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Correlations of Serum MMP-9, TIMP-1, TGF-β and bFGF Levels with Atrial Fibrillation in Patients without Structural Heart Disease

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ABSTRACT The researchers aimed in highlighting the correlations of serum MMP-9, TIMP-1, TGF-β1 and bFGF levels with atrial fibrillation (AF) within diseased persons showing no structural heart ailment. A total of 236 eligible diseased persons had been categorized within AF category (n=168) and non-AF category (n=68). Left atrial diameter (LAD), left ventricular end-diastolic dimension (LVEDD) and left ventricular ejection fraction (LVEF) had been measured through cardiac ultrasonography. Comparing concerning non-AF category, AF class had larger LAD and smaller LVEF (P<0.01). MMP-9 and TGF-β1 levels had been higher while TIMP-1 level remained less within AF class as compared to non-AF class (P<0.01). Age, LVEF and left atrial MMP-9/TIMP-1 remained free correlative factors of AF (P<0.01). MMP-9/TIMP-1 remained definitely correlated concerning LAD within AF class (r=0.509, P<0.01). Left atrial MMP-9/TIMP-1 remained higher within diseased persons regarding persistent AF (P<0.01). Left atrial MMP-9/TIMP-1 is correlated with AF within diseased persons having no structural heart ailment, particularly in the case of persistent AF.

INTRODUCTION

Atrial fibrillation (AF) belonging to ordinary category of arrhythmia regarding experimental exercise, going to be the main cardiovascular ailment which affects the health of the people at a large scale. Expansion and progression of AF are associated with electrical and organizational renovation atrial tissues (Jansen et al. 2018). A longer duration of AF suggests severer structural remodeling, which is more likely to induce persistent AF. Electrical remodeling can be reversed by antiarrhythmic drugs that can also restore and maintain sinus rhythm. Besides, radiofrequency ablation is able to block the electrical conduction between the atria and veins to prevent AF. However, the above two approaches cannot reverse structural remodeling.

It is well-accepted that structural remodeling is mainly related to transforming growth aspects (TGFs), renin-angiotensin system, inflammatory and oxidative stress factors, matrix metalloproteinases (MMPs), tissue inhibitors of metalloproteinases (TIMPs), etc. (Pellegrino et al. 2013; Xiao et al. 2020). Balance within MMPs, TIMPs determines content concerning extracellular matrix (ECM), TGF-β1 does a crucial part within signal cascade amplification for expansion and progression of myocardial fibrosis (Kim et al. 2011). Thus, corresponding drugs for structural remodeling-related factors, together with angiotensin-converting enzyme inhibitors, angiotensin receptor antagonists, MMP inhibitors, can reverse this process. MMP-9/TIMP-1 ratio raised up as inner diameter the left atrium increases (Soran et al. 2012). The dynamic balance within MMPs, TIMPs predominantly inhibits the excessive dilapidation concerning collagen. The level of MMP-9 is the main marker of extracellular collagen degradation, which gradually increases within different periods of AF (paroxysmal, persistent to permanent AF) (Veidal et al. 2012). Therefore, the degree of left atrial structural remodeling can be predicted by measuring serum MMP-9/TIMP-1 ratio and TGF-β1 level in AF patients without structural heart disease. The

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results may be of great significance for screening AF patients, maintaining sinus rhythm and improving prognosis (Sangaralingham et al. 2016).

Objective

Scope of this work had been measuring the serum planes MMP-9, TIMP-1, TGF-β1, basic fibroblast growth factor (bFGF) within left or right atrium of AF diseased persons, and to investigate their correlations within diseased persons without operational heart ailment.

MATERIAL AND METHODS

Subjects

The ethical committee of the hospital acknowledges the importance of undertaken work and all diseased persons provided their consent in writing to be the part of this work. 236 diseased persons who were diagnosed as arrhythmia and treated with radiofrequency ablation from January 2018 to December 2019 were selected, including 168 patients diagnosed as AF by body surface or Holter electrocardiogram (including 72 cases of persistent AF and 96 cases of paroxysmal AF) and 68 patients with paroxysmal supraventricular tachycardia or pre-excitation syndrome (left accessory atrioventricular pathway on electrocardiogram). Before surgery, routine electrocardiogram, echocardiography and blood tests for liver and kidney functions were carried out, and patients with structural heart disease, acute coronary syndrome, concurrent infectious or non-infectious inflammatory diseases, tumors, immune system diseases or severe liver or kidney dysfunction were excluded. The enrolled diseased persons had been categorized within AF class(n=168), non-AF class (n=68).

