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Exploring the Novel Potential of Serum SIRT1 and TIMP3 as Biomarkers for In-Stent Restenosis Following Percutaneous Transluminal Angioplasty and Stenting in Arteriosclerosis Obliterans Patients

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This study aimed to investigate the potential of serum Sirtuin 1 (SIRT1) and tissue inhibitor of metalloproteinase 3 (TIMP3) levels as biomarkers for in-stent restenosis (ISR) in arteriosclerosis obliterans (ASO) patients following percutaneous transluminal angioplasty (PTA) and stenting. A total of 256 ASO patients who underwent successful PTA with stent implantation were included. Serum levels of SIRT1 and TIMP3 were assessed at baseline and 4 weeks post-procedure. After 6 months, 65 patients were identified with ISR. Significant differences were noted in serum SIRT1 and TIMP3 levels between ISR and non-ISR groups at 4 weeks. TIMP3 had a higher AUC (0.782, 95% CI: 0.726-0.831) than SIRT1 (0.737, 95% CI: 0.678-0.789) for predicting ISR at 6 months. Correlation analysis showed a positive association between SIRT1 and TIMP3 levels in ISR patients at 4 weeks, but not in non-ISR patients. Multivariate analysis revealed diabetes (OR = 1.436, 95% CI: 1.205-1.925) and carotid stenosis (OR = 4.551, 95% CI: 1.364-15.185) significantly increased ISR risk, while lower SIRT1 (OR = 0.985, 95% CI: 0.978-0.992) and TIMP3 (OR = 0.574, 95% CI: 0.464-0.710) levels were significantly associated with ISR. Serum SIRT1 and TIMP3 levels at 4 weeks post-procedure are significant predictors of ISR in ASO patients following PTA and stenting. Lower SIRT1 and TIMP3 levels correlate with higher ISR risk. These findings suggest that monitoring serum SIRT1 and TIMP3 levels could be a valuable tool in predicting ISR, which could inform clinical decisions and patient management.

Keywords: arteriosclerosis obliterans; biomarkers; in-stent restenosis; sirtuin 1; tissue inhibitor of metalloproteinase 3
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Introduction

Percutaneous transluminal intervention (PTA) is a minimally invasive procedure used to treat narrow or blocked blood vessels, typically arteries, without the need for open surgery (Zhao et al. 2022). In some cases, stent placement during PTA helps keep the artery open, a technique known as stent placement (Lee et al. 2024). However, a major limitation of stent usage is in-stent restenosis (ISR), the primary cause of stent failure, necessitating reintervention in a significant proportion of patients within a few years, presenting a substantial management challenge (Haybar et al. 2020; Erdogan et al. 2022). ISR is primarily driven by two mechanisms: excessive neointimal hyperplasia, characterized by smooth muscle cell proliferation and extracellular matrix formation, and neo-atherosclerosis, marked by lipid-laden macrophage accumulation and potential plaque rupture (Marazzi et al. 2007; Pelliccia et al. 2023). Key risk factors for ISR include patient-related factors such as diabetes mellitus, chronic kidney disease, and smoking; genetic predispositions affecting response to stents; anatomic factors like small vessel size and complex lesion morphology; and procedural factors such as stent underexpansion and malposition (Pelliccia et al. 2023). Despite its potential to restore blood flow and alleviate symptoms in arteriosclerosis obliterans (ASO) patients, the efficacy of PTA is often hampered by the occurrence of restenosis (Kawarada et al. 2020). This highlights the complexity of ASO and underscores the critical need for robust prognostic biomarkers to identify individuals at elevated

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risk for ISR.

