (PLT) levels, plasma C-reactive Protein (CRP) levels, the Erythrocyte Sedimentation Rate (ESR), Lactate Dehydrogenase (LDH) levels, Triglyceride (TG) levels, total cholesterol levels, High-Density Lipoprotein (HDL) levels, Low-Density Lipoprotein (LDL) levels, and Fasting Blood Sugar (FBS) levels, measured at the pathophysiology laboratory of Ayatollah Taleghani Hospital in Tehran.

RNA extraction and cDNA synthesis

An additional 10 milliliters of whole blood collected, and a previously described method was used to isolate Peripheral Blood Mononuclear Cells (PBMCs) (14). The fresh blood was centrifuged at 4000 RPM for 5 minutes, and the resulting serum preserved at -80 °C. Total RNA was then extracted from the PBMCs utilizing an RNA extraction kit (Roje, Tehran, Iran) according to the manufacturer's guidelines. The extracted RNA subsequently converted into complementary DNA (cDNA) using an RT ROSET KIT cDNA synthesis kit (Roje, Tehran, Iran) following the instructions provided by the manufacturer.

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Gene expression analysis

We assessed the expression levels of three lncRNAs, MALAT1, LINC00305, and ANRIL, by an SYBR Green qPCR Master Mix kit from Azmaaksir (Azmaelixir, Tehran, Iran). qPCR performed using a real-time PCR Step One Plus™ instrument (Applied Biosystems, Foster City, CA, USA). Each run included a negative control sample to prevent contamination and a standard sample (housekeeping gene B2M) to compare the expression levels of the studied genes. Two-step real- time PCR was performed using the following conditions: one cycle at 95°C for 15 min; 40 cycles of 95°C for 15 s, and 60°C for 60 s. The melting curve profile and agarose gel electrophoresis were performed to verify the specificity of the primers and the authenticity of the PCR products. The primers list is also mentioned in the table 1 below.

Table 1. List of primer pairs.

Name	Sequence (5' → 3')	
	Forward	Reverse
ANRIL	TCGAGGAACAGCTAAGTGTC	CAGCACACCTAACAGTGATG
Linc00305	GAACCAGACTGCCCTTCAC	TTGACCTTTGTAAGTGTGGCTG
MALAT1	AAGTGCTTTAAGAGGCGGCG	TCTGCGGTTTCCTCAAGCTC

The expression levels of three long noncoding RNAs, namely, MALAT1, LINC00305, and ANRIL, in the PBMCs of CSF patients were compared with those in the PBMCs of healthy controls. We used the comparative-delta Ct method to calculate expression levels in each sample. This method included adjusting the efficiency of the normalizer gene (B2M) and the target gene.

Statistical analysis

In this study, SPSS 22.0 software (IBM, Armonk, NY, USA) and GraphPad Prism 9.0 (GraphPad Software, Jolla, CA, USA) were used for the statistical analyses. The Shapiro–Wilk test was employed to assess normality through normality plots. The mean (±SD) was used to present quantitative data. Qualitative data are presented as frequencies (percentages). The independent sample t-test and chi-square test were used to compare the two groups. Additionally, logistic regression analysis was conducted to identify the predictors of CSF by calculating the odds ratios (ORs) and 95% confidence intervals (CIs).

Unpaired t-test or nonparametric Mann–Whitney U test were utilized to identify DEGs between the CSF group and controls. The -delta Ct data were plotted as individual values, including the means with 95% confidence intervals (CIs). Additionally, the Spearman's rank correlation coefficient was used to measure correlations between gene expression levels in both patient and control samples, considering the non-normal distribution of some data. Receiver Operating Characteristic (ROC) curves were constructed to evaluate the diagnostic power of expression levels for differentially expressed lncRNA genes. Youden's J parameter was used to determine the optimal threshold. Furthermore, the correlation between lncRNA expression levels and other variables was evaluated using the Pearson's correlation coefficient. All tests were two-sided, and a significance level of $p< 0.05$ was considered to indicate statistical significance.