Collection of Blood Samples

Before radiofrequency ablation, 4 mL of blood was collected from the left and right atria of patients as follows. After local anesthesia with 10 ml/L lidocaine, venipuncture was conducted on the right axilla, a 6F sheath was inserted, and a Cordis10 coronary sinus mapping electrode was placed through the sheath. Afterwards, right femoral vein puncture was performed twice under local infiltration anesthesia with 10 ml/L lidocaine. Following the placement of the first long guidewire, a Swartz L1 long sheath had been positioned along long guidewire. Then a BRK needle had been positioned within accurate atrium over long sheath to collect blood. Afterwards, the interatrial septum was successfully punctured using the BRK needle, and the puncture needle and sheath core were directly pulled out. A Swartz R0 long sheath (St. Jude Medical, Inc.) was thereafter placed along another long guidewire. The BRK needle placed through the sheath was used to puncture the interatrial septum. Subsequently, the puncture needle and sheath core were withdrawn after successful puncture, and blood was collected from the left atrium.

Double Antibody ELISA

Collected Blood sample was centrifuged, and then the superior coating fluid had been composed, subpackage, stockpiled in a refrigerator at -80°C. Double antibody ELISA was performed to detect the points MMP-9, TIMP-1, TGF-β1 and bFGF using corresponding kits (Roche, Switzerland).

Statistical Analysis

SPSS23.0 software has been employed to measure data in statistics way. Quantitative stuff had been represented mean ± standard deviation, intergroup comparisons were conducted by the independent t test. Numerical stuff had been articulated percentage; intergroup assessments had been carried out with the χ^2 test. Correlations were studied by Spearman's analysis. $P<0.05$ had been taken sufficient in statistics way.

RESULTS

Univariate Analysis Results

No noteworthy modifications were detected within AF and non-AF classes regarding terms concerning gender, heart rate, hypertension, triglyceride, fasting blood glucose and Left Ventricular end Diastolic Dimension (LVEDD). Age and

left atrial diameter (LAD) had been pointedly higher (age: (54.2 ± 7.9) years vs. (45.8 ± 8.7) years, LAD: (36 ± 7) mm vs. (31 ± 4) mm, P<0.01), while left ventricular ejection fraction (LVEF) remained less within AF class than those within non-AF class ((61 ± 8)% vs. (68 ± 7)%, P<0.01).

The left atrial serum MMP-9 phase remained on the top within AF class than that in non-AF class ((3.15 ± 0.47) μg/L vs. (2.38 ± 0.47) μg/L, P<0.01), whereas the two groups had similar right atrial MMP-9 levels. Compared with non-AF group, AF group had significantly decreased TIMP-1 level (right atrium MMP-9: (14.38 ± 2.19) μg/L vs. (12.02 ± 2.31) μg/L, left atrium TIMP-1: (19.45 ± 3.26) μg/L vs. (13.21 ± 4.16) μg/L, P<0.01) and raised TGF-β1 level (left atrium TGFβ-1: (4.38 ± 1.56) μg/L vs. (6.78 ± 1.62) μg/L, right atrium TGFβ-1: (3.76 ± 0.34) μg/L vs. (8.14 ± 0.68) μg/L, P<0.01). Besides, the two groups had similar bFGF levels. The serum MMP-9/TIMP-1 ratio of the left atrium remained notably on the top as compared to right atrium in AF group (0.31 ± 0.07 vs. 0.25 ± 0.06, P<0.01) (Table 1).

Multivariate Logistic Regression Analysis Results

Multivariate logistic regression examination was performed concerning AF being nondependent variable and influences concerning statistical implication within univariate analysis being free variables. Then above variables were incorporated into the regression equation to exclude confounding factors. As a result, the independent related factors for AF in patients without structural heart disease were age (OR (95% CI): 1.109 (1.019-1.204), P<0.01), LVEF (OR (95% CI): 0.849 (0.722-0.954), P<0.01), left atrial MMP-9/TIMP-1 ratio (OR (95%CI): 1.235 (1.028-1.504), P<0.01) (Table 2).

Correlation between MMP-9/TIMP-1 Ratio and LAD

Results of correlation analysis suggested that there was a positive correlation between MMP-9/TIMP-1 ratio, LAD within AF class (r=0.509, P<0.01) (Table 3).