In recent years, attention has shifted toward exploring the potential of serum biomarkers in predicting restenosis after PTA (Li et al. 2018; Lian et al. 2021). Tissue inhibitor of metalloproteinase 3 (TIMP3), a protease with high expression in the heart, plays a crucial role in extracellular matrix turnover, maintaining equilibrium with matrix metalloproteinases, and exhibiting a protective role in coronary artery disease (CAD) and myocardial infarction (Chen et al. 2022). Stents with biosynthetic coatings effectively promote TIMP3 adenovirus transduction and transcription, reducing neointimal proliferation and confirming its role in preventing ISR (Wu et al. 2003). The type III deacetylase sirtuin 1 (SIRT1) plays a protective role in cardiovascular health by enhancing endothelial function, suppressing inflammation through NF-κB inhibition, promoting endothelial nitric oxide synthase activity, and inhibiting foam cell formation by reducing low-density lipoprotein uptake and promoting reverse cholesterol transport (Caribe et al. 2020; DiNicolantonio et al. 2022). The negative correlation between SIRT1 and HIF-1α expression in hypoxic vascular smooth muscle cells (VSMCs) suggests a potential role of SIRT1 in modulating neointima formation, a key process in restenosis (Bae et al. 2013). Guo et al. (2020) suggested that interventions targeting SIRT1, like resveratrol, might mitigate restenosis by modulating SIRT1-related pathways, reducing neointimal formation in high-fat-fed insulin-resistant rats, and enhancing insulin sensitivity and vascular function. Furthermore, resveratrol inhibits restenosis post-balloon angioplasty by suppressing vascular adventitia fibroblasts' proliferation, migration, and trans-differentiation through SIRT1-mediated regulation of the TGF-β1-SMAD3-NOX4 pathway (Li et al. 2024).

Therefore, this study aims to comprehensively investigate the potential of serum SIRT1 and TIMP3 as biomarkers for restenosis in ASO patients' post-PTA with stenting, addressing the critical need for effective prognostic indicators in the management of ISR.

Materials and Methods

Ethical statement

This study was conducted following approval from our hospital's ethics committee (2023-80K), and informed consent was obtained from all participants.

Study population

This retrospective analysis, conducted within the framework of a prospective study, utilized data gathered from 256 consecutive patients treated at our hospital between 2020 and 2023. All patients, aged 18 years or older and diagnosed with ASO (Takahara 2021), underwent successful PTA with stent implantation (Yang 2017). All enrolled patients were administered long-term aspirin therapy at a dosage of 100 mg/day, in addition to 75 mg of clopidogrel per day for a duration of three months post-angioplasty and stenting with bare-metal nitinol stents. Exclusion criteria included documented intolerance or adverse reactions to aspirin or clopidogrel, current or previous use of vitamin K antagonists (e.g., warfarin, phenprocoumon, acenocoumarol), ticlopidine, dipyridamole, or nonsteroidal anti-inflammatory drugs. Furthermore, patients with a personal or family history of bleeding disorders, malignant paraproteinemias, myeloproliferative disorders, heparin-induced thrombocytopenia, severe hepatic impairment, known qualitative defects in platelet function, recent major surgical procedures within one week before enrollment, platelet counts below 100,000 or above 450,000/mL, and hematocrit levels less than 30% were also excluded from the study.

Follow-up imaging and ISR definition

All patients underwent a duplex ultrasonography (US) examination within 48 hours after the procedure. After discharge, follow-up duplex US was conducted at the 1-month mark. Subsequently, at 6 months post-procedure, patients were monitored using ankle brachial index (ABI), duplex US and diagnostic angiography examinations, as determined by a vascular surgeon with at least ten years of experience, based on clinical symptoms and ultrasound findings. Patients were contacted to inquire about any clinical symptoms reported during the follow-up period via phone calls. ISR occurrence is typically observed between 3 and 6 months post-stent placement (Tomberli et al. 2024). After the 6-month follow-up period, 65 patients were identified as having in-stent restenosis (ISR group), while the remaining 191 patients were categorized as the non-ISR group. ISR has been defined as a reduction $\geq$ 50% of the luminal diameter within the previously stented segment or the vessel segments 5 mm proximal and distal to the stent (the "stent edges") (Cvirn et al. 2012; Alfonso et al. 2022), as assessed by the lower limb angiography.

Enzyme-linked immunosorbent assay (ELISA)

Serum levels of TIMP-3 and SIRT1 were assessed using ELISA before and at 4 weeks post-procedure. Blood samples were centrifuged at 3,000 rpm for 15 min to separate the serum that were later processed in the laboratory. The human TIMP-3 ELISA Kit (catalog number EH458RB) exhibited inter-batch variation < 12%, intra-batch variation < 10%, sensitivity of 0.05 ng/mL, and a detection range of 0.05-10 ng/mL. The human SIRT1 ELISA Kit (catalog number EH427RB) demonstrated inter-batch variation < 12%, intra-batch variation < 10%, sensitivity of 1.23 ng/mL, and a detection range of 1.23-300 ng/mL. These ELISA kits were sourced from Thermo Fisher Scientific Inc. (Shanghai, China).

