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New mechanisms and drugs for the treatment of cardiovascular disease with diabetes

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Published in
Frontiers in Cardiovascular Medicine

ISSN 1664-8714
ISBN 978-2-83251-819-9
DOI 10.3389/978-2-83251-819-9

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New mechanisms and drugs for the treatment of cardiovascular disease with diabetes

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Citation

Li, J., Huang, Y., Pan, X., Yu, J., eds. (2023). *New mechanisms and drugs for the treatment of cardiovascular disease with diabetes*. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-83251-819-9

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frontiers | Frontiers in Cardiovascular Medicine

TYPE Editorial
PUBLISHED 20 February 2023
DOI 10.3389/fcvm.2023.1144858

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Cardiovascular Pharmacology and Drug Discovery, a section of the journal Frontiers in Cardiovascular Medicine

RECEIVED 15 January 2023
ACCEPTED 26 January 2023
PUBLISHED 20 February 2023

CITATION
Zhang S, Li J, Huang Y, Yu J and Pan X-F (2023) Editorial: New mechanisms and drugs for the treatment of cardiovascular disease with diabetes. *Front. Cardiovasc. Med.* 10:1144858. doi: 10.3389/fcvm.2023.1144858

Editorial: New mechanisms and drugs for the treatment of cardiovascular disease with diabetes

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KEYWORDS

diabetes, cardiovascular diseases, mechanisms, drugs, cardiovascular therapeutics, oxidative stress, inflammation, SGLT2 inhibitors

Editorial on the Research Topic
New mechanisms and drugs for the treatment of cardiovascular disease with diabetes

Cardiovascular diseases (CVDs) are a leading cause of deaths worldwide (1). Globally, the prevalence of CVDs was estimated to be 608 million cases in 2020. CVDs and diabetes are closely linked: CVDs are major complications for diabetes and share multiple risk factors with diabetes. Concomitant CVDs and diabetes thus should be treated as a substantial subgroup in both clinical treatments and research.

Risk factors for CVDs and related medical conditions

The presence of uterine fibroids has been reported to associate with hypertension (2). Li B. et al. examined the association between uterine fibroids and CVDs and whether the association was modified by prevalent diabetes using cross-sectional data for 5,509 women from the National Health and Nutrition Examination Survey (NHANES) 1999–2006. Women with uterine fibroids were 1.44 times as likely to have CVDs compared with those without uterine fibroids. Although a statistical interaction was not provided in the article, it seemed that the positive association between uterine fibroids and CVDs was noted only in participants without diabetes but not in those with diabetes. Metabolic syndrome is a constellation of metabolic risk factors for CVDs. Using echocardiography and high-definition impedance cardiography combined with exercise tolerance test, Chen J. et al. found significant structural alteration, apparent overburden of left ventricular work index, and pre- and afterload in non-CVD patients with metabolic syndrome compared with those without metabolic syndrome. Vulnerable plaques (VPs) could contribute to the onset of coronary heart disease (3). It is essential to detect vulnerable plaques early in order to prevent CVDs. Neutrophil-lymphocyte ratio (NLR) is a marker of

chronic inflammation, while non-high-density lipoprotein cholesterol (non-HDL-C) is thought to contribute to the formation and development of coronary atherosclerosis. In a cross-sectional analysis among 204 patients with type 2 diabetes, Huang, Yang et al. found that both NLR and non-HDL-C showed independent associations and favorable prediction for coronary artery VPs. If validated in other studies, non-HDL-C and NLR may have the potential to be used as simple predictors for VPs.

Ferroptosis is regulated cell death that is driven by iron-dependent phospholipid peroxidation. Evidence is accumulating that ferroptosis could be involved in multiple diseases. In a review, Guo et al. summarized the current evidence of ferroptosis in the development of CVDs, including cardiomyopathy, atherosclerosis, acute myocardial infarction, myocardial ischemia/reperfusion injury, and heart failure, and assessed the potential underlying mechanisms. In addition, they briefly related their narratives to potential ferroptosis-targeting treatments of CVDs. Pyroptosis is a programmed process that results in non-inflammatory cell death (4), and recent evidence suggests that it has a potential role in diabetic cardiomyopathy (Lu Y. et al.). On this front, Jia et al. reviewed current evidence on pyroptosis mechanisms and pyroptosis-targeting pre-clinical and clinical treatments for diabetic cardiomyopathy.

Cardiovascular effects of antiglycemic treatments

Since diabetes predisposes to CVDs, whether antiglycemic drugs could be potentially effective for reducing risk of CVDs is still inconclusive (5). Chen C. et al. used real-world data to assess a similar topic in 1,356 patients with diabetes from the NHANES 2017–2020. Oral metformin use was associated with improved cardiovascular health as defined by 5 indicators (smoking, body mass index, physical activity, blood pressure, and total cholesterol). While sodium-glucose co-transporter 2 inhibitor (SGLT2i) has been reported to reduce risks of major adverse cardiovascular events and heart failure hospitalization in patients with type 2 diabetes (6), the potential mechanisms are still elusive. Xi et al. examined cardiovascular effects of 12-week treatment of empagliflozin (a frequently used SGLT2i) in 24 male rats with streptozocin-induced diabetes through a multi-omics approach. Empagliflozin treatment ameliorated lipid accumulation and mitochondrial damage in the myocardium of diabetic rats. In a separate animal study, Zhan et al. examined the mechanism by which dapagliflozin, another SGLT-2i, reduced the risk of atrial fibrillation in diabetes. They found that dapagliflozin alleviated atrial remodeling and reduced the inducibility of atrial fibrillation in rats with streptozocin-induced diabetes, partly through the toll-like receptor 4, interleukin receptor-associated kinase 1, tumor necrosis factor receptor-associated factor 6, and nuclear factor-kappa B inflammatory pathway. For patients with both coronary heart disease and diabetes, glycemic control could be consequential. Education and support platforms may improve compliance to lifestyle improvement and medication use in patients with diabetes. In a parallel-group, open-label, randomized clinical trial among 160 patients with both coronary heart disease and type 2 diabetes, Zhong et al. found that education and support (educational materials and reminders in response to individual blood glucose) through the WeChat group function led to greater reductions in HbA1C, fasting blood glucose, and systolic blood pressure than usual care, which highlights a convenient and low-cost way to improve self-management in coronary heart disease and diabetes.

Treatments for CVDs complicated by diabetes

Statins are recommended for use in primary and secondary prevention of CVDs in patients with diabetes (7). However, few studies assessed the risk-benefit profiles of statins in patients with concomitant acute myocardial infarction (AMI) and diabetes. In a sample of 1315 patients with AMI and diabetes from the Medical Information Mart Intensive Care-IV (MIMIC-IV), Lu X. et al. showed that statin users had 72% lower in-hospital mortality and 86% lower intensive care unit (ICU) mortality than non-users, and had similar benefits for subgroups with or without hyperlipidemia. In the same study population, Huang, Zhang et al. showed that anti-embolism device therapy was associated with 50% lower 28-day mortality and 52% lower ICU mortality, compared with no such therapy. Left ventricular thrombus (LVT) is a common complication of AMI, and its treatment is still not standardized. Zhu et al. reported successful treatment of splenic infarction and bilateral renal infarction due to multiple peripheral embolization of LVT in a patient with AMI and diabetes using oral anticoagulant combined with percutaneous coronary intervention (PCI). In another case report, Shen, Liao et al. showed that intracoronary artery retrograde thrombolysis (ICART) combined with PCI improved myocardial reperfusion in a patient with ST-segment elevation myocardial infarction (STEMI) and massive thrombus formation complicated with diabetes and hypertension, even if the myocardial infarction exceeded 12 h. In addition, Shen, Wang et al. published a protocol for a randomized controlled trial to investigate whether ICART is more effective than thrombus aspiration or percutaneous transluminal coronary angioplasty in improving myocardial perfusion in 286 patients with STEMI undergoing PCI. The claimed first study of its type could provide evidence for the efficacy and safety of ICART in STEMI patients receiving PCI. The triglyceride-glucose (TyG) index has been reported to be a surrogate biomarker for insulin resistance and be closely related to cardiovascular diseases. In secondary analyses of data from a prospective study among 241 STEMI patients with high thrombus burden, Yu et al. investigated the relationship between the TyG index and post-PCI quantitative flow ratio (QFR), an alternative modality to reflect residual coronary ischemia (8, 9). The authors showed that a high TyG index was an independent risk factor for post-PCI QFR $\leq$ 0.92 (a cut-off regarded as indicating high risk for adverse cardiovascular events in the work) in STEMI patients (Yu et al.). Although findings from this study were still primitive, it may support the evidence that insulin resistance could be suggestive of residual coronary ischemia in STEMI patients. While the prevalence of dilated cardiomyopathy (DCM) increases, there is still a lack of evidence for the optimal clinical management of recovered DCM (10). Li P. et al. proposed an open-label randomized controlled trial to assess the safety and efficacy of halved vs. original doses of neurohumoral blockades (i.e., "angiotensin converting enzyme inhibitor/angiotensin-receptor blocker/angiotensin receptor-neprilysin inhibitor + beta-blockers")

for patients with recovered DCM. Evidence from this trial could assist decision making for dosages of neurohumoral blockades.

In conclusion, this Research Topic covers a wide variety of articles on etiology, treatments, and prognosis of concomitant CVDs and diabetes. The collective evidence could potentially facilitate clinical research and practice in CVDs complicated by diabetes.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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frontiers | Frontiers in Cardiovascular Medicine

MINI REVIEW
published: 23 May 2022
doi: 10.3389/fcvm.2022.911564

Roles of Ferroptosis in Cardiovascular Diseases

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Ferroptosis is an iron-dependent regulated cell death characterized by lipid peroxidation and iron overload, which is different from other types of programmed cell death, including apoptosis, necroptosis, autophagy, and pyroptosis. Over the past years, emerging studies have shown a close relation between ferroptosis and various cardiovascular diseases such as atherosclerosis, acute myocardial infarction, ischemia/reperfusion injury, cardiomyopathy, and heart failure. Herein, we will review the contributions of ferroptosis to multiple cardiovascular diseases and the related targets. Further, we discuss the potential ferroptosis-targeting strategies for treating different cardiovascular diseases.

Keywords: ferroptosis, atherosclerosis, acute myocardial infarction, cardiomyopathy, heart failure

OPEN ACCESS

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Specialty section:
This article was submitted to Science and Environmental Communication, a section of the journal Frontiers in Cardiovascular Medicine

Received: *02 April 2022*
Accepted: *02 May 2022*
Published: *23 May 2022*

Citation:
Guo YT, Zhang W, Zhou X, Zhao S, Wang J, Guo Y, Liao Y, Lu H, Liu J, Cai Y, Wu J and Shen M (2022) Roles of Ferroptosis in Cardiovascular Diseases. Front. Cardiovasc. Med. 9:911564. doi: 10.3389/fcvm.2022.911564

INTRODUCTION

Cardiovascular diseases include hypertension, atherosclerosis, acute myocardial infarction (AMI), arrhythmia, cardiomyopathy, valvular heart diseases, congenital cardiovascular diseases and heart failure (1), which are the leading causes of disability and death in the world (2). Cardiomyocyte death is a basic pathological process in the progression of cardiovascular diseases. Understanding the mechanism of cardiomyocyte death can provide support for protecting cardiac function.

Ferroptosis, which was proposed by Dixon et al. (3), is a non-apoptotic form of cell death. Ferroptosis is characterized by lipid peroxidation and iron overload. Its morphological features mainly involve mitochondrial changes encompassing mitochondria shrinkage, increased mitochondria membrane density, crista destruction, and outer membrane rupture, but not nucleus morphological changes. Ferroptosis is a new pattern of programmed cell death that differs from several other forms of regulated cell death in various aspects, including morphology, biochemistry, and immune status (**Table 1**).

Recently, several studies have found various significant factors of ferroptosis and revealed a range of complex regulatory mechanisms in the progression of ferroptosis involving iron metabolism, lipid metabolism, and amino acid metabolism (**Figure 1**). In the iron metabolism pathways, transferrin receptor 1 (TfR1) transport extracellular Fe^{3+} to the nucleus and convert it into Fe^{2+}, which is released from the nucleus through divalent metal transporter 1 (DMT1), triggering the Fenton reaction, activating lipoxygenases, and promoting the generation of lipid peroxides, resulting in ferroptosis (4, 5). Amino acid metabolism involves vital regulatory factors, including system X_C^- (consisting of two subunits SLC3A2 and SLC7A11) (6, 7) and glutathione peroxidase 4 (GPX4). Inhibitors of system X_C^- decrease the uptake of cystine and reduce cysteine and

suppress glutathione (GSH) production, further inactivating GPX4 (8) and reducing the conversion of GSH to glutathione disulfide (GSSG) (9), which will result in lipid peroxidation and ferroptosis in amino acid metabolism. By activating acyl-CoA synthetase long-chain family member four (ACSL4) and lysophosphatidylcholine acyltransferase three (LPCAT3), polyunsaturated fatty acids (PuFAs) induce lipid peroxidation and promote ferroptosis (10).

Over the years, researches on the link between ferroptosis and clinical diseases have been gradually improved, with cancer and neurodegenerative diseases being the focus (11–19). Recent studies have demonstrated ferroptosis participates in the genesis and development of cardiovascular diseases. We discuss the roles and potential mechanisms of ferroptosis in cardiovascular diseases in this article and hopefully provide an effective strategy for the treatment of cardiovascular diseases.

FERROPTOSIS AND CARDIOVASCULAR DISEASES

Ferroptosis and Cardiomyopathy

Cardiomyopathy is a group of myocardial diseases caused by heterogeneous factors, leading to myocardial and/or cardiac electrical dysfunction, with high mortality (20).

Doxorubicin (DOX), also known as adriamycin, is the second-generation anthracycline chemotherapy drug, a commonly used antitumor agent with fatal cardiotoxicity. Its most serious side effect is cardiomyopathy, called doxorubicin-induced cardiomyopathy (DIC) (21). Tadokoro et al. (22) found that mitochondria-dependent ferroptosis plays an essential role in DIC. DOX down-regulated GPX4 and caused excessive lipid peroxides production in mitochondria through the DOX-Fe^{2+} complex, resulting in mitochondria-dependent ferroptosis. GPX4 overexpression in mitochondria or iron chelates targeting Fe^{2+} can ameliorate doxorubicin-induced ferroptosis. Furthermore, this study showed that apoptosis is also a major form of doxorubicin-induced cardiomyocyte death. And two death forms are independent of each other. The combination of ferrostatin-1 (Fer-1) and zVAD-FMK to inhibit ferroptosis and apoptosis could completely prevent doxorubicin-induced cardiomyocyte death in rats. In addition, Fang et al. (23) showed that DOX significantly up-regulated heme oxygenase-1 (Hmox1) through NF-E2-related factor 2 (NRF2), induced local heme degradation, leading to the release of free iron, and further inducing ferroptosis in mouse myocardial tissue. Zinc protoporphyrin IX (ZnPP), a competitive inhibitor of Hmox1, reduced DOX-induced ferroptosis. These results suggest that Hmox1 plays an important role in doxorubicin-induced ferroptosis and cardiomyopathy. This study also found that ferroptosis inhibitor Fer-1 or dexrazoxane (DXZ) prevented lipid peroxidation and DIC by maintaining mitochondrial function. However, MitoTEMPO, a mitochondria-targeted antioxidant, can alleviate DIC by specifically clearing lipid peroxidation in mitochondria. These studies show that DOX-induced cardiotoxicity is closed with mitochondrial iron overload and subsequent ferroptosis. In 2021, He et al. (24) proved *in vitro* and *in vivo* that ferroptosis, autophagy, and apoptosis are related to DOX-induced cardiotoxicity. Epigallocatechin-3-gallate(EGCG)is a polyphenol compound in green tea and is also a natural antioxidant. EGCG up-regulated AMP-activated protein kinaseα2 (AMPKα2), activated adaptive autophagy, reduced iron deposition, inhibited reactive oxygen species (ROS) overproduction and rectified abnormal lipid metabolism, thereby reversing ferroptosis in DIC. Similarly, in a recent article, Sun et al. (25) demonstrated potent antioxidant melatonin inhibited mitochondrial lipid peroxidation and ameliorated doxorubicin-induced cardiac ferroptosis. In summary, we know that many forms of cell death are involved in DIC, among which ferroptosis is a pivotal one. Thus, targeting ferroptosis might be an effective treatment for DIC in cancer patients.

Diabetic cardiomyopathy (DCM) is defined as a disorder of cardiac structure and function in patients with diabetes in the absence of coronary artery disease, hypertension, valvular heart diseases, and other conventional cardiovascular risk factors (26). Excessive overproduction of ROS is regarded as an essential mechanism for the occurrence and development of diabetic cardiomyopathy (27), and the accumulation of lipid ROS induced ferroptosis (28). Therefore, ferroptosis is more likely to be involved in DCM. Some studies have supported that administration of ferroptotic inhibitors coenzyme Q_{10} and Vitamin E in diabetic animals might protect the myocardium by suppressing oxidative stress (29, 30). GPX4 is one of the crucial regulators of ferroptosis, and GPX4 deficiency induced lipid peroxidation and resulted in myocardial metabolic disturbance in high-fat, high-sucrose diet mice (31). Conversely, GPX4 overexpression could alleviate mitochondrial dysfunction and protect the hearts from diabetic damage (32). A recent study has identified that ferroptosis exerts a pivotal effect on the pathogenesis of DCM. NRF2 agonist sulforaphane inhibited lipid peroxidation via AMPK/NRF2 pathways, which suppressed ferroptosis and prevented DCM (33). These findings suggest that ferroptosis has a substantial impact on DCM.

Sepsis cardiomyopathy is a severe life-threatening complication caused by sepsis (34). Li et al. (35) found ferroptosis is involved in the progression of sepsis cardiomyopathy. Their experiments showed that ferroptotic inhibitor Fer-1 or iron chelates DXZ mitigated lipopolysaccharide (LPS)-induced ferroptotic cell death in sepsis cardiomyopathy model, while ferroptosis inducers sorafenib and erastin exacerbated LPS-induced myocardial injury.

In conclusion, ferroptosis plays a crucial role in the pathogenesis of cardiomyopathy, and ferroptosis inhibitors are expected to be a novel therapeutic strategy for cardiomyopathy.

Ferroptosis and Atherosclerosis

Atherosclerosis is a chronic inflammatory disease involving the main and middle arteries (36). Martinet et al. (37) suggested that intraplaque hemorrhage, iron deposition, and lipid peroxidation are common pathological features of an advanced stage of human atherosclerotic plaque. Guo et al. (38) have found that overexpression of GPX4 inhibited lipid peroxidation and delayed the pathological process of atherosclerosis in $ApoE^{-/-}$mouse. And lipid peroxidation accumulation is one of

TABLE 1 | Comparison of different forms of programmed cell death.

