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Diagnostic value of carotid artery ultrasound and hypersensitive C-reactive protein in Type 2 diabetes mellitus patients with acute myocardial infarction in Chinese population

Juan Li, MSc[a], Liangliang Wang, BSc[b], Qian Wang, BSc[b,*], Zhaoqin Xin, BSc[b], Yilin Liu, BSc[a], Qinghua Zhao, BSc[b]

Abstract

Hypersensitive C-reactive protein (hs-CRP) is reported to be significant risk indicators not only for the development of cardiovascular disease, but also for the development or progression of type 2 diabetes. The objective of this study was to analyze the significance of hs-CRP in type 2 diabetes mellitus (T2DM) combined with acute myocardial infarction (AMI).

Fifty patients with both T2DM and AMI, 50 patients with T2DM alone, and 50 healthy subjects (control group) were selected.

Operating characteristic (ROC) analysis revealed that the sensitivity, specificity, accuracy, and critical value in the diagnosis of T2DM combined with AMI using hs-CRP level were 84.6%, 75.9%, 0.856, and 7.34 mg/L, respectively. For using vulnerable plaque rate, these were 92.7%, 95.3%, 0.923, and 0.52, respectively.

hs-CRP play a significant role in the early diagnosis of T2DM combined with AMI.

Abbreviations: AMI = acute myocardial infarction, AS = atherosclerosis, AUC = area under the curve, CAG = coronary arteriography, hs-CRP = hypersensitive C-reactive protein, ROC = operating characteristic, T2DM = type 2 diabetes mellitus.

Keywords: acute myocardial infarction, carotid artery ultrasound, hypersensitive C-reactive protein, multiple logistic regression analysis, type 2 diabetes mellitus

1. Introduction

The incidences of type 2 diabetes mellitus (T2DM), tumor, cardiac, and cerebral vascular diseases in China occupy the top 3 in the non-communicable diseases.[1] Meanwhile, T2DM is cardiovascular "risk equivalents," with acute myocardial infarction (AMI) being the most common among T2DM complications.[2] Atherosclerosis (AS) is the main pathogenesis of AMI as well as the primary target vessel lesion of T2DM.[3] C-reactive protein is a glycosylated polyprotein, which is the main index to reflect the changes of inflammation. Recently interest has been focused on simple biochemical marker for high-sensitivity C-reactive protein (hs-CRP) have been shown to be especially promising. The level and change of hs-CRP, which was more accurate, was a sensitive index demonstrating the level of inflammation in the early stage of human body.[4] hs-CRP is associated with inflammation, which is suspected to play an important role in the pathophysiology of left ventricular (LV) remodeling. The ultrasonic examination carotid arteriosclerosis (CAS) was one of main approaches to diagnose vasculopathy, which could correctly evaluate plaque burden and plasminogen activator (PA) of carotid artery.[5] Although type 2 diabetes is a multifactorial metabolic disease, recent evidence suggests that chronic subclinical inflammation plays an important role in the development of type 2 diabetes.[6,7] hs-CRP, as an elevated circulating level of subclinical inflammatory markers, is reported to be significant risk indicators not only for the development of cardiovascular disease, but also for the development or progression of type 2 diabetes.[8] Thus, the possibility exists that the level of hs-CRP may contribute to the early diagnosis of T2DM in patients combined with AMI. However, to the best of our knowledge, there have been no studies that investigated the effect of level of hs-CRP simultaneously on the early diagnosis in type 2 diabetic patients combined with AMI. This study aimed to analyze the results of serum hs-CRP and carotid artery ultrasound in patients with T2DM combined with AMI, in order to provide reference for early diagnosis and prognosis evaluation of the disease.

2. Materials and methods

2.1. Object data

The study population consisted of patients with T2DM and AMI recruited from the medical outpatient clinic affiliated to our hospital from January 2014 to January 2016. In the present study, 50 patients with simple T2DM and 50 healthy subjects

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[a] Special Inspection Department, [b] Ultrasound Department, Liaocheng People's Hospital, Shandong, China.

