

Original Article

Expression of MMP-9 and hs-CRP in the serum of patients with viral myocarditis

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Abstract: Objective: This study was designed to evaluate the expression of high sensitivity C-reactive protein (hs-CRP) and matrix metalloproteinase (MMP-9) in patients with viral myocarditis (VM), and its clinical significance. Methods: A total of 126 patients with VM admitted to our hospital were included as the study subjects, placed into the observation group and compared with 120 healthy objects who received physical examination in our hospital in the same period as the control group. To test hs-CRP and MMP-9 in both groups, particle-enhanced turbidimetric immunoassay (PETIA) and enzyme linked immunosorbent assay (ELISA) were adopted, respectively. The two groups were compared for the expression of hs-CRP and MMP-9 in serum and the positive rate. Results: (1) In the observation group, the main symptoms in the 126 patients with VM included 92 with fever, 8 with hidrosis, 3 with shortness of breath, 50 with nausea and vomiting, 48 with discomfort of the precordial area, 43 with general fatigue, 100 with abdominal pain, and 32 with expiratory dyspnea. (2) In the observation group, the main signs in the patients with VM included 126 with a low and obtuse first heart sound (S1), 92 with arrhythmia, 95 had sychnosphygmia, 12 with bradycardia, 45 with proiosystole and 3 with shock. (3) In the observation group, the hs-CRP and MMP-9 positive rates were 84.13% and 92.06%, and the measured hs-CRP and MMP-9 were (12.42±3.45) mg/L and (39.89±5.68) µg/L, respectively; while those of in the control group were 3.33%, 8.33%, (1.81±0.18) mg/L, and (28.02±1.28) µg/L, respectively: which were significantly different (P<0.05). Conclusion: Patients with VM have markedly elevated hs-CRP and MMP-9 in their serum, which can be used as important auxiliary indices in the clinical diagnosis of VM.

Keywords: Viral myocarditis, serum, hs-CRP, MMP-9, positive rate

Introduction

Viral Myocarditis (VM) is an angiocardiopathy with higher clinical incidence and manifests as diffuse or localized myocarditis due to viral infection [1]. In recent years, it has been reported that more and more patients suffer from VM, of which most cases cleared without treatment, but some extended and resulted in various arrhythmias or even third degree or high atrioventricular block [2, 3].

In recent years, with significant improvements in the technical level of detection, clinically it was found that various viruses contribute to myocarditis [4], and VM is diagnosed by comprehensive means including signs, medical history, myocardial enzymes, and electrocardiogram. At the current stage, hs-CRP and MMP-9 are extensively applied in the diagnosis and

treatment of angiocardiopathy [5]. MMP is a zinc-dependent endogenous proteolytic enzyme, whose activity change and expression contribute to pumping hypofunction and ventricular remodeling significantly, and may stimulate the synthesis of immature cell collagen fiber, and finally result in cardiac insufficiency and myocardial matrix remodeling [6, 7]. CRP is an acute-phase protein whose synthesis in the liver is boosted by inflammation. hs-CRP refers to a low-concentration CRP measured by hypersensitive means and therefore is easily detected [8].

The study aims to analyze the expression of hs-CRP and MMP-9 in the serum of patients with VM, and assess its clinical significance by tests against the VM group and the healthy controls receiving physical examination, in order to provide scientific basis for better clinical diagnosis of VM.

Materials and methods

Materials

In total, 126 patients with VM being treated in our hospital from June 2017 to June 2019 were included as the study subjects and formed the observation group. (1) Inclusion criteria: patients who have been diagnosed with VM [9] and provided informed consent were included. The study was approved by the medical ethic committee of our hospital. (2) Exclusion criteria: patients with congenital heart disease (CHD), toxic myocarditis (TM), and rheumatic myocarditis (RM) were excluded. The 126 study subjects consisted of 78 males and 48 females with ages between 23 and 75 and a mean age of (58.18±1.28). As the controls, 120 healthy subjects receiving physical examination in the hospital were also included on the conditions that they were neither infected nor treated with hormones. The controls included 68 males and 52 females with ages between 24 and 76 and a mean age of (58.25±1.16).

Methods

ELISA: Three ml blood was drawn from all study subjects, 2 d after their hospitalization in a fasted state, blood was let stand for 10-20 min at room temperature and centrifuged at 3000 r/min. While part of the serum was tested for hs-CRP and MMP-9 immediately, the rest was stored in freezer at -20°C for future use. hs-CRP was measured by PETIA with test kits from DiaSys (Shanghai) Co., Ltd., according to the instructions and the measurement criterion of >3 mg/L for positive results. MMP-9 is measured by ELISA with test kits from R&D Co., Ltd. according to the instructions.