Statistical analysis

Data were analyzed using GraphPad Prism 8.0. Count data were expressed as n and analyzed using the chi-square test. Measurement data were presented as mean ± SD (for normal distribution) or median (IQR, for non-normal distri-

bution) based on the Shapiro-Wilk test for normality. Paired t-tests or Wilcoxon matched-pairs signed rank tests were used for within-group comparisons of measurement data before and after treatment. Between-group comparisons of measurement data were conducted using t-tests or Mann-Whitney U tests. The diagnostic value of serum SIRT1 and TIMP3 levels for ISR at 4 weeks post-procedure was evaluated using ROC curve analysis (MedCalc software). The correlation between serum SIRT1 and TIMP3 levels at 4 weeks post-procedure in the ISR and non-ISR groups was assessed using Spearman analysis. Univariate logistic regression was performed to assess the association between variables and ISR. Variables with $P < 0.100$ and those judged to be of clinical importance were included in a multivariate logistic regression to identify independent predictors of ISR. Results were presented as odds ratios (OR) with 95% confidence intervals (CI). A P value < 0.05 was considered statistically significant.

Results

Serum SIRT1 and TIMP3 levels in ASO patients with ISR

The baseline characteristics of the study population were comparable between the ISR (n = 65) and non-ISR (n = 191) groups (all $P > 0.05$, Table 1). As shown in Fig. 1, the baseline levels of SIRT1 were comparable between the ISR and non-ISR groups ($P > 0.05$). However, following treatment, significant differences were observed. Median SIRT1 levels in the ISR group were consistently lower than

Table 1. Baseline Patient Characteristics.

Variable	ISR Group (n = 65)	Non-ISR Group (n = 191)	*P*
Sex			0.433
Male	43 (66.2%)	137 (71.7%)	
Female	22 (33.8%)	54 (28.3%)	
Age (years)	74 (69-83)	73 (64-80)	0.119
Length of Stay (days)	10 (6-19)	12 (8-17)	0.411
History			
Diabetes Mellitus	28 (43.1%)	97 (50.8%)	0.316
Hypertension	41 (63.1%)	139 (72.8%)	0.158
Coronary Artery Disease	9 (13.8%)	33 (17.3%)	0.568
Atrial Fibrillation	11 (16.9%)	17 (8.9%)	0.105
Stroke	18 (27.7%)	44 (23.0%)	0.503
Rheumatoid Arthritis	8 (12.3%)	13 (6.8%)	0.191
Carotid Stenosis	9 (13.8%)	12 (6.3%)	0.068
Hyperhomocysteinemia	6 (9.2%)	8 (4.2%)	0.202
Procedure Status			0.322
Elective	52 (80.0%)	164 (85.9%)	
Urgent	13 (20.0%)	27 (14.1%)	
Treated lesions			0.568
Aortic	2 (3.1%)	4 (2.1%)	
Aorto-iliac	2 (3.1%)	16 (8.4%)	
Iliac	39 (60.0%)	111 (58.1%)	
Ilio-femoral	11 (16.9%)	24 (12.6%)	
Femoral	11 (16.9%)	36 (18.8%)	
Uni/bilateral			0.756
Unilateral	44 (67.7%)	134 (70.2%)	
Bilateral/aortic	21 (32.3%)	57 (29.8%)	
Number of stents			0.999
1	27 (41.5%)	81 (42.4%)	
2	18 (27.7%)	53 (27.7%)	
3	14 (21.5%)	40 (20.9%)	
>3	6 (9.2%)	17 (8.9%)	
Type of stent			0.07
Bare-metal stents (BMS)	49 (75.4%)	120 (62.8%)	
Drug-eluting stents (DES)	16 (24.6%)	71 (37.2%)	
Stent length (mm)	83.8 (51.1-105.3)	71.6 (43.8-94.7)	0.116
Stent diameter (mm)	6.3 (4.0-7.8)	6.4 (4.4-6.4)	0.111

