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Expressions of vascular endothelial cadherin and soluble Fas ligand in patients with viral myocarditis and their correlations

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ABSTRACT

Background: We aimed to explore the expressions of vascular endothelial cadherin (VE-cadherin) and soluble Fas ligand (sFasL) in patients suffering from viral myocarditis (VMC), and the correlations between them.

Methods: Fifty VMC patients diagnosed between January 2022 and December 2023 were selected as the subjects (experimental group), and an equal number of participants receiving health examination were included as a control group. The experimental group was then subdivided into mild, moderate, and severe subgroups according to their clinical symptoms. Peripheral blood samples were collected to detect the serum levels of VE-cadherin and sFasL through enzyme-linked immunosorbent assay, and the differences in their levels were compared. The clinical baseline data and cardiac function indicators (left ventricular systolic function as well as early and late left ventricular diastolic function) were acquired.

Results: The ejection fraction of the inferior ventricular septum showed no significant difference between the two groups ($p>0.05$), but there were significant differences in the remaining indicators ($p<0.05$). The levels of VE-cadherin and sFasL in the experimental group were significantly higher than those in the control group ($p<0.05$). The severe VMC group had significantly elevated VE-cadherin and sFasL levels compared with those of moderate and mild groups, and the moderate group had significantly raised levels compared to those of the mild group ($p<0.05$). VE-cadherin level was positively correlated with sFasL level.

Conclusions: The elevation of serum VE-cadherin and sFasL levels may be associated with myocardial inflammatory response and cardiac function damage. VE-cadherin and sFasL are potential biomarkers of VMC for early diagnosis and treatment evaluation.

Keywords: correlation, expression, soluble Fas ligand, vascular endothelial cadherin, viral myocarditis

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INTRODUCTION

Viral myocarditis (VMC), a viral infection-induced myocardial inflammatory response, is one of the major causes of cardiomyopathy and heart failure [1,2]. Despite certain progress in the clinic, there are still plenty of uncertainties in the pathogenesis and diagnostic markers of VMC. Vascular endothelial cadherin (VE-cadherin) is an intercellular adhesion protein widely existing in endothelial cells [3], which plays a vital role in maintaining the integrity and stability of endothelial cells. Soluble Fas ligand (sFasL) is capable of inducing cell apoptosis upon binding to Fas receptor [4]. Previous studies have pointed out that there may be changes in VE-cadherin and sFasL expressions during myocarditis [5,6], but their specific action mechanisms and relationship have not been fully elucidated. Hence, this study was designed to investigate the expression levels of VE-cadherin and sFasL in VMC patients and analyze their correlation. The in-depth research on the roles of these two molecules in VMC can facilitate the understanding of the pathogenesis and provide new targets and strategies for the diagnosis and treatment of the disease.

In this study, therefore, we herein detected the expression levels of VE-cadherin and sFasL as well as cardiac function indicators in the myocardial tissues of VMC patients, and analyzed their correlations, aiming to provide references for VMC diagnosis and treatment.

METHODS

Subjects

The present study was approved by the ethics committee of Kunming Municipal Hospital of Traditional Chinese Medicine on January 5th, 2022 (approval No. KMHTCM202201003). Fifty VMC patients visiting the hospital between January 2022 and December 2023

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were recruited as an experimental group, and an equal number of healthy subjects who received physical examinations in the same period were assigned to a control group. The experimental group was then subdivided into mild, moderate, and severe subgroups according to their clinical symptoms. Mild VMC: Patients with mild symptoms such as minor arrhythmias or chest pain without significant cardiac dysfunction. Moderate VMC: Patients exhibiting more pronounced symptoms, such as moderate arrhythmias, chest pain, and reduced left ventricular function. Severe VMC: Patients with severe arrhythmias, significant reduction in left ventricular function, heart failure symptoms, or cardiogenic shock [7].