Observation indices

(1) Analyze the main symptoms and signs of patients with VM. The main symptoms and signs of VM were analyzed, including fever, sweating, shortness of breath, nausea and vomiting, precordial discomfort, fatigue, abdominal pain and dyspnea. The signs mainly included a low and obtuse S₁, arrhythmia, tachycardia, bradycardia, prephase contraction and shock. The proportion of patients with each symptom and sign was analyzed.

(2) The positive rates of hs-CRP and MMP-9 in the two groups were compared. The detected

result of hs-CRP >3 mg/L indicated the result to be positive, while that of ≤3 mg/L indicated the result to be negative. MMP-9 positive criteria: Observation of the brown-yellow granules observed in the cytoplasm, and the final positive judgment was determined according to the proportion of positive cells and the degree of staining. Positive cells ≤10% were recorded as 1 score, 10 to 50% as a score of 2, and above 50% as a score of 3. Staining degree negative was recorded as 0 score, light yellow as 1 score, medium yellow 2 scores, brown yellow as 3 scores. The result of two integral multiplication <3 was considered negative, while that of ≥3 was considered positive.

(3) Test results of hs-CRP and MMP-9 were compared in both the observation group and the control group. The operation process in the methods was used for the measurement and the results were recorded.

(4) The levels of hs-CRP and MMP-9 in patients with different severity of myocardial injury were compared in the observation group. The degree of myocardial injury was measured according to the heart function classification New York Heart Association (NYHA): Grade I: having heart disease but no restriction on physical activity; Grade II: mildly limited physical activity; Grade III: significantly limited physical activity; Grade IV: unable to engage in any physical activity.

Statistical analysis

Statistical analysis was performed with SPSS 22.0. In case of numerical data expressed as Mean ± Standard Deviation, comparison studies were carried out through independent-samples T test for data which were normally distributed, and Mann-Whitney U test for data which were not normally distributed, paired *t* test for pre-and-pro comparison in the group; in case of nominal data expressed as [n (%)], comparison studies were carried out through X² test for intergroup comparison. For all statistical comparisons, significance was defined as *P*<0.05.

Results

Analysis of general data in the two groups

The proportion of males and females in the observation group was not significantly different from that in the control group (*P*>0.05), and the average age in the observation group had

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Table 1. Comparison of general materials in observation group and control group [n (%)]/($\bar{x} \pm s$)

Materials	Observation Group (n=126)	Control Group (n=120)	t/ χ^2	P
Gender (n)				
Male	78 (61.90)	68 (56.67)	0.699	0.403
Female	48 (38.10)	52 (43.33)		
Age (y)	58.18 \pm 1.28	58.06 \pm 1.16	0.791	0.430
Weight (kg)	65.38 \pm 4.19	66.22 \pm 5.04	0.851	0.162
Height (cm)	163.85 \pm 10.49	166.59 \pm 12.12	0.385	0.251

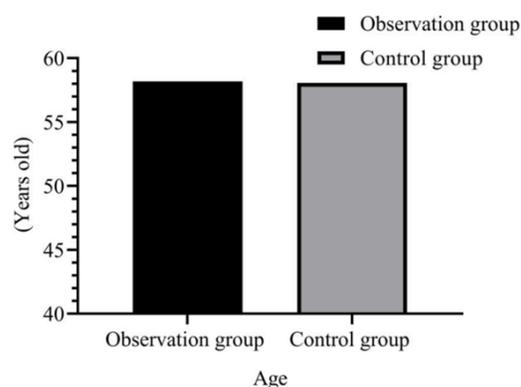


Figure 1. Comparison between the observation group and the control group for age. The average age in the observation group had no significant difference compared with the control group ($P > 0.05$).

no significant difference compared with the control group ($P > 0.05$) (Table 1 and Figure 1).

Analysis of main symptoms of the observation group

Amongst the 126 patients with VM, 92 (73.02%) complained of fever, and 8 (6.35%) were found with hidrosis, 3 (2.38%) with shortness of breath, 50 (39.68%) with nausea and vomiting, 48 (38.10%) with discomfort of the precordial area, 43 (34.13%) with general fatigue, 100 (79.37%) with abdominal pain, and 32 (25.40%) with expiratory dyspnea (Table 2).