Cell death	Morphological features	Biochemical changes	Immune status
Ferroptosis	Mitochondria shrinkage, increased mitochondria membrane density, crista destruction, and outer membrane rupture, but not nucleus morphological changes	Lipid peroxidation and iron overload	Pro-inflammatory
Apoptosis	Cell shrinkage, chromatin condensation, plasma membrane blebbing without rupture, formation of apoptotic bodies, cytoskeletal disintegration	DNA fragmentation	Anti-inflammatory (mostly)
Necroptosis	Cytoplasm and organelles swelling, formation of necrosome, plasma membrane rupture, and release of cell contents	ROS production, random degradation of DNA, damage-associated molecular patterns (DAMPs) release, R1PK1, R1PK3 and MLKL phosphorylation	Anti-inflammatory
Autophagy	Formation of double- membraned autophagic vesicles, normal membrane and nucleus	Increased lysosomal activity, LC3-I to LC3-II conversion, P62 degradation	Anti-inflammatory (mostly)
Pyroptosis	Cytoplasm swelling, formation of pyroptotic bodies, plasma membrane rupture, release of cell contents, and unaffected mitochondrial integrity	Activation of caspase and GSDMD, pro-inflammatory factors release	Pro-inflammatory (mostly)

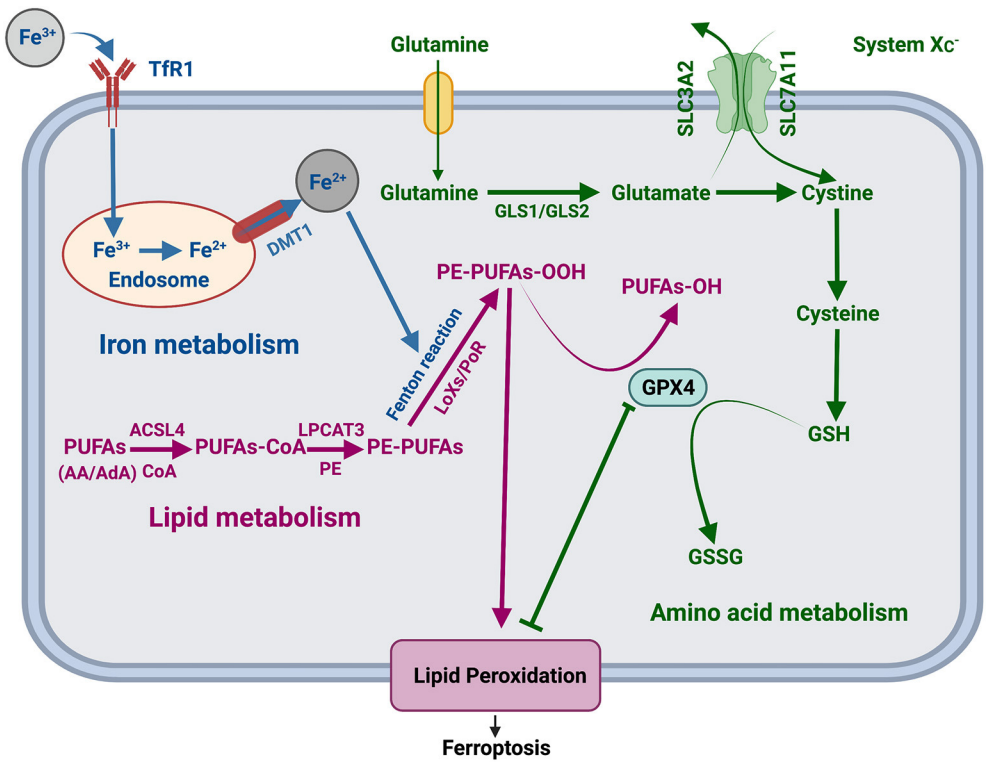

FIGURE 1 | Regulatory mechanism of ferroptosis. TfR1, transferrin receptor 1; DMT1, divalent metal transporter 1; LOXs, lipoxygenases; POR, cytochrome P450 oxidoreductase; PUFAs, polyunsaturated fatty acids; AA, arachidonic acid; AdA, adrenal acid; CoA, coenzyme A; PE, phosphatidylethanolamine; ACSL4, acyl-CoA synthetase long-chain family member 4; LPCAT3, lysophosphatidylcholine acyltransferase 3; SLC7A11, solute carrier family 7 member 11; SLC3A2, solute carrier family 3 member 2; GSH, glutathione; GSSG, glutathione disulfide.

the characteristics of ferroptosis, so we speculate that ferroptosis plays an essential role in the initiation and development of atherosclerosis. CD98 heavy chain (CD98hc), also named solute carrier family 3 member 2 (SLC3A2), is a component of the antiporter system X_C^-. Inhibitor of system X_C^- triggered endoplasmic reticulum stress and resulted in ferroptosis, while

the expression of CD98hc in vascular smooth muscle cells contributed to the stable formation of atherosclerotic plaque (39, 40). There is direct evidence that ferroptosis occurs in the development of atherosclerosis. Ferroptotic inhibitor Fer-1 delayed the progression of atherosclerosis by reducing endothelial dysfunction, lipid peroxidation and iron content in mouse aortic endothelial cells (41). It is well known that diabetes can be complicated with vascular diseases, which include atherosclerosis. A study by Meng et al. (42) indicated that ferroptosis is involved in the occurrence and development of atherosclerosis in diabetes mellitus. In the cell models treated with high glucose, and high lipids, Hmox1 deficiency reduced iron overload, ROS production and lipid peroxidation to inhibit ferroptosis in endothelial cells. Hmox1 may be a therapeutic target for diabetic atherosclerosis. Based on those studies, we know that ferroptosis has an essential effect on atherosclerosis. Targeting ferroptosis may provide new ideas for the treatment of atherosclerosis.

Ferroptosis and Acute Myocardial Infarction

The clinical definition of AMI refers to myocardial injury with abnormal cardiac biomarkers detected in the condition of acute myocardial ischemia (43). Park et al. (44) found that the down-regulation of GPX4 induced ferroptosis during AMI, resulting in cardiomyocyte death and myocardial injury. Baba et al. (45) showed that mechanistic target of rapamycin (mTOR) suppressed cell death, ferroptosis and improved left ventricular remodeling by reducing the production of ROS. MiR-23a-3p is a kind of enriched miRNAs in exosomes derived from mesenchymal stem cells (MSCs) (46). It was reported that DMT1 is a miR-23a-3p target gene. Ferroptosis occurred in the hypoxic cardiomyocytes and infarcted myocardium. MSCs exosomes derived from human umbilical cord blood inhibited ferroptosis via miR-23a-3p/DMT1 axis and mediated myocardial repair in AMI mice (47). In the above studies, ferroptosis has been implicated in the initiation and development of AMI. Inhibition of ferroptosis has been provide novel tactics for the precise treatment of myocardial infarction. Meanwhile, Through machine learning, Huang et al. (48) filtered out ferroptosis-related genes (FRGs) specifically expressed in the peripheral blood of AMI patients. In this study, they also proposed a diagnostic model composed of mitogen-activated protein kinase 3 (MAPK3), WD repeat domain phosphoinositide-interacting protein 2 (WIPI2) and voltage-dependent anion channel three (VDAC3) and provided a new direction for early diagnosis of AMI.

Since diabetes mellitus significantly inhibits the establishment of collateral circulation of ischemic myocardium, aggravating myocardial injury, patients with diabetes comorbiditied with AMI have higher incidence and mortality of coronary heart disease (49). Diabetes increases ROS production in the infarcted myocardium (50), and ROS are considered as essential signals of ferroptosis (51). We hypothesize that ferroptosis might be involved in the pathological process of diabetes comorbiditied with AMI. However, it has not been reported explicitly whether ferroptosis participates in diabetes comorbiditied with AMI, and further studies are needed.

Ferroptosis and Myocardial Ischemia/Reperfusion Injury

Myocardial ischemia/reperfusion injury (I/RI) refers to the pathological process of aggravated myocardial damage caused by reperfusion within a certain period of time after partial or complete acute occlusion of coronary artery. Tang et al. (52) proposed that up-regulation of ubiquitin-specific protease 7 (USP7) activated the protein 53 (p53)/TfR1 pathway to promote ferroptosis in the I/RI rat model. Increased oxidized phosphatidylcholines (OxPCs) caused mitochondrial dysfunction and disrupted calcium transients and resulted in extensive cardiomyocyte death via ferroptosis during myocardial I/RI. Intervention to OxPCs could prevent ferroptosis in I/RI patients (53). These findings supported that ferroptosis might play a significant role in the pathogenesis of myocardial I/RI. Pretreating mice with ferroptotic inhibitor Fer-1, DXZ or liproxstatin-1 (Lip-1) could alleviate myocardial injury after ischemia/reperfusion (23, 54). The latter was mainly achieved by reducing mitochondrial ROS production, increasing GPX4 level, and decreasing voltage-dependent anion channel 1 (VDAC1) level (54). Anthocyanins can be found in most plants and cyanidin-3-glucoside (C3G) is a major type of anthocyanins. Anthocyanins have strong antioxidant activity, which can effectively scavenge free-radical and protect the heart (55). C3G suppressed the promotion of ras synthetic lethal 3 (RSL3) on ferroptosis. C3G reduced the Fe^{2+}content, down-regulated TfR1 and up-regulated ferritin heavy chain1 (FTH1), inhibited ferroptosis and alleviated myocarial injury in I/RI models (56). Likewise, Xanthohumol (XN) isolated from Humulus lupulus had also been shown to protect ischemic/reperfusion myocardium from ferroptosis (57). Besides, exosomal long noncoding RNA (lncRNA) MIR9-3 host gene (Mir9-3hg) derived from bone MSCs mitigated ferroptosis in I/RI mice by regulating pumilio RNA binding family member two (Pum2)/peroxiredoxin 6 (PRDX6) axis and showed cardioprotective effects both *in vitro* and *in vivo* (58). These exciting findings have further broadened therapeutic approaches for ferroptosis in I/RI.

Recent studies have demonstrated the pathological process of diabetic I/RI is relevant to ferroptosis. Wang et al. (59) discovered that diabetes exacerbated I/RI via decreasing AMPK, inducing oxidative stress associated with NADPH oxidase 2 (NOX2) and programmed cell death including ferroptosis. Meanwhile, Li et al. (60) found that restraining ferroptosis could reduce endoplasmic reticulum stress and oxidative stress damage and delay the progression of diabetic I/RI. Nevertheless, the role of ferroptosis in diabetes I/RI needs to be better elucidated.

Ferroptosis also participates in I/RI related to heart transplantation. Ferroptosis mediated I/RI after heart transplantation by recruiting neutrophils to the transplanted heart. Inhibition of ferroptosis before transplantation can alleviate reperfusion injury, reduce left ventricular remodeling, and improve the prognosis of heart transplant recipients (61).

Ferroptosis and Heart Failure

Heart failure is a set of clinical syndromes in which cardiac output is inadequate due to various structural and functional abnormalities of the heart (62). The loss of cardiomyocytes plays a crucial part in the development of heart failure. Programmed cell death, such as autophagy and ferroptosis, occurs in the heart failure stage. Knockdown of toll-like receptor 4 (TLR4) or NADPH oxidase 4 (NOX4) restrained ferroptosis and autophagy, which attenuated the loss of cardiomyocytes and delayed the progression of heart failure (63). Moreover, ferroptosis has been observed in heart failure resulted from pressure overload. The model of heart failure was established by aortic coarctation in this research. Antioxidant puerarin could inhibit ferroptosis via increasing GPX4 and ferritin heavy chain 1 (FTH1), and down-regulating expression of NOX4, which could improve cell viability in rats, reduce death of H9C2 cardiomyocytes treated with erastin or isoproterenol (ISO) and retard the development of heart failure (64). Nitenberg et al. (65) demonstrated abnormal myocardial iron probably exists in diabetic heart failure. Iron chelator deferoxamine can improve coronary microcirculation in patients with type two diabetes by suppressing the increase of oxygen radicals, which may be a novel target for reversing deterioration of cardiac function in patients with diabetic heart failure. Nevertheless, the toxicity and short half-life of deferoxamine affect its application in improving cardiac function for clinical patients with diabetic heart failure. Thus, the role of ferroptosis in heart failure remains to be further studied.

Ferroptosis and Other Cardiovascular Diseases

Hypertension is a common cardiovascular disease. Currently, there are few works on the relationship between hypertension and ferroptosis. A research by Yang et al. (66) showed that reductions of GPX4 and GSH in the brains of hypertensive rats led to lipid peroxidation and iron overload, inducing hypertensive brain injury. Elabela is an endogenous ligand for apelin receptor, which is primarily expressed in the cardiac microvascular endothelial cells (CMVECs). Zhang et al. (67) studied the effect of elabela on hypertension. They found that elabela inhibited cardiac oxidative stress, inflammation, fibrosis, and ferroptosis in Angiotensin II (Ang-II) treated CMVECs and hypertensive mice to suppress hypertensive ventricular remodeling. Hence, we guess that ferroptosis might be involved in hypertension and result in the damage to hypertensive target organs.

Aortic dissection (AD), also known as aortic dissecting aneurysm (ADA), is a type of cardiovascular diseases with high mortality (68). Zou et al. (69) revealed that ferroptosis is an important pathological mechanism of Stanford type A aortic dissection (TAAD). Some ferroptosis-related genes mediated

TABLE 2 | The role of ferroptosis in various cardiovascular diseases.

Diseases	Characteristics or changes	Pathways or signals	References
DIC	Excess lipid peroxides production in mitochondria	Down-regulation of GPX4 expression	Tadokoro et al. (22)
DIC	Up-regulation of Hmox1 expression	NRF2/Hmox1 pathway	Fang et al. (23)
DCM	Lipid peroxidation	Advanced Glycation end-products (AGEs) inhibited SLC7A11 expression and ferritin, decreased GSH expression and increased unstable iron levels.	Wang et al. (33)
Sepsis cardiomyopathy	Iron overload and excessive ROS in mitochondria	NCOA4 expression increased, interacted with ferritin, activated SFXN1 expression, and transferred Fe^{2+} to mitochondria	Li et al. (35)
Diabetic Atherosclerosis	Iron overload, ROS increased, down-regulation of GPX4 and SCL7A11, lipid peroxidation and together resulted in ferroptosis in endothelial cells	Hmox1 increased	Meng et al. (42)
AMI	Accumulation of lipid peroxides	Down-regulation of GPX4	Park et al. (44)
AMI	GSH level decreased, iron deposition, Fe^{2+} level increased, excessive lipid peroxides and ROS	DMT1 overexpression	Song et al. (47)
I/RI	Up-regulation of USP7, p53 and TfR1	USP7 / p53 / TfR1 pathway	Tang et al. (52)
I/RI	Mitochondrial dysfunction, calcium transients blocked and contractile dysfunction	Loss of GPX4 activity	Stamenkovic et al. (53)
Diabetic I/RI	A increase in myocardial oxidative stress, apoptosis, pyroptosis and ferroptosis	Nox2 activation mediated through AMPK suppression	Wang et al. (59)
Diabetic I/RI	The interaction between endoplasmic reticulum stress and ROS caused cardiomyocytes injury	ATF4-CHOP pathway	Li et al. (60)
I/RI related to heart transplantation	Neutrophils recruitment to impaired myocardium	TLR4/TRIF pathway	Li et al. (61)

DIC, doxorubicin-induced cardiomyopathy; GPX4, glutathione peroxidase 4; Hmox1, heme oxygenase-1; NRF2, NF-E2-related factor 2; DCM, Diabetic cardiomyopathy; AGEs, advanced glycation end-products; ROS, reactive oxygen species; NCOA4, nuclear receptor coactivator 4; SFXN1, siderofexin; SCL7A11, solute carrier family 7 member 11; AMI, acute myocardial infarction; GSH, glutathione; DMT1, divalent metal transporter 1; I/RI, ischemia/reperfusion injury; USP7, ubiquitin-specific protease 7; p53, protein 53; TfR1, transferrin receptor 1; Nox2, NADPH oxidase 2; AMPK, AMP-activated protein kinase; ATF4, Activating transcription factor 4;CHOP, C/EBP homologous protein; TLR4, toll-like receptor 4; TRIF, TIR domain-containing adapter-inducing interferon-β.

ferrptosis in cells and influenced the development of TAAD. Smooth muscle cell (SMC) loss is an important mechanism of aortic dissection. Ferroptosis participated in SMC loss and AD progression. BRD4770 is a new ferroptosis inhibitor, which suppressed inflammatory response, reduced lipid peroxidation and inhibited ferroptosis in SMC of AD mice to prevent the formation of aortic dissection (70, 71).

In addition, recent studies have indicated a possible link between ferroptotic death and arrhythmia. Iron overload caused the occurrence of arrhythmia via promoting mitochondrial ROS generation and membrane potential depolarization, and mitochondrial dysfunction is one of the main characteristics of ferroptosis (72). Frequent alcohol consumption is known to increase the risk of atrial fibrillation (73). Regular drinking promoted ferroptosis via iron overload and increased the incidence of atrial fibrillation. Ferroptosis inhibitor Fer-one, reduced the susceptibility to atrial fibrillation induced by frequent drinking in mice (74). Hence, we supposed that ferroptotic cell death might be a latent target for arrhythmia therapy in the future.

DISCUSSION

Ferroptosis is a novel regulated cell death, which has received much attention in recent years. We discuss the roles of ferroptosis in cardiomyopathy, atherosclerosis, acute myocardial infarction, ischemia, and reperfusion injury, heart failure, hypertension, arrhythmia and aortic dissection in this review (**Table 2**). But the roles of ferroptosis in other cardiovascular diseases, including valvular heart disease, have been rarely studied, which require further researches. Besides, except for iron chelators DXZ and deferiprone (DFP) authorized by FDA are used in treating DIC and AMI (75, 76), a majority of researches of ferroptosis in cardiovascular diseases have only been confirmed in the cell and animal models, with relatively limited clinical evidence. Thus, clinical investigations are essential for the application of ferroptosis in cardiovascular diseases. Furthermore, ferroptotic inhibitors are greatly limited in the human body due to their toxicity, instability and short half-life. And it is urgent to develop non-toxic and long-acting inhibitors targeting ferroptosis.

A series of researches showed that ferroptosis and other types of programmed cell death take part in cardiovascular diseases together (22, 24, 59, 63). Whether there is a crosstalk between ferroptosis and other cell death forms in various cardiovascular diseases is unclear and needs further researches, which is crucial for reducing cardiomyocyte death and broadening the treatment models of cardiovascular diseases. Liu et al. (77) found that self-assembly indocyanine green-Lecithin (ICG/LECI) can be used to enhance magnetic resonance/ photoacoustic (MR/PA) imaging and reduce iron toxicity, opening the way for personalized diagnosis and treatment for iron overload patients. FRGs specifically expressed in the peripheral blood of AMI patients also provided a new direction for early diagnosis of AMI. However, more attention needs to be paid to the development of testing methods suitable for routine clinical diagnosis of ferroptosis, and the introduction of biomarkers of ferroptosis characteristics is expected to provide helps for the early identification and diagnosis of cardiovascular disease.

In conclusion, ferroptosis plays a key role in the progression of cardiovascular diseases, and the roles of ferroptosis in cardiovascular diseases remain to be further studied. We can anticipate that diagnostic tools and therapeutic drugs based on ferroptosis will greatly help in the diagnosis and treatment of cardiovascular diseases in the future.

