TNF Receptor 1/2 Predict Heart Failure Risk in Type 2 Diabetes Mellitus Patients

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SUMMARY

Inflammation plays an important role in heart failure and diabetes mellitus. Traditional serum markers have limited predictive value in heart failure and diabetes. TNFR1 and TNFR2 (TNFR1/2) have been proven to be strongly associated with heart failure and diabetes complications. This study aimed to assess the association of sTNFR1 and sTNFR2 levels and incidental HF risk in diabetes patients.

We detected the mRNA, protein, and serum expression of TNFR1/2, their downstream signaling pathway protein NF-kB, and JNK expression and some traditional serum inflammatory markers in a heart failure group without diabetes mellitus or abnormal glucose tolerance (n = 84), a diabetes mellitus group without heart failure (n = 86), and a heart failure with diabetes mellitus group (n = 86).

TNFR1/2 were significantly higher in patients with heart failure and diabetes mellitus based on mRNA expression to protein expression and serum expression. However, there were no differences in mRNA, protein, and serum levels of TNFR1/2 between the HF group and DM group. Furthermore, there were no differences between the groups in some traditional serum inflammatory markers.

This study demonstrated higher expressions of TNFR, NF-kB, and JNK in patients with heart failure and diabetes mellitus. Compared with traditional serum markers, TNFR1 and TNFR2 are associated with heart failure risk in type 2 diabetes mellitus patients. (Int Heart J 2017; 58: 245-249)

Key words: TNFR, Inflammation, Arrhythmia, Metabolism

As a final outcome of most heart and vascular diseases, heart failure (HF) is a global problem with a high incidence and a low 5-year survival rate. 1 Heart failure is caused by damage or overloading of the myocardium and heart muscle due to ischemia, hypertension, atrial fibrillation, and cardiomyopathy. 2-4 In a previous study, inflammation was shown to play a key role in the pathogenesis of HF and a have a strong association with risk of heart failure by direct effects on myocardial function. 5-8 Tumor necrosis factor (TNF) in particular is well known to have harmful effects on cardiomyocyte contractility in HF by inducing reactive oxygen species formation, cardiomyocyte hypertrophy, and apoptosis. 9,10

Diabetes mellitus (DM), is an independent risk factor of coronary artery disease in terms of serious long-term complications. DM has also been shown to be affected by multi-inflammatory factors such as toll like receptors, interleukin, TNF, and other cytokine factors. 11-13 TNF-α is associated with the progression of diabetes via pancreatic β-cell destruction. Further study in a TNF related apoptosis-inducing ligand (TRAIL) gene deletion model found a significantly higher risk of diabetes incidence. 14,15

Tumor necrosis factor receptor (TNFR) is a trimeric cytokine receptor that binds tumor necrosis factors. 16 Studies have shown that TNFR1/2 act as immune mediators and regulate the inflammatory cascade. TNFR1 is the receptor that mediates cell injury apoptosis and death through nuclear factor kB, however, TNFR2 may cause cell migration, proliferation, and restoration to fight the harmful effects of TNFR1. 17,18

An independent association between TNFR1/2 and the risk of HF in diabetes patients has not yet been rigorously established. The aim of this study was to assess the association between sTNFR1 and sTNFR2 levels and incidental HF risk in diabetes patients.

METHODS

Population characteristics: All volunteers provided informed consent and were recruited from the First Affiliated Hospital of Medical College of Xi’an Jiaotong University and The Second...
Affiliated Hospital of Wenzhou Medical University. The study was approved by the institutional ethics committee of the Medical College of Xi’an Jiaotong University.

The subjects were divided into 3 groups: 1) HF group without diabetes mellitus or abnormal glucose tolerance (n = 84); 2) DM group without HF (n = 86); and 3) HF with DM (n = 86). Unless clinically contraindicated, HF patients had non-ischemic HF for at least 5 years, while DM patients had DM for at least 15 years and were on insulin therapy.