Results

In this research, an evaluation was conducted on 72 patients in the CSF group and 71 individuals in the normal coronary group. The case group consisted of 46 men and 26 women, with an average age of 54.9

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years. The control group included 46 men and 25 women, and the mean age was 56 years. Table 2 presents the demographic and baseline characteristic assessments between the control and CSF groups, revealing no significant difference except for a higher BMI in the case group (p = 0.017). The results of the serum tests for the samples in the CSF and normal groups, based on the mean and standard deviation, are reported in Table 3. The mean WBC, RBC, Hb, LDH, LDL, and serum FBS levels and the percentage of neutrophils were significantly greater in the case group than in the control group (p<0.05).

Table 2. Comparison of demographic and baseline characteristics in control and CSF groups.

Variables	Controls (n=71)	CSF (n=72)	P-value
Age (year)*	56.05 ± 10.59	54.97 ± 10.78	0.54
Sex**			
Male	46 (64.7%)	46 (63.8%)	0.91
Female	25 (35.3%)	26 (36.2%)	
BMI (kg/m^2) *	24.15 ± 2.86	25.36 ±3.11	0.017
Smoking **			
Yes	13 (18.3%)	18 (25%)	0.33
No	58 (81.7%)	54 (75%)	
Diabetes mellitus (n, %)	18 (25.4%)	22 (30.6%)	0.48
Hypertension (n, %)	34 (47.9%)	40 (55.6%)	0.36
Hyperlipidemia (n, %)	9 (12.7%)	4 (5.6%)	0.14

* The data is shown as mean ± SEM. The quantitative variables were compared using the student t-test. ** The Chi-square test and frequency (percent) were used to compare the qualitative variables. Abbreviation: BMI, Body mass index

Table 3. Biochemical parameter comparison between the CSF and control groups.

Variables	Controls (n=71)	CSF (n=72)	P-value
WBC (10^3/mm^3)	6.33 ± 1.48	8.09 ±2.21	**<0.0001**
NEUT (10^3/mm^3)	55 ± 7.19	64.84 ± 10.85	**<0.0001**
RBC (10^3/mm^3)	4.27 ± 0.38	4.87 ±0.68	**<0.0001**
Hb (g/dl)	12.47 ± 1.19	14.13 ± 1.93	**<0.0001**
PLT	20776.56 ± 40688.5	221041.6 ± 63482	0.14
ESR (mm/hr)	12.11 ± 7	13.33 ±8.11	0.33
CRP (mg/L)	4.45 ± 5.38	4.72 ± 4.69	0.74
LDH (mg/dl)	350.12 ± 70.35	380.3 ± 98.27	**0.037**
TG (mg/dl)	136.43 ± 65.85	152.11 ± 62.21	0.14
Chol (mg/dl)	159.15 ± 41	153.8 ± 34.28	0.4
HDL (mg/dl)	43.66 ± 10.3	52.06 ± 48.51	0.15
LDL (mg/dl)	90.12 ± 26.86	102.3 ± 30.03	**0.012**
FBS (mg/dl)	106.52 ± 19.1	123.04 ± 32.12	**0.0002**

Abbreviation: WBC, White blood cell; NEUT, Neutrophils; RBC, Red blood cell; Hb, Hemoglobin; PLT, Platelet count; ESR, Erythrocyte sedimentation rate; CRP, C-reactive protein; LDH, Lactate dehydrogenase; TG, Triglyceride; Chol, Cholesterol; HDL, High-density lipoprotein; LDL, Low-density lipoprotein; FBS, Fasting blood sugarThe data is shown as mean ± SEM. The student t-test was used to compare quantitative variables.

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TIMI Frame Count (TFC) showed that the mean values in the LAD, LCX, and RCA vessels were 39.3, 25.5, and 28.6, respectively. Additionally, these numbers were reported to be 19.2, 18.90, and 18.94 in the normal group, indicating that they were significantly ($p < 0.0001$) greater in the case group than in the control group (Table 4).

Table 4. TIMI blood flow evaluation in the control and CSF groups.

Variables	Controls (n=71)	CSF (n=72)	P-value
TFC of LAD	19.21 ± 2.77	39.3 ± 13.41	<0.0001
TFC of LCX	18.9 ± 2.48	25.5 ± 7.47	<0.0001
TFC of RCA	18.94 ± 2.22	28.61 ± 11.34	<0.0001
Mean TFC	19.01 ± 2.49	30.97 ± 10.74	<0.0001

Abbreviation: TIMI, Thrombolysis in myocardial infarction; TFC, TIMI frame count; LAD, left anterior descending; LCX, left circumflex artery; RCX, right coronary artery; Mean TFC, Mean TIMI frame count The mean ± SD was used to express continuous variables with a normal distribution.