AUTHOR CONTRIBUTIONS

MS: conceived and designed the review. YuG, WZ, XZ, SZ, JWa, YiG, YL, HL, and JL: collected the literatures. YuG, WZ, and MS: wrote the manuscript. MS, JWu, and YC: reviewed and edited the manuscript. JWu: revised the manuscript and the language. All authors contributed to the article and approved the submitted version.

FUNDING

This study was supported by Hainan Science and Technology Project (ZDYF2020123, ZDYF2020027, and ZDKJ2019012), Hainan Province Clinical Medical Center, National Key R & D Plan (2020YFC2004706), and National Natural Science Foundation of China (Fund No. 81500202).

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers | Frontiers in Cardiovascular Medicine

ORIGINAL RESEARCH
published: 20 June 2022
doi: 10.3389/fcvm.2022.927768

Predictive Value of Non-high-Density Lipoprotein Cholesterol and Neutrophil-Lymphocyte Ratio for Coronary Artery Vulnerable Plaques in Type 2 Diabetes Mellitus

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Specialty section:
This article was submitted to Cardiovascular Therapeutics, a section of the journal Frontiers in Cardiovascular Medicine

Received: 25 April 2022
Accepted: 30 May 2022
Published: 20 June 2022

Citation:
Huang X, Yang S, Zhao Q, Chen X, Pan J, Lai S, Ouyang F, Deng L, Du Y, Li X, Hu Q, Guo B and Liu J (2022) Predictive Value of Non-high-Density Lipoprotein Cholesterol and Neutrophil-Lymphocyte Ratio for Coronary Artery Vulnerable Plaques in Type 2 Diabetes Mellitus. Front. Cardiovasc. Med. 9:927768. doi: 10.3389/fcvm.2022.927768

Background: Patients with diabetes have an increased risk of developing vulnerable plaques (VPs), in which dyslipidemia and chronic inflammation play important roles. Non-high-density lipoprotein cholesterol (non-HDL-C) and neutrophil-lymphocyte ratio (NLR) have emerged as potential markers of both coronary artery VPs and cardiovascular prognosis. This study aimed to investigate the predictive value of non-HDL-C and NLR for coronary artery VPs in patients with type 2 diabetes mellitus (T2DM).

Methods: We retrospectively enrolled 204 patients with T2DM who underwent coronary computed tomography angiography between January 2018 and June 2020. Clinical data including age, sex, hypertension, smoking, total cholesterol, low-density lipoprotein cholesterol, HDL-C, triglyceride, non-HDL-C, glycated hemoglobin, neutrophil count, lymphocyte count, NLR, and platelet count were analyzed. Multivariate logistic regression was used to estimate the association between non-HDL-C, NLR, and coronary artery VPs. Receiver operating curve analysis was performed to evaluate the value of non-HDL-C, NLR, and their combination in predicting coronary artery VPs.

Results: In our study, 67 patients (32.84%) were diagnosed with VPs, 75 (36.77%) with non-VP, and 62 (30.39%) with no plaque. Non-HDL-C and NLR were independent risk factors for coronary artery VPs in patients with T2DM. The areas under the ROC curve of non-HDL-C, NLR, and their combination were 0.748 [95% confidence interval (CI): 0.676–0.818], 0.729 (95% CI: 0.650–0.800), and 0.825 (95% CI: 0.757–0.887), respectively.

Conclusion: Either non-HDL-C or NLR could be used as a predictor of coronary artery VPs in patients with T2DM, but the predictive efficiency and sensitivity of their combination would be better.

Keywords: type 2 diabetes mellitus, coronary heart disease, coronary computed tomography angiography, vulnerable plaque, neutrophil-lymphocyte ratio, non-high-density lipoprotein cholesterol

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is one of the most prevalent chronic non-communicable diseases worldwide, with an estimated 415 million people aged 20–79 years having diabetes worldwide, according to 2017 data from the International Diabetes Federation (1, 2). Diabetic vascular diseases are the most common and serious chronic complications of diabetes, and coronary heart disease (CHD) is one of the leading causes of death in patients with T2DM. Recently, large scale meta-analyses showed that in individuals with mild elevated hyperglycemia, the risk of cardiovascular disease and worse prognosis was increased (3–6). The pathological basis of CHD is the presence of vulnerable plaques (VPs). When ruptured, they can cause secondary thrombosis, resulting in acute severe stenosis or occlusion of the lumen and ultimately resulting in clinical acute coronary events (7). Therefore, early identification of VPs is crucial to preventing cardiovascular events.

Atherosclerosis is a long-term chronic inflammatory process, novel inflammatory biomarkers may be useful for evaluation of the severity and prognosis of CHD (8, 9). Recently, the neutrophil-lymphocyte ratio (NLR) has been considered as a marker for chronic inflammation (10). NLR reflects the balance between neutrophils and lymphocytes in the body and can, therefore, relate to the systemic inflammatory response. Given its relationship with inflammation, NLR is considered a novel marker for the clinical prediction of cardiovascular events (11).

Patients with T2DM often present with mixed dyslipidemia, and low treatment compliance rate, which are risk factors for complicated CHD. Although lipid-lowering therapy can control the plasma low-density lipoprotein cholesterol (LDL-C) level in patients with CHD and reduce the risk of cardiovascular events, recent studies have shown that traditional lipid indexes such as LDL-C cannot fully reflect the actual scenario of lipid metabolism in CHD patients with T2DM. Therefore, novel and more specific lipid metrics related to cardiovascular disease pathology are needed to predict cardiovascular events better, and non-HDL-C is considered to play an essential role in the formation and development of coronary atherosclerosis.

Coronary artery computed tomography angiography (CCTA) can rapidly and accurately assess the degree of coronary artery stenosis and identify the morphology and components of coronary atherosclerotic plaques. VPs diagnosed by CCTA are highly consistent with pathology and have good predictive value for future cardiovascular events (12, 13), making their detection feasible in studies on the VPs of coronary atherosclerosis. Although a few previous studies have shown a close relationship between NLR or non-HDL-C and coronary artery VPs, atherosclerosis due to diabetes is different from atherosclerosis caused by other risk factors (14). Research on NLR or non-HDL-C and CCTA imaging in relation to T2DM is scarce.

Therefore, this study was conducted to explore whether the correlation between NLR, non-HDL-C, and VPs of coronary artery in patients with T2DM using CCTA. The possibility of using coronary artery VPs as a predictive marker of cardiovascular events in patients with T2DM was also explored.

MATERIALS AND METHODS

Patient Selection and Grouping

We retrospectively collected data from 213 patients diagnosed with T2DM and examined by CCTA in Shunde Hospital of Southern Medical University between January 2018 and June 2020. The inclusion criteria were as follows: (1) Patients who met the 2017 American Diabetes Association diagnostic criteria (15); (2) Patients who were diagnosed with T2DM more than 5 years ago; (3) Patients who received CCTA and completed the VPs assessment; (4) Patients with complete clinical data. The exclusion criteria were as follows: (1) Patients who were diagnosed with type 1 diabetes or other types of diabetes or acute complications of diabetes; (2) Patients with acute infection; (3) Patients with severe cardiac insufficiency, arrhythmia, and acute myocardial infarction; (4) Patients with severe valvular heart disease, cardiomyopathy, rheumatic heart disease, congenital heart disease, severe liver insufficiency (Child-Pugh class C, or Alanine aminotransferase, ALT$>$250 U/L, or Total bilirubin, TBil$>$115 μ mol/L), severe renal insufficiency (glomerular filtration rate, RGF$<$30 mL/min), and malignant tumors; (5) Patients with poor quality of CCTA image that was insufficient for further analysis. According to plaque and plaque vulnerability, patients were divided into the no plaque group, non-vulnerable plaque group, and VP group. All patients provided written informed consent, and the local ethics committee approved the study.

Scanning Protocol

All patients were scanned by a dual-source CT (SOMATOM Definition Flash, Siemens, Germany). Contrast-enhanced CT imaging was performed after 40 s delay following intravenous administration of 70 mL of iodinated contrast material (Ultravist 350, Bayer Schering Pharma, Berlin, Germany) at a rate of 5.0 mL/s with a pump injector (Ulrich CT Plus 150, Ulrich Medical, Ulm, Germany) after routine pre-contrast CT, followed by infusion of 50 mL of saline at the same infusion rate. The parameters were as follows: 120 kV, 90 kV; 320 mAs; rotation time, 0.33 s; detector collimation: 32 mm × 2 mm × 0.6 mm; pitch = 0.20–0.28 mm (automatic adjustment according to heart rate changes); slice thickness = 0.75 mm; slice gap = 0.5 mm; field of view (FOV) = 260 × 260 mm; matrix = 512 × 512.

Imaging Processing and Analysis

All CCTA scans were evaluated for the presence of non-evaluable segments. According to the American Heart Association classification, coronary arteries were divided into 16 segments (16). Coronary plaques were defined as structures of at least 2 mm^2 areas within and/or adjacent to the artery lumen, clearly distinguishable from the vessel lumen, and surrounded by pericardial tissue.

A Siemens post-processing workstation (Syngo.Via VB10, Siemens, Germany) was used to reconstruct coronary arteries for all patients. Maximum intensity projection, curved planar reformation (CRP), volume rendering, and other post-processing methods were used to analyze the images by radiologists. The

TABLE 1 | Baseline clinical characteristics for T2DM patients.

The patients' baseline characteristics				
Characteristics	**Vulnerable plaque (*n* = 67)**	**Non-vulnerable plaque (*n* = 75)**	**No plaque (*n* = 62)**	***P***
Age, mean ± *SD*, years	65.3 ± 8.8a	66.0 ± 9.2a	60.3 ± 10.5	0.001
Sex, no. (%)				
Male	37 (35.92)	37 (35.92)	29 (28.16)	0.612
Female	30 (29.71)	38 (37.62)	33 (32.67)	
Hypertension, no. (%)	52 (34.90)	60 (40.27)[a]	37 (24.83)	0.017
Smoking, no. (%)	13 (52.00)	6 (24.00)	6 (24.00)	0.089
Total cholesterol (mg/dl)	208.7 ± 38.7[ab]	189.4 ± 46.4	174.0 ± 46.4	<0.001
LDL-C (mg/dl)	123.7 ± 34.8[ab]	104.4 ± 34.0	92.7 ± 31.3	<0.001
HDL-C (mg/dl)	47.9 ± 10.4[a]	52.2 ± 31.3	57.2 ± 13.1	<0.001
Triglycerides (mg/dl)	184.3 ± 108.9[b]	134.6 ± 78.9	147.0 ± 92.1	0.006
Non-HDL-C (mg/dl)	160.8 ± 39.5[ab]	137.2 ± 39.6	116.8 ± 34.6	<0.001
HbA1c (%), mean ± *SD*	6.7 ± 1.4	6.9 ± 0.8	6.5 ± 0.8	0.063
Neutrophil ($10^3/\mu l$)	4.89 ± 1.83[ab]	4.21 ± 1.24	4.00 ± 1.63	0.003
Lymphocyte ($10^3/\mu l$)	1.92 ± 0.53	1.93 ± 0.65	1.99 ± 0.59	0.764
NLR (%), mean±*SD*	3.06 ± 1.36	2.24 ± 1.15	2.01 ± 0.79	<0.001
Platelet ($10^3/\mu l$)	205.20 ± 56.31[ab]	210.23 ± 67.60	211.29 ± 69.04	0.846
FBG (mmol/L)	7.44 ± 2.29	6.88 ± 2.41	6.46 ± 2.02	0.108
Insulin (*n*, %)	17 (25.4%)	17 (22.7%)	20 (32.3%)	0.213
OADs (*n*, %)	26 (38.8%)	32 (42.7%)	36 (58.1%)	0.068
Statins (*n*, %)	39 (58.2%)[b]	42 (56.0%)[b]	44 (71.0%)	0.165

[a] *P means compared with non-plaque group.*
[b] *P means compared with the non-vulnerable plaque group.*
NLR, neutrophil-lymphocyte ratio; SD, standard deviation; FBG, fasting blood glucose.

TABLE 2 | Multifactorial logistic regression analysis of coronary artery vulnerable plaques in T2DM patients.

Variables	B	S.E	Wald X^2	*P*	OR	95% CI
Multivariable analysis						
Age	0.038	0.021	3.32	0.068	1.039	0.997–1.082
Hypertension	0.361	0.451	0.64	0.424	1.435	0.592–3.476
Total cholesterol	0.029	0.336	0.007	0.932	1.029	0.533–1.987
LDL-C	0.072	0.4	0.032	0.857	1.075	0.491–2.354
HDL-C	−1.046	0.678	2.382	0.123	0.351	0.093–1.327
Triglycerides	0.104	0.194	0.288	0.592	1.110	0.758–1.625
Non-HDL-C	0.916	0.326	7.908	0.005	2.500	1.32–4.735
Neutrophil	0.153	0.138	1.217	0.27	1.165	0.888–1.528
NLR	0.692	0.191	13.077	<0.001	1.998	1.373–2.907

NLR, neutrophil-lymphocyte ratio; OR, odds ratio; CI, confidence interval.

imaging features of VPs include spotty calcification, positive reconstruction, low attenuation plaque, and napkin ring sign (NRS) (17). CCTA imaging data were analyzed and evaluated independently by two radiologists with more than 10 years of experience in cardiovascular disease imaging. Disagreements between the radiologists were resolved by consensus and, if necessary, by consultation with a third radiologist.

Clinical Characteristics

The following clinical characteristics were determined: age, sex, height, weight, hyperlipidemia history, hypertension history (blood pressure > 140/90 mmHg), smoking history, and drinking history. Blood samples were obtained from fasting venous blood for 8–12 h on the next morning. An automated blood cell counter (XE-2100, Sysmex, Kobe, Japan) was used for analysis according to the instructions, and NLR was calculated from the results. Biochemical indexes including total cholesterol (TC), TG, LDL-C, high-density lipoprotein cholesterol (HDL-C), and HbA1c (%) were measured using an automatic biochemical analyzer (Cobas-8000, Roche, Basel, Switzerland). Non-HDL-C was calculated by the formula (18): Non-HDL-C = TC–LDL-C. Two independent radiologists retrospectively reviewed the clinical characteristics with more than 10 years of experience in cardiovascular disease imaging.

Statistical Analysis

SPSS26.0 and R software 3.60[1] were used for statistical analysis. The Shapiro-Wilk test was used to ascertain the normality of the measurement data, which are expressed as mean ± standard deviation (X ± S). One-way ANOVA was used for statistical analysis of variance, and the Bonferroni correction method was used to compare the three groups. Statistical data are expressed as percentages, and statistical analysis was conducted using the χ^2-test/Fisher's exact test. Independent risk factors for VPs were obtained by univariate and multivariate logistic regression. The receiver operating characteristic (ROC) curve

[1] https://www.r-project.org/

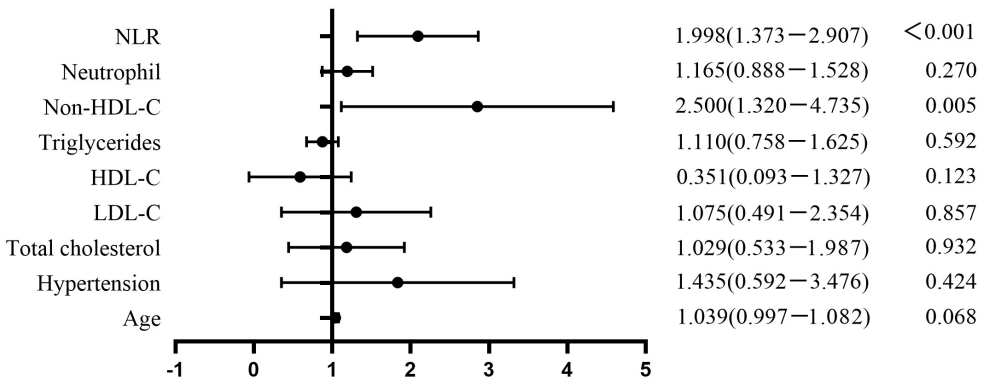

FIGURE 1 | Forest plots show the comparison of risk factors for coronary artery vulnerable plaques in T2DM patients. Non-HDL-C (OR 2.500, 95%CI: 1.32–4.735) and NLR (OR 1.998, 95%CI: 1.373–2.907) were independent risk factors for coronary artery vulnerable plaques.

and the area under the ROC curve (AUC) were used to evaluate the predictive value. A P-value < 0.05 was considered to indicate statistical significance.

RESULTS

Patients and Clinical Characteristics

After excluding nine patients (four due to missing image data, three due to allergy to contrast agent, and two due to phobia at the time of examination), 204 patients diagnosed with T2DMwere selected (mean age 63.1 ± 9.8 years; range, 34–85 years), and 103 (50.49%) were males. VPs was seen in 67 patients (32.84%), non-VP in 75 (36.77%), and no plaque in 62 patients (30.39%). Univariate analysis showed significant differences in age, hypertension, TC, LDL-C, HDL-C, TG, non-HDL-C, neutrophil count, and NLR among the three groups. Comparisons of patient clinical characteristics between the three groups are shown in **Table 1**.

Risk Factors for Vulnerable Plaques

Multivariate logistic regression analysis was performed considering indexes found significant in the univariate analysis as independent variables. These included age, hypertension, TC, LDL-C, HDL-C, TG, non-HDL-C, neutrophil count, and NLR. Non-HDL-C [odds ratio (OR): 2.500, 95% CI: 1.32–4.735] and NLR (OR: 1.998, 95% CI: 1.373–2.907) were independent risk factors for VPs (**Table 2** and **Figure 1**).

Diagnostic Performance of Different Lipid Indexes for Vulnerable Plaques

Table 3 and **Figure 2** show the ROC curves of non-HDL-C, NLR, and their combination in predicting coronary artery VPs in patients with T2DM. Non-HDL-C combined with NLR achieved the highest performance, with AUC 0.825 (95% CI: 0.757–0.887), sensitivity 82.1%, and specificity 70.8%, followed by non-HDL-C, with AUC 0.748 (95% CI: 0.676–0.818), sensitivity 0.701, and specificity 0.708, and NLR, with AUC 0.729 (95% CI: 0.650–0.800, sensitivity 0.776, and specificity 0.577).

DISCUSSION

We identified two risk factors associated with coronary artery VPs in patients with T2DM: non-HDL-C concentration and NLR. HDL-C is an independent protective factor. Non-HDL-C combined with NLR achieved the best predictive performance, with an AUC 0.825 (95% CI: 0.757–0.887). Non-HDL-C and NLR have been shown to be of clinical value in predicting coronary artery VPs in patients with T2DM.