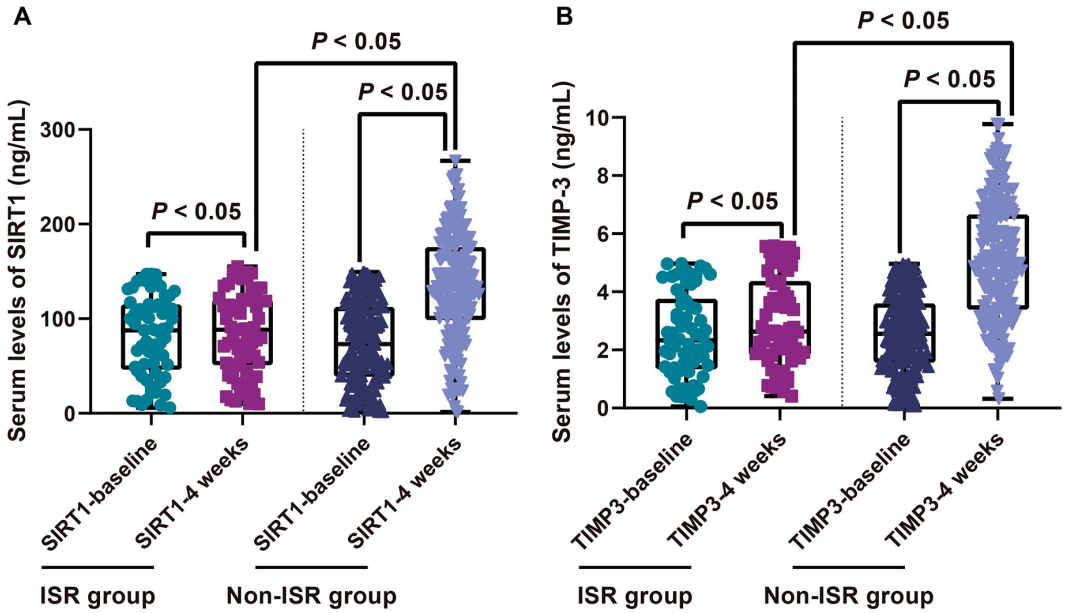

Fig. 1. Serum sirtuin 1 (SIRT1) and tissue inhibitor of metalloproteinase 3 (TIMP3) levels in arteriosclerosis obliterans (ASO) patients with in-stent restenosis (ISR).
Serum SIRT1 (A) and TIMP3 (B) levels were detected using Enzyme-linked immuno sorbent assay (ELISA) in ASO patients with and without ISR.

those in the non-ISR group at 4 weeks post-procedure (88.3 ng/mL vs. 133.7 ng/mL) ($P < 0.05$), suggesting a potential association between lower SIRT1 levels and in-stent restenosis. Similarly, while baseline TIMP3 levels did not significantly differ between groups, median TIMP3 levels in the ISR group were consistently lower than in the non-ISR group at 4 weeks post-procedure (4 weeks post-procedure: 2.63 ng/mL vs. 5 ng/mL) ($P < 0.05$), indicating a potential link between lower TIMP3 levels and in-stent restenosis.

Serum SIRT1 and TIMP3 levels at 4 weeks as predictors of in-stent restenosis in ASO patients following PTA and stenting

The Table 2 and Fig. 2 presented the diagnostic performance of serum SIRT1 and TIMP3 levels at 4 weeks as predictors of in-stent restenosis. For SIRT1, the AUC was 0.737 (95% CI: 0.678-0.789), indicating moderate predictive capability. The sensitivity of SIRT1 was 78.46% (95% CI: 66.5%-87.7%), with a specificity of 59.16% (95% CI: 51.8%-66.2%). In contrast, TIMP3 demonstrated a higher AUC of 0.782 (95% CI: 0.726-0.831), signifying better predictive ability. TIMP3 exhibited a sensitivity of 56.92% (95% CI: 44.0%-69.2%) and a specificity of 85.34% (95% CI: 79.5%-90.0%). These findings underscored the potential utility of TIMP3 as a more accurate predictor compared to SIRT1 in identifying in-stent restenosis.

Table 2. Serum Sirtuin 1 (SIRT1) and Tissue Inhibitor of Metalloproteinase 3 (TIMP3) Levels at 4 Weeks as Predictors in Arteriosclerosis Obliterans (ASO) Patients with In-Stent Restenosis (ISR).