As shown in Figure 1, the expression of MALAT1, LINC00305, and ANRIL significantly differed between the two groups. The findings showed that the expression of MALAT1 was lower in the case group

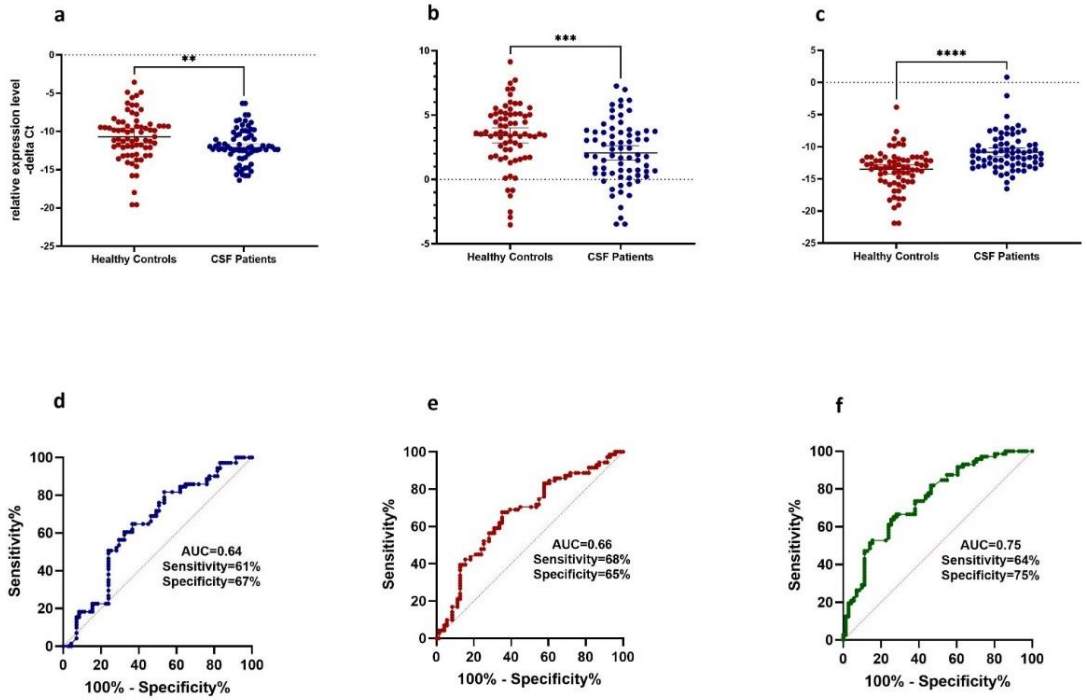

Fig. 1. The expression of MALAT1, LINC00305, and ANRIL in CSF from patients was examined. (a-c) The expression of MALAT1, LINC00305, and ANRIL in PBMCs from the control and CSF groups was detected using qRT−PCR. Delta Ct data are represented as individual values, including the means with 95% CIs. (d-f) The potential of MALAT1, LINC00305, and ANRIL as predictive tools for CSF patients was assessed through Receiver Operating Characteristic (ROC) curve analysis. The data, expressed as mean ± SD, were significantly different from those of the control group (**p < 0.01; ***p < 0.001; ****p < 0.0001). Abbreviations: CSF, coronary slow flow; PBMCs, peripheral blood mononuclear cells; ROC, receiver operating characteristic; AUC, area under the ROC curve

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($p < 0.01$), as was that of LINC00305 ($p < 0.001$). However, the expression level of ANRIL was significantly greater in the CSF group ($p < 0.0001$). Additionally, the potential of MALAT1, LINC00305, and ANRIL as predictive tools for CSF patients were assessed through ROC curve analysis, as shown in Figure 1. The sensitivity and specificity of the three lncRNAs reported in Figure 1 and the area under the ROC curve (AUC) values for MALAT1, LINC00305, and ANRIL were 0.64, 0.66, and 0.75, respectively.