A diagnosis of heart failure was made based on a left ventricular ejection fraction (< 50%) and 1 or more of the following criteria: cardiomegaly, hepatojugular reflex, neck vein distention, paroxysmal nocturnal dyspnea or orthopnea, pulmonary rales, and third heart sound (S3 gallop rhythm). Diabetic ketoacidosis, acute virus/bacterial infection disease, acute heart failure, diabetic ketoacidosis, acute virus/bacterial infection disease, coronary artery disease, ischemia heart disease, stroke, advanced liver disease, neoplastic disease, autoimmune disease, or other inflammatory disease.

TNFR1/2 mRNA expression: Target mRNA was isolated from peripheral blood mononuclear cells (PBMC) in blood samples using Trizol (Invitrogen Corp., Carlsbad, CA). cDNA templates were obtained by reverse transcription using a First Strand cDNA Synthesis Kit (Fermentas, MBLCA). Human TNFR1, TNFR2, and β-actin mRNA levels were quantified by 2-step real time RT-PCR. The 2–ΔΔCt method was used to calculate the data.

For TNFR1, the sequences of the forward and reverse primers were 5’–TCT CAG TGG CAA GAC ATG TCG-3’ and 5’–TTG TGC AGA TTA GGA CCG-3’, respectively, and for TNFR2 they were 5’–GGG GTA CCA TGG CCG CCG TGCC CCG TCT GGT-3’ and 5’–GAA GCT TGT GCG CAC TGC GCC CAG TGC TCC CTG CAG CTG-3’, respectively.

Immunoblotting: Total protein extraction was extracted from peripheral blood and separated by 10% SDS-PAGE. After protein blotting, the polyvinylidene fluoride (PVDF) membranes were blocked in 5% skim milk. The PVDF membranes were immersed overnight at 4°C in anti-NF-κB (ab16502, Abcam, USA) diluted 1:1000, anti-JNK (c-Jun N-terminal kinase) (ab179461, Abcam) diluted 1:1000, anti-TNFR1 (Anti-TNFRSF1A, ab111119, Abcam) diluted 1:1000, anti-TNFR2 (ab15563, Abcam) diluted 1:1000 with bovine serum albumin. After activation with secondary antibody, immunoblots were immersed in enhanced chemiluminescence reagent. The loading amount was modified by β-actin protein.

Serum biomarker measurements: Serum was separated from blood samples and frozen at -70°C. TNF-α (HSTA50 kit, R&D Systems), sTNFR1 (DRT100 kit, R&D Systems), sTNFR2 (DRT200 kit, R&D Systems), MCP-1, IL-10, and IL-8 (Quantikine ELISA Kit, R&D Systems) were detected according to the respective protocols.

### Table. Subject Characteristics

<table>
<thead>
<tr>
<th>Age (yr)</th>
<th>Gender (M/F)</th>
<th>Smoking (%)</th>
<th>Drinking (%)</th>
<th>Hypertension (%)</th>
<th>WHR</th>
<th>BMI (kg/m²)</th>
<th>FBG (mmol/L)</th>
<th>TC (mmol/L)</th>
<th>TG (mmol/L)</th>
<th>HDL (mmol/L)</th>
<th>LDL (mmol/L)</th>
<th>Scr (μmol/L)</th>
<th>BUN (mmol/L)</th>
<th>cTnI (ng/mL)</th>
<th>HbA1c (%)</th>
<th>hs-CRP (mg/L)</th>
<th>CK-MB (IU/L)</th>
<th>NT-proBNP (pg/mL)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>65 ± 3</td>
<td>42/42</td>
<td>56%</td>
<td>27%</td>
<td>34%</td>
<td>1.13 ± 0.052</td>
<td>28.56 ± 4.23</td>
<td>4.13 ± 1.75</td>
<td>3.98 ± 1.18</td>
<td>1.66 ± 0.43</td>
<td>1.12 ± 0.29</td>
<td>1.97 ± 0.33</td>
<td>64.52 ± 11.23</td>
<td>5.17 ± 1.32</td>
<td>0.22 (0.10, 0.31)</td>
<td>6.2 ± 0.12</td>
<td>1.79 (1.46, 2.35)</td>
<td>11.25 ± 1.34</td>
<td>512.36 ± 17.44</td>
<td>0.826</td>
</tr>
<tr>
<td>65 ± 3</td>
<td>43/43</td>
<td>57%</td>
<td>25%</td>
<td>37%</td>
<td>1.17 ± 0.068</td>
<td>28.74 ± 3.82</td>
<td>4.22 ± 1.61</td>
<td>4.07 ± 1.09</td>
<td>1.45 ± 0.38</td>
<td>1.23 ± 0.17</td>
<td>1.88 ± 0.27</td>
<td>72.15 ± 14.76</td>
<td>5.32 ± 1.47</td>
<td>0.24 (0.10, 0.31)</td>
<td>6.5 ± 0.14</td>
<td>1.81 (1.32, 2.17)</td>
<td>12.37 ± 1.26</td>
<td>197.64 ± 16.83</td>
<td>1</td>
</tr>
<tr>
<td>68 ± 5</td>
<td>43/43</td>
<td>56%</td>
<td>30%</td>
<td>35%</td>
<td>1.18 ± 0.076</td>
<td>29.46 ± 5.13</td>
<td>4.09 ± 1.53</td>
<td>4.12 ± 1.21</td>
<td>1.62 ± 0.51</td>
<td>1.19 ± 0.24</td>
<td>2.01 ± 0.31</td>
<td>68.29 ± 16.61</td>
<td>5.62 ± 1.41</td>
<td>0.31 (0.10, 0.31)</td>
<td>6.4 ± 0.16</td>
<td>1.84 (1.15, 2.03)</td>
<td>11.97 ± 1.61</td>
<td>533.81 ± 20.15</td>
<td>0.954</td>
</tr>
</tbody>
</table>