Analysis of main signs of the observation group

All the 126 (100%) patients with VM had low and obtuse first heart sounds (S1), 92 (73.02%) had arrhythmia, 95 (75.39%) had sychnosphygmia, 12 (9.52%) had bradycardia, 45 (35.71%) had proiosystole and 3 (2.38%) had signs of shock (Table 2).

Comparison between the observation group and the control group for expression of hs-CRP and MMP-9

The hs-CRP and MMP-9 positive rates in the observation group were 84.13% and 92.06%, while those of in the control group were 3.33% and 8.33%, which had statistical differences ($P < 0.05$, Table 3).

Comparison between the observation group and the control group for measured hs-CRP

The measured hs-CRP in the observation group was (12.42 \pm 3.45) mg/L, while that of in the control group was (1.81 \pm 0.18) mg/L, which had statistical differences ($P < 0.05$, Table 4; Figure 2).

Comparison between the observation group and the control group for measured MMP-9

The measured hs-CRP in the observation group was (39.89 \pm 5.68) μ g/L, while that of in the control group was (28.02 \pm 1.28) μ g/L, which had a statistical difference ($P < 0.05$, Table 5; Figure 3).

Comparison of the levels of hs-CRP and MMP-9 in patients with different severities in the observation group

Among the 126 cases in the observation group, 47 cases were Grade I for myocardial injury, 59 cases in Grade II, and 20 cases in Grade III. For Grade III patients, hs-CRP level was (7.85 \pm 1.13) mg/L, and MMP-9 level was (30.65 \pm 2.18) μ g/L. For Grade II patients, hs-CRP level was (10.68 \pm 1.25) mg/L, and MMP-9 level was (35.46 \pm 3.51) μ g/L. For Grade I patients, hs-CRP level was (13.62 \pm 1.38) mg/L, and MMP-9 level was (41.26 \pm 5.67) μ g/L. The higher the severity of myocardial injury, the higher the hs-CRP and MMP-9 levels in patients, and the difference was statistically significant ($P < 0.05$) (Figure 4).

Discussion

Commonly found in clinic, VM can be attributed to various viruses, including poliovirus, Echovirus, Coxsackie B (the main contributor) [10] and A viruses, which lead to upper respiratory tract and enterovirus infection in most cases.

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Table 2. Comparison between the observation group and the control group in symptoms and signs [n (%)]

Materials	Observation Group (n=126)	Control Group (n=120)	χ^2	P
Main symptoms				
Fever	92 (73.02)	2 (1.67)	132.524	<0.000
Hidrosis	8 (6.35)	0 (0.00)	7.875	0.005
Nausea and vomiting	50 (39.68)	1 (1.67)	56.448	<0.000
Discomfort of the precordial area	48 (38.10)	0 (0.00)	56.797	<0.000
Abdominal pain	43 (34.13)	2 (3.33)	43.330	<0.000
Expiratory dyspnea	100 (79.37)	0 (0.00)	160.470	<0.000
Signs				
Low and obtuse S1	126 (100.00)	0 (0.00)	246.000	<0.000
Arrhythmia	92 (73.02)	0 (0.00)	139.963	<0.000
Sychnosphymia	95 (75.39)	1 (1.67)	143.607	<0.000
Bradycardia	12 (9.52)	1 (1.67)	9.274	0.002
Proiosystole	45 (35.71)	0 (0.00)	52.452	<0.000
Signs of shock	3 (2.38)	0 (0.00)	2.892	0.089

Table 3. Comparison between the observation group and the control group for positive rates of hs-CRP and MMP-9 [n (%)]

Group	Cases (n)	hs-CRP	MMP-9
Observation Group	126	106 (84.13)	116 (92.06)
Control Group	120	2 (3.33)	10 (8.33)
χ^2		169.697	125.658
P		0.0001	0.0012

Table 4. Comparison between the observation group and the control group for measured hs-CRP ($\bar{x} \pm s$, mg/L)

Group	Measured hs-CRP
Observation Group (n=126)	12.42±3.45
Control Group (n=120)	1.81±0.18
t	33.642
P	0.000