Furthermore, univariate linear regression analysis was conducted to assess the correlation between the expression levels of MALAT1, LINC00305, and ANRIL and the TFC of LAD, the TFC of LCX, and the TFC of RCA. These results are reported in Figures 2-4. Notably, the decrease in the relative expression of MALAT1 and LINC00305 was associated with increased TFC in the LAD coronary artery ($p < 0.05$). However, there were no significant changes in the relative expression levels of these genes in the other vessels. Conversely, the increase in the relative expression of ANRIL was significantly correlated with an increase in TFC across all three vessels ($p < 0.05$). Additionally, the Spearman's correlations between the expression levels of the three lncRNAs in the case group are presented in Figure 5.

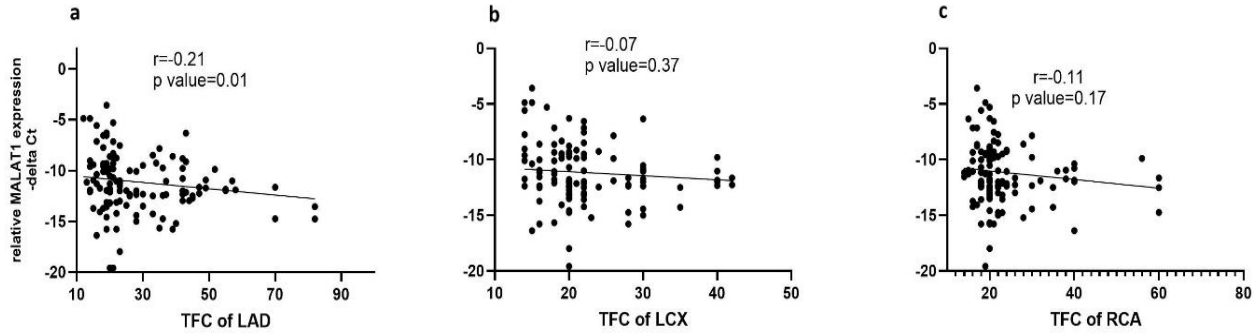

Fig. 2. The relationship between the expression of MALAT1 and three different grades of CSF TIMI was examined. Correlations between MALAT1 expression and the TFC of the LAD (a), the TFC of the LCX (b), and the TFC of the RCA were assessed using univariate linear regression analysis.

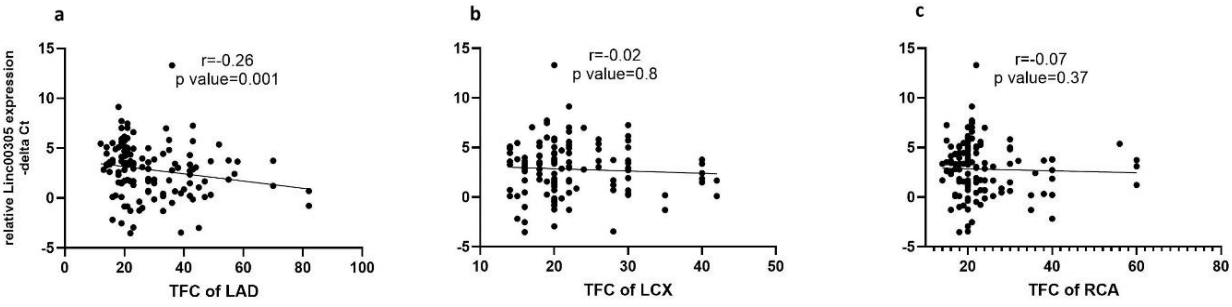

Fig. 3. The relationship between the expression of LINC00305 and three different grades of CSF TIMI was examined. The correlations between LINC00305 expression and the TFC of LAD (a), the TFC of LCX (b), and the TFC of RCA were assessed using univariate linear regression analysis. CSF, coronary slow flow; TIMI, thrombolysis in myocardial infarction; TFC, TIMI frame; mean TFC, mean TIMI frame count.

