Expression of lncRNA APF in Peripheral Blood of Patients with Acute Myocardial Infarction Caused by Coronary Heart Disease and its Clinical Significance

Haitao Zeng, MD, Fangxing Hu, MD, Yong Duan, MD, Hui Li, MD and Yong Wang, MD

Summary

Coronary heart disease (CHD) is the leading cause of death from cardiovascular disease. This study investigated the expression and clinical significance of long noncoding RNA (lncRNA) autophagy promoting factor (APF) in peripheral blood of patients with acute myocardial infarction (AMI) caused by CHD. Patients with angina pectoris (AP) \((n = 80)\) and AMI \((n = 96)\) and other patients \((n = 60)\) with precordial discomfort but no CHD were included. The serum levels of lncRNA APF, MIAT, MALAT1, H19, CHAST, CDR1AS, miR-188-3p, and cardiac troponin I (cTnI)/creatine kinase (CK)/creatine kinase isozymes (CK-MB) were detected using reverse transcription-quantitative polymerase chain reaction or enzyme-linked immunosorbent assay. Patients with AMI were divided into high/low expression groups based on the median level of APF, and the clinical baseline indicators of patients with AMI were compared. The correlation between lncRNA APF and cTnI/CK/CK-MB/miR-188-3p was analyzed using Pearson analysis, and the clinical value of lncRNA APF was evaluated using the receiver operating characteristic curve. The levels of lncRNA APF, MIAT, MALAT1, H19, CHAST, and CDR1AS in patients with AMI were increased, whereas there were no differences in patients with AP. The APF levels in patients with AMI were higher than MIAT, MALAT1, and CHAST, whereas there were no differences between APF and H19 and CDR1AS. In patients with AMI, the high level of lncRNA APF was correlated with the history of smoking/drinking. Moreover, lncRNA APF was positively correlated with cTnI/CK/CK-MB levels and negatively correlated with miR-188-3p. LncRNA APF has high diagnostic efficacy for AMI. Overall, lncRNA APF is highly expressed in patients with AMI caused by CHD and has high diagnostic efficacy for AMI.

Key words: Long noncoding RNA, Receiver operating characteristic curve, Correlation, Diagnostic efficacy, Cardiac troponin I, Creatine kinase, Creatine kinase isozymes

Coronary heart disease (CHD), also known as atherosclerotic heart disease, is a multi-factorial disease. It is characterized by coronary artery endothelial injury, which leads to coronary artery remodeling and stenosis in the form of chronic inflammation. Stroke diseases such as acute myocardial infarction (AMI) caused by CHD are currently the leading cause of death and disability in the global population. CHD is caused by genetic and environmental factors and their interactions, and a large number of studies have confirmed that diabetes mellitus, hypertension, dyslipidemia, and obesity are routine risk factors for CHD, but these external factors can only partially explain the cause of CHD. In addition to environmental and genetic factors, there is growing evidence that epigenetic changes may play a key role in the development of CHD. Therefore, it has become a new challenge to further understand the pathogenesis of CHD and to search for markers for early diagnosis of CHD.

Long noncoding RNAs (IncRNAs) represent a class of transcripts over 200 nucleotides in length that are important components of the human genome and are associated with cardiovascular development, aging, and diseases. IncRNAs have been identified in cardiomyocytes and are essential for heart development. Many of these IncRNAs play a role in pluripotency regulation and activation of heart-specific genes in particular and have been shown to be associated with CHD risk and AMI. It has been reported that IncRNAs are present in the blood and may serve as diagnostic markers for cardiovascular diseases. However, no specific IncRNA has been found that can be used to effectively identify early CHD. Auto-
phagocytosis and cardiovascular remodeling. However, there are few novel therapeutic strategies and diagnostic tools for myocardial infarction. Therefore, the purpose of this study was to investigate the expression and clinical significance of lncRNA APF in peripheral blood of patients with CHD.

Methods

Ethics statement: This study was approved by the Ethics Committee of Changsha Third Hospital, and all sample collection and data investigation were signed and approved by the patients and their families, which was in accordance with the Helsinki Declaration of the World Medical Association.