Table 1: Clinical data of AF and non-AF classes

Item	*AF group (n=168)*	*Non-AF group (n=68)*	*P*
Male (%)	120 (71.00)	48 (71.00)	>0.05
Age (year)	54.2 ± 7.9	45.8 ± 8.7	<0.01
Heart rate (beat/min)	78 ± 11	77 ± 10	>0.05
Hypertension	64 (38.10)	16 (23.53)	>0.05
Triacylglycerol (mmol/L)	2.12 ± 0.24	2.09 ± 0.23	>0.05
Fasting blood glucose (mmol/L)	5.79 ± 0.75	5.81 ± 0.80	>0.05
LAD (mm)	36 ± 7	31 ± 4	<0.01
LVEDD (mm)	46 ± 7	47 ± 5	>0.05
LVEF (%)	61 ± 8	68 ± 7	<0.01
Left atrial MMP-9 (μg/L)	3.15 ± 0.47	2.38 ± 0.47	<0.01
Right atrial MMP-9 (μg/L)	2.59 ± 0.59	2.85 ± 0.62	<0.01
Left atrial TIMP-1 (μg/L)	13.21 ± 4.16	19.45 ± 3.26	<0.01
Right atrial TIMP-1 (μg/L)	12.02 ± 2.31	14.38 ± 2.19	<0.01
Left atrial TGFβ-1 (μg/L)	6.78 ± 1.62	4.38 ± 1.56	<0.01
Right atrial TGFβ-1 (ìg/L)	8.14 ± 0.68	3.76 ± 0.34	<0.01
Left atrial bFGF (μg/L)	349.28 ± 21.35	347.56 ± 20.38	>0.05
Right atrial bFGF (μg/L)	367.21 ± 23.27	365.27 ± 24.36	>0.05
Left atrial MMP-9/TIMP-1	0.31 ± 0.07	0.19 ± 0.08	<0.01
Right atrial MMP-9/TIMP-1	0.25 ± 0.06	0.26 ± 0.06	>0.05

Source: Authors

Table 2: Multivariate logistic regression analysis results

Factor	*β*	*Wald* χ^2	*P*	*OR (95% CI)*
Age	0.102	6.387	0.005	1.109 (1.019-1.204)
LEVF	-0.128	7.012	0.009	0.849 (0.722-0.954)
Left atrial MMP-9/TIMP-1	0.211	4.398	0.008	1.235 (1.028-1.504)

Source: Authors

Table 3: Correlation between MMP-9/TIMP-1 ratio and LAD

	LAD (mm)	
MMP-9/TIMP-1	*r*	*P*
	0.509	<0.01

Source: Authors

Serum Levels of MMP-9, TIMP-1, TGF-β1 and bFGF within Diseased Persons Concerning Persistent and Paroxysmal AF

The left atrial MMP-9/TIMP-1 ratio suggestively high within diseased persons concerning insistent AF associated with that within cases relating paroxysmal AF (0.36±0.07 vs. 0.26±0.07, P<0.01). The serum points of MMP-9, TIMP-1, TGF-β1, bFGF had no substantial variances within diseased persons regarding persistent, paroxysmal AF (Table 4).

DISCUSSION

In this study, the development of AF was positively correlated with age, being consistent with the findings of preceding scholarships (Halvorsen et al. 2014; Li et al. 2015). Probably, degeneration occurs in the atrial structure with aging, giving rise to atrial fibrosis and enlargement, that is, the resulting atrial structural remodeling induces AF. In addition, LAD of AF class remained pointedly larger as compared to non-AF class, possibly because AF changed the cellular and subcellular structures of the atria, further altering the anatomical structure and eventually resulting in atrial enlargement. Lenaerts et al. found that rapid electric activity of the atria led to Ca^{2+} overloading in cells, which activated calpains and triggered degradation of contractile proteins including troponin (Tn) T and Tn I, then affecting the contractile function of the atria, increasing the load therein and resulting in passive expansion (Lenaerts et al. 2009). Moreover, LVEF remained pointedly lesser within AF class as compared to non-AF class. In the case of AF, fast and irregular activation disenables the regular, harmonious and effective contraction of the atria, resulting in poor ventricular filling, impaired ventricular systolic function and reduced ejection fraction (Smaardijk et al. 2018).

As the most important proteinase system of collagen degradation, MMPs modulate the dynamic balance between collagen degradation and recombination. TIMPs are endogenous specific inhibitors of MMPs. TIMP-1 can form a reversible complex with MMP-9 to impede the activation of MMPs and their degradation of ECM (Stanciu et al. 2018). MMP-9/TIMP-1 ratio varies within different periods of AF (Liu et al. 2016). During the initial stage of AF, activity of MMPs is markedly elevated, the content of myocardial fibrillar collagen is observably reduced, the level of TIMPs remains unchanged, and myocardial remodeling progresses to cardiac cavity expansion. In the late stage of AF, the activity of MMPs and the content of myocardial fibrillar collagen change following opposite trends, the level of TIMP-1 rises, and myocardial remodeling develops towards collagen accumulation. Herein, in AF group, appearance left atrial MMP-9 signally increased, TIMP-1 level decreased, and MMP-9/TIMP-1 ratio remained preeminent, being pos-

Table 4: Serum levels of MMP-9, TIMP-1, TGF-β1 and bFGF within diseased persons regarding persistent and paroxysmal AF