Parameter	Serum SIRT1 levels	Serum TIMP3 levels
Area under the ROC curve (AUC)	0.737	0.782
Standard Error (SE)	0.0322	0.031
95% Confidence Interval (CI)	0.678 to 0.789	0.726 to 0.831
z Statistic	7.356	9.097
Significance Level (P value)	< 0.001	< 0.001
Youden Index (J)	0.376	0.423
Associated Criterion	≤ 121.73 ng/mL	≤ 2.8 ng/mL
Sensitivity (%)	78.46 (66.5 - 87.7)	56.92 (44.0 - 69.2)
Specificity (%)	59.16 (51.8 - 66.2)	85.34 (79.5 - 90.0)
Positive Likelihood Ratio (+LR)	1.92 (1.55 - 2.38)	3.88 (2.60 - 5.81)
Negative Likelihood Ratio (-LR)	0.36 (0.23 - 0.59)	0.5 (0.38 - 0.67)

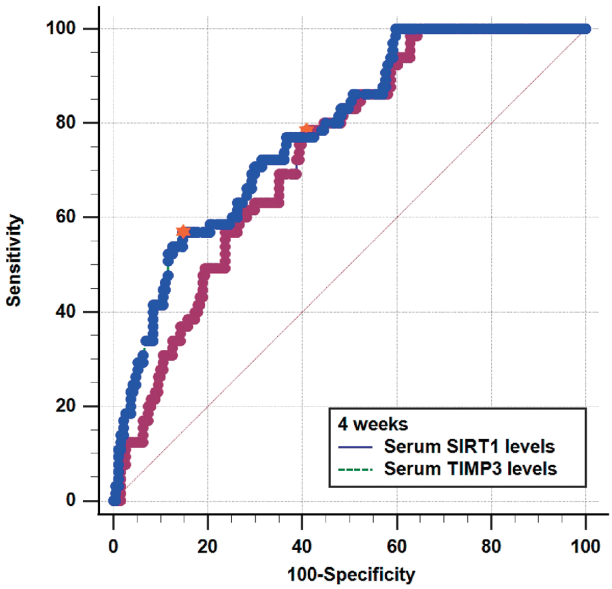

Fig. 2. Serum SIRT1 and TIMP3 levels at 4 weeks post-procedure predicted ISR in ASO patients after PTA and stenting.
Sirtuin 1 (SIRT1); Tissue Inhibitor of Metalloproteinase 3 (TIMP3); Arteriosclerosis Obliterans (ASO); Percutaneous Transluminal Angioplasty (PTA); In-Stent Restenosis (ISR).

Correlation between serum levels of SIRT1 and TIMP3 at 4 weeks post-procedure in ASO patients with or without ISR following PTA and stenting

As illustrated in Fig. 3, correlation analysis revealed a significant positive association between serum SIRT1 and TIMP3 levels at 4 weeks post-procedure in ISR patients (Spearman's r = 0.630, 95% CI: 0.450-0.761, $P < 0.001$). However, in the non-ISR group, there was no significant correlation observed between serum SIRT1 and TIMP3 levels (Spearman's r = 0.056, 95% CI: –0.091-0.200, $P = 0.444$), indicating that changes in serum SIRT1 levels may not directly correlate with TIMP3 levels in patients without ISR.

Comparison of clinical characteristics between low and high serum SIRT1 and TIMP3 levels at 4 weeks post-procedure among ISR group patients

Supplementary Tables S1, S2 compared the clinical characteristics between low and high levels of SIRT1 and TIMP3 at 4 weeks post-procedure among ISR group patients, based on median serum levels. No significant differences were found in age, length of hospital stay, hypertension, coronary artery disease, atrial fibrillation, stroke, rheumatoid arthritis, carotid stenosis, hyperhomocysteinemia, procedure status, treated lesions, unilateral vs. bilateral/aortic lesions, number of stents, stent length, or stent diameter between the subgroups (all $P > 0.05$). However, TIMP3 levels at 4 weeks post-procedure were significantly higher in the high SIRT1 group compared to the low SIRT1 group (4.26 ± 1.06 ng/mL vs. 1.76 ± 0.78 ng/mL, $P < 0.001$). Additionally, diabetes mellitus was more prevalent in the low SIRT1 group (63.6% vs. 21.9%, $P = 0.001$). Similarly, significant differences were observed in SIRT1 levels at 4 weeks post-procedure between the low and high TIMP3 groups (107.27 ± 33.54 ng/mL vs. 66.01 ± 40.82 ng/mL, $P < 0.001$). Diabetes mellitus was also more prevalent in the low TIMP3 group compared to the high TIMP3 group ($P = 0.024$). These findings highlighted the significant association of serum SIRT1 and TIMP3 levels with diabetes mellitus among ISR patients.