Study subjects: A total of 176 patients aged from 40 to 75 years who underwent coronary angiography or coronary computed tomography (CT) in Changsha Third Hospital from February 2019 to February 2020 were selected according to the exclusion criteria, which were defined as the CHD group, and the CHD group was divided into two subgroups: the angina pectoris (AP) group (n = 80) and the AMI group (n = 96). Patients (n = 60) who were admitted to the hospital due to precordial discomfort (PD) but without coronary artery disease confirmed by coronary CT angiography were selected as the control group. The subjects were hospitalized within 12 hours of onset, and blood collection was completed. They did not receive any relevant treatment before hospitalization, and their clinical data were complete.

Inclusion and exclusion criteria: Inclusion criteria were as follows: patients who met the following diagnostic criteria for coronary angiography were diagnosed with CHD. The left main artery, left anterior descending artery, left circumventing artery, and right coronary artery were the main vessels; the standard Judkins method was adopted; each vessel had at least three multi-position projections; and stenosis of one or more vessels > 50%. According to the diagnostic guidelines, patients with CHD were divided into the AMI group and the AP group. Inclusion criteria for AMI were as follows: according to the 2010 Guidelines for the Diagnosis and Treatment of Acute ST-Segment Elevation Myocardial Infarction and the 2012 Guidelines for the Diagnosis and Treatment of Non-ST-Segment Elevation Acute Coronary Syndrome, the patients with typical prolonged chest pain accompanied by continuous changes in the standard 12-lead electrocardiogram (ECG) or creatine kinase (CK) and creatine kinase isozymes (CK-MB) greater than 2 times the upper limit of normal values were included. Inclusion criteria for the AP group were as follows: the patients with resting AP in the last 24 hours, accompanied by transient obvious ischemic ST-T changes in ECG, but CK content was less than 2 times the upper limit of normal value, and the patients with ST-elevation or new Q wave during the observation period were not included; or the patients had typical labor AP with worsening or resting angina attacks within the last 6 months.

Exclusion criteria were as follows: patients complicated with liver, kidney, and other important organ dysfunction; patients complicated with malignant tumors, other chronic diseases, neuromotor system diseases, or cardiogenic shock; patients complicated with valvular heart disease, severe arrhythmia, or blood infectious diseases; and patients with CHD with severe complications during hospitalization.

Sample collection: We recorded the following data of enrolled subjects at admission as follows: gender, age, family history, smoking history, drinking history, diabetes mellitus, systolic blood pressure (SBP), diastolic blood pressure (DBP), total cholesterol, and body mass index (BMI). Within 12 hours of onset, 8 mL of venous blood was collected from the CHD group and the control group in an ethylenediaminetetraacetic acid-K2 anticoagulant vacuum tube (GMS13017.2; Genmed Sciences Inc., Shanghai, China). High-speed centrifugal stratification (2000 g, 10 minutes) was completed within 3 hours after blood collection, and the separated serum was stored in a refrigerator at −80°C.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR): The total RNA was extracted from serum by TRIzol (YT2188; Yita Biotechnology Co. Ltd., Beijing, China), and the concentration and purity of total RNA were determined by ultraviolet spectrophotometer. RT was performed using the AMV reverse transcription kit (RP1200-100; Solarbio, Beijing, China) according to the provided manufacturer’s instructions. The RT-qPCR was performed using SYBR Green PCR Master Mix