Generally speaking, the clinical symptoms in patients with VM are closely associated with the disease regions and the prevalence. While patients with mild conditions have no clinical symptoms, patients with severe cases may have cardiac shock, heart failure and sudden death [1, 11]. As a rule, patients with TM may have a history of enteric infection or upper respiratory tract infection 1 to 3 weeks before onset, which manifests as abdominal pain, vomiting, nausea, lassitude, pharyngalgia, gen-

eral aches, fever, edema, expiratory dyspnea, dizziness, chest pain, chest tightness, and palpitations. In a small number of patients, cardiac shock and symptoms of heart failure are expected [12, 13]. Amongst the 126 patients with VM in the present study, 73.02% complained of fever, and 6.35% were found with hidrosis, 2.38% with shortness of breath, 39.68% with nausea and vomiting, 38.10% with discomfort of the precordial area, 34.13% with general fatigue, 79.37% with abdominal pain, and 25.40% with expiratory dyspnea. Next, analysis of clinical signs found that all the 126 (100%) patients with VM had low and obtuse first heart sounds (S1), 92 (73.02%) had arrhythmia, 95 (75.39%) had sychnosphymia, 12 (9.52%) had bradycardia, 45 (35.71%) had proiosystole, and 3 (2.38%) had signs of shock; indicating that higher attention and future detailed examinations are necessary for patients with clinical symptoms of nausea and vomiting, fever, and abdominal pain, or with arrhythmia or low and obtuse S1 in medical examination, to make a definite diagnosis.

CPR is a serum β globulin synthesized in the liver and exists in blood with a relative molecular mass between 115 kDa and 140 kDa [14]. It is an acute protein assisting the C-polysaccharide attached on the cell walls of pneumococci to produce a precipitation reaction, for which, it earns the name of C-reactive

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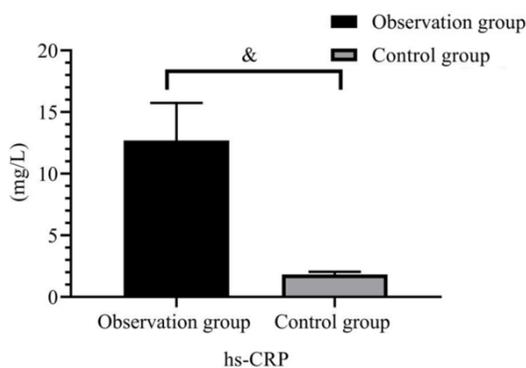


Figure 2. Comparison between observation group and control group for hs-CRP levels. The measured level of hs-CRP in the observation group was higher than that in the control group ($P < 0.05$). & indicated $P < 0.05$ when the two groups were compared.

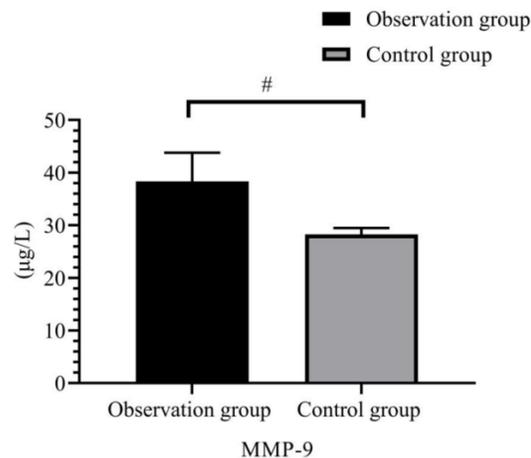


Figure 3. Comparison between observation group and control group for MMP-9 levels. The measured level of MMP-9 in the observation group was higher than that in the control group ($P < 0.05$). # indicated $P < 0.05$ when the two groups were compared.

Table 5. Comparison between the observation group and the control group for measured MMP-9 ($\bar{x} \pm s$, $\mu\text{g/L}$)

Group	Measured MMP-9
Observation Group (n=126)	39.89±5.68
Control Group (n=120)	28.02±1.28
t	22.356
P	0.000

protein. Under the action of calcium ions, CRP can be bound with polysaccharide substances in pathogens such as bacteria and fungi to activate the complement system and affiliate to cells, in order to boost the release of mediators of inflammation, and to enhance phagocytosis of macrophages, facilitating target cell lysis [15]. In general, a normal human body contains extremely low amount of CRP [16], which can be up regulated to a certain degree by interleukin-1 (IL-1) and interleukin-6 (IL-6). Where the tissues are damaged and the body was invaded by microbes, the amount of CRP synthesized in the liver will increase considerably [17], and its contents rises when the body is hurt or suffering from a fever or in patients with autoimmune diseases or chronic inflammations. The changes of CRP are closely related to the disease status but without individual differences. When damaged, due to various reasons, the body will experience a series of acute-phase reactions, leading to elevated CRP concentration in the serum which has a clear association with the severity of inflammation. At the current stage, CRP is not only used as a clinical means