Epidemiological statistics have shown that CHD is the main cause of death among cardiovascular diseases. However, its pathogenesis is not completely understood, and the most common causes are endothelial cell injury, inflammatory reaction, hemodynamic changes, lipid metabolism disorder, immune factors, and genetic factors. DM is a risk factor for CHD. The incidence and mortality of cardiovascular events in patients with diabetes are much higher than those in the general population. Hyperglycemia can directly damage the intima layer of blood vessels, resulting in the deposition of LDL-C and other lipid substances in the intima and the activation of various inflammatory cells. Subsequently, neutrophils secrete inflammatory mediators such as interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α), which attract smooth muscle cells and macrophages for phagocytosis, resulting in vascular endothelial dysfunction (19, 20), and anti-inflammatory medicine or cytokines can improved the vascular endothelial dysfunction and decreased the risk of CHD (21, 22). Atherosclerotic plaques gradually form on the vascular wall. In the United States, about 25% of patients with CHD over 35 years old develop complications of diabetes (23). Patients with diabetes carry a 2–4 times greater risk of CHD than patients without diabetes, and about 75% of deaths among patients with diabetes are caused by coronary artery ischemia (24, 25). Esposito et al. observed predominant VPs in patients with diabetes

TABLE 3 | Predictive value of different lipid indicators for vulnerable plaques.

	Accuracy	Sensitivity	Specificity	*P*	F1_score	AUC (95% CI)
Non-HDL-C+NLR	0.745	0.821	0.708	0.003[a]	0.679	0.825 (0.757–0.887)
Non-HDL-C	0.706	0.701	0.708	0.697[b]	0.61	0.748 (0.676–0.818)
NLR	0.642	0.776	0.577	0.001[c]	0.588	0.729 (0.650–0.800)

NLR, neutrophil-lymphocyte ratio; AUC, area under the curve; CI, confidence interval.
[a]*P means compared with Non-HDL-C.*
[b]*P means compared with NLR.*
[c]*P means compared with Non-HDL-C+NLR.*

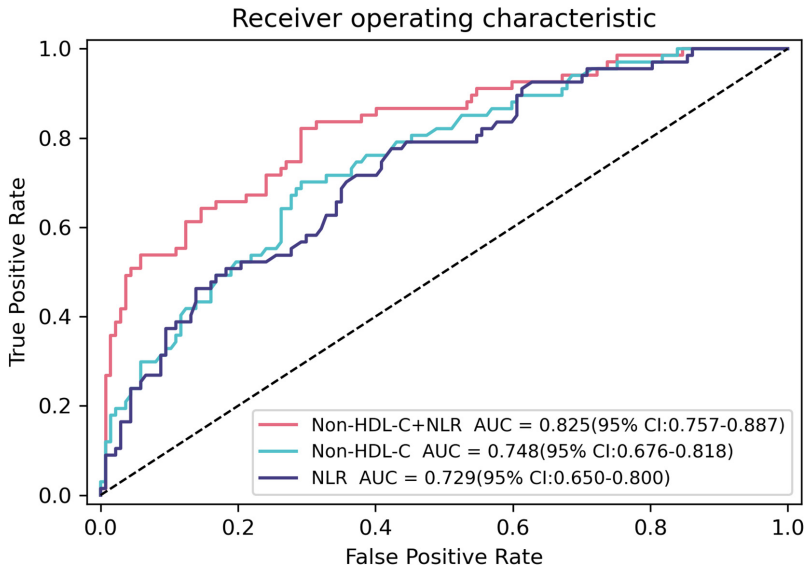

FIGURE 2 | Receiver operating characteristic curves for predicting coronary artery vulnerable plaques using baseline Non-HDL-C, NLR, and their combination in T2DM patients. The AUC of Non-HDL-C, NLR, and their combination were 0.748 (95% CI: 0.676–0.818), 0.729 (95% CI: 0.650–0.800), and 0.825 (95% CI: 0.757–0.887), respectively.

(26). They concluded that T2DM influences the type and stability of atherosclerotic plaques, and patients with T2DM might have atherosclerotic patterns different from those seen in patients without diabetes. Patients with diabetes mainly have elevated TG, low HDL-C, and insulin resistance (IR), which promotes inflammatory vascular injury and induces the occurrence and development of CHD (27). On the one hand, the decrease in HDL-C levels will undoubtedly lead to an increase in non-HDL-C levels, reverse the reduction of the TC effect, and increase the risk of VPs in patients with diabetes. On the other hand, the increase in TG leads to an increase in TRL, which is closely related to coronary artery calcification (28). Therefore, non-HDL-C should be added to routine lipid assessment to evaluate coronary atherosclerosis (28).

In our study, univariate analysis showed that age, hypertension, TC, LDL-C, HDL-C, TG, non-HDL-C, neutrophil, and NLR showed statistically significant differences, but only non-HDL-C and NLR showed statistically significant differences among the three groups in multivariate analysis ($P < 0.05$). Non-HDL-C (OR: 2.500, 95%:1.32–4.735) and NLR (OR: 1.998, 95%:1.373–2.907) are significant risk factors for coronary artery VPs in patients with T2DM. Lowering serum LDL-C levels can delay the progression of atherosclerotic plaque formation and induce plaque regression (29). However, some patients with high residual cardiovascular risk, such as diabetes, metabolic syndrome, and obesity, carry a significantly increased risk of plaque formation (30). By focusing only on LDL-C levels, those at high risk for T2DM may be overlooked, increasing the risk of CHD in these patients. Currently, European guidelines for dyslipidemia management recommend that the main target of lipid regulation in patients with T2DM is LDL-C<2.6 mmol/L, and the secondary target is non-HDL-C<3.4 mmol/L (31). Non-HDL-C is a better risk estimation indicator than LDL-C, particularly in patients with T2DM, higher levels of TG, and metabolic syndrome (32). Wu et al. found that non-HDL-C was a risk factor for VPs (33). Their results indicated that VPs should be assessed more carefully in patients with high levels of non-HDL-C so that atherosclerotic cardiovascular disease events can be prevented, aligning well with our research.

NLR was found to be another risk factor for coronary artery VPs in patients with T2DM. However, not many studies on NLR and coronary artery VPs, especially in patients with T2DM, have been conducted. A chronic inflammatory response

is closely related to diabetic macrovascular lesions (34), and inflammatory response plays an essential role in the formation and rupture of VPs. In existing plaques, neutrophils gather around new or damaged plaques, promoting the release of inflammatory cytokines and activating monocytes to transform them into macrophages. This, in turn, accelerates the formation and shedding of new plaques. VPs in the coronary artery can rupture and lead to thrombosis, which can cause acute coronary syndrome (35). Under inflammatory conditions in patients with T2DM, the number of CD8+T lymphocytes is reduced, accompanied by the imbalance in lymphocyte function and subpopulation ratio, thereby decreasing immunity. Thus, chronic inflammation persists. Chronic low-grade inflammation eventually leads to IR and insulin secretion dysfunction, promoting the occurrence of T2DM and its complications (36). NLR is relatively constant compared to absolute counts of neutrophils and lymphocytes, reflecting a balance between inflammatory activators and inflammatory regulators (37). Yun et al. found that CAD patients with a high NLR are at a higher risk of developing VPs and extensive inflammation, leading to acute coronary events (38). They suggested that NLR can be used as a valuable tool to detect significant atherosclerosis and VPs in patients with CAD. Therefore, the early monitoring of NLR can directly affect the occurrence and development of coronary artery events in patients with T2DM, especially the occurrence and development of coronary artery VPs.

The ROC curve results of this study showed that the AUCs of non-HDL-C and NLR for predicting coronary VPs in patients with T2DM were 0.748 and 0.729, respectively, and the AUC of the combination of the two parameters for predicting coronary artery VPs in patients with T2DM was 0.825. The predictive value of non-HDL-C combined with NLR was significantly higher than that achieved when using non-HDL-C or NLR. Therefore, the detection of non-HDL-C and NLR in CAD patients with T2DM facilitates the evaluation of the vulnerability of plaques, thereby improving the prognosis and quality of life of patients.

This study was subject to several limitations. First, this was a retrospective study performed at a single center. Second, this study does not investigate the relationship between different VP types and non-HDL-C and NLR in patients with T2DM and CHD. Finally, a prospective study should be performed to validate the findings.

CONCLUSION

In conclusion, this study demonstrates that elevated serum non-HDL-C and NLR were independent risk factors for coronary artery VPs in patients with T2DM, and elevated HDL-C is an independent protective factor. Both NLR and non-HDL-C can be used to predict the development of VPs in patients with T2DM, and their combination achieves better predictive efficacy, with higher sensitivity and accuracy, providing a reference basis for the early diagnosis and treatment of VPs of the diabetic coronary artery.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Medical Ethics Committee of Shunde Hospital, Southern Medical University. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

XH, SY, JL, BG, FO, and QH: conception and design. XH, QZ, XC, JP, SL, and FO: acquisition of data. XH, SY, BG, FO, LD, YD, XL, and QH: analysis and interpretation of data. XH, SY, BG, and JL: drafting or revising the article. All authors contributed to the article and approved the submitted version.

FUNDING

This research was supported by grants of Foshan Self-Funded Science and Technology project (2020001005216, 2017AB003623, and 2017AB003683), the Scientific Research Foundation for the Younger researchers of Shunde Hospital (SRSP2018010), the Scientific Research Foundation for the Younger researchers of Southern Medical University (PY2018N116), and Guangdong Medical Science and Technology Research Fund (A2020395, A2020089, and A2021483).

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frontiers | Frontiers in Cardiovascular Medicine

ORIGINAL RESEARCH
published: 12 July 2022
doi: 10.3389/fcvm.2022.949113

Metformin Protects Cardiovascular Health in People With Diabetes

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OPEN ACCESS

Edited by:
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Southern Medical University, China

Reviewed by:
Zhongheng Zhang,
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Specialty section:
This article was submitted to Cardiovascular Therapeutics, a section of the journal Frontiers in Cardiovascular Medicine

Received: 20 May 2022
Accepted: 20 June 2022
Published: 12 July 2022

Citation:
Chen C, Yuan S, Zhao X, Qiao M, Li S, He N, Huang L and Lyu J (2022) Metformin Protects Cardiovascular Health in People With Diabetes. Front. Cardiovasc. Med. 9:949113. doi: 10.3389/fcvm.2022.949113

Background: Metformin is the most commonly used drug for patients with diabetes, but there is still some controversy about whether it has a protective effect on cardiovascular health. We therefore used the National Health and Nutritional Examination Survey (NHANES) database to analyze the impact of metformin use on cardiovascular health in patients with diabetes.

Methods: We extracted the demographic data and laboratory test results of all people with diabetes in the NHANES database from January 2017 to March 2020. The outcomes were seven indicators of cardiovascular health from the American Heart Association, each was scored as 0, 1, and 2 to represent poor, moderate, and ideal health statuses, respectively. The scores for the indicators (excluding diet and glycemic status) were summed, and the sum score was then considered to indicate unhealthy (0–5) or healthy (>5). Multivariate logistic regression analysis was used, and subgroup analyses were performed by age, alcohol consumption, education, and marital status.

Results: This study included 1,356 patients with diabetes, among which 606 were taking metformin. After adjusting for all included variables, oral metformin in patients with diabetes had a protective effect on the cardiovascular health of patients (OR = 0.724, 95% CI = 0.573–0.913, $P = 0.007$). Subgroup analysis indicated that metformin protects the cardiovascular health of people with diabetes more clearly in those who are young (OR = 0.655, 95% CI = 0.481–0.892, $P = 0.007$), married (OR = 0.633, 95% CI = 0.463–0.863, $P = 0.003$), and drink alcohol (OR = 0.742, 95% CI = 0.581–0.946, $P = 0.016$).

Conclusion: This study found that metformin has a protective effect on the cardiovascular health of patients with diabetes. The study findings support the general applicability of metformin.

Keywords: metformin, cardiovascular health, logistic regression, NHANES, subgroup analysis

BACKGROUND

Diabetes is a chronic disease caused by insufficient insulin secretion or by insulin utilization dysfunction in the body. As the course of the disease prolongs, long-term hyperglycemia toxicity will have adverse effects on other tissues and organs, thereby causing corresponding complications. Blood vessels are important target organs of diabetes, which can lead to cardiovascular disease if damaged. It is one of the ten most common causes of death worldwide, and in 2017 about 425 million people had diabetes worldwide (1, 2), thereby seriously affecting human health.

Metformin is currently the first-choice antiglycemic drug for patients with diabetes (3). Its mechanism of action is mostly to restore adenylyl cyclase inhibition using insulin through the G proteins of the liver cell membrane, reduce hepatic gluconeogenesis and hepatic glucose output, promote anaerobic glycolysis, increase the uptake and utilization of glucose by tissues such as skeletal muscle, inhibit or delay glucose absorption in the gastrointestinal tract, and improve glucose metabolism. It has also been recently found that metformin can increase the GLP-1 concentration in blood, and increase insulin sensitivity. Studies of metformin have also recently found that it can increase fibrinolysis and improve blood lipid concentrations, and plays a pivotal role in patients with diabetes.

Cardiovascular mortality has declined recently. However, research indicates that in 2019, the number of cardiovascular deaths worldwide reached 18.6 million, and it is still the most common cause of death worldwide (4). Studies have found that diabetes is an important risk factor for cardiovascular disease and death (5). It is therefore particularly important to pay attention to the cardiovascular health of patients with diabetes.

The cardiovascular effects of metformin in patients with diabetes have been controversial (6, 7). We therefore extracted the medication status of patients with diabetes from the National Health and Nutritional Examination Survey (NHANES) database to study the impact of the metformin on the patient's cardiovascular health, with the aim of improving to the cardiovascular health among people with diabetes.

METHODS

Data Sources

The NHANES is a nutritional status study program of all populations in the United States that combines interviews and physical examinations (8). The survey annually examines a nationally representative sample of 5,000 people, with interviews covering demographics, socioeconomics, diet, and health-related questions, including medical, dental, and physiological measurements, as well as laboratory measurements by medical professionals (9). Importantly, the NHANES project information and its survey data are updated on the website in a timely manner and are freely available to the public.

Research Variables

This study included subject data from March 2017 to March 2020, in which patients with diabetes were identified based on questionnaires and laboratory tests that included (1) providing information on the presence of diabetes in the questionnaire, and (2) a glycated hemoglobin level of 6.5% and fasting blood glucose at ≥126 mg/dL (10). The main parameters included in the study were age, sex, race, marital status, education, BMI, physical activity, smoking and alcohol statuses, metformin use, and related laboratory indicators.

Cardiovascular index	poor	Intermediate	Ideal
Current smoking	yes	Former≤12 mouth	Never or quit>12 mouth
Body mass index	≥30 kg/m2	25–29.9 kg/m2	<25 kg/m2
Physical activity	None	1–149 min/wk moderate intensityor 1–74 min/wk vigorousintensity or 1–149 min/wkModerate+vigorous	≥150 min/wk moderateintensity or≥75min/wk vigorousintensity or≥150min/wkModerate+vigorous
Total cholesterol	≥240 mg/dL	200–239 mg/dL	?200 mg/dL
Blood pressure	SBP≥140 orDBP≥90 mm Hg	SBP 120–139 or DBP80–89 mm Hg	<120/<80 mm Hg

	Unhealthy	Healthy
Cardiovascular Score Category	0-5	≥5

FIGURE 1 | Cardiovascular health definition.

The outcome variable was cardiovascular health, the definition of the latest cardiovascular health is not just for diseases. Health is a more extensive concept, and it should also include the body, psychological and social functions, and other components. Therefore, we consider using cardiovascular health more than using cardiovascular disease alone. It is measured by 7 indicators to measure cardiovascular health. It is measured by 7 indicators (smoking, BMI, physical activity, empty blood sugar, blood pressure, cholesterol, diet) (11). Since there is no dietary data for 19–20 years in the NHANES database, and our study target was on patients with diabetes, we used the remaining five indicators to define cardiovascular health (**Figure 1**). According to the definition of the AHA (American Heart Association), each index of cardiovascular health is scored as ideal, moderate, or poor. We assigned a value of 0 for poor, 1 for moderate, and 2 for ideal, giving a total score of 0–10. Total scores of ≥5 and 0–5 were used to indicate healthy and unhealthy cardiovascular fitness, respectively.

The race, marital status, and education of the subjects were classified by the data codes in the database. Smoking status, physical activity, blood pressure, BMI, and total cholesterol were all classified as defined in **Figure 1**. Alcohol drinkers were defined as consuming at least 12 alcohol intake per year. Laboratory data are presented as continuous variables.

Statistical Analysis

Continuous and categorical variables of baseline data were presented as mean ± standard-deviation values and count and percentage values, respectively. Multivariate logistic regression was applied to metformin use in patients with diabetes to analyze its relationship with cardiovascular health outcomes. The logical regression model has adjusted the age, gender, race, marriage, education level, drinking, and some laboratory data. To further test this relationship, we performed subgroup analyses by age, marital status, and alcohol use, and introduced interaction effects using the Wald test.

All statistical analyses in the article were performed using R software, all tests were two-sided, and the significance cutoff was $P = 0.05$.

TABLE 1 | Baseline information.

Variable	No-Metformin	Metformin	*P*
Total	749	606	
Sex			0.117
Male	397 (53)	347 (57.3)	
Female	352 (47)	259 (42.7)	
Age	62 (51.71)	63 (55.71)	0.029
Age			0.066
<65	450 (60.1)	334 (55.1)	
≥65	299 (39.9)	272 (44.9)	
Race			<0.001
Mexican American	85 (11.3)	92 (15.2)	
Other hispanic	80 (10.7)	62 (10.2)	
Non-hispanic white	264 (35.2)	179 (29.5)	
Non-hispanic black	226 (30.2)	157 (25.9)	
Other Race	94 (12.6)	116 (19.1)	
Education level			0.384
< 9th grade	71 (9.5)	75 (12.4)	
9–11th grade	99 (13.2)	72 (11.9)	
High school graduate	203 (27.1)	149 (24.6)	
Some college or AA degree	244 (32.6)	196 (32.3)	
College graduate or above	132 (17.6)	114 (18.8)	
Marital status			0.032
Married/living with partner	428 (57.1)	386 (63.7)	
Widowed/divorced/separated	232 (31)	167 (27.6)	
Never married	89 (11.9)	53 (8.7)	
Drinking			0.243
Yes	672 (89.7)	555 (91.6)	
No	77 (10.3)	51 (8.4)	
Albumin	3.9 (3.7, 4.2)	4 (3.8, 4.2)	<0.001
Haematuria	16 (12, 20)	15 (12, 20)	0.269
Serum creatinine	0.9 (0.7, 1.2)	0.9 (0.7, 1)	<0.001
Glucose	122 (102, 156)	126 (103, 162)	0.257
Blood urea nitrogen	0.4 (0.3, 0.5)	0.4 (0.3, 0.6)	0.824
Total protein	7.1 (6.8, 7.4)	7.1 (6.8, 7.5)	0.78
Uric acid	0.6 (4.6, 6.7)	5.5 (4.6, 6.8)	0.799

RESULTS

Table 1 lists the baseline data of all subjects according to whether or not they took metformin. This study included 1,355 subjects, and similar numbers of people took or did not take metformin. There were slightly more males than females, and the mean age was around 62 years. The table also lists demographic data such as race and marital status, as well as related laboratory indicators.