Table 1. Primer Sequence

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward (5’→3’)</th>
<th>Reverse (5’→3’)</th>
</tr>
</thead>
<tbody>
<tr>
<td>APF</td>
<td>CTCCTCATATGTTGTTCAAG</td>
<td>GTGTCATAAAGTGAACCTGAA</td>
</tr>
<tr>
<td>MIAT</td>
<td>TAGCTCGAGGCTTTTCACTTGCAGCG</td>
<td>TCAAGAAATGCGGACCCAGAT-AGGCCACCTTGT</td>
</tr>
<tr>
<td>MALAT1</td>
<td>GTGGCACAATGTTCGTTTTG</td>
<td>AGGGAAATGGCCCATGTTG</td>
</tr>
<tr>
<td>H19</td>
<td>TCTCGAGGCGGGCAATTAC</td>
<td>GCTGTCCTTAGGGAGATC</td>
</tr>
<tr>
<td>CHAST</td>
<td>TGGGTGAGGGAAGGAAAAG</td>
<td>ATGGCAATGACCTGTTC</td>
</tr>
<tr>
<td>CDR1AS</td>
<td>TCTGCTGCTTCCCAACATC</td>
<td>AGATCGACACACTGGAACG</td>
</tr>
<tr>
<td>GAPDH</td>
<td>TGTTGCGTCGTTGGATCTGA</td>
<td>CCTGCTTCCACCATCTG</td>
</tr>
<tr>
<td>miR-188-3p</td>
<td>ATTATGGGCTCCCCACATGCAAGG</td>
<td>ATCCAGTGCAAGGTGGAG</td>
</tr>
<tr>
<td>U6</td>
<td>GCTCCCGAGCACATATACTAA</td>
<td>AACGCTTCAGAATTTGGT</td>
</tr>
</tbody>
</table>


**Results**

**Clinical baseline characteristics of the subjects:** A total of 236 subjects were enrolled in this study, including 80 patients with AP, 96 patients with AMI, and 60 control cases with PD but no coronary artery lesions in the same period. The clinical baseline characteristics of the AP group, the AMI group, and the control group were compared and analyzed. The results showed that there were no significant differences in gender, age, smoking history, diabetes mellitus and CHD, patients with AMI were divided into three groups: AP, AMI, and control group. The expression of lncRNA APF, MIAT, MALAT1, H19, CHAST, and CDR1AS in the serum was quantitatively analyzed by the 2−ΔΔCT method.

**Table II. Comparison of Clinical Baseline Characteristics**

<table>
<thead>
<tr>
<th></th>
<th>AP group</th>
<th>AMI group</th>
<th>Control group</th>
<th>P value*</th>
<th>P value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cases</td>
<td>80</td>
<td>96</td>
<td>60</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gender (male/female)</td>
<td>44/36</td>
<td>59/37</td>
<td>34/26</td>
<td>0.8442</td>
<td>0.5529</td>
</tr>
<tr>
<td>Age (years)</td>
<td>59.12 ± 7.04</td>
<td>60.46 ± 7.62</td>
<td>58.73 ± 6.29</td>
<td>0.7349</td>
<td>0.143</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.14 ± 2.23</td>
<td>25.13 ± 2.16</td>
<td>24.73 ± 1.68</td>
<td>0.2351</td>
<td>0.2238</td>
</tr>
<tr>
<td>Family history (cases, %)</td>
<td>42 (52.5%)</td>
<td>51 (53.13%)</td>
<td>28 (46.67%)</td>
<td>0.4945</td>
<td>0.5108</td>
</tr>
<tr>
<td>Smoking history (cases, %)</td>
<td>21 (26.25%)</td>
<td>72 (75%)</td>
<td>18 (30%)</td>
<td>0.6243</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>History of drinking (cases, %)</td>
<td>46 (57.5%)</td>
<td>82 (85.42%)</td>
<td>8 (13.33%)</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Diabetes mellitus (cases, %)</td>
<td>32 (40%)</td>
<td>65 (67.71%)</td>
<td>21 (35%)</td>
<td>0.5461</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>128.86 ± 9.39</td>
<td>152.49 ± 9.54</td>
<td>115.92 ± 8.23</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>89.38 ± 7.06</td>
<td>105.67 ± 7.65</td>
<td>76.84 ± 6.35</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>5.48 ± 1.20</td>
<td>6.05 ± 1.24</td>
<td>4.39 ± 1.16</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>

BMI indicates body mass index. Measurement data were expressed as means ± standard deviation, and unpaired t test was used for comparisons between groups. *P value represented the comparison between the AP group and the control group. †P value represented the comparison between the AMI group and the control group.