Inclusion and exclusion criteria

Inclusion criteria: 1) patients meeting the clinical diagnostic criteria of VMC [8]; 2) those with obvious symptoms of myocarditis including chest tightness and palpitation; 3) those with myocarditis features on electrocardiogram and echocardiogram; 4) those without interference from other heart diseases.

Exclusion criteria: 1) patients accompanied with other systemic diseases; 2) those with severe heart failure or cardiogenic shock; 3) those with severe hepatic or renal impairment; 4) those with a history of immunosuppressant use; 5) pregnant or lactating women.

Collection of blood samples

Before collection, the patients were informed of the collection procedure and provided their consent. Peripheral blood samples (5 mL in volume) were collected using blood collection needles and EDTA tubes (Becton Dickinson, USA). Blood was drawn from the patients immediately upon hospital admission. After collection, the samples were processed within 30 minutes and centrifuged at 1,500 ×g for 10 minutes at 4°C using Sorvall ST 16 Centrifuge (Thermo Fisher Scientific, USA) to separate the plasma. The plasma was then aliquoted and stored at-80°C for subsequent analysis.

Measurement of VE-cadherin and sFasL levels

Enzyme-linked immunosorbent assay kits manufactured by Medical & Biological Laboratories Co., Ltd. (Japan) were used for measurement in strict accordance with the instructions.

Testing of cardiac function indicators (left ventricular systolic function plus early and late left ventricular diastolic function)

Apex SP-6 SPECT instrument (Elscint, Israel) and a low-energy all-purpose parallel-hole collimator were employed to examine the left ventricular systolic function and early and late left ventricular diastolic function, respectively.

Statistical analysis

The normality of measurement data was tested by SPSS 26.0 software. The normally distributed measurement data were expressed in the format of ($x \pm s$) and subjected to the independent-samples t-test for comparison between groups. Count data were represented as [n (%)], and the $\chi 2$ test was performed. The correlation between VE-cadherin and sFasL in VMC patients was investigated by Pearson analysis. α=0.05 was determined as the threshold of significance.

RESULTS

Baseline data

There were no statistically significant differences in the baseline data between the two groups (p>0.05) (Table 1).

Parameters related to left ventricular systolic function and early / late left ventricular diastolic function of patients

The experimental group presented significantly lower levels of parameters related to left ventricular systolic function as well as early and late left ventricular diastolic function than the control group. Among them, the ejection

Table 1. Baseline data

Indicator		Control group (n=50)	Experimental group (n=50)	Statistical value	*p* value
Age (year)		28.36±8.62	29.13±7.46	0.478	0.634
Gender	Male	26 (52.00)	23 (46.00)	0.360	0.549
	Female	24 (48.00)	27 (54.00)		
Hypertension	Yes	11 (22.00)	10 (20.00)	0.060	0.806
	No	39 (78.00)	40 (80.00)		
Diabetes mellitus	Yes	9 (18.00)	7 (14.00)	0.298	0.585
	No	41 (82.00)	43 (86.00)		
Educational background	Senior high school and below	21 (42.00)	24 (48.00)	0.414	0.814
	Junior college	17 (34.00)	16 (32.00)		
	Undergraduate or above	12 (24.00)	10 (20.00)		
Marital status	Married	44 (88.00)	46 (92.00)	0.444	0.505
	Unmarried	6 (12.00)	4 (8.00)		

fraction [EF (%)] of the inferior ventricular septum showed no statistically significant difference between the two groups (p>0.05), but differences of statistical significance were observed for the other indicators (p<0.05) (Table 2).

VE-cadherin and sFasL levels

The levels of VE-cadherin and sFasL in the experimental group were significantly higher than those in the control group (p<0.05) (Table 3).

VE-cadherin and sFasL levels in experimental groups with varying severity of disease

The severe group had significantly elevated VE-cadherin and sFasL levels compared with the moderate and mild groups, and the moderate group exhibited significantly raised levels by contrast to the mild group, with statistically significant differences between the two groups (p<0.05) (Table 4).

Correlation between VE-cadherin and FasL

It was revealed by Pearson correlation analysis that VE-cadherin was positively correlated with FasL (Figure 1).