Item	*Paroxysmal AF (n=96)*	*Persistent AF (n=72)*	*P*
Left atrial MMP-9 (μg/L)	2.98 ± 0.49	3.45 ± 0.42	<0.01
Right atrial MMP-9 (μg /L)	2.60 ± 0.58	2.58 ± 0.61	>0.05
Left atrial TIMP-1 (μg /L)	13.20 ± 4.09	13.22 ± 4.16	>0.05
Right atrial TIMP-1 (μg /L)	11.97 ± 2.31	12.08 ± 2.19	>0.05
Left atrial TGFβ-1 (μg /L)	6.76 ± 1.61	6.79 ± 1.54	>0.05
Right atrial TGFβ-1 (μg /L)	8.14 ± 0.68	3.76 ± 0.34	<0.01
Left atrial bFGF (μg /L)	349.28 ± 21.35	347.56 ± 20.38	>0.05
Right atrial bFGF (μg /L)	367.21 ± 23.27	365.27 ± 24.36	>0.05
Left atrial MMP-9/TIMP-1	0.26 ± 0.07	0.36 ± 0.07	<0.01

Source: Authors

itively associated with LAD. Accordingly, left atrial MMP-9/TIMP-1 ratio was related with development of AF and atrial expansion, and AF in patients without structural heart disease was in the early stage, that is, mainly collagen degradation.

As the center of signal cascade that affects the occurrence of myocardial fibrosis, TGF-β1 can induce and amplify fibrotic signals (Kim et al. 2011; Chen et al. 2013). ECM increases significantly upon AF (Lin et al. 2007), but TGF-β1 hardly increases in the case of heart failure complicated with AF (Behnes et al. 2011). Hence, TGF-β1 participates in the early stage of AF, and specifically acts on atrial cells, inducing and faciliating the accumulation of ECM and atrial fibrosis. Structural remodeling during AF is typified by the interaction between paracrine signaling protein TGF-β1 and fibroblasts. TGF-β1 is capable of enhancing the viability of fibroblasts. Induced by angiotensin II, TGF-β1 is synthesized to promote construction concerning angiotensin II and other pro-fibrotic influences, forming a constructive feedback regulation. These profibrotic signals trigger ECM remodeling, induce local inflammation and fibroblast proliferation, and lead to collagen deposition and impulse conduction hinder (Suthahar et al. 2017). In this study, TGF-β1 level was obviously raised in AF group, implying that TGF-β1 was interrelated concerning development of AF within diseased persons showing no organizational heart ailment. TGF-β1 has been associated with AF and atrial fibrosis in patients with rheumatic heart disease (Xiao et al. 2010). Herein, patients without structural heart disease had smaller LAD and higher TGF-β1 level than those of cases with structural heart disease (Kim et al. 2011), suggesting that the former oness may have atrial fibrosis.

As a growth factor expediting fibroblast division and collagen deposition, bFGF mainly facilitates the proliferation of fibroblasts and the synthesis of ECM collagen by modulating TGF-β1 (Polejaeva et al. 2016). In the present study, AF and non-AF groups had similar bFGF levels, indicating that atrial fibrosis was milder in patients without structural heart disease than that of cases with structural heart disease. The cases enrolled in this study were AF patients who received radiofrequency ablation, so the sample size was small, which may influence the results.

CONCLUSION

In conclusion, left atrial MMP-9/TIMP-1 proportion is associated concerning AF within diseased persons showing no organizational heart ailment, which increases within patients regarding persistent AF compared with that in cases with paroxysmal AF. Therefore, it is a predictive factor for the persistence of AF.

RECOMMENDATIONS

In AF class, notable variation found within left, right atrial MMP-9/TIMP-1 ratio. In addition, based on multivariate analysis, the left atrial MMP-9/TIMP-1 ratio remained free related aspect for AF within diseased persons showing no organizational heart illness. This may be because the level of cytokines in venous blood or left atrial blood represents different meanings in the case of AF due to different serum clearance rates of diverse cytokines. For cytokines with a high serum clearance rate (for example, MMP-9 and TIMP-1), the level in left atrial or arterial blood is more suitable, while for those with a low serum clearance rate (for example, TGF-β1 and bFGF), the content in venous blood is proper. Hence, detecting serum MMP-9/TIMP-1 ratio non-invasively is of great significance for monitoring gradation of atrial fibrosis within AF sufferers, guiding medication and improving experimental prognosis of victims.

LIMITATIONS

Multicenter studies with larger sample sizes are still in need.

ABBREVIATIONS

AF: Atrial fibrillation; bFGF: basic fibroblast growth factor; ECM: extracellular matrix; LAD: left atrial diameter; LVEDD: left ventricular end-diastolic dimension; LVEF: left ventricular ejection fraction; MMP: matrix metalloproteinase; TGF: transforming growth factor; TIMP: tissue inhibitor of metalloproteinase; Tn: troponin.

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