**Enzyme-linked immunosorbent assay (ELISA):** Serum levels of cardiac troponin I (cTnI), CK, and CK-MB were detected by ELISA. The specific procedures were strictly in accordance with the instructions of cTnI kits (SEKH-0400; Solarbio), CK kit (CK-E11022; Sino Best Biological Technology Co. Ltd., Shanghai, China), and CK-MB kit (E02861; Wksubio, Shanghai, China).

**Statistical analysis:** Data were analyzed and mapped using GraphPad Prism 8.0.1 Software (GraphPad Software Inc., San Diego, CA, USA). Measurement data were presented as mean ± standard deviation, and one-way analysis of variance (ANOVA) was used for analysis among groups, Tukey’s multiple comparison test was used for the post hoc test, and unpaired t test was used for comparisons between two groups. Enumeration data was expressed as number of cases and percentage. Chi-square test was used for comparison between groups. Pearson linear correlation analysis was used for correlation analysis. The receiver operating characteristic (ROC) curve was used to evaluate the diagnostic efficiency of the parameters and obtain the cut-off point values. The area under the curve (AUC) of lncRNA APF was obtained to evaluate its predictive value for CHD. The P value was obtained from a bilateral test, and a value of P < 0.05 indicated that the difference is statistically significant.
Figure 1. Comparison of lncRNA APF levels in peripheral blood of patients with CHD. A–F: The expression of lncRNA APF, MIAT, MALAT1, H19, CHAST, and CDR1AS in peripheral blood of patients with CHD and control group was detected by RT-qPCR. G: Comparison of APF and MIAT, MALAT1, H19, CHAST, and CDR1AS levels in peripheral blood of patients with AMI. One-way ANOVA was used for comparisons between groups, and Tukey’s multiple comparisons test was used for the post hoc test. **P < 0.01.

Table III. Correlation between High and Low Levels of lncRNA APF in Peripheral Blood of Patients with AMI and Clinical Baseline Indicators

<table>
<thead>
<tr>
<th>Factors</th>
<th>AMI (n = 96)</th>
<th>LncRNA APF High (n = 48)</th>
<th>LncRNA APF Low (n = 48)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smoking history (cases, %)</td>
<td>72 (75.00)</td>
<td>46 (95.83)</td>
<td>26 (54.17)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>History of drinking (cases, %)</td>
<td>82 (85.42)</td>
<td>45 (93.75)</td>
<td>37 (77.08)</td>
<td>0.0207</td>
</tr>
<tr>
<td>Diabetes mellitus (cases, %)</td>
<td>65 (67.71)</td>
<td>36 (75.00)</td>
<td>29 (60.42)</td>
<td>0.1265</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>152.49 ± 9.54</td>
<td>153.45 ± 10.12</td>
<td>151.25 ± 8.70</td>
<td>0.2645</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>105.67 ± 7.65</td>
<td>106.16 ± 7.99</td>
<td>104.95 ± 7.17</td>
<td>0.4496</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>6.05 ± 1.24</td>
<td>6.21 ± 1.24</td>
<td>5.67 ± 1.19</td>
<td>0.0527</td>
</tr>
</tbody>
</table>

into the high expression group and the low expression group according to the median value (2.61) of lncRNA APF level. Then, the correlation between AMI and clinical baseline indicators was analyzed. The results showed that the high expression group had more people with smoking history and drinking history (all $P < 0.01$), but had no correlation with diabetes mellitus, SBP, DBP, and total cholesterol ($P > 0.05$) (Table III).

Correlation analysis of lncRNA APF expression with cTnI, CK, CK-MB, and miR-188-3p: At present, cTnI is a serum marker widely used in the clinical diagnosis of AMI, but it is susceptible to the effects of cardiac failure, atrial fibrillation, and other serious and chronic heart diseases. cTnI, CK, and CK-MB are biomarkers that can reflect the degree of myocardial injury. In addition, lncRNA APF has been reported to promote autophagy and myocardial infarction by targeting miR-188-3p. The levels of cTnI, CK, and CK-MB in peripheral blood of the AMI group and the control group were detected by ELISA, and the levels of miR-188-3p in peripheral blood of each group were detected by RT-qPCR. The results showed that compared with the control group, the levels of cTnI (5.94 ± 1.34), CK (126.83 ± 34.92), and CK-MB (115.42 ± 20.84) in peripheral blood of the AMI group were significantly increased, whereas the levels of miR-188-3p (0.50 ± 0.34) were decreased (Figure 2A-D, all $P < 0.01$). Further, Pearson analysis showed that the lncRNA APF in peripheral blood of patients with AMI was positively correlated with cTnI, CK, and CK-MB levels and negatively correlated with miR-188-3p levels (Figure 2E-H, all $P < 0.0001$, $r$ = 0.8827, 0.8861, 0.8744, −0.8033).