DISCUSSION

Myocarditis is a heart disease in which the myocardium is infected or in an inflammatory state. VMC has the clinical manifestations of arrhythmia [9-11], chest pain, dyspnea, fatigue and weakness, and digestive system symptoms. Regarding the hazards [12,13], it can trigger heart failure, that is, myocarditis leads to myocardial function decline and heart failure at last, thus affecting the normal pumping function of the heart. Besides, it can cause arrhythmia, including tachyarrhythmia and atrial fibrillation, thereby elevating the risk of sudden cardiac death. Moreover, long-term myocarditis may also impair the myocardial structure and function, consequently influencing heart health. Hence, this study investigated the expression of VE-cadherin and sFasL in VMC patients and the correlation between VE-cadherin and sFasL, aiming to provide potential insights for diagnosis and treatment.

In this study, the levels of these parameters were significantly reduced in the experimental group compared with those in the control group, suggesting that VMC had a serious influence on cardiac function. Additionally, VE-cadherin and sFasL had abnormal expression levels in the experimental group. Therefore, the decline of left ventricular function in VMC patients may be associated with the abnormal expressions of VE-cadherin and sFasL.

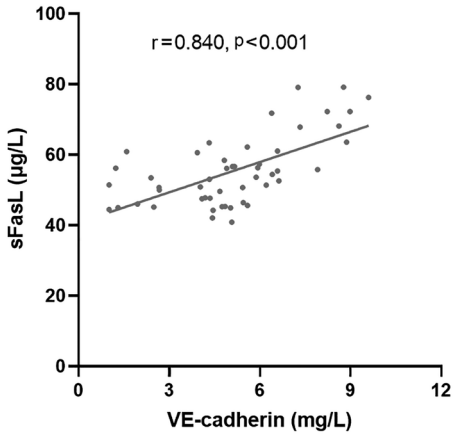

Figure 1. Correlation between VE-cadherin and FasL.

Table 2. Parameters of left ventricular systolic function

Indicator	Control group (n=50)	Experimental group (n=50)	Statistical value	*p* value
Overall EF (%)	63.45±4.52	56.82±6.41	5.977	0.001
EF (%) of lateral wall	74.16±9.22	60.15±5.47	9.241	0.001
EF (%) of inferior wall	87.26±8.43	83.16±7.94	2.504	0.014
EF (%) of cardiac apex	85.16±10.26	79.23±9.16	3.049	0.003
EF (%) of inferior ventricular septum	65.48±11.26	63.19±10.26	1.063	0.290
Peak filling rate (PFR) (s-1)	3.35±0.51	2.81±0.63	4.711	0.001
Time to PFR (s)	0.15±0.02	0.17±0.02	5.000	0.000
PFR/time to PFR	26.43±6.42	23.51±5.73	2.399	0.018
1/3 filling fraction	0.65±0.35	0.47±0.19	3.196	0.002

Table 3. VE-cadherin and sFasL levels

Indicator	Control group (n=50)	Experimental group (n=50)	Statistical value	*p* value
VE-cadherin (mg/L)	2.12±0.12	6.36±1.58	18.921	0.001
sFasL (μg/L)	22.13±7.29	73.41±6.84	36.273	0.001

Table 4. VE-cadherin and sFasL levels in the experimental group with varying severity of disease

Indicator	Mild group (n=26)	Moderate group (n=15)	Severe group (n=9)	Statistical value	*p* value
VE-cadherin (mg/L)	3.76±2.15	5.26±1.04	8.23±1.03	22.772	0.001
sFasL (μg/L)	49.37±5.22	55.62±5.11	72.28±5.27	65.011	0.001