WHR indicates waist-to-hip ratio; BMI, body mass index; FBG, fasting blood glucose; TC, total cholesterol; TG, triglycerides; HDL, high-density lipoprotein; LDL, low-density lipoprotein; Scr, serum creatinine; BUN, blood urea nitrogen; cTnI, cardiac troponin I; HbA1c, glycated hemoglobin; hs-CRP, high-sensitivity C-reactive protein; CK-MB, creatine kinase isoenzyme MB; and NT-proBNP, N-terminal pro-brain natriuretic peptide. Values are mean ± SD or n (%) or median and interquartile range.
Statistical analysis: SPSS 13.0 software was used for all analyses. Data are presented as the mean (SD) for continuous variables, median and interquartile range for nonparametric values, and percentage for categorical variables. After performing Scheffé’s test, Spearman correlations were used to compare the continuous data among the groups. *P < 0.05 was taken as being a statistically significant difference.

RESULTS

Subject characteristics: This study included 84 heart failure patients without diabetes mellitus or abnormal glucose tolerance, 86 diabetes mellitus patients without heart failure, and 86 heart failure patients with diabetes mellitus. As the data show in the Table, there were no significant differences in age, gender, WHR, BMI, FBG, TC, TG, HDL, LDL, SCr, BUN, cTnI, hs-CRP, or CK-MB. However, NT-proBNP was significantly higher in the HF group and HF+DM group than in the DM group (P = 0.0058).

TNFR1/2 mRNA expression in different groups: Quantified by RT-PCR, TNFR1/2 mRNA expression was measured in the 3 groups as shown in Supplemental Figure 1. In the DM+HF group, TNFR1 (2.87 ± 0.14) and TNFR2 (2.74 ± 0.16) were both significantly higher than in the DM group (TNFR1, 1.26 ± 0.17; TNFR2, 1.31 ± 0.13) (P = 0.0169) or HF group (TNFR1, 1.28 ± 0.16; TNFR2, 1.34 ± 0.18) (P = 0.0193). However, neither TNFR1 mRNA expression (P = 0.782) nor TNFR2 expression (P = 0.643) was significantly different between the DM group and HF group.

TNFR1/2 and signaling pathway protein expression: In Supplemental Figure 2, TNFR1/2, NF-kB, and JNK protein expression was detected by Western blotting using peripheral blood samples. As the data show, TNFR1/2, NF-kB, and JNK protein expression in patients with heart failure and diabetes mellitus was significantly higher than that in the HF group (TNFR1, P = 0.021; TNFR2, P = 0.019; NF-kB, P = 0.008; JNK, P = 0.011) or DM group (TNFR1, P = 0.017; TNFR2, P = 0.022; NF-kB, P = 0.013; JNK, P = 0.015). However, TNFR1/2, NF-kB, and JNK protein expression showed no differences between the HF group and DM group (TNFR1, P = 0.342; TNFR2, P = 0.411; NF-kB, P = 0.278; JNK, P = 0.315).