[*] Correspondence: Qian Wang, No. 67, Dongchang West Road, Liaocheng City, Shandong Province, 252000, China (e-mail: gzjyche@sina.com)

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(control group) were selected. All subjects were conformed to the diagnostic criteria of T2DM and AMI. Diagnosis of T2DM was performed according to the criteria of American Diabetes Association[9]: patients who have one of the following criteria were considered as T2DM: fasting plasma glucose (FPG) ≥126 mg/dL, blood sugar (BS) 2-hour pp ≥200 mg/dL, and hemoglobin A1c (HbA1c) ≥6.5%. In addition, patients who have one or more of the following symptoms suggestive of AMI were recruited: de novo acute myocardial infarction patients admitted to the hospital between 24 and 48 hours; patients had elective percutaneous coronary intervention (PCI) 1 week after onset of symptoms; patients who did not have previous coronary artery bypass surgery, dilated cardiomyopathy, valvular heart disease, and/or heart transplantation. The criteria for exclusion were other types of heart diseases, such as heart failure, cardiac valve disease, cardiomyopathy, complications with cerebrovascular disease, liver and kidney dysfunction. Baseline data of participants in 3 groups were comparable, except fasting blood-glucose and HbA1C (Table 1). All the procedures were in accordance with the ethical standards of Liaocheng People's Hospital. The study protocol was approved by the ethics committee of Liaocheng People's Hospital and all patients signed a consent form before the follow-ups.

2.2. Study methods

2.2.1. Detection of hs-CRP level with ELISA. Fasting elbow vein blood 3 to 5 mL was collected and stored in heparin anticoagulation tube, which was centrifuged with a $2500 \times g$ for 15 minutes. Thereafter, the supernatant was taken and stored under –20 °C in refrigerator. Specific procedures were as follows: natural thawing under room temperature; dilution and incubation of standard substances: 40 μL diluent was added into 10 μL standard substance and well shook up. Fourty microliter diluent and 10 μL sample were added into the hole of measured sample before sealing, and were incubated under 37 °C. Plate washing: The plate was washed 4 times by Microplate Washer after the removal of microplate sealer, with static time for 60 seconds each time and incubated with enzyme supplementation. Enzyme supplementation: 50 μL enzyme-reagent were added and incubated for 30 minutes under 37 °C. Plate rewashing: The plate was continuously washed 4 times by microplate washer, with static time for 40 seconds each time. Color development and termination: color development reagent A solution (50 μL) and B solution (50 μL) were added in each standard orifice, mixed, kept away from light and incubated for 15 minutes under 20 °C, 50 μL stop buffer was added eventually. Optical density (OD) value was read with microplate reader within 15 minutes and corresponding sample concentration was calculated. Reagents were purchased from Sigma Co., St. Louis, MO.

2.3. Echocardiography

Subjects were in supine posture, fully exposing their neck with padding pillow, tilting their head back, and slightly lifting their mandible toward the contralateral side. With the use of Color Doppler ultrasound detector (high frequency linear array probe, 5–12 MHz frequency), probe was placed in cervical region of participants, and crosswise scan towards cranial region in sequence was performed, with checking points including bifurcation of common carotid artery, internal carotid artery, external carotid artery, and common carotid artery (distal, medial, proximal part). Cross and sagittal sections were used to determine artery stenosis and bilateral symmetry. After selecting measuring point approximately 1.5 cm away from the distal part of the bifurcation of common carotid artery, vertical dimension between inner surface of intima of artery and outer surface of tunicae media, namely intima-media thickness (IMT), was measured. Quantity, form, and internal echo of plaques were observed and lumen area (LA), plaque area (PA), maximum dose (Dmax), minimum dose (Dmin), and ecart inferieur (EI = [Dmax − Dmin]/Dmax) were calculated. Plaques were classified into stable type and vulnerable type in accordance with their echo characteristics. Stable plaque had a smooth surface, with over 50% regions presenting homogenous or strong echo. The echo intensity was stronger than that in adventitia tissue and was accompanied by acoustic shadowing in posterior. Vulnerable plaque had a rough surface, with over 50% regions presenting heterogenous, low echo, and irregular low echo dark region or/and ulcer plaque.

2.4. Statistical analysis

SPSS 19.0 (IBM, International Business Machines Corporation; New York) was applied for data analysis. Quantitative data with normal distribution were presented as mean ± standard deviation, comparison among groups with the use of one-way analysis of variance (ANOVA), analysis, pairwise comparison by means of least-significant difference test (LSD-t). Qualitative data were presented as rate with chi-squared test. Influencing factor analysis was performed using Logistic regression model, by screening with step-back technique (inclusion criteria: $\alpha \leq 0.10$, exclusion criteria: $\alpha < 0.05$). Diagnostic sensitivity and specificity were analyzed with ROC, and accuracy was presented by means of area under the curve (AUC). $P < .05$ meant the difference was statistically significant, all for the two-sided test.