Moreover, the expression level of VE-cadherin was significantly elevated in VMC patients in proportion to the severity, which may be closely related to the pathogenesis of VMC. Firstly, viral infections can activate the immune system to release inflammatory factors and exacerbate inflammatory responses [14]. In this case, VE-cadherin expression may be distinctly up-regulated. Inflammatory factors like tumor necrosis factor-α and interleukin-1β can promote VE-cadherin expression to affect the adhesion and permeability of vascular endothelial cells [15]. Secondly, viral infections may directly affect the function and stability of vascular endothelial cells, which in turn induce the abnormal expression of VE-cadherin [16]. In addition, viral infections may cause apoptosis and abnormal proliferation of vascular endothelial cells, thereby affecting the expression of VE-cadherin. Consistently, VE-cadherin disruption has been reported to occur in response to inflammation, leading to endothelial barrier breakdown and vascular leakage, which are key contributors to VMC severity [17]. Furthermore, increased levels of soluble VE-cadherin fragments in patients with inflammatory conditions reflect endothelial dysfunction and have been linked to worse clinical outcomes [18]. These findings support the hypothesis that VE-cadherin dysfunction plays a pivotal role in VMC by promoting endothelial permeability and subsequent myocardial injury.

The role of sFasL in apoptosis is also well established, with elevated levels contributing to increased cardiomyocyte apoptosis in VMC patients [19]. In this study, sFasL expression level was significantly up-regulated in VMC patients along with the increased severity. This phenomenon may be closely associated with the viral infection-triggered immune response and cell apoptosis. In the case of VMC, virus particles invade and replicate in cardiomyocytes to give rise to pathological changes therein, and simultaneously activate the immune system to release massive inflammatory mediators [20]. Such inflammatory mediators not only directly damage cardiomyocytes, but also affect the regulation of apoptotic pathways, leading to the up-regulation of sFasL. Moreover, viral infection-triggered cell apoptosis is a crucial link in the pathogenesis of VMC [21]. In this process, sFasL is involved in the regulation of cell apoptosis as an important apoptotic signaling molecule, and the up-regulation of its expression level may be a self-protection mechanism against viral infections [22]. Besides, the up-regulation of sFasL may be associated with immune dysfunction in VMC patients as well [23]. Likewise, sFasL up-regulation has been associated with both immune-mediated damage and viral infection-induced apoptosis, leading to worsened cardiac outcomes [24]. In particular, sFasL has been identified as a key regulator of apoptosis in cardiomyocytes, and its increased expression in VMC is correlated with disease severity. This supports our findings that sFasL plays a key role in myocardial damage during the progression of VMC.

Additionally, we herein found a positive correlation between the expression levels of VE-cadherin and sFasL in VMC patients, suggesting a possible interaction between the two biomarkers which are both implicated in the pathogenesis of VMC. Further correlation analysis revealed that the concurrent up-regulation of VE-cadherin and sFasL may be closely correlated with VMC severity. This is consistent with previous literature indicating that disruption of the endothelial barrier, mediated by VE-cadherin cleavage, can exacerbate apoptotic signaling and inflammation [25]. This relationship underscores the complex interplay between endothelial injury and apoptosis in the pathogenesis of VMC, confirming the importance of these markers in assessing disease severity.

Nevertheless, there are some limitations in this study. First, the relatively small sample size may cause selection bias. Second, only VMC rather than other types of myocarditis was studied. Third, this study merely focused on the expression levels of VE-cadherin and sFasL, and their specific regulatory mechanisms and interactions remain to be further investigated.

CONCLUSIONS

In conclusion, the expressions of VE-cadherin and sFasL are altered significantly in VMC patients, which drop with the disease severity. VE-cadherin is positively correlated with sFasL in the serum. Future studies should focus on elucidating the molecular mechanisms underlying their interaction and exploring the potential of VE-cadherin and sFasL as therapeutic targets for managing VMC severity.

ABBREVIATIONS

EF- ejection fraction

PFR- peak filling rate

sFasL- soluble Fas ligand

VE-cadherin- vascular endothelial cadherin

VMC- viral myocarditis

AUTHORS' CONTRIBUTION

FL – data analysis

HH – study design, data analysis

NL – data collection

RL- writing

XW- data collection

CONFLICT OF INTEREST

None to declare.

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