Serum inflammatory marker expression: To examine the differences in the inflammatory conditions of the 3 groups, the expressions of various serum inflammatory markers are presented in the Figure. There were no differences in TNF-α, MCP-1, IL-8, or IL-10 among the 3 groups (TNF-α, P = 0.563; MCP-1, P = 0.479; IL-8, P = 0.382; IL-10, P = 0.763). Also, there was no difference in TNFR1/2 between the HF group and DM group (TNFR1, P = 0.532; TNFR2, P = 0.477). However, the soluble TNFR1 and 2 levels were significantly higher in patients with heart failure with DM (TNFR1, P = 0.0121; TNFR2, P = 0.0153).
DISCUSSION

In this study, we demonstrated TNFR1/2 was significantly higher in patients with heart failure and diabetes mellitus in terms of mRNA expression to protein expression and serum expression. However, there were no significant differences in the mRNA, protein, and serum levels of TNFR1/2 between the HF group and DM group. Furthermore, there were no differences in various traditional serum inflammatory markers between the groups. We therefore believe that TNFR1/2 is associated with heart failure risk in type 2 diabetes mellitus patients.

A long history of diabetes mellitus can cause a series of complications such as coronary atherosclerotic disease, diabetic kidney disease, and other microvascular diseases. Furthermore, diabetes is associated with coronary artery stenosis and sudden cardiac death in patients hospitalized with heart failure.

As past research has shown, inflammation is a common factor in all stages of diabetes mellitus and heart failure. TNFR1 plays a pro-inflammatory and apoptotic role via activated the NF-kB and JNK signaling pathways. In contrast, TNFR2 has an anti-inflammatory and tissue repair effect by counteracting the effect of TNFR1 on NF-kB and JNK. Although NF-kB and JNK are chronically activated in heart failure and diabetes mellitus, there is still disagreement as to whether they have a protective or detrimental role. However, TNFR1/2 are important bridges for extracellular and intracellular inflammatory responses.

There is still controversy with regards to the cause-effect relationship between TNFR1/2 and HF in DM. TNFR1 is expressed in nearly all cell types (except erythrocytes), while TNFR2 is found primarily in cells of the immune system and myocardial cells. Previous research suggests TNFR1/2 signaling is complex; TNFR1 could aggravate while TNFR2 ameliorates chamber remodeling and hypertrophy due to their different effects on NF-kB, JNK, inflammatory activation, and apoptosis.

In our study, we compared inflammatory expression in different groups. The baseline characteristics of the subjects showed that they had a longer history of DM compared to HF. However, there were no significant differences in TNFR1/2 or NF-kB and JNK signaling pathway protein between the DM group and HF group. Surprisingly, TNFR1/2 expression was much higher in the DM+HF group compared with patients only suffering from heart failure or diabetes mellitus. Both heart failure and DM2 are multi-factorial inflammatory diseases. DM2 constitutes a group of damage effects including inflammation that augment and deteriorate myocardial injury and cardiovascular incident risk. Thus, higher TNFR1/2 expression in the DM+HF group may be due to inflammatory overlap of DM and HF. This difference does not appear in traditional serum inflammatory expression due to their complex signal pathway. Although there were major differences in the medications taken between the groups, there are no published results with regard to the impacts of ACEI, β-blockers, diuretics, and insulin on TNFR1/2.

In summary, this study has demonstrated higher expressions of TNFR, NF-kB, and JNK in patients with heart failure and diabetes mellitus. Compared with traditional serum markers, TNFR1 and TNFR2 are associated with heart failure risk in type 2 diabetes mellitus patients.

DISCLOSURE

Conflict of interest: The authors declare that they have no conflicts of interest.

Statement of human and animal rights: All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (the institutional ethics committee of Shaanxi Provincial People’s Hospital, China) and with the Helsinki Declaration of 1975, as revised in 2008.

REFERENCES


**Supplemental Files**

Supplemental Figures 1, 2
Please see supplemental files; https://www.jstage.jst.co.jp/article/ihj/58/2/58_16-236/_article/supplement