Univariate and multivariate logistic regression analysis of predictors for ISR following PTA and stenting

Table 3 displayed the results of univariate and multivariate logistic regression analyses. In the univariate analysis, age showed a trend towards significance ($P = 0.110$, OR = 1.025, 95% CI: 0.994-1.056), as did atrial fibrillation ($P = 0.078$, OR = 2.085, 95% CI: 0.921-4.722) and carotid stenosis ($P = 0.061$, OR = 2.397, 95% CI: 0.96-5.985). Additionally, the type of stent used (BMS vs. DES) approached significance ($P = 0.067$, OR = 1.812, 95% CI: 0.959-3.423). The serum levels of SIRT1 and TIMP3 at 4 weeks post-procedure were highly significant predictors,

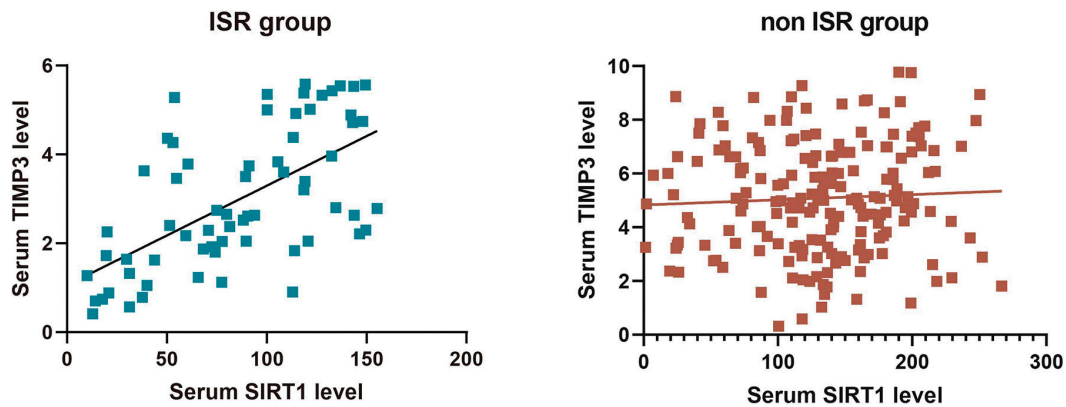

Fig. 3. Correlation between serum SIRT1 and TIMP3 levels at 4 weeks post-procedure in ASO patients with/without ISR after PTA and stenting.
Sirtuin 1 (SIRT1); Tissue Inhibitor of Metalloproteinase 3 (TIMP3); Arteriosclerosis Obliterans (ASO); Percutaneous Transluminal Angioplasty (PTA); In-Stent Restenosis (ISR).

Table 3. Univariate and multivariate logistic regression analysis of predictors for ISR following PTA and stenting.

Variable	Univariate Analysis		Multivariate Analysis	
	P	OR (95%CI)	*P*	OR (95%CI)
Age (years)	0.110	1.025 (0.994-1.056)	0.055	1.039 (0.999-1.080)
Sex (Male vs. Female)	0.396	0.770 (0.422-1.407)		
Diabetes (Yes vs. No)	0.284	1.033 (0.416-1.293)	0.031	1.436 (1.205-1.925)
Hypertension (Yes vs. No)	0.141	0.639 (0.352-1.160)	0.371	0.708 (0.332-1.509)
Coronary heart disease (Yes vs. No)	0.520	0.769 (0.347-1.708)	0.118	0.432 (0.151-1.236)
Atrial fibrillation (Yes vs. No)	0.078	2.085 (0.921-4.722)	0.233	1.852 (0.673-5.099)
Cerebral infarction (Yes vs. No)	0.450	1.279 (0.675-2.425)		
Rheumatoid arthritis (Yes vs. No)	0.169	1.922 (0.758-4.870)		
Carotid stenosis (Yes vs. No)	0.061	2.397 (0.960-5.985)	0.014	4.551 (1.364-15.185)
Hyperhomocysteinemia (Yes vs. No)	0.132	2.326 (0.776-6.977)		
Procedure Status (Elective vs. Urgent)	0.263	0.659 (0.317-1.369)		
Treated lesions	0.776	1.044 (0.775-1.407)	0.302	1.237 (0.826-1.853)
Uni/bilateral	0.709	1.122 (0.613-2.055)		
Number of stents	0.882	1.022 (0.771-1.354)	0.817	1.045 (0.718-1.521)
Type of stent (BMS vs. DES)	0.067	1.812 (0.959-3.423)	0.130	1.860 (0.834-4.152)
Stent length (mm)	0.118	1.007 (0.998-1.017)	0.059	1.011 (1.000-1.023)
Stent diameter (mm)	0.118	0.898 (0.784-1.028)	0.335	0.917 (0.768-1.094)
Length of Stay (days)	0.417	1.019 (0.974-1.065)		
SIRT1 (baseline)	0.232	1.004 (0.997-1.011)		
TIMP3 (baseline)	0.808	0.975 (0.792-1.199)		
SIRT1 (4 weeks)	< 0.001	0.984 (0.979-0.990)	< 0.001	0.985 (0.978-0.992)
TIMP3 (4 weeks)	< 0.001	0.551 (0.456-0.666)	< 0.001	0.574 (0.464-0.710)