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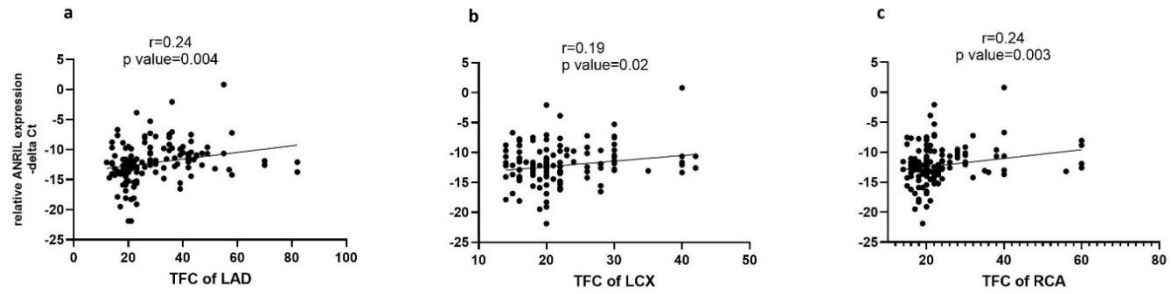

Fig. 4. The relationship between the expression of ANRIL and three different grades of CSF TIMI was examined. The correlations between ANRIL expression and the TFC of the LAD (a), the TFC of the LCX (b), and the TFC of the RCA were assessed using univariate linear regression analysis. TFC, TIMI frame count; TFC, mean TIMI frame count.

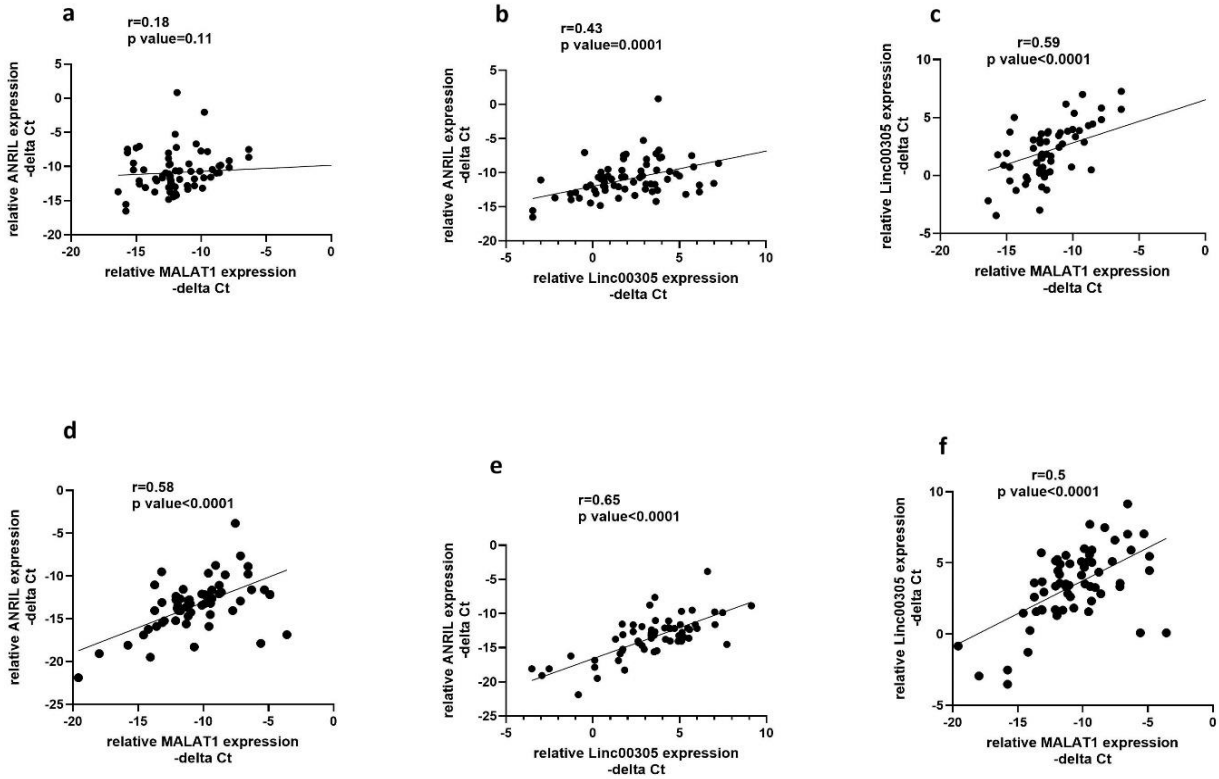

Fig. 5. Spearman's correlations were used to analyze the RNA expression levels of three lncRNAs among the CSF group (a-c) and control group (d-f).