High expression of lncRNA APF in peripheral blood can assist in the diagnosis of AMI: In the above studies, we have confirmed that lncRNA APF was significantly overexpressed in peripheral blood of patients with AMI, and that it was positively correlated with cTnI, CK, and CK-MB levels. Based on these two points, we further studied the diagnostic value of lncRNA APF in AMI using the ROC curve. The results showed that the AUC of lncRNA APF in the diagnosis of AMI was 0.9793 (95% CL: 0.963-0.995), the optimal cut-off value was 1.875, the sensitivity was 83.33%, and the specificity was 100% (Figure 3). These results were indicative that lncRNA APF
Figure 2. Correlation analysis of lncRNA APF expression with cTnI, CK, and CK-MB. The levels of myocardial injury markers in peripheral blood of the AMI group and the control group were detected by ELISA. A: The level of cTnI. B: The level of CK. C: The level of CK-MB. D: The level of miR-188-3p was detected by RT-qPCR. The correlation between lncRNA APF and myocardial injury markers in peripheral blood of patients with AMI was analyzed by Pearson analysis. E: Correlation between lncRNA APF and cTnI. F: Correlation between lncRNA APF and CK. G: Correlation between lncRNA APF and CK-MB. H: Correlation between lncRNA APF and miR-188-3p. Unpaired t test was used to compare the two groups, \( \text{**} P < 0.01 \), compared with the control group.

Figure 3. ROC curve analysis of lncRNA APF in the diagnosis of AMI. The diagnostic value of lncRNA APF level in peripheral blood in patients with AMI was analyzed using the ROC curve. Level > 1.875 can assist in the diagnosis of AMI.

Discussion

AMI is a common cardiac emergency worldwide and is characterized by myocardial necrosis, but the genetic mechanism of AMI remains largely unknown.\(^{23}\) As a lncRNA, autophagy promoter is involved in cardiomyocyte loss and extracellular matrix remodeling.\(^{13}\) Meanwhile, as a new autophagy regulator, APF can induce autophagy death to increase the size of myocardial infarction.\(^{14}\) Our study found that lncRNA APF was highly expressed in patients with AMI and had a high diagnostic efficacy for AMI.

A total of 236 subjects were included in this study, including patients with AP (\( n = 80 \)), patients with AMI (\( n = 96 \)), and control patients (\( n = 60 \)). Studies have shown that age, gender, smoking, drinking, diabetes, hypertension, dyslipidemia, and BMI are common risk factors associated with AMI.\(^{24,25}\) First, we analyzed the clinical baseline characteristics of enrolled patients and found that the proportion of patients with AP with drinking history was higher than that of patients with PD, and that SBP, DBP, and total cholesterol were increased; in patients with AMI, SBP, DBP, and total cholesterol were increased, and the proportion of smoking/drinking history and diabetes mellitus was higher than that of patients with PD. These preliminarily showed that SBP, DBP, elevated blood lipid total cholesterol, smoking and drinking history, and diabetes are risk factors for AMI.