3. Results

3.1. Comparison on the level of hs-CRP among groups

As shown in Fig. 1 below, the level of serum hs-CRP in T2DM combined with AMI group was significantly higher than that in

Table 1

Comparison on patients' baseline data in 3 groups.

Items	Control group (n = 50)	T2DM group (n = 50)	T2DM combined with AMI group (n = 50)	F/χ^2	P
Male/Female	28/22	27/23	26/24	0.161	.923
Age, y	56.6 ± 8.4	55.9 ± 7.6	56.4 ± 7.3	0.185	.867
Smoking (n [%])	18 (36.0)	21 (42.0)	23 (46.0)	1.045	.593
MSBP, mm Hg	133.4 ± 6.4	135.9 ± 6.5	138.5 ± 7.2	0.432	.659
MDBP, mm Hg	83.7 ± 3.8	87.5 ± 4.7	88.6 ± 5.6	0.523	.641
BMI, kg/m^2	22.6 ± 2.7	22.5 ± 2.6	22.6 ± 2.8	0.326	.758
FBG, mmol/L	4.3 ± 1.5	7.2 ± 1.6	7.3 ± 1.5	5.624	.027
HbA1C (%)	5.3 ± 0.7	6.7 ± 0.5	6.8 ± 0.6	5.127	.033

BMI = body mass index, FBG = fasting blood glucose, HbA1C = hemoglobin A1c, MDBP = mean diastolic blood pressure, MSBP = mean systolic blood pressure.

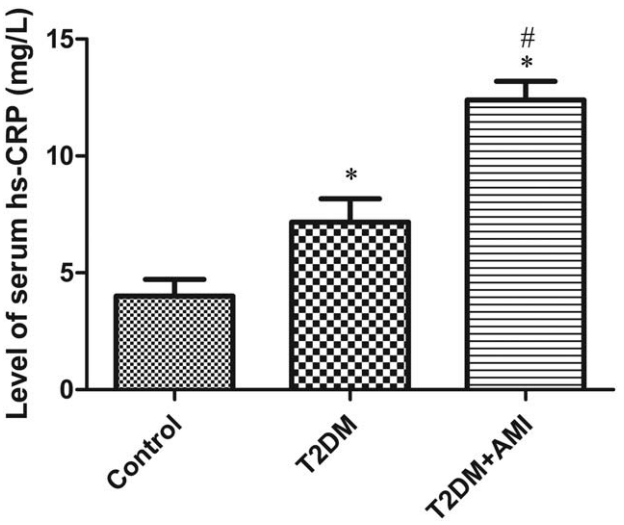

Figure 1. Comparison on the level of hs-CRP among groups. hs-CRP = hypersensitive C-reactive protein.

T2DM group, with control group being the lowest. The difference was statistically significant ($P < .05$).

3.2. Comparison on the carotid artery plaque among groups

The IMT, PA, PA/LA, Dmax, ecart inferieur (EI), and plaque quantity in T2DM combined with AMI group were all higher than those in T2DM group, with control group being the lowest. The vulnerable plaques increased while stable plaques decreased (Table 2).

3.3. Multiple logistic regression analysis in T2DM combined with AMI group

With patients' baseline data (sex, age, smoking, blood pressure, body mass index [BMI], fasting blood-glucose, and HbA1C), the level of serum hs-CRP, and ultrasound detection results (IMT, LA, PA, PA/LA, Dmax, Dmin, EI, plaque quantity, and vulnerable plaque rate) as independent variables, and T2DM combined with AMI as dependent variables, both of them were included in the model, which drew a conclusion that the level of serum hs-CRP, IMT, PA/LA, EI, and vulnerable plaque rate were independent risk factors of the occurrence of T2DM combined with AMI (Table 3).

3.4. ROC analysis on diagnosis of hs-CRP and vulnerable plaque rate

The level of serum hs-CRP and vulnerable plaque rate (diagnosis indexes) and T2DM combined with AMI (diagnosis result) were included in ROC analysis, which came to a conclusion that the sensitivity, specificity, accuracy, and critical value in the diagnosis of T2DM combined with AMI using hs-CRP level were 84.6%, 75.9%, 0.856 ($P < .001$) and 7.34 mg/L, respectively. The sensitivity, specificity, accuracy, and critical value in the diagnosis of T2DM combined with AMI using vulnerable plaque rate were 92.7%, 95.3%, 0.923 ($P < .001$), and 0.52, respectively (Fig. 2).