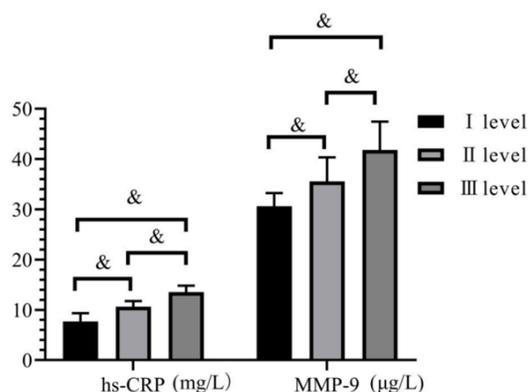


Figure 4. Comparison of the levels of hs-CRP and MMP-9 in patients with different degrees of myocardial injury. The hs-CRP and MMP-9 levels of Grade II and III patients were higher than those of the Grade I patients ($P < 0.05$), and the hs-CRP and MMP-9 levels of Grade III patients were higher than those of the Grade II patients ($P < 0.05$). & indicated $P < 0.05$ when the two groups were compared.

to identify and discriminate viral infection and bacterial infection, but also as a key inflammation marker for extensive application in judgment of some kinds of inflammation [18, 19]. With more and more in-depth clinical studies on CRP, hs-CRP emerged to test the low-level CRP by hypersensitive means. In this study, hs-CRP was measured in healthy subjects and patients with VM. The positive rate of hs-CRP in the observation group was 84.13%, which was higher than 3.33% in the control group ($P < 0.05$).

The measured value of hs-CRP in the observation group was (12.42 ± 3.45) mg/L, which was significantly higher than (1.81 ± 0.18) mg/L in the control group ($P < 0.05$), suggesting that hs-CRP could be used as an important auxiliary index in clinical diagnosis of VM. Paeschke et al. [20] found that the serum hs-CRP level of children in the acute stage of VM was significantly higher than that of the healthy control group ($P < 0.05$), which was consistent with the results of this study, and also proved that hs-CRP is of great value in the judgment and diagnosis of VM. hs-CRP has the same biological characteristics as CRP, but has higher sensitivity, and has been widely used in clinical diagnosis and treatment of a variety of diseases. In the clinical diagnosis of VM, cardiac troponin and myocardial enzyme levels are usually detected, but the positive rate and specificity need to be further improved. Viral infection is the main cause of VM, hence this disease is also one of the myocardial inflammatory lesions [20].

In this study, the positive rate of MMP-9 in the observation group was 92.06%, which was higher than 8.33% in the control group ($P < 0.05$). The measured value of MMP-9 in the observation group was (39.89 ± 5.68) μ g/L, which was significantly higher than (28.02 ± 1.28) μ g/L in the control group ($P < 0.05$), suggesting that MMP-9 could be used as an important marker reflecting myocardial damage in VM and play a certain role in the diagnosis of VM. MMP-9 plays an important role in extracellular matrix (ECM) remodeling [21]. Studies showed that MMP-9 can play an important role in angiogenesis by releasing a large amount of vascular endothelial growth factor, effectively regulate the activity of cytokines and other proteases, promote α -1 anti-trypsin degradation, and increase the collagenolytic activity of collagen cells MMP-13 [22]. The increase of volume load will promote the activation of mast cells and secrete a large amount of endothelin-1 to promote mast cell degranulation, increase MMP activity, promote the degradation of ECM and remodeling of myocardium, and then it can be inferred that the volume load can be effectively reflected by the level of serum MMP-9. When myocarditis occurs, MMP will be activated, collagen structure will be destroyed, and the connection between the myocardium will be in a loose state. Under the action of continuous ventricular wall pressure, the configuration of

the left ventricle will also change accordingly, thereby affecting the function of the left ventricle. An B et al. [23] studied the mouse model of VM and found that ECM degradation could increase the mobility of cardiac cells, expand the cardiac cavity and damage the function of the pump, which was an important cause of myocardial collagen remodeling and cardiac dysfunction. In conclusion, the contents of hs-CRP and MMP-9 in the serum of patients with VM will elevate significantly, which can be used as a key assistant index in the clinical diagnosis of VM.

However, the study results are not typically representative as fewer study subjects are included. Future studies shall focus on this aspect and be based on a larger sample size, longer period, and more comprehensive coverage, in order to further explore the expression of hs-CRP and MMP-9 in the serum of patients with VM, and its clinical significance.

Disclosure of conflict of interest

None.

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