with SIRT1 levels ($P < 0.001$, OR = 0.984, 95% CI: 0.979-0.990) and TIMP3 ($P < 0.001$, OR = 0.551, 95% CI: 0.456-0.666) showing a strong inverse relationship with ISR. In the multivariate analysis, age remained a near-significant predictor of ISR ($P = 0.055$, OR = 1.039, 95% CI: 0.999-1.080). Diabetes showed a significant association with increased ISR risk ($P = 0.031$, OR = 1.436, 95% CI: 1.036-1.992), as did carotid stenosis ($P = 0.014$, OR = 4.551, 95% CI: 1.364-15.185). Stent length also approached significance ($P = 0.059$, OR = 1.011, 95% CI: 1.000-1.023). The inverse relationship of SIRT1 levels ($P < 0.001$, OR = 0.985, 95% CI: 0.978-0.992) and TIMP3 levels ($P < 0.001$, OR = 0.574, 95% CI: 0.464-0.710) at 4 weeks post-procedure with ISR remained highly significant. These results underscore the roles of diabetes, carotid stenosis, and the post-procedural levels of SIRT1 and TIMP3 as key factors influencing ISR.

Discussion

The outcomes of this investigation shed light on the potential significance of serum biomarkers, particularly SIRT1 and TIMP3, in anticipating ISR in ASO patients' post-PTA with stent insertion. Our findings underscore a notable correlation between serum levels of SIRT1 and TIMP3 four weeks post-procedure among ISR patients, implying a possible interplay or mutual influence between these biomarkers during ISR development.

Stent-induced injury to the arterial wall triggers thrombotic and inflammatory processes, culminating in the hyperproliferation of smooth muscle cells and the formation of neointimal stenosis, thereby contributing to ISR pathogenesis (Lichtenberg et al. 2017; Yang et al. 2020). SIRT1, recognized for its cardiovascular health-related functions, undergoes downregulation as part of the acute inflammatory response (Strycharz et al. 2018; Singh and Ubaid 2020). SIRT1 may play a role in post-interventional restenosis; for example, its upregulation by peroxisome proliferator-activated receptor δ (PPARδ) inhibits vascular smooth muscle cell migration and proliferation, potentially attenuating restenosis and atherosclerosis (Hwang et al. 2016). Poldip2, involved in cell cycle regulation, may influence restenosis through the SIRT1 pathway (Brown et al. 2014). Moreover, the protective effects of SIRT1 against neointimal proliferation and restenosis post-angioplasty have been evidenced through its anti-inflammatory and anti-proliferative mechanisms (Huang et al. 2022). Additionally, TIMP3, known for its involvement in extracellular matrix turnover, may hinder neointimal hyperplasia and restenosis by inhibiting matrix metalloproteinases and promoting smooth muscle cell apoptosis (Johnson et al. 2005). The expression of TIMP-3 mRNA, influenced by growth factors and cytokines, could modulate basement membrane degradation, potentially contributing to restenosis post-angioplasty (Fabunmi et al. 1996). Overexpression of TIMP-3 via a novel adenovirus vector could mitigate smooth muscle cell activity and migration, offering a promising therapeutic