4. Discussion

Metabolic disorder is caused by elevated blood glucose in patients with T2DM, which leads to abnormal deposition of lipid in the end arterium, hyperplasia of intima smooth muscle cells resulting in thickening and fibrosis of end arterium. Together with platelet aggregation hyperfunction, the thrombogenesis increases, promoting the formation of atherosclerosis, which leads to hypoxia-ischemia and necrosis of myocardial cells.[10] The morbidity of

Table 2

Comparison on the carotid artery plaque among groups.

Items	Control group (n = 50)	T2DM group (n = 50)	T2DM combined with AMI group (n = 50)
IMT, mmol/L	0.72 ± 0.13	1.32 ± 0.38	1.83 ± 0.42
LA, mm^2	5.62 ± 0.73	5.67 ± 0.84	5.58 ± 0.79
PA, mm^2	0.54 ± 0.17	1.28 ± 0.23	1.87 ± 0.35
PA/LA	0.19 ± 0.05	0.24 ± 0.07	0.38 ± 0.09
Dmax, mm	0.15 ± 0.04	0.27 ± 0.06	0.36 ± 0.08
Dmin, mm	0.06 ± 0.02	0.05 ± 0.02	0.04 ± 0.02
EI	0.55 ± 0.12	0.82 ± 0.15	0.89 ± 0.17
Plaque quantity	1.2 ± 0.3	2.1 ± 0.4	2.8 ± 0.6
Vulnerable plaque rate	0.24 ± 0.08	0.46 ± 0.12	0.67 ± 0.18

Dmax = maximum dose, Dmin = minimum dose, EI = (Dmax − Dmin)/Dmax, IMT = intima-media thickness, LA = lumen area, PA = plaque area.

Table 3

Multiple logistic regression analysis in T2DM combined with AMI group.

Factor	β	Wald	*P*	OR	95% CI
hs-CRP	0.125	5.627	.021	1.638	0.865–2.132
IMT	0.147	5.127	.024	1.324	0.639–2.235
PA/LA	0.169	4.698	.028	1.126	0.524–2.538
EI	0.123	4.852	.026	1.215	0.785–3.202
Vulnerable plaque rate	0.215	6.235	.016	2.532	1.659–3.254

CI = confidence interval, Dmax = maximum dose, Dmin = minimum dose, EI = (Dmax − Dmin)/Dmax, IMT = intima-media thickness, LA = lumen area, OR = odds ratio, PA = plaque area.

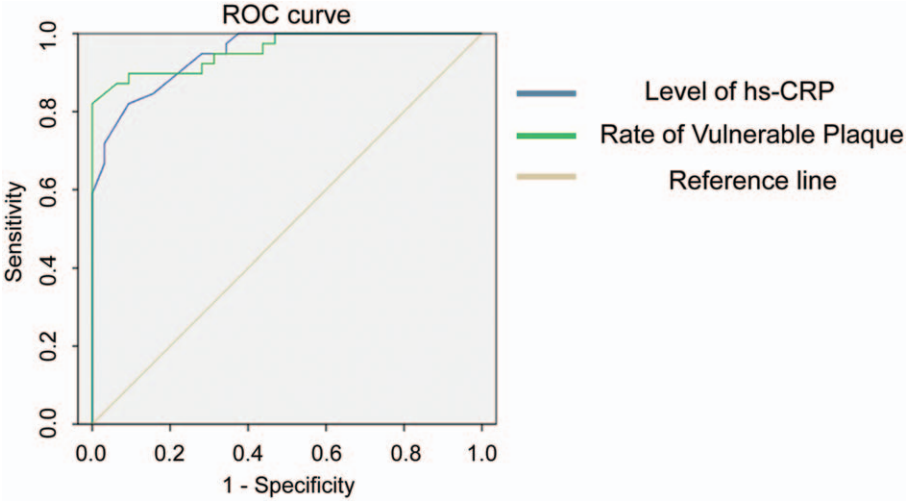

Figure 2. ROC analysis on diagnosis of hs-CRP and vulnerable plaque rate. hs-CRP = hypersensitive C-reactive protein, ROC = receiver operating characteristic.