Figure 2 is a forest diagram of the relationship between metformin use and cardiovascular disease in patients with diabetes. A logical regression model that adjusts all variables (including age, gender, race, marriage, education level, albumin, hematuria, serum creatinine, glucose, blood urea nitrogen, total protein, uric acid) to determine the effect of metformin use on cardiovascular disease in patients with diabetes. Results show that taking metformin is related to the protection of cardiovascular health in patients (OR: 0.724; 95%CI: 0.573, 0.913; $P = 0.007$).

Figure 3 shows the results of a subgroup analysis that further verified the effect of metformin on cardiovascular health in people with diabetes. After adjusting for all confounding factors, it can be seen that there were significant differences between the young (OR = 0.651, 95% CI = 0.478–0.886, $P = 0.006$), married (OR = 0.628, 95% CI = 0.459–0856, $P = 0.003$), and alcohol-drinking (OR = 0.750, 95% CI = 0.587–0.967, $P = 0.020$) populations.

We also independently analyzed the interactions of metformin with age, marital status, and alcohol consumption, which were all not significant ($P = 0.104$, $P = 0.238$, and $P = 0.311$, respectively).

DISCUSSION

The study data of patients with diabetes from 2017 to 2020 were extracted from the NHANES database

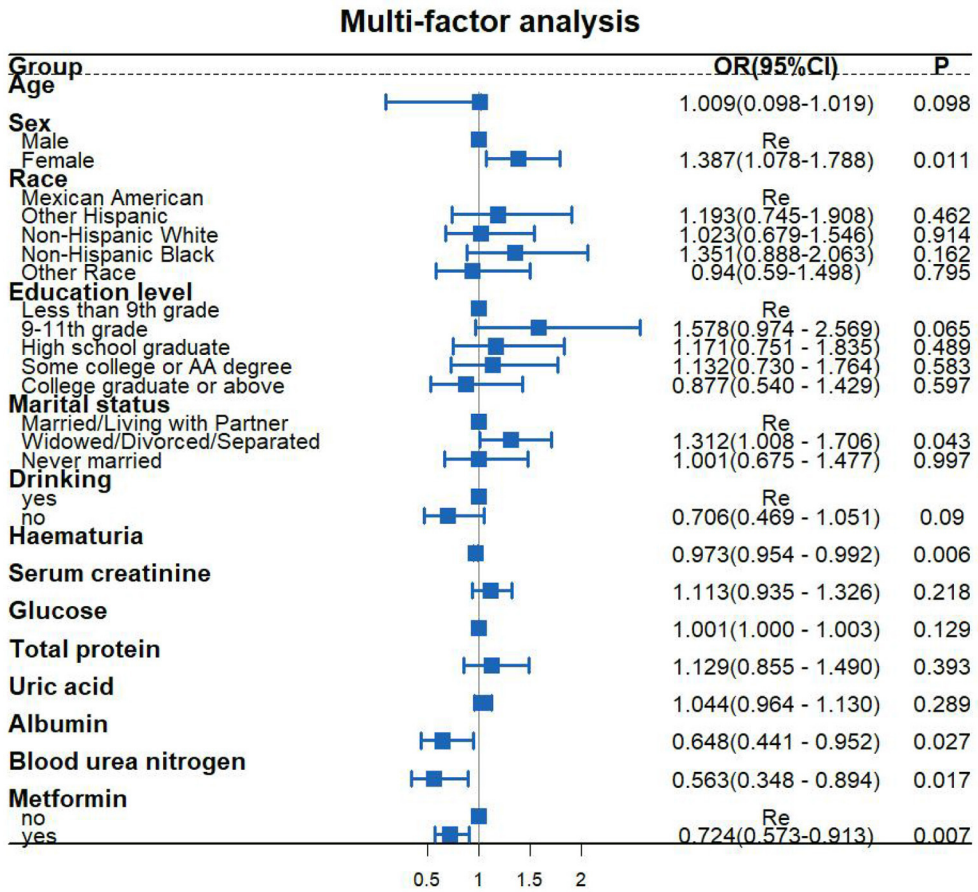

FIGURE 2 | Multi-factor analysis.

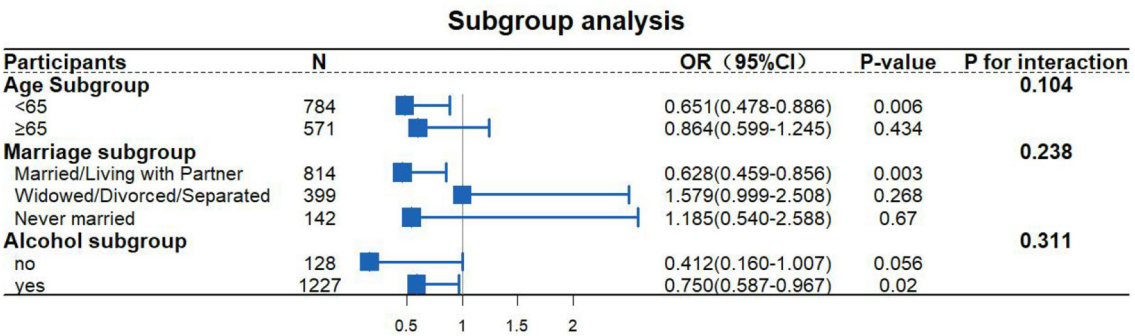

FIGURE 3 | Subgroup analysis.

to explore researchers' use of metformin affects their own cardiovascular health. Our results indicated that metformin patients taking metformin are related to better cardiovascular protection, and the results in the subsequent subgroup analyses were more prominent in the younger, married, alcohol-drinking, and highly educated populations.

Studies have found that people with diabetes often also experience cardiovascular events (12, 13). It is therefore of great significance to study the effect of diabetes treatment on cardiovascular events (14). Metformin is the drug of choice for the clinical treatment of patients with diabetes (15). However, recent research findings on the relationship between metformin and cardiovascular events have been controversial (6, 7). The

present study found that metformin has a significant protective effect on cardiovascular health. There have been previous studies on metformin use with cardiovascular disease or death as outcomes, but few studies have included cardiovascular health as an outcome. Our study therefore analyzed the relationship between metformin use and cardiovascular disease in patients with diabetes in greater depth.

Several previous prospective randomized controlled trials (16–18) have provided strong evidence for the cardiovascular protection of metformin. However, there is no exact description of the protective mechanism of metformin on the cardiovascular system. In the literature, metformin is considered to reduce the transformation of monocytes to macrophages and the formation of endothelial activation markers (19, 20), both of which are early events in atherosclerosis. Body weight and the fat distribution are also risk factors for cardiovascular health, as found by a study comparing metformin with placebo (21). Metformin can significantly reduce the body weight, and there is also evidence that metformin induces modest changes in blood lipid levels, especially in cholesterol, and triglyceride regulation (22, 23). Long-term maintenance of high blood sugar levels in the body may cause sugars to stick to cellular proteins (24). Metformin promotes a combination of oxidative stress and inflammation, a process called sugar oxidation, which is also responsible for diabetes complications, and metformin neutralizes the intermediates (25) to inhibit the glucose oxidation process, while reducing the occurrence of cardiovascular events (26).

To further demonstrate the protective effect of metformin on cardiovascular events, we performed subgroup analyses by age, marital status, and alcohol use. We found that metformin protects cardiovascular health more significantly in younger populations, mostly because older populations can have greater physiological decline and arterial stiffness, often accompanied by complications such as atherosclerosis and stroke (27, 28), which greatly impact cardiovascular health (29). These may be factors affecting metformin expression in the cardiovascular health of elderly patients with diabetes. Unsatisfactory social relationships can lead to poor habits, in turn leading to the occurrence of psychological and physical diseases (30). A recent prospective study found that loneliness can also have a great impact on cardiovascular health, including in people who are divorced or widowed (31), thereby affecting metformin expression in cardiovascular health. Our subgroup analysis of alcohol use found the relationship to be more meaningful in people who drink alcohol, but because most of the included subjects consumed alcohol, the results for people who did not drink alcohol could be erroneous. Moderate alcohol drinking has positive effects on cardiovascular health (32, 33), and taking metformin has a protective effect on cardiovascular health in people who drink alcohol. It can therefore be speculated that moderate alcohol drinking in patients with diabetes and taking metformin can have complementary effects on cardiovascular health.

Strengths of this study included (1) the subjects being randomly invited to participate in the NHANES, which is a very nationally representative population, and (2) the cardiovascular health scoring system being based on the AHA definition, which ensures the reliability of the study. The study also had some limitations. Because it had a cross-sectional design, we cannot accurately infer the causal relationship between metformin and cardiovascular health. Due to the restrictions of the NHANES database, we cannot analyze the combination of other anti -sugar drugs and metformin drugs. Also, cardiovascular health may be affected by many other factors, and so bias may have been present in the research results.

CONCLUSION

This study found that metformin patients taking metformin are related to better cardiovascular protection. This protective effect was more pronounced in people who were younger, married, and drank alcohol. Our findings may therefore further support the use of metformin by people with diabetes.

DATA AVAILABILITY STATEMENT

Publicly available datasets were analyzed in this study. This data can be found here: (Nhanes) https://www.cdc.gov/nchs/nhanes/index.htm.

ETHICS STATEMENT

Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

JL conceptualized the research aims and planned the analyses. CC and SY guided the literature review and wrote the first draft of the paper and the other authors provided comments and approved the final manuscript. XZ and MQ extracted the data from the NHANES database. XZ, SL, NH, LH, and MQ participated in data analysis and interpretation. All authors contributed to the article and approved the submitted version.

FUNDING

This study was supported by Guangdong Provincial Key Laboratory of Traditional Chinese Medicine Informatization (2021B1212040007).

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers | Frontiers in Cardiovascular Medicine

TYPE Original Research
PUBLISHED 19 July 2022
DOI 10.3389/fcvm.2022.948924

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Cardiovascular Therapeutics, a section of the journal Frontiers in Cardiovascular Medicine

RECEIVED 20 May 2022
ACCEPTED 27 June 2022
PUBLISHED 19 July 2022

CITATION
Huang X, Zhang L, Xu M, Yuan S, Ye Y, Huang T, Yin H and Lyu J (2022) Anti-embolism devices therapy to improve the ICU mortality rate of patients with acute myocardial infarction and type II diabetes mellitus. *Front. Cardiovasc. Med.* 9:948924. doi: 10.3389/fcvm.2022.948924

Anti-embolism devices therapy to improve the ICU mortality rate of patients with acute myocardial infarction and type II diabetes mellitus

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Background: Anti-Embolism (AE) devices therapy is an additional antithrombotic treatment that is effective in many venous diseases, but the correlations between this medical compression therapy and cardiovascular arterial disease or comorbid diabetes mellitus (DM) are still controversial. In this study we investigated the association between compression therapy and intensive care unit (ICU) mortality in patients with a first acute myocardial infarction (AMI) diagnosis complicated with type II DM.

Methods: This retrospective cohort study analyzed all patients with AMI and type II DM in the Medical Information Mart for Intensive Care-IV database. We extracted the demographics, vital signs, laboratory test results, comorbidities, and scoring system results of patients from the first 24 h after ICU admission. The outcomes of this study were 28-day mortality and ICU mortality. Analyses included Kaplan–Meier survival analysis, Cox proportional-hazards regression, and subgroup analysis.

Results: The study included 985 eligible patients with AMI and type II DM, of who 293 and 692 were enrolled into the no-AE device therapy and AE device therapy groups, respectively. In the multivariate analysis, compared with no-AE device therapy, AE device therapy was a significant predictor of 28-day mortality (OR = 0.48, 95% CI = 0.24–0.96, $P = 0.039$) and ICU mortality (OR = 0.50, 95% CI = 0.27–0.90, $P = 0.021$). In addition to age, gender and coronary artery bypass grafting surgery, there were no significant interactions of AE device therapy and other related risk factors with ICU mortality and 28-day mortality in the subgroup analysis.

Conclusions: Simple-AE-device therapy was associated with reduced risks of ICU mortality and 28-day mortality, as well as an improvement in the benefit on in-hospital survival in patients with AMI complicated with type II DM.

KEYWORDS

anti-embolic therapy, acute myocardial infarction, type II diabetes mellitus, mortality, ICU

Introduction

The prevalence of type II diabetes mellitus (DM) is increasing greatly worldwide. The number of patients with diabetes has been predicted to increase to 300 million by 2025 (1). DM is currently the most-serious factor contributing to heart failure and reinfarction prognoses caused by acute myocardial infarction (AMI) in cardiovascular diseases. It has been classified as a marker of a poor prognosis after AMI (2, 3). AMI caused by type II DM is the complication with the highest mortality and disability rate among diabetes-induced diseases, which has received widespread attention from both governments and the general public. Back in 2010, the NATIONAL Institute for Health and Clinical Excellence (NICE) guidelines recommended the combined use of mechanical prophylaxis (AES, foot impulse devices, intermittent pneumatic compression devices), whether a mechanical device used to prevent venous thromboembolism, as a simple non-invasive medical device, can also prevent ICU patients with acute myocardial infarction complicated with diabetes who lack certain activity, is worth exploring and studying its possibility (4, 5).

During the course of the continuous disease progression, patients with diabetes and AMI are prone to large or small microvascular neuropathies and cardiomyopathies due to coronary heart disease (6). Especially under the conditions of high blood sugar, along with the vascular endothelial cell injury, lipid deposition in macrophages and cause atherosclerosis, vascular smooth-muscle proliferation, and blood high condensation conditions to thrombosis, easy to increase vascular stenosis or blocked, so as to make the myocardial ischemia in partial necrosis (7, 8), eventually leading to heart failure or life-threatening cardiac shock. Thus, the early prevention of lower limb vein edema and periodic peripheral edema therefore appears to be particularly important in controlling the incidence of complications (9, 10). In anti-embolism (AE) treatment, which includes the removal of anticoagulation, thrombi therapy, and simple-AE-device auxiliary therapy (11), simple-AE-device therapy (including elastic stockings, ACE wraps, and compression sleeves) (4, 12, 13) is generally made most patients with diabetes and AMI more compliant than other early anti-embolism interventions, which is due to its simple, non-invasive, and convenient characteristics, and the ability to wear the device and use it in daily life. Most (90%) of patients with diabetes and AMI can improve their survival by enhancing the compression of the package to dissolve the fibrin discharged from the vein to relieve swelling (14, 15).

However, AE device therapy has long been controversial due to concern about endangering arterial circulation under high pressures (7, 16, 17). In order to solve this problem, this study compared the risk of type II DM with other established risk factors for death. The results suggested that simple-AE-device therapy has a specific prognostic impact on patients with type II DM and AMI. This retrospective cohort study examined the associations of AE device therapy alone with ICU mortality and 28-day mortality in type II DM with AMI.

Methods

Data source

A large, single-center, public database called the Medical Information Mark for Intensive Care (MIMIC-IV) (18–20) was used in this study. It was approved by the Massachusetts Institute of Technology (Cambridge, MA) and Beth Israel Deaconess Medical Center (Boston, MA). Because the present study performed an analysis of third-party anonymized publicly available data with pre-existing institutional review board (IRB) approval, approval from the IRB of our institution was not needed. In the database, the true identity information of the patients are hidden. Obtaining informed consent from patients was therefore not needed. After completing the online course of the National Institutes of Health and passing the examination for the protection of human study participants, all of the authors obtained a certificate to access the database (record ID: 45351934).

Population selection criteria

According to ICD-9 and ICD-10 codes, a total of 6,314 AMI records were included in MIMIC-IV database, including 3,383 records of patients with type II diabetes. Patients were excluded based on the following criteria: (1) multiple ICU admissions, (2) younger than 18 years, (3) ICU stay shorter than 24 h, or (4) treated using an AE device for <24 h. The follow-up duration was 28 days after the time of admission, and the survival status was observed at discharge. The final cohort included 985 patients, 293 and 692 of who were enrolled into the no-AE device therapy and AE device therapy groups, respectively. According to the above inclusion criteria, we extracted relevant information using Structured Query Language (SQL) in the Navicat Premium (version 15.0) program by identifying the subject_ids of the study population. The flow chart of included patients is illustrated in Figure 1.

Data extraction

Data were extracted using SQL with PostgreSQL tools (version 15.0). Extracted data included demographics, vital signs, comorbidities, llaboratory tests within the first 24 h of ICU admission. The initially selected laboratory measurements included age, sex, weight, Acute Physiology

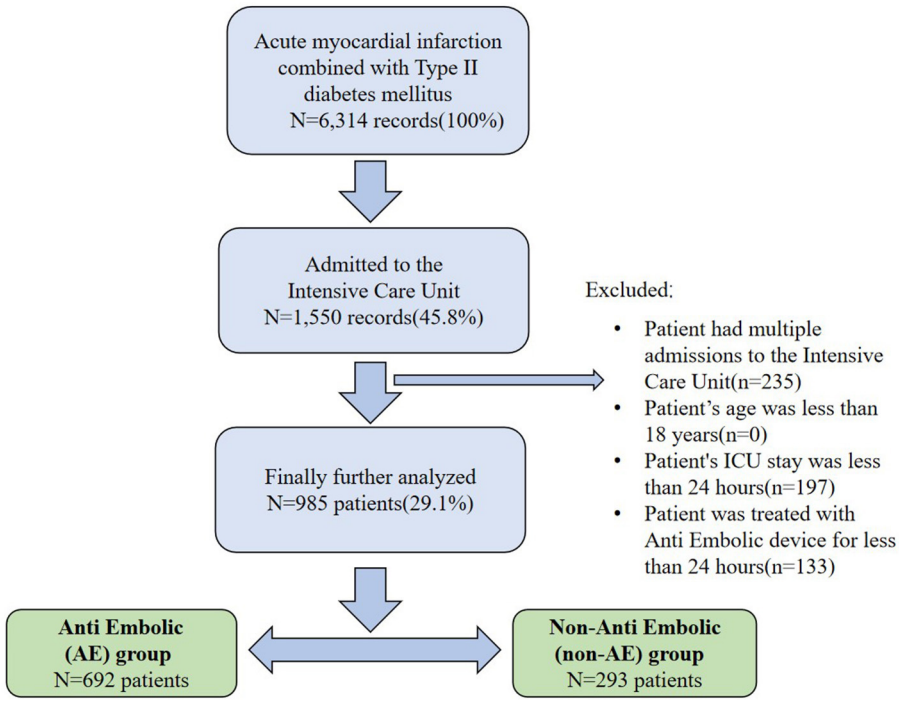

FIGURE 1
Flowchart of study population selection.

Score III (APSIII), ethnicity, first care unit, ventilator and vasopressor use, continuous renal replacement therapy (CRRT) use, percutaneous coronary intervention (PCI), coronary artery bypass grafting (CABG), antiplatelets, anticoagulation, hypertension, congestive heart failure, peripheral vascular disease, cerebrovascular disease, chronic pulmonary disease, renal disease, liver disease, malignant cancer, mean blood pressure (MBP), heart rate, respiratory rate, mean oxygen saturation (SpO_2), temperature, maximum troponin T, creatine kinase-myocardial band (CKMB), white blood cells (WBCs), hemoglobin, platelets, potassium, creatinine, blood urea nitrogen, maximum glucose, international normalized ratio (INR), alanine transaminase (ALT), urine output, anion gap (AG), and lactate. The endpoints of our study were 28-day mortality and ICU mortality.