Additionally, the logistic regression analysis demonstrated a significantly positive correlation between BMI, LDL, LDH, and FBS levels and CSF incidence (OR > 1, $p < 0.05$). On the other hand, the expression level of LINC00305 had an inverse correlation with CSF incidence (OR: 0.83, p value: 0.008), but that of ANRIL showed a positive correlation (OR: 1.43, p value < 0.0001) (Table 5).

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Table 5. Results of the logistic regression analysis for variables related to CSF.

Variables	OR	95 % CI	P-value
Age	0.99	0.96-1.02	0.99
Male	0.96	0.48-1.9	0.91
Hypertension	1.36	0.7-2.62	0.36
Diabetes mellitus	1.3	0.62-2.69	0.49
smoker	1.48	0.66-3.32	0.33
BMI	1.14	1.02-1.28	**0.019**
LDL	1.01	1.003-1.03	**0.014**
LDH	1.004	1-1.008	**0.041**
TG	1.004	0.99-1.009	0.15
HDL	1.02	0.99-1.05	0.13
FBS	1.02	1.01-1.04	**0.001**
Chol	0.99	0.98-1.005	0.4
MALAT1 expression	0.91	0.81-1.02	0.1
LINC00305 expression	0.83	0.72-0.95	**0.008**
ANRIL expression	1.43	1.21-1.68	**<0.0001**

Abbreviation: BMI, Body mass index; LDL, Low-density lipoprotein; LDH, Lactate dehydrogenase; TG, Triglyceride; HDL, High-density lipoprotein; FBS, Fasting blood sugar; Chol, Cholesterol

Discussion

The etiology and pathology of CSF have not been completely defined. However, the current understanding suggests that CSF is associated with endothelial disorders in coronary vessels, inflammatory responses, defects in small vessel reactivity, subclinical atherosclerosis, and platelet dysfunction (15). Although epigenetic modifications in inflammatory processes and endothelial dysfunction have significant effects on the function of endothelium, which leads to coronary artery disease (16), the complete mechanisms of epigenetic regulation of endothelial dysfunction and inflammation in the CSF remain unclear. Considering the broad role of lncRNAs in epigenetics, the present study evaluated the expression patterns of ANRIL, MALAT1, and LINC00305, which play a proven role in inflammation and endothelial dysfunction.

In addition, to better understand the role of inflammation and inflammatory processes in the etiology of CSF, several serum markers, mainly those that are altered during inflammatory processes, were investigated in this study. Therefore, this study was designed to compare the serum levels of MALAT1, ANRIL, and LINC00305 and the corresponding laboratory parameters between patients with CSF and healthy individuals without coronary artery disease.

Numerous lncRNAs play a role in inflammatory processes at all stages. However, the present study specifically investigated lncRNAs that participate in inflammatory pathways and have established roles in endothelial function, including ANRIL, MALAT1, and LINC00305 (9).

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Various studies have reported varying results regarding the association of ANRIL with inflammation. Moreover, ANRIL is associated with elevated levels of the inflammatory C - reactive protein (CRP), which is considered a marker for CAD, in the plasma of patients with periodontitis (17). Other studies have also suggested that increased expression of ANRIL can elevate inflammatory factor levels, whereas decreased ANRIL expression may reduce inflammatory marker levels. These studies have shown that IL-1 can strengthen atherosclerosis (AS), and independent clinical associations exist between IL-6 and AS. Both of these markers increase with the upregulation of ANRIL. Furthermore, increased ANRIL expression leads to enhanced Endothelial Cell (EC) apoptosis in the coronary arteries of mice, while decreased ANRIL expression causes the opposite effect. EC apoptosis plays an important role in AS development, ultimately contributing to cardiovascular events (18).

ANRIL is activated through the NF-kB pathway and plays a crucial role in the expression of inflammatory genes regulated by the NF-kB pathway. Significant protection against arterial stiffness is provided when NF-κB signaling in endothelial cells is inhibited. As a result, ANRIL can serve as a downstream modulator of the NF-kB pathway. Investigating whether ANRIL can be used as a more specific target for medication development in this context warrants further examination (19).