RNA can be used as a new biomarker of AMI, including miRNAs and lncRNAs.\(^{26}\) LncRNA is a set of RNA transcripts containing more than 200 nucleotides,
which cannot be translated into proteins, but are involved in regulating many life activities, including gene transcription, epigenetic inheritance, and protein expression.\(^{27,28}\) Additionally, lncRNAs are involved in the regulation of heart development and the pathogenesis of heart failure and cardiovascular aging.\(^{29,30}\) LncRNAs such as MIAT, MALAT1, H19, CHAST, and CDRIAS have been reported to be elevated in patients with AMI, which can be considered as biomarkers of AMI.\(^{31-33}\) By regulating miR-188-3p, lncRNA APF affects ATG7\(^{\#}\) level, autophagy death, and myocardial infarction.\(^{43}\) Moreover, lncRNA APF is involved in post-ischemia myocardial remodeling.\(^{44}\) Hence, we hypothesize that it may be a potential target and diagnostic tool for new therapeutic strategies for myocardial infarction and heart failure, but its clinical significance in AMI has not been reported. To explore the level of lncRNA APF in patients with AMI, we detected the expression of lncRNA APF in the peripheral blood of the subjects. We found that there was no significant change in the level of lncRNA APF in the peripheral blood of patients with AP and PD, whereas the level of lncRNA APF in the peripheral blood of patients with AMI was increased. The expression of lncRNA APF is upregulated in myocardial infarction cells.\(^{45}\) In summary, these indicated that lncRNA APF is highly expressed in the peripheral blood of patients with AMI.

To further study the correlation between lncRNA APF and CHD, patients with AMI were divided into the high expression group and low expression group, and the correlation analysis of clinical baseline indicators was conducted. The results showed that the patients with high expression of lncRNA APF were more likely to have a history of smoking and drinking. The two risk factors of smoking and drinking were reported to be positively correlated with AMI.\(^{33}\) As a whole, this indicated that the level of lncRNA APF is correlated with the history of smoking and drinking in patients with AMI. The higher level of lncRNA APF suggested a greater proportion of people with a smoking and drinking history.

Previous studies have shown that cTnI, CK, and CK-MB are markers that can reflect the degree of myocardial injury.\(^{2,12}\) By detecting the levels of cTnI, CK, and CK-MB in the peripheral blood of patients with AMI, we found that the levels of cTnI, CK, and CK-MB in the peripheral blood of patients with AMI were increased. Cardiac injury markers CK, CK-MB, and cTnI were highly expressed in patients with AMI.\(^{2}\) Additionally, our results showed that lncRNA APF was positively correlated with cTnI, CK, and CK-MB levels in peripheral blood of patients with AMI. Briefly, high expression of lncRNA APF may also reflect myocardial injury.

Furthermore, we studied the diagnostic value of lncRNA APF in AMI. We plotted the ROC curve and found that the AUC of lncRNA APF in the diagnosis of AMI was 0.9793, the optimal cut-off value was 1.875, the sensitivity was 83.33%, and the specificity was 100%. Some lncRNAs are associated with AMI and can be used as blood biomarkers for AMI detection.\(^{43}\) To the best of our knowledge, all these results first suggested that lncRNA APF level could assist in the diagnosis of AMI.

In conclusion, this study mainly found that lncRNA APF expression has high diagnostic efficiency for AMI, which provides a certain reference for the clinical diagnosis of AMI. However, we only analyzed the relationship between lncRNA APF and clinical indicators of AMI in a relatively small study population. In the future, a larger sample size will be included to conduct ROC curve analysis on the diagnostic efficacy of lncRNA APF for AMI. Moreover, we did not analyze the combined diagnostic value of lncRNA APF with other possible factors and lncRNAs, so more research directions need to be explored. Therefore, in the future, we need to further study the molecular mechanism of the high expression of lncRNA APF in AMI and the combined diagnostic value of lncRNA APF with other possible factors and lncRNAs.

Disclosure

Conflicts of interest: All authors declare that there is no conflict of interests in this study.

Ethics approval: This study was approved by the Ethics Committee of Changhai Third Hospital, and all sample collection and data investigation were signed and approved by the patients and their families, which was in accordance with the Helsinki Declaration of the World Medical Association.

Availability of data and materials: All the data generated or analyzed during this study are included in this published article.

Authors’ contributions: HTZ contributed to the study concepts, study design, and definition of intellectual content; FXH and HTZ contributed to the literature research; YD contributed to the manuscript preparation; HL contributed to the manuscript editing and review; HL and YW contributed to the experimental studies and data acquisition. All authors read and approved the final manuscript.

References