Statistical analysis

The covariates of the no-AE device therapy and AE device therapy groups were compared using the chi-square or Fisher's exact tests as appropriate. Continuous variables are represented by mean and standard deviations or medians and interquartile ranges (IQR) (21, 22).

Subgroup analyses were performed to access the associations of AE device therapy with 28-day mortality and ICU mortality, and included age, sex, PCI, CABG, antiplatelets, and anticoagulation therapy. The data were analyzed using R software (http://www.R-project.org). Cox proportional-hazards regression models with increasing covariates were established to analyze the effects of multiple factors on survival time and clinical status. Kaplan–Meier survival analysis was used to examine differences in ICU mortality among groups. Log-rank tests were used to further compare differences among groups. A probability value of $P < 0.05$ was considered statistically significant, and all probability values were two-sided.

Results

Baseline results

This study enrolled 985 eligible patients, of whom 875 were survivors and 110 were non-survivors. The characteristics of the patients in the no-AE device therapy and AE device therapy groups are summarized in Table 1. Differences were found between the groups in the first care unit, ventilator and vasopressor use, PCI, CABG, antiplatelets, anticoagulation, cerebrovascular disease, MBP, mean heart rate, mean SpO_2, maximum troponin T, CKMB, WBCs, hemoglobin, glucose, ALT, urine output, and lactate ($P < 0.05$).

TABLE 1 Baseline characteristics of the study population.

	Non-AE-therapy	AE-therapy	*p*-value
N	293	692	
Age, years	71.00 (63.00, 80.00)	71.50 (63.00, 79.00)	0.863
Gender, *n* (%)			0.401
Male	173 (59.0)	430 (62.1)	
Female	120 (41.0)	262 (37.9)	
Weight, kg	86.05 (72.90, 99.00)	84.10 (71.12, 97.68)	0.218
APSIII	44.00 (35.00, 61.00)	49.00 (36.00, 68.00)	0.003
Ethnicity, *n* (%)			0.988
White	174 (59.4)	413 (59.7)	
Others	119 (40.6)	279 (40.3)	
First careunit, *n* (%)			<0.001
CCU	266 (90.8)	449 (64.9)	
Others	27 (9.2)	243 (35.1)	
Ventilator, *n* (%)			<0.001
No	204 (69.6)	230 (33.2)	
Yes	89 (30.4)	462 (66.8)	
Vasopressor, *n* (%)			0.001
No	214 (73.0)	426 (61.6)	
Yes	79 (27.0)	266 (38.4)	
CRRT, *n* (%)			0.719
No	288 (98.3)	676 (97.7)	
Yes	5 (1.7)	16 (2.3)	
PCI, *n* (%)			<0.001
No	128 (43.7)	601 (86.8)	
Yes	165 (56.3)	91 (13.2)	
CABG, *n* (%)			<0.001
No	290 (99.0)	438 (63.3)	
Yes	3 (1.0)	254 (36.7)	
Antiplatelet, *n* (%)			0.028
No	7 (2.4)	41 (5.9)	
Yes	286 (97.6)	651 (94.1)	
Anticoagulation, *n* (%)			0.008
No	20 (6.8)	89 (12.9)	
Yes	273 (93.2)	603 (87.1)	
Hypertension, *n* (%)			0.589
No	173 (59.0)	423 (61.1)	
Yes	120 (41.0)	269 (38.9)	
Congestive heart failure, *n* (%)			0.879
No	114 (38.9)	264 (38.2)	
Yes	179 (61.1)	428 (61.8)	
Peripheral vascular disease, *n* (%)			0.426
No	241 (82.3)	585 (84.5)	
Yes	52 (17.7)	107 (15.5)	
Cerebrovascular disease, *n* (%)			0.002
No	259 (88.4)	552 (79.8)	
Yes	34 (11.6)	140 (20.2)	
Chronic pulmonary disease, *n* (%)			1.000
No	220 (75.1)	519 (75.0)	
Yes	73 (24.9)	173 (25.0)	
Renal disease, *n* (%)			0.563
No	170 (58.0)	386 (55.8)	
Yes	123 (42.0)	306 (44.2)	
Liver disease, *n* (%)			0.962
No	269 (91.8)	633 (91.5)	
Yes	24 (8.2)	59 (8.5)	
Malignant cancer, *n* (%)			0.136
No	280 (95.6)	642 (92.8)	
Yes	13 (4.4)	50 (7.2)	
Mbp mean, mmHg	76.88 (69.31, 83.67)	74.64 (69.71, 79.77)	0.016
Heart rate mean, beats/min	79.51 (70.50, 88.00)	83.19 (73.88, 91.72)	<0.001
Respiratory rate mean, beats/min	19.04 (17.24, 21.21)	19.03 (16.95, 21.09)	0.516
SpO_2 mean, %	96.63 (95.36, 97.96)	97.33 (96.09, 98.50)	<0.001
Temperature mean, °C	36.79 (36.61, 36.95)	36.80 (36.60, 37.02)	0.529
Troponin T Max, ng/mL	2.17 (0.80, 5.80)	0.86 (0.27, 2.74)	<0.001
CKMB, ng/mL	21.00 (6.00, 75.75)	9.00 (4.00, 26.00)	<0.001
WBC, K/uL	10.70 (8.00, 14.20)	9.70 (7.60, 13.20)	0.011
Hemoglobin, g/dl	11.20 (9.80, 12.95)	10.80 (9.10, 12.40)	0.003
Platelet, K/uL	208.50 (163.00, 263.75)	199.00 (150.00, 254.50)	0.052
Potassium, mEq/L	4.30 (3.90, 4.70)	4.30 (3.90, 4.70)	0.612
Creatinine, mg/dL	1.30 (0.90, 2.00)	1.30 (0.90, 2.02)	0.792
Urea Nitrogen, mg/dL	26.50 (17.00, 40.00)	26.00 (17.00, 43.00)	0.976
Glucose max, mg/dl	254.50 (194.75, 329.00)	221.00 (186.75, 280.00)	<0.001
INR	1.20 (1.10, 1.40)	1.20 (1.10, 1.40)	0.580
ALT, IU/L	31.00 (18.00, 73.25)	26.00 (16.00, 48.00)	0.002

TABLE 1 Continued

	Non-AE-therapy	AE-therapy	*p*-value
Urine output, ml/s	1720.00 (1075.00, 2465.00)	1450.00 (911.50, 2191.25)	0.003
Anion Gap, mEq/L	16.00 (14.00, 20.00)	16.00 (14.00, 19.00)	0.565
Lactate, mmol/L	1.90 (1.30, 2.70)	1.60 (1.20, 2.40)	0.002
Day 28-mortality, *n* (%)			
0	240 (81.9)	598 (86.4)	0.086
1	53 (18.1)	94 (13.6)	
ICU-mortality, *n* (%)			
0	252 (86.0)	623 (90.0)	0.085
1	41 (14.0)	69 (10.0)	

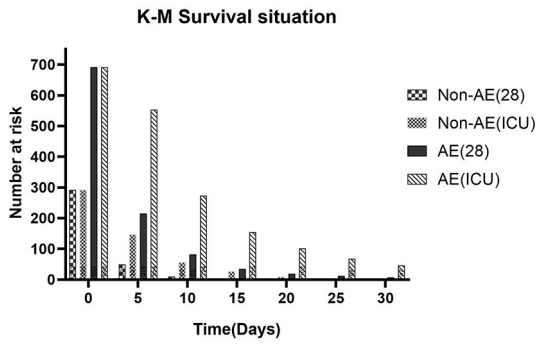

FIGURE 2
K–M survival situation about AE-device therapy and outcomes at different times.

Kaplan–meier survival curve analysis

The Kaplan–Meier curves of all examined categorical variables are illustrated in Figure 2. Those in the AE device therapy group had a higher probability of survival than did those in the no-AE device therapy group. We analyzed the different survival conditions between the two groups according to the time nodes obtained using the Kaplan–Meier survival curve. Survival at 28 days and in the ICU was significantly more likely in the AE device therapy group than in the no-AE device therapy group when the follow-up time was changed.

Cox proportional-hazards models

A Cox proportional-hazards regression model was constructed to further investigate the effects of multiple variables on survival time and outcome, and to estimate the hazard ratios (HR) for 28-day mortality and ICU mortality. As listed in Table 2, compared with no-AE device therapy, AE device therapy was a significant predictor of 28-day mortality (OR = 0.48, 95% CI = 0.24–0.96, $P = 0.039$) and ICU mortality (OR = 0.50, 95% CI = 0.27–0.90, $P = 0.021$) after adjusting for covariates.

TABLE 2 Analysis of the associations between AE-device therapy and outcomes.

	Non-AE-device therapy	AE-device therapy	
	HR (95%CI)	HR (95%CI)	*p*-value
ICU-mortality			
Unadjusted	Reference	0.32 (0.22, 0.48)	<0.001
Adjusted	Reference	0.46 (0.23, 0.93)	0.030
Day28 mortality			
Unadjusted	Reference	0.44 (0.31, 0.62)	<0.001
Adjusted	Reference	0.49 (0.27, 0.89)	0.020

Analysis of the associations between AE-device therapy and outcomes.
HR, hazard ratio; CI, confidence interval.
Models were derived from Cox proportional hazards regression models.
Model I was not adjusted for covariates.
Model II covariates were adjusted for Age, Weight, Ethnicity, Gender, First_careunit, APSIII, Anion_Gap, Heart_rate_mean, CKMB, WBC, Respiratory_rate_mean, Mbp_mean, SpO_2_mean, Temperature_mean, Troponin_T_Max, Hemoglobin, Glucose_max, INR, Platelet, Potassium, Creatinine, Urea_Nitrogen, ALT, Urine_output, Lactate, Anti_Embolic, Antiplatelet, Anticoagulation, Congestive_heart_failue, Renal_disease, Malignant_cancer, Liver_disease, PCI, CABG, Ventilator, Vasopressor, CRRT, Peripheral_vascular_disease, Cerebrovascular_disease, Chronic_pulmonary_disease, Hypertensionid.

Subgroup analyses

A subgroup analysis was applied to the main influencing variables in this study to determine the associations of AE device therapy with 28-day mortality and ICU mortality (Table 3). There were no significant interactions in most strata in the subgroup analyses ($P = 0.146$). Nevertheless, patients who were ≥65 years old, female, and received PCI, CABG, or anticoagulation (heparin and warfarin) therapy had significantly higher risks of 28-day mortality and ICU mortality.

Discussion

As a strong risk factor for cardiovascular disease, type II DM (1, 23) is a common clinical endocrine and metabolic disease that is primarily characterized by hyperglycemia. Long-term hyperglycemia caused by impaired insulin secretion leads to the chronic dysfunction of various tissues, and sustained

TABLE 3 Subgroup analysis of the associations between AE-therapy and outcomes.

	ICU-mortality		Day 28-mortality	
	HR (95%CI)	*p*-value	HR (95%CI)	*p*-value
Age, years				
≤65 ($n = 300$)	NA		NA	
>65 ($n = 685$)	0.32 (0.13, 0.77)	0.010	0.35 (0.17, 0.71)	0.003
Gender, *n* (%)				
Male ($n = 603$)	0.52 (0.21, 1.31)	0.164	0.57 (0.27, 1.21)	0.146
Female ($n = 382$)	0.02 (0.01, 0.04)	<0.001	0.06 (0.03, 0.12)	<0.001
PCI, *n* (%)				
No ($n = 729$)	0.42 (0.15, 1.15)	0.092	0.43 (0.20, 0.90)	0.025
Yes ($n = 256$)	NA		NA	
CABG, *n* (%)				
No ($n = 728$)	0.47 (0.23, 0.95)	0.036	0.50 (0.28, 0.91)	0.023
Yes ($n = 257$)	NA		NA	
Antiplatelet, *n* (%)				
No ($n = 48$)	NA		NA	
Yes ($n = 937$)	0.67 (0.32, 1.42)	0.302	0.58 (0.31, 1.09)	0.093
Anticoagulation, *n* (%)				
No ($n = 109$)	NA		NA	
Yes ($n = 876$)	0.49 (0.24, 1.02)	0.058	0.47 (0.25, 0.89)	0.020

Subgroup analysis of the associations between AE-therapy and outcomes.
Hazard ratio (95% CI): from Cox proportional hazards regression models. The covariate adjustment was consistent with Model II in Table 2.

ischemia and hypoxia leads to myocardial necrosis of varying degrees. Due to the greatly damaged vascular endothelial cells of patients with high blood glucose, lipid deposition in the vascular wall leads to arteriosclerosis. Increased platelet adhesion often results in blood in the hypercoagulable state becoming thrombotic in arterioles, thus blocking blood vessels and aggravating luminal stenosis (24–26). Therefore, in patients with AMI and DM—who are prone to coronary artery branch stenosis or intramyocardial coronary artery stenosis—a collateral circulation disorder can cause extensive infarction, making early preventive AE device therapy for this population particularly important. Therefore, simple-AE-device treatment has received considerable attention in recent cardiovascular research (27).

Considering that AE-device therapy is closely associated with AMI complicated with DM, we selected 985 CCU patients from a large critical-care database (MIMIC-IV) and adjusted for numerous potential confounders, including APSIII, CRRT, CABG, and PCI. Survival appeared to be more likely in the AE-therapy group than in the no-AE device therapy group, with significant differences in ICU mortality and 28-day mortality. Below we summarize the findings and contributions made.

In the past 30 years, some large randomized clinical trials have shown that the main methods to prevent and treat AMI caused by venous thromboembolism include Catheter-directed thrombolysis and endovascular treatment (28, 29), which can clear the disease to a certain extent, greatly reduce the burden of thrombosis, protect vascular and valve functions, and thus reduce the incidence of recurrence of myocardial infarction. However, in actual clinical applications (30), because there are very strict indications and contraindications for thrombolysis or endovascular therapy in patients with deep vein thrombosis (DVT), especially in patients with diabetes, while AE device therapy is non-invasive and convenient, a certain degree of compression can ameliorate limb pain and swelling in DVT ideally, thus greatly improving the compliance of patients, which can allow treatment alongside daily activities as well. This finding was similar to the results of the present study, suggesting that applying therapy with a simple embolization device to patients with AMI and DM can influence mortality outcomes. A subgroup analysis indicated that female patients older than 65 years often have adverse cardiovascular outcomes compared with the remaining AMI population. Especially for diabetic patients with a history of CABG and PCI who have taken anticoagulant

drugs for a long time, using AE device therapy can protect the early prognosis of patients, which has definite clinical significance (31).

Previous studies (31, 32) have shown that venous valve function can be improved by applying pressure to leg tissues and blood vessels to support blood pumping to the calf muscles, speeding blood flow back from the legs to the heart, and reducing the risk of thrombosis and embolism. Based on the present study, considering the changes of platelet and coagulation function in patients with AMI combined with DM and the increased risk of thrombosis, AE device therapy is of great significance for these patients, and can greatly reduce the risk of recurrent myocardial infarction or adverse cardiovascular outcomes at an early stage. Simple compression devices (4, 12, 33) including elastic stockings, ACE wraps, and compression sleeves are well-suited for patients with diabetes and AMI, and mild compression can provide them with a certain degree of comfort. In contrast, AE devices have long been clinically controversial because of the risk to the arterial circulation caused by long-term high pressure compression. The long-term application of high degrees of compression may indeed lead to ischemic skin injury, and may even cause accidental injury (16, 34). We therefore compared different use durations and clinical stasus of patients using simple AE devices. As the Kaplan–Meier Survival Curve analysis showed that these devices were most commonly used for 0–20 days, with the longest use time not exceeding 3 months. The use of simple AE compression devices must be accompanied by appropriate management and monitoring, and interventions should be performed in the early stages of discomfort or ischemic injury so as to remove patient concerns about the potential risks of AE device therapy and improve the compliance of patients with AMI complicated with DM using this treatment method. It has to be mentioned that this study is the first to focus on ICU patients with AMI with type II diabetes from the perspective of adjuvant therapy, which can not only improve the compliance of ICU patients, but also provide a new reference for how to effectively support the prognostic treatment of patients with diabetes complicated with AMI.

Limitations

This was the first study of the correlation between AE device therapy and AMI complicated with DM. However, this study did have some limitations. First, the study had a single-center retrospective design, and so there was selection bias for the population and covariate factors, which would be overcome by a prospective multicenter design. Second, we only extracted certain laboratory indicators and scores from patients with AMI complicated with DM who were admitted to ICUs, and did not analyze the dynamic changes of indicators and scores, which could directly reflect the prognosis of patients. Third, the MIMIC-IV database lacks the different use times of specific simple-AE-device therapies, so it was not possible to compare the efficacy of AE device therapy among different populations, which means that the study was not detailed or comprehensive. Fourth, as a retrospective study, the number of patients included was not large, which means that there are many uncertainties when attempting to generalize its conclusions to other populations, such as the specific degree of compression used in the socks in AE device therapy, compliance with AE device therapy, and follow-up of patients after discharge. Fifth, although we have made our best efforts to control for bias using multivariate models, moreover, subgroup analysis is limited by confounding factors, there are likely to be many other undiscovered factors. Finally, the study was subject to the standard limitations of large public databases, and so further studies—especially with a multicenter, large-scale, prospective design—are needed to remove these.

Conclusions

Our findings demonstrated that the 28-day mortality and ICU mortality risks in the AE device therapy group were obviously lower than those in the no-AE device therapy group. This suggests that AE device therapy can improve the poor prognoses of patients with AMI complicated with type II DM, and that simple AE devices appear to be a fruitful direction for future research to improve poor prognoses. However, the present findings need to be confirmed in large prospective multicenter studies.

Data availability statement

Publicly available datasets were analyzed in this study. This data can be found here: This data can be found here: The data were available on the MIMIC-IV website at https://mimic-iv.mit.edu/.

Ethics statement

All procedures performed in studies involving human participants were in accordance with the Ethical Standards of the Institutional and National Research Committee and with the 1964 Helsinki declaration and its later amendments or comparable Ethical Standards.

Author contributions

XH and LZ created the study protocol, performed the statistical analyses, and wrote the first manuscript draft. MX conceived the study and critically revised the manuscript. SY and YY assisted with the study design and performed data collection. TH assisted with data collection and manuscript editing. HY assisted with manuscript revision and data confirmation. JL contributed to data interpretation and manuscript revision. All authors read and approved the final manuscript.