The rs1333048 polymorphism in the ANRIL gene has been associated with various cardiovascular conditions, but its specific role in CSF remains unclear. Studies have shown that ANRIL polymorphisms, including rs1333048, are linked to coronary artery disease (CAD) (11, 20). Moreover, research suggests that ANRIL genetic variants may influence the risk of Acute Coronary Syndrome (ACS) (21). Therefore, while the exact relationship between rs1333048 in ANRIL and CSF specifically has not been directly studied, the broader implications of ANRIL polymorphisms in cardiovascular and inflammatory diseases warrant further explorations in the context of CSF.

To our knowledge, no study has investigated the expression levels of ANRIL in patients with CSF, and the present study revealed that the lncRNA ANRIL is expressed at higher levels in CSF patients than in normal individuals. Additionally, the present study demonstrated that the ANRIL level is positively correlated with the incidence of CSF, the ROC curve analysis also revealed that it could be used as an excellent predictive molecular marker.

Reportedly, MALAT1 plays a role in micro- and macro-vascular complications by turning on inflammatory mediators in the endothelium; for example, some inflammatory molecules, such as TNFα and IL6, have shown to increase MALAT1 upregulation. On the other hand, reduced levels of MALAT1 increase the risk of atherosclerotic lesion in animal models and are correlated with human atherosclerotic disease (22).

The present study revealed that MALAT1 was significantly expressed in the control group. Although this difference was not as significant as that of the other two lncRNAs, the logistic regression analysis revealed no significant correlation between MALAT1 and CSF incidence. The findings of the study by Zhao et al. were different from those of the present study. They reported that MALAT1 exhibited high expression in CSF patients. Further, endothelial function was improved upon MALAT1 knockdown. MALAT1 is involved in the regulation of endothelial disorder in CSF through the miR-181b-5p-MEF2A-ET-1 axis, potentially representing a novel therapeutic target for CSF (23). We acknowledge the conflicting results

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regarding MALAT1 expression in our study compared to those of Zhao et al.'s study. Potential reasons for this discrepancy could include differences in study populations (ethnicity, geographic location), methodologies (sample collection, RNA extraction, and qPCR protocols), or disease stages of the CSF patients. Further investigations across diverse cohorts employing standardized protocols and accounting for potential confounders are required to validate the expression pattern of MALAT1 in CSF patients.

LINC00305 was overexpressed in the PBMCs of individuals with atherosclerotic plaques. Its overexpression led to increased expression of inflammatory genes and a reduction in the expression of contractile markers. LINC00305 activates inflammatory genes through the NF-κB pathway (24). As immune-related lncRNAs, lncRNAs promote the inflammation underlying atherosclerosis (25). Additionally, further studies have revealed that the interaction of LINC00305 with Lipocalin-Interacting Membrane Receptor (LIMR) leads to increased NF-κB activation (12, 26). In the present study, LINC00305 was significantly downregulated in the CSF group. Despite its role in promoting inflammation, the downregulation of LINC00305 in CSF patients could be a compensatory mechanism to counteract excessive inflammatory responses. Alternatively, the reduced expression of LINC00305 might result from dysregulation of upstream signaling pathways or transcriptional regulatory networks that govern its expression in CSF.

Our evaluation of the ROC curves demonstrated that these three lncRNAs displayed moderate discriminatory capacity in discerning CSF patients, with AUCs ranging from 0.64 to 0.75. These AUCs suggest that these lncRNAs can act as diagnostic indicators for CSF patients, especially when utilized alongside other molecular markers. Identifying dependable diagnostic indicators is crucial for the prompt identification and precise diagnosis of CSF phenomenon, which can enhance patients' quality of life.

Figure 5 shows the correlation between the expression levels of lncRNAs, which shows that the expression of all three lncRNAs can be correlated. These associations indicate potential coregulatory mechanisms or functional interactions between these lncRNAs in the CSF.

The other findings of the current study demonstrated that the mean serum levels of WBC, RBC, Hb, LDH, LDL, and FBS and the percentage of neutrophils were significantly higher in individuals with CSF than in normal individuals. Mohammadzad et al. also reported the significantly elevated levels of hemoglobin, platelets, cholesterol, HDL, RBC, and triglycerides in patients with CSF. In contrast, the WBC and FBS levels did not differ significantly between the two groups (27). A study by Huang et al. conducted on a Chinese population showed similar findings, with no significant association between a history of diabetes or high blood pressure and CSF (28). Further, apart from the parameters studied in the current study, a substantial increase in hematocrit, RBC count, eosinophils, and basophils was observed in CSF patients compared to those with normal coronary blood flow.