avenue for conditions associated with neointimal hyperplasia such as ISR (White et al. 2013). In our study, diminished serum levels of SIRT1 and TIMP3 in ISR patients following PTA and stenting in ASO compared to non-ISR patients imply their involvement in the mechanisms underlying restenosis in ASO patients and their potential predictive value for ISR post-PTA and stenting in ASO patients. Regarding diagnostic performance, SIRT1 exhibited a sensitivity of 78.46% and a specificity of 59.16%, whereas TIMP3 showed a sensitivity of 56.92% and a specificity of 85.34%. Diagnostic analysis further emphasizes the potential utility of TIMP3 as a more accurate predictor of ISR post-PTA and stenting in ASO patients compared to SIRT1. The higher AUC and specificity of TIMP3 indicate its superior discriminatory ability in identifying patients at elevated risk of ISR, offering potential clinical implications for risk stratification and personalized management strategies in ASO patients following PTA and stenting.

In our study, we observed a significant positive correlation between serum SIRT1 and TIMP3 levels, suggesting a potential synergistic mechanism in ISR development. This interplay or mutual influence between SIRT1 and TIMP3 appears to be specific to ISR progression. Supporting this, recent studies have shown that in type 2 diabetes, reduced TIMP3 expression in atherosclerotic plaques leads to increased activity of ADAM17 and MMP9, a process regulated by SIRT1 (Cardellini et al. 2009). Additionally, SIRT1 activation prevents high glucose-induced downregulation of renal tubular ACE2 expression in diabetic kidney disease by modulating the TIMP3/ADAM17 pathway (Guo et al. 2024). Notably, this correlation was not observed in the non-ISR group, suggesting that the SIRT1-TIMP3 interplay is specific to ISR-related pathophysiological processes following PTA and stenting in ASO. It is plausible that SIRT1-mediated suppression of inflammation and oxidative stress may contribute to the upregulation of TIMP3 expression, thereby inhibiting vascular remodeling and reducing the risk of restenosis. Moreover, in our analysis, diabetes mellitus was significantly more prevalent in the low SIRT1 and low TIMP3 groups among ISR patients, highlighting the association of these markers with diabetes. Multivariate analysis further identified diabetes and carotid stenosis as significant risk factors for increased ISR risk. The inverse relationship between SIRT1 and TIMP3 levels at 4 weeks post-procedure and ISR was also highly significant, underscoring the roles of these markers in ISR development. These results underscore the roles of age, diabetes, carotid stenosis, and the post-procedural levels of SIRT1 and TIMP3 as key factors influencing ISR.

However, our study has several limitations that need to be acknowledged. Firstly, the levels of SIRT1 and TIMP3 sampled before intervention may not effectively predict the development of ISR. These biomarkers lack practical utility in identifying patients at risk of ISR before stenting, which limits their value in guiding pre-emptive interventions. Secondly, we need to establish the independence of SIRT1 and TIMP3 levels from procedure-related variables and inflammatory markers to confirm their reliability as ISR biomarkers. The observed correlation between SIRT1 and TIMP3 levels in ISR patients might reflect a shared response to inflammation rather than a direct interaction. Thus, further research is necessary to validate their independence and association with inflammation in ISR prediction. Thirdly, the retrospective design of our study, despite the prospective data collection, precludes establishing causality. Additionally, we did not measure the longitudinal trends of these markers, limiting our ability to predict ISR effectively. Future studies should incorporate longitudinal measurements of these markers to better understand their temporal dynamics and improve ISR prediction. Finally, the relatively small sample size of our study may introduce unaccounted confounding factors, limiting the generalizability of the results. Larger-scale prospective studies are needed to validate these findings and enhance their applicability.

Conclusion

Our study elucidates the potential of serum SIRT1 and TIMP3 levels as biomarkers for predicting ISR in patients with ASO following PTA and stenting. We observed that lower serum levels of SIRT1 and TIMP3 at 4 weeks post-procedure were significantly associated with ISR development, with TIMP3 demonstrating superior predictive ability compared to SIRT1. Additionally, diabetes mellitus and carotid stenosis emerged as significant predictors of ISR in multivariate analysis, further highlighting their critical role in ISR pathogenesis.

Author Contributions

Han-Jun Wang contributed to the conception, design, data acquisition, analysis, interpretation of data, and drafting of the manuscript. Sheng-Yuan Mao contributed to the design, data acquisition, critical revision of the manuscript for important intellectual content, and final approval of the version to be submitted.

Conflict of Interest

The authors declare no conflict of interest.

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Supplementary Files

Please find supplementary file(s);
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