Funding

This study was supported by Guangdong Provincial Key Laboratory of Traditional Chinese Medicine Informatization (2021B1212040007).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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frontiers | Frontiers in Cardiovascular Medicine

TYPE Case Report
PUBLISHED 22 July 2022
DOI 10.3389/fcvm.2022.962127

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Cardiovascular Therapeutics, a section of the journal Frontiers in Cardiovascular Medicine

RECEIVED 05 June 2022
ACCEPTED 27 June 2022
PUBLISHED 22 July 2022

CITATION
Shen M, Liao Y, Wang J, Zhou X, Guo Y, Nong Y, Guo Y, Lu H, Jin R, Wang J, Fu Z, Li D, Zhao S and Tian J (2022) Intracoronary artery retrograde thrombolysis combined with percutaneous coronary interventions for ST-segment elevation myocardial infarction complicated with diabetes mellitus: A case report and literature review.
Front. Cardiovasc. Med. 9:962127.
doi: 10.3389/fcvm.2022.962127

Intracoronary artery retrograde thrombolysis combined with percutaneous coronary interventions for ST-segment elevation myocardial infarction complicated with diabetes mellitus: A case report and literature review

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Background: The management of a large thrombus burden in patients with acute myocardial infarction and diabetes is still a worldwide problem.

Case presentation: A 74-year-old Chinese woman presented with ST-segment elevation myocardial infarction (STEMI) complicated with diabetes mellitus and hypertension. Angiography revealed massive thrombus formation in the mid-segment of the right coronary artery leading to vascular occlusion. The sheared balloon was placed far from the occlusion segment and urokinase (100,000 u) was administered for intracoronary artery retrograde thrombolysis, and thrombolysis in myocardial infarction (TIMI) grade 3 blood flow was restored within 7 min. At last, one stent was accurately implanted into the culprit's vessel. No-reflow, coronary slow flow, and reperfusion arrhythmia were not observed during this process.

Conclusion: Intracoronary artery retrograde thrombolysis (ICART) can be effectively and safely used in patients with STEMI along with diabetes mellitus and hypertension, even if the myocardial infarction exceeds 12 h (REST or named ICART ClinicalTrials.gov number, ChiCTR1900023849).

KEYWORDS

diabetes mellitus, myocardial infarction, intracoronary retrograde thrombolysis, thrombosis, reperfusion preconditioning

Background

Coronary heart disease and acute myocardial infarction (AMI) are major contributors to global morbidity and mortality. In the meantime, type 2 diabetes (T2DM) is a major risk factor for coronary heart disease and AMI (1–3). Patients with T2DM are at high risk of myocardial infarction and have a poor prognosis after myocardial infarction, especially after STEMI (4). Acute hyperglycemia promotes coagulation and induces platelet aggregation, resulting in increased thrombus burden in STEMI, and a worse prognosis (5). High thrombus burden combined with diabetes remains an important predictor of poor prognosis after STEMI (6).

Primary percutaneous coronary intervention (PPCI) remains the preferred reperfusion strategy for AMI (7). But the management of intracoronary thrombus is still a great challenge in PPCI Thrombus aspiration seems to be a promising strategy in the past years. However, routine thrombus aspiration combined with PPCI has been proved unable to improve clinical outcomes in patients with STEMI. Even in patients with a high thrombus burden, routine thrombus aspiration does not improve outcomes after 1 year and is associated with increased stroke incidence. As such, there is still a long way to go to solve the problem of intracoronary thrombosis, especially in patients with a large thrombus burden.

Here we reported a case of diabetic patients with STEMI. Intracoronary artery retrograde thrombolysis (ICART) combined with PPCI was successfully used to realize micro-perfusion, micro-flow, and micro-opening to produce reperfusion preconditioning, thereby reducing reperfusion injury, no-reflow, and slow blood flow. Slow blood flow has a certain promotion value. ICART has strong application value.

Case presentation

A 74-year-old woman with sudden chest pain lasting for 17 h was transferred to the emergency department. She had been suffering from diabetes for more than 8 years. Though treated with metformin, the blood glucose was still poorly controlled as evidenced by a fasting blood glucose fluctuating between 8.8 and 10.6 mmol/L, and postprandial blood glucose fluctuating between 9.7 and 13 mmol/L. She also suffered from hypertension for 10 years and took nifedipine controlled-release tablets, and her blood pressure was well controlled. Blood pressure was 145/98 mmHg and pulse rate 103 beats per minute. The electrocardiogram showed ST elevation 0.05–0.2mV in leads II, III, and aVF (**Figure 1A**). Emergency examination showed that myoglobin reached 359.4 ng/ml, creatine kinase index was 1,094 U/L, creatine kinase isoenzyme index was 167.1 U/L, troponin T was 0.736 ng/ml (the normal reference value: 0–0.1 ng/mL), N-terminal pro-B-type natriuretic peptide (NT-proBNP) was 2235 pg/mL. Cardiac ultrasound showed that the motion of the left ventricular inferior wall was slightly weakened and a small amount of mitral regurgitation. No moist rales were heard on bilateral lung auscultation. The patient was diagnosed with acute inferior ST-segment elevation myocardial infarction, Killip grade 1. The patient had myocardial infarction for 17 h. Although it had been more than 12 h, she still had chest pain, indicating that there was still new myocardial necrosis. Therefore, we chose emergency angiography and PPCI if necessary. Aspirin 300 mg and clopidogrel 600 mg were chewed and swallowed, then the patient bypassed the CCU and went directly to the catheterization laboratory for coronary angiography immediately. The treatment was approved by the Hainan Hospital of PLA General Hospital ethics committee and informed consent was signed.

Figure 2 shows the pattern of ICART combined with PPCI in the treatment of this case of acute inferior ST-segment elevation myocardial infarction. Coronary angiography showed that the proximal and middle distal stenosis of the anterior descending artery were 60 and 50%, respectively. Diffuse atherosclerosis from proximal to distal circumflex branch with stenosis. The distal part was the narrowest, and the degree of stenosis was 90%. Mild stenosis was found in the proximal segment of the right coronary artery (RCA), and thrombosis occurred in the middle segment, resulting in vascular occlusion (**Figure 3A**). Bivalirudin was pumped intravenously for anticoagulation.

Guiding catheter JR4 was connected to the opening of the right coronary artery. A run-through guidewire was used through the culprit artery to the distal end of the occluded RCA. The end of the Sprinter Legend 2 mm × 15 mm balloon was cut off, leaving only the proximal metal ring as a marker. Then the cut balloon was inserted along the run-through guidewire to the distance of the occluded coronary artery (**Figure 3B**). 100,000 units of urokinase, 15 ml physiological saline, and 5 ml iopromide were mixed to form a 20 ml cocktail, which could be called a visual thrombolytic. Subsequently, 1 mL visualized thrombolytic was bolus-injected through the cut balloon, repeated every 30 s (**Figure 3B**). During the injection of a visualized thrombolytic agent, the thrombolytic agent mixed with the contrast agent in the occluded lumen could visualize the occluded blood vessel and exert the reverse thrombolytic function through the thrombolytic agent. After thrombolysis with 70,000 u urokinase for 7 min, right coronary angiography showed that the coronary thrombosis disappeared completely and TIMI

Abbreviations: PCI, percutaneous coronary interventions; PPCI, primary percutaneous coronary interventions; STEMI, ST-segment elevation myocardial infarction; TIMI, thrombolysis in myocardial infarction; ICART, intracoronary artery retrograde thrombolysis; AMI, acute myocardial infarction; T2DM, type 2 diabetes; NT-proBNP, N-terminal pro-B-type natriuretic peptide; PLA, People's Liberation Army; RCA, right coronary artery; CHD, coronary heart disease; MACE, major adverse cardiovascular events; I/RI, ischemia/reperfusion injury.

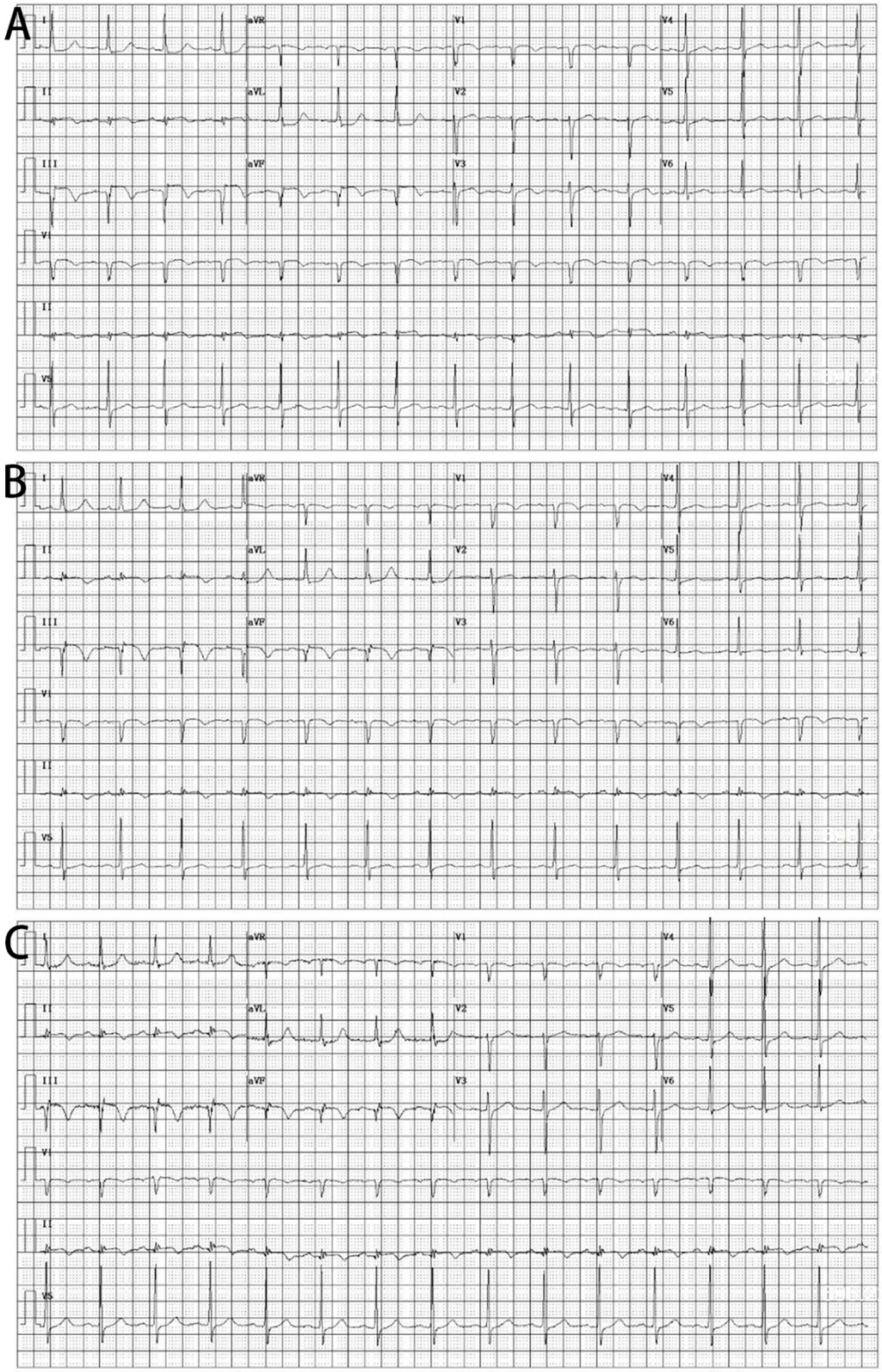

FIGURE 1
Electrocardiograms. **(A)** In the emergency room. **(B)** After intracoronary artery retrograde thrombolysis (ICART). **(C)** 7 days after ICART.

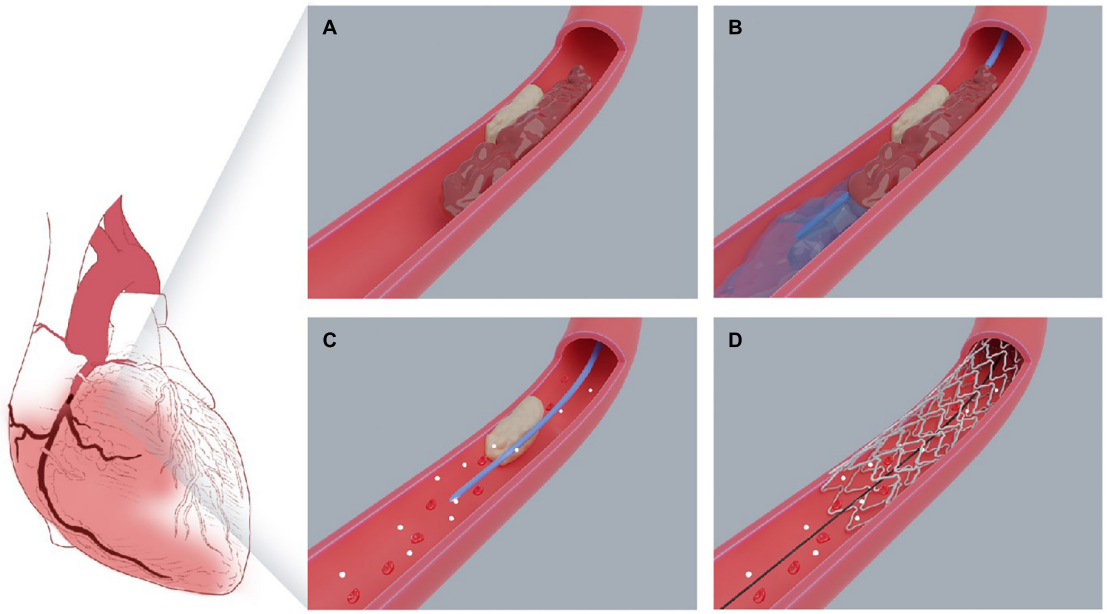

FIGURE 2
Pattern diagram of intracoronary artery retrograde thrombolysis system. **(A)** The right coronary plaque ruptured, leading to thrombosis. **(B)** The sheared balloon was placed far away from the occluded segment, and the "visual thrombolytic agent" was slowly injected into the occluded site through the sheared balloon. **(C)** The occluded thrombus was completely dissolved and the blood flow recovered. **(D)** The stent was accurately implanted into the lesion.

blood flow recovered to grade 3 (**Figure 3C**). After accurate positioning, a 2.75 mm × 15 mm stent was directly released by 10 atm pressure. A 3 mm × 12 mm post-dilation balloon was used to dilate at 12 atm for 15 s with less than 5% residual stenosis. Right coronary blood flow returned to TIMI grade 3 (**Figure 3D**). During the operation, we measured ACT to evaluate the bleeding risk. After 20 min, ACT was 254 s; after 30 min, ACT was 265 s.

The patient's chest pain symptoms were completely relieved, and the ST segment did not decrease very obviously half an hour after the operation (**Figures 1B,C**). Bivalirudin was continued for 4 h postoperatively. Tirofiban was applied sequentially for 36 h. The patient regularly took aspirin (100 mg/day), clopidogrel (75 mg/day), rosuvastatin (10 mg/day), bisoprolol (5 mg/day), nicorandil (15 mg/day), and other drugs. For diabetes treatment, the patient received insulin to control blood sugar during the acute phase. On the second postoperative day, the patient started taking metformin hydrochloride (500 mg, tid) regularly to control blood sugar. After treatment, the patient's fasting blood glucose fluctuated between 6.2 and 7.6 mmol/L and postprandial blood glucose fluctuated between 9.2 and 10.3 mmol/L. The patient had no complications such as hemorrhage and stroke after ICART combined with PCI and was discharged from the hospital 7 days later, and the ST segment did not decrease very obviously.

Discussion and conclusion

The results of this study demonstrate that ICART combined with PPCI is feasible and can improve myocardial reperfusion in 18-h among patients with STEMI along with diabetes.

Diabetes is seriously affecting public health worldwide, and the number of people with diabetes will continue to increase as obesity and population aging gradually increase (8). The prevalence of adult type 2 diabetes was 8.8% worldwide in 2017, and this proportion is expected to grow to 9.9% by 2045 (9). People with diabetes exhibit a higher risk of cardiovascular complications, while AMI is the primary cause of death in patients with diabetes (10). Diabetic patients with a history of myocardial infarction have a risk of more than 40% of recurrent myocardial infarction (11). Therefore, coronary heart disease (CHD) patients with diabetes need more aggressive treatment to reduce the risk of myocardial infarction compared with CHD in patients without diabetes (12). In addition to being associated with increased cardiovascular risk, T2DM may influence the choice of multiple treatments for CHD, especially myocardial infarction.

Plaque rupture or plaque erosion leads to intracoronary thrombus formation, which in turn causes coronary artery occlusion and ST-segment elevation myocardial infarction (13). PPCI has been shown to have great advantages in establishing effective and early recanalization of infarct-related arteries,

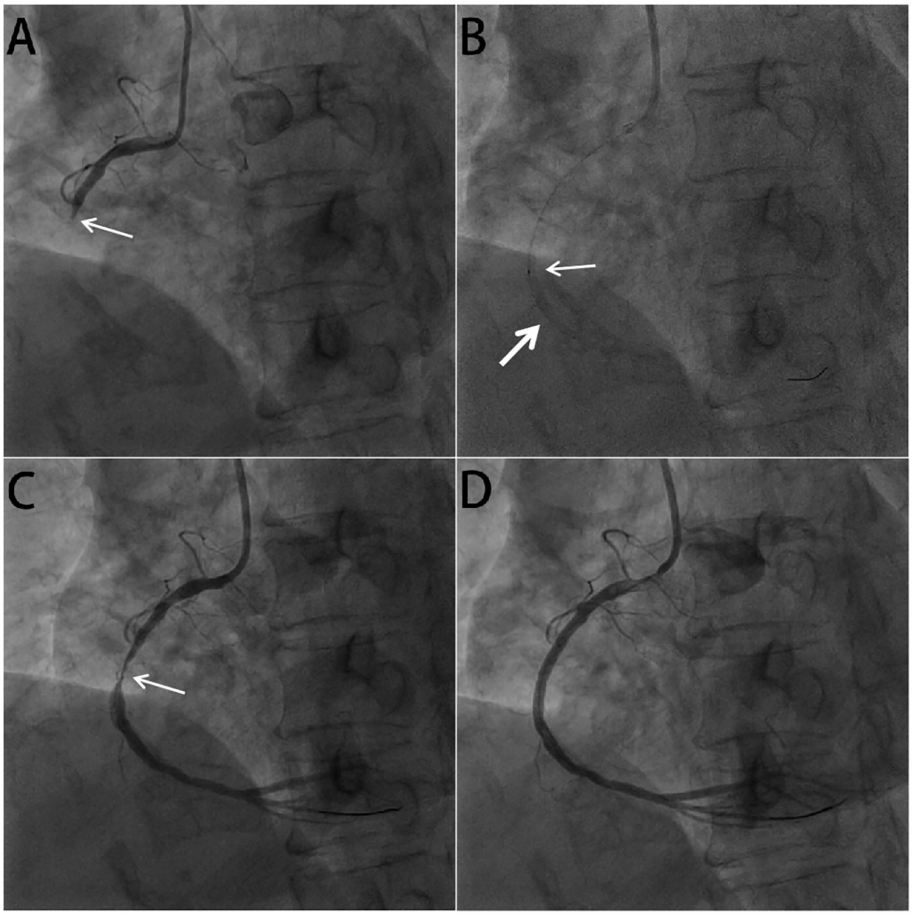

FIGURE 3
Coronary angiogram in acute right coronary arterial occlusion. **(A)** The basal angiogram showed total occlusion of the right coronary artery distal segment with thrombus image. The arrow showed the occlusion. **(B)** The procedure of intracoronary artery retrograde thrombolysis (ICART) through the sheared balloon. The distal thrombus was gradually dissolved. The fine arrow showed the tip of the sheared balloon, and the coarse arrow indicated the thrombolytic agent with contrast agent to fill the occluded lumen. **(C)** After thrombolysis, the culprit lesion (arrow) in the middle of the right crown was observed. **(D)** A stent was implanted in the RCA to achieve revascularization.

reducing major adverse cardiovascular events (MACE), and improving survival (14–16). However, intracoronary thrombus remains the bane of interventional cardiologists (17, 18). Failure to recanalize, suboptimal outcomes, distal embolization, no-reflow, and impaired myocardial perfusion are some of the unresolved difficulties that frequently arise during PCI in patients with a high intracoronary thrombus burden, indicating an unmet need (19, 20). In the high target lesion SYNTAX score lesions receiving balloon predilation, a maximum predilation pressure >10 atm was associated with a higher risk of no-reflow (21).