AMI in patients with T2DM is much higher than that in patients without T2DM.[11]

As an acute phase protein secreting by liver, hs-CRP is a sensitive index of inflammatory response, which plays a part in the formation and development of AS. During the formation of AS plaque, inflammatory factors of interleukin-6 and tumor necrosis factor-α were activated via macrophage aggregation, and the factors in return induced hepatocyte to synthesize more hs-CRP, resulting in gradual anterior development of AS plaque and a series of chain reaction, with a higher level of hs-CRP especially in plaque rupture.[12] The mechanism of action included: the effect on lipid core of plaque[13]: there was a positive correlation between end arterial lipid deposition and hs-CRP level. Immune reaction which is generated by hs-CRP could reach its peak in plaque rich in lipid; the effect on plaque thickness[14]: hs-CRP promoted the apoptosis of vascular smooth muscle cell and lowered the synthesis of matrix in fibrous cap, which leads to the thinness of fibrous cap and increase of plaque brittleness; effect on inflammatory response[15]: inflammatory response which activated by hs-CRP could promote macrophage's elevated secretion of matrix metalloproteinase, which intensified degradation of matrix in fibrous cap, reduced the thickness of fibrous cap, and increased the instability of plaque. Based on the results, it can be seen that the level of serum hs-CRP in T2DM combined with AMI group was significantly higher than that in T2DM group, with the control group being the lowest, indicating the level of serum hs-CRP could reflect the severity of diseases.

The early diagnostic approach of T2DM patients with AMI includes coronary arteriography (CAG), dual-source CT, and carotid artery ultrasonography. Traditional CAG is traumatic and risky, while CT examination contains extensive radiation and renal toxicity of contrast agents. Carotid artery ultrasonography has the features of non-invasive, no radiation damage, and clearly imaging, thus receiving wide usage in clinic. With short imaging time, full demonstration of arterial tree structure and traceability towards elongated branch, the ultrasonography which has a preferable adaptability in patients with fast heartbeat, is the most common screening tool.[16,17] The study indicated that the IMT, PA, PA/LA, Dmax, EI, and plaque quantity in T2DM combined with AMI group were all higher than those in T2DM group, with control group being the lowest; the vulnerable plaques increased while stable plaques decreased.

Lipid in patients with T2DM gradually accumulated in intima and formed plaque. When the incrassation of IMT reached over 1.5 mm, plaque burden was evidently increased, which accompanied by significant increase of PA, decreased nutrition supply of plaque and secondary ischemia, necrosis and rupture in the plaque, resulting in acute complete occlusion in distal coronary.[18,19] Echo in plaque region by this time was characterized by heterogeneity and low echo. During IMT thickening that caused by a little lipid deposition in the early stage, intima was lacking in smoothness, PA increase was not evident, and plaque echo showing hyperechoic, indicated that steady progressing plaque was mainly formed, and compensatory adjustments could be proceeded in human body to lower the occurrence of AMI.[20] Therefore, early examination of characteristic and intervention on plaque in carotid artery can lower the occurrence of serious adverse cardiac events and improve prognosis.[21]

5. Limitation

A very small sample size is an evident limitation of this study which is due to the limited availability of data within the short duration of the study. In addition, the consent from the patients was not easy as many of them were unaware of the importance of the study and hence such cases were rejected to participate in the study. Further research is required to observe whether there is a beneficial strategy to reduce the risk of AMI in Type 2 diabetic patients with high levels of hs-CRP.

6. Conclusion

According to the further analysis in this study, it concluded that serum hs-CRP level, IMT, PA/LA, EI, and vulnerable plaque rate were independent risk factors of the occurrence of T2DM combined with AMI. The level of hs-CRP and vulnerable plaque rate had a high sensitivity, specificity, and accuracy in the diagnosis of T2DM combined with AMI. In conclusion, carotid artery ultrasound and hs-CRP have played a significant role in the early diagnosis ofT2DM combined with AMI.

Author contributions

Conceptualization: Juan Li, Liangliang Wang.
Data curation: Juan Li, Liangliang Wang, Zhaoqin Xin.
Formal analysis: Juan Li, Liangliang Wang, Qian Wang, Qinghua Zhao.
Investigation: Juan Li, Liangliang Wang, Qian Wang, Yilin Liu.
Methodology: Juan Li, Liangliang Wang, Zhaoqin Xin.
Project administration: Qian Wang.
Validation: Qian Wang.
Writing – original draft: Juan Li, Liangliang Wang, Zhaoqin Xin, Yilin Liu, Qinghua Zhao.
Writing – review & editing: Juan Li, Liangliang Wang.

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