Furthermore, a positive correlation was demonstrated between these parameters and the occurrence of CSF, although the underlying mechanisms remain unclear. Danaei et al. reported higher total cholesterol levels in the CSF group and noted significantly greater levels of TNFa, IL8, and IL1b in the CSF group (14). In the current study, BMI was higher in the CSF group. Kopetz *et al*. also reported that the mean BMI of individuals in the CSF group was greater (29). Consequently, the present study and other research indicate that although there are differences in the levels of various inflammatory markers across studies, all of them

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suggest that these inflammatory markers are elevated in the CSF. However, the underlying cause of this phenomenon remains unknown. In the present study, various markers were considered, but other laboratory parameters still require comprehensive evaluation in a single study. Moreover, it is recommended that future studies assess the levels of serum markers over long intervals in CSF patients to reach a more precise conclusion regarding their role.

The dysregulation of lncRNAs involved in inflammatory processes, such as ANRIL, MALAT1, and LINC00305, in CSF patients could be a consequence of epigenetic modifications or alterations in the activity of transcriptional regulators. Epigenetic mechanisms, including DNA methylation, histone modifications, and chromatin remodeling, can influence the expression of lncRNAs and contribute to the observed expression patterns in CSF. The inflammatory milieu in CSF patients may trigger specific epigenetic changes that lead to the upregulation of ANRIL and downregulation of MALAT1 and LINC00305. Alternatively, preexisting epigenetic signatures or genetic variations in the regulatory regions of these lncRNAs could predispose individuals to altered expression levels, potentially contributing to the development of CSF.

Furthermore, the interplay between lncRNAs and transcription factors involved in inflammatory signaling pathways, such as NF-κB, can affect the observed expression patterns. LncRNAs can modulate the activity of transcription factors by acting as decoys, guides, or scaffolds, thereby influencing the expression of downstream target genes involved in inflammatory responses. In addition, the elevated serum levels of inflammatory markers, such as WBC, neutrophil percentage, RBC, Hb, LDH, LDL, and FBS, observed in CSF patients could be a consequence of the dysregulated expression of lncRNAs involved in inflammatory pathways. Conversely, these serum markers might influence the expression of lncRNAs through feedback mechanisms or by altering the cellular microenvironment.

Investigating the epigenetic landscapes, transcriptional regulatory networks, and the interplay between lncRNAs and inflammatory signaling pathways in CSF patients can provide valuable insights into the underlying molecular mechanisms involved. Moreover, the involvement of multiple pathogenic mechanisms, such as endothelial dysfunction, inflammatory processes, and hypoxia, could have caused the observed complex expression patterns. Understanding the cell type-specific and tissue-specific regulatory mechanisms governing these lncRNAs in the context of CSF is crucial for elucidating their precise roles and functions.

In the present study, although the groups were tried to be close to each other in terms of age and sex, but considering the importance of equalizing these factors in gene expression studies, it is suggested that in future studies that investigate the expression level of these genes or functional studies that examine the pathways involved in the slow flow phenomenon, participants as cases and controls compared in the same age and gender groups.

Although the present study provides valuable insights, further research is warranted to validate these findings in larger cohorts and across diverse populations. Integrating multiomics approaches, including transcriptomics, epigenomics, and proteomics, can help unravel the complex interplay between lncRNAs, inflammatory pathways, and other molecular mechanisms underlying the development and progression of the CSF phenomenon.

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In conclusion, the dysregulation of ANRIL, MALAT1, and LINC00305 in CSF patients indicates their probable involvement in the pathogenesis of the CSF phenomenon. Moreover, the lncRNA ANRIL exhibited increased expression in CSF patients, unlike MALAT1 and LINC00305, whose expression decreased. These long noncoding RNAs can be potential diagnostic biomarkers for CSF, displaying moderate discriminatory ability in distinguishing CSF patients from normal coronary patients. The associations between the expression levels of these genes suggest potential coregulatory mechanisms for CSF incidence. Additionally, the present study revealed that specific serum markers, such as WBC, neutrophil percentage, RBC, Hb, LDH, LDL, and FBS, are elevated in patients with CSF.

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Conflicts of Interest

None declared.

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