To reduce thrombus burden in patients with STEMI, a number of methods are used during PPCI, such as distal protection devices (22, 23), thrombus aspiration (6, 24, 25), and glycoprotein IIb/IIIa antagonists (26, 27).

However, several studies, including the AIMI, PROMISE, and EMERALD trials (28), found that distal protection devices were not protective and even detrimental to myocardial perfusion and eventual infarct size.

In patients with a high thrombus burden, routine thrombus aspiration did not improve outcomes at 1 year and was associated with an increased rate of stroke (6, 29). Thrombus aspiration does not appear to be associated with an improvement in clinical outcomes regardless of ischemic time (30). We speculate that this is directly related to reperfusion injury caused by thrombus aspiration, intracoronary artery thrombosis is still a nightmare for interventional cardiologists.

Due to the detrimental effects of acute ischemia/reperfusion injury (I/RI) on the heart, myocardial reperfusion has been referred to as "a double-edged sword" (31). I/RI causes cardiomyocyte death and may in fact cause up to 50% of the final myocardial infarction size (32).

Reperfusion injury is mainly characterized by myocardial stunning, reperfusion-induced arrhythmias, coronary no-reflow

phenomenon, and lethal myocardial reperfusion injury (32–34). The above-mentioned problems are the primary problems of opening the occluded blood vessels, that is, how to reduce the thrombus burden and reperfusion injury. Ischemic postconditioning is helpful (35, 36), but due to the heavy thrombus burden, repeated balloon dilation can also lead to thrombus detachment, resulting in slow blood flow or no-reflow. At the same time, during ischemic postconditioning, the culprit's blood vessels are opened first. In fact, obvious reperfusion has occurred in this process and obvious reperfusion injury occurs. Post-treatment is some remedial measures after reperfusion injury.

In this patient, intracoronary artery retrograde thrombolysis was used. In this process, the thrombolytic agent is administered through the sheared balloon to generate micro-blood flow, micro-opening, and micro-perfusion, which can be called reperfusion preconditioning. This concept has not been proposed yet. Just like a beggar who has been hungry for a long time, if a large amount of meat or broth is suddenly given, it may actually kill the beggar. For patients with STEMI, if the occluded blood vessel is suddenly opened by thrombus aspiration or balloon dilation, it will lead to significant reperfusion injury, malignant arrhythmia, and extensive myocardial necrosis. If the blood flow is fine and opened gradually, the myocardial necrosis will be reduced, and the incidence of malignant arrhythmia will be reduced.

The advantage of intracoronary artery retrograde thrombolysis lies not only in reperfusion preconditioning but also in thrombolysis, showing the distal vascular bed, which is also of great benefit to judging whether the guide wire is in the true cavity, slow blood flow, and no-reflow. At the same time, it can clearly show the lesions, which is very helpful for the accurate selection of stents. On the other hand, the thrombus in the coronary artery is dissolved, which reduces the risk of cerebral infarction caused by thrombus shedding during thrombus aspiration.

This medical record is equivalent to the addition of thrombolytic agents during emergency PCI, but the dosage of urokinase is only 1/15 of the conventional dosage, which is very small. No significant bleeding risk was observed in this patient. Generally speaking, intravenous thrombolysis can be considered for ST-elevation myocardial infarction with an onset of less than 12 h. In this case, 18 h after myocardial infarction, the thrombus can still be dissolved by intracoronary artery retrograde thrombolysis, which reflects that thrombolytic agents have a good effect on the slightly older thrombus as well.

In conclusion, intracoronary reverse thrombolysis is safe and effective for diabetic patients with STEMI with high thrombus load for more than 18 h, and it is an important treatment option. However, based on the application of double suppositories and anticoagulants, the addition of thrombolytics will increase the risk of bleeding relatively. Whether it is suitable for people with high bleeding risk remains to be further observed and more samples will be included. Additionally, the effect on TIMI blood flow needs further observation, whether it can really reduce slow blood flow and no-reflow. In addition, whether it can improve cardiac function and improve ejection fraction remains to be a randomized controlled trial with large sample size.

Ethics statement

This study has been approved by the Ethics Committee Board of the Chinese PLA General Hospital, Beijing, China. Written informed consent was obtained from the patient for their participation in this case report. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

JHW, JW, XZ, YTG, and YN carried out patient management and data collection. MS, DL, SZ, and YG drafted the manuscript and edited the figures. JT, SZ, RJ, HL, and YL performed the angioplasty. JT and ZF critically revised the manuscript for important intellectual content. All authors read and approved the final manuscript.

Funding

This study was supported by the Hainan Science and Technology Project (ZDYF2020123, ZDYF2017096, and ZDYF2020027), the National Key R&D Plan (2020YFC2004706), and Open Subject of the National Clinical Research Center of Geriatrics Disease NCRCG-PLAGH-2018014 (MS).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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frontiers | Frontiers in Cardiovascular Medicine

TYPE Original Research
PUBLISHED 08 August 2022
DOI 10.3389/fcvm.2022.976656

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Cardiovascular Therapeutics, a section of the journal Frontiers in Cardiovascular Medicine

RECEIVED 23 June 2022
ACCEPTED 20 July 2022
PUBLISHED 08 August 2022

CITATION
Lu X, Zhang L, Li S, He D, Huang T, Lin H, Yin H and Lyu J (2022) Association between statin use and the prognosis of patients with acute myocardial infarction complicated with diabetes.
Front. Cardiovasc. Med. 9:976656.
doi: 10.3389/fcvm.2022.976656

Association between statin use and the prognosis of patients with acute myocardial infarction complicated with diabetes

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Background: Type 2 diabetes leads to an increase in the prevalence of lipid abnormalities, which increases the risk of cardiovascular disease. Therefore, current guidelines generally recommend the use of moderate or high-intensity statins in patients with type 2 diabetes. There are still few studies on the overall risk benefit balance of statins for acute myocardial infarction (AMI) patients with diabetes. Compared with other types of lipid-lowering drugs, the advantage of statins for the prognosis of patients with AMI has not yet been determined. We investigated the effects of statins and non-statins on intensive care unit (ICU) and inpatient mortality in patients with AMI and diabetes.

Methods: This study retrospectively collected all patients with AMI and diabetes in the Medical Information Mart Intensive Care-IV database. We assessed ICU and in-hospital mortality rates during hospitalization in both groups. The clinical end point was in-hospital mortality and ICU mortality. Kaplan-Meier and Cox proportional-hazards regression models were applied to analyze the correlation between the two groups and the outcomes.

Results: Data on 1,315 patients with AMI and diabetes were collected, among which 1,211 used statins during hospitalization. The overall in-hospital mortality of patients with AMI and diabetes was 17.2%, and the total ICU mortality was 12.6%. The in-hospital mortality was lower for the statin group than for the non-statin group (13.9% and 55.8%, respectively). Kaplan-Meier survival curves demonstrated that survival probability was higher in the statin group than in the non-statin group. In the cohort without hyperlipidemia, the statin group had lower risks of ICU death (HR = 0.12, 95% CI = 0.04–0.40) and in-hospital death (HR = 0.36, 95% CI = 0.16–0.84) compared with the non-statin group.

Conclusions: Statins can significantly reduce ICU and in-hospital mortality rates in patients with AMI and diabetes. Even in the population without

10.3389/fcvm.2022.976656

hyperlipidemia, statins can still reduce the mortality in patients with AMI and diabetes.

KEYWORDS

statin, acute myocardial infarction, diabetes, lipid-lowering drugs, cardiovascular disease

Introduction

Increases in the incidence rates of obesity, metabolic syndrome, and diabetes have led to cardiovascular disease (CVD) becoming the most common disease leading to death and decreased quality of life, and this adverse situation may further escalate in the near future (1). Diabetes and dyslipidemia are independent risk factors related to the incidence of atherosclerotic CVD (2). The risk of death due to CVD is 3- to 6-fold higher in patients with diabetes than in those without diabetes (3). Lipid-lowering therapy for patients with diabetes is therefore an important measure for reducing the CVD risk. The UK Prospective Diabetes Study identified elevated low-density lipoprotein (LDL) cholesterol as the leading coronary risk factor in patients with diabetes (4). Statins are 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors with the primary function of reducing endogenous LDL cholesterol. Some previous studies found that statins exert fascinating pleiotropic effects in addition to reducing LDL cholesterol, such as anti-inflammatory, antithrombotic, and antioxidant effects (5), which can improve vascular function and improve ventricular remodeling (6). There is evidence that statins can reduce the risk of various cardiovascular events in patients with diabetes (7) resulting in statins becoming the first choice of lipid-lowering drugs for reducing CVD risk. Type 2 diabetes leads to an increase in the prevalence of lipid abnormalities, which increases the risk of CVD. Therefore, current guidelines generally recommend the use of moderate or high-intensity statins in patients with type 2 diabetes (8, 9). However, there is still controversy about whether statins are important in acute myocardial infarction (AMI) patients with diabetes, and there are still few studies. Some retrospective registration studies showed that the statin group showed lower major adverse cardiac events, all-cause mortality, cardiac death than the non-statin group (10, 11). However, studies have shown that the beneficial effect of statins in AMI patients with diabetes has not been confirmed (12). Most previous studies have focused exclusively on the protective effect of statins on cardiovascular events, and so the overall risk–benefit balance of statins for patients with AMI and diabetes needs to be reassessed. Compared with other types of lipid-lowering drugs, the advantage of statins for the prognosis of patients with AMI has yet to be determined. We therefore hypothesized that patients with AMI and diabetes who receive statins have lower intensive care unit (ICU) and in-hospital mortality rates than those who do not receive lipid-lowering drugs. We tested this hypothesis using the Medical Information Mart Intensive Care-IV (MIMIC-IV) database.

Methods

Data source and population

This was a retrospective study based on version 1.0 of the MIMIC-IV database, which a vertical, single-center database that includes all patients admitted to the Beth Israel Deaconess Medical Center (BIDMC) emergency department or ICU during 2008–2019 (13). We obtained access to the database after completing the recognized "Protecting Human Research Participants" course. The institutional review boards of BIDMC and MIT approved any researcher meeting the data user requirements to use the MIMIC-IV database, and exempted them from the requirement to obtained informed consent from patients. This study included all patients with AMI complicated with diabetes in the database, and excluded patients younger than 18 years. We only extracted the information of patients hospitalized and admitted to ICU for the first time, and excluded those with multiple hospitalization records (Figure 1).

Data extraction

Structured Query Language was used to extract the following information from the database: age, gender, weight, ethnicity, acute physiology score-III (APSIII), first care unit, ventilator and vasopressor use, continuous renal replacement therapy (CRRT), percutaneous coronary intervention (PCI), and coronary artery bypass grafting (CABG) (14). Major comorbidities included diabetes, hyperlipidemia, hypertension, congestive heart failure, peripheral vascular disease, cerebrovascular disease, chronic pulmonary disease, renal disease, malignant cancer, and liver disease. The average values of the following vital signs were collected: mean blood pressure (MBP), heart rate, respiratory rate, temperature, and peripheral capillary oxygen saturation (SpO_2) within 24 h of ICU admission. The following first laboratory test results in the ICU were collected: white

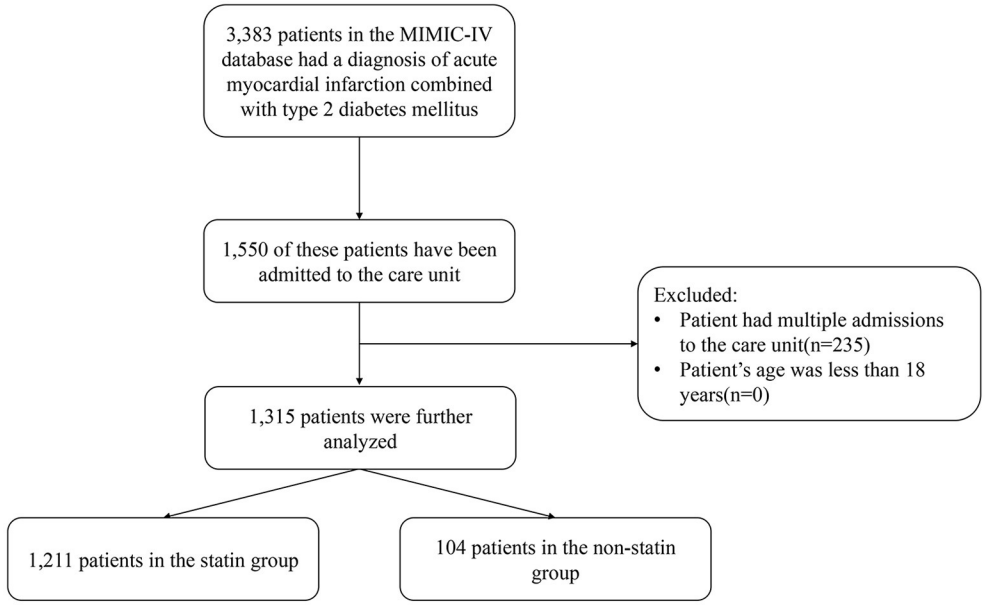

FIGURE 1
Flow chart for study participants.

blood cell (WBC), hemoglobin, platelet, red cell distribution width (RDW), anion gap, potassium, calcium total, creatinine, urea nitrogen, glucose, international normalized ratio (INR), urine output, total bilirubin, lactate, and the peak myocardial infarction markers of troponin T and creatine kinase isoenzyme (CKMB). The end point of this study was whether patients died in hospital, and the secondary outcome was ICU mortality.

Statistical analyses

We first used the multiple imputation method to supplement variables with <20% missing data using the R software "mice" package. The patients in this study were divided into statin and non-statin groups according to whether they had been treated with an antihyperlipidemic agent (HMG-CoA reductase inhibitor). After the data cohort was determined, all categorical variables were expressed in numbers and percentages, and chi-square and Fisher's exact tests were used to determine the differences between the two groups. All continuous variables were expressed as median and interquartile range values, and differences between the two groups was determined using the Mann-Whitney U test. Kaplan-Meier and Cox proportional-hazards regression models were applied to analyze the correlation between the two groups and the outcomes. Log-rank tests were performed as non-parametric analyses to compare the survival distributions of the two groups. Two Cox models were constructed: model 1 had no adjustments, and model 2 was adjusted for all of the above-mentioned covariates. All statistical analyses were performed using R software (version 4.0.1), and $P < 0.05$ (two-sided) was considered indicative of statistical significance.

Results

Baseline characteristics

We finally included and analyzed 1,315 patients with AMI and diabetes from the MIMIC-IV database, among which 1,211 patients used statins during hospitalization (statins group) and 104 did not (non-statin group). The baseline data of the two groups are listed in Table 1. The overall in-hospital mortality of patients with diabetes complicated with AMI was 17.2%, and the total ICU mortality rate was 12.6%. The in-hospital mortality rate was significantly lower in the statin than in the non-statin group (13.9 and 55.8%, respectively; $P < 0.001$), as was the ICU mortality rate (9.3 and 51.0%, respectively; $P < 0.001$). In the statin group, the proportions of those who received CRRT and vasoactive drugs were lower (1.8 and 5.8%, respectively; $P = 0.02$), and the proportions of those that received PCI (26.3 and 12.5%, respectively; $P = 0.003$) and CABG (29.4 and 4.8%, respectively; $P < 0.001$) were higher. There were more patients with hyperlipidemia in the statin than the non-statin group (66.6 and 45.2%, respectively; $P < 0.001$).

TABLE 1 The baseline data of the statins group and non-statin group.

	Statin group $n = 1,211$	Non-statin group $n = 104$	p
Age (year)	71.00 (63.00, 79.00)	75.00 (63.75, 83.00)	0.064
Gender (%)			0.01
Male	766 (63.3)	52 (50.0)	
Female	445 (36.7)	52 (50.0)	
Ethnicity (%)			0.908
White	712 (58.8)	60 (57.7)	
Others	499 (41.2)	44 (42.3)	
Weight (kg)	84.82 (71.26, 99.12)	78.03 (69.16, 93.82)	0.025
APSIII	44.00 (34.00, 61.00)	67.00 (49.00, 93.75)	<0.001
First care unit (%)			<0.001
CCU	923 (76.2)	45 (43.3)	
others	288 (23.8)	59 (56.7)	
Vasopressor (%)			<0.001
No	855 (70.6)	46 (44.2)	
Yes	356 (29.4)	58 (55.8)	
Ventilator (%)			0.109
No	571 (47.2)	40 (38.5)	
Yes	640 (52.8)	64 (61.5)	
CRRT (%)			0.02
No	1,189 (98.2)	98 (94.2)	
Yes	22 (1.8)	6 (5.8)	
PCI (%)			0.003
No	893 (73.7)	91 (87.5)	
Yes	318 (26.3)	13 (12.5)	
CABG (%)			<0.001
No	855 (70.6)	99 (95.2)	
Yes	356 (29.4)	5 (4.8)	
Comorbidities			
Diabetes complicated (%)			0.632
No	698 (57.6)	63 (60.6)	
Yes	513 (42.4)	41 (39.4)	
Hyperlipidemia (%)			<0.001
No	404 (33.4)	57 (54.8)	
Yes	807 (66.6)	47 (45.2)	
Hypertension (%)			0.507
No	722 (59.6)	66 (63.5)	
Yes	489 (40.4)	38 (36.5)	
Congestive heart failure (%)			0.874
No	508 (41.9)	45 (43.3)	
Yes	703 (58.1)	59 (56.7)	
Peripheral vascular disease (%)			1
No	1,021 (84.3)	88 (84.6)	
Yes	190 (15.7)	16 (15.4)	
Cerebrovascular disease (%)			0.095
No	1,015 (83.8)	80 (76.9)	
Yes	196 (16.2)	24 (23.1)	
Chronic pulmonary disease (%)			0.627
No	923 (76.2)	82 (78.8)	
Yes	288 (23.8)	22 (21.2)	
Renal disease (%)			0.841
No	694 (57.3)	58 (55.8)	
Yes	517 (42.7)	46 (44.2)	
Liver disease (%)			<0.001
No	1,127 (93.1)	85 (81.7)	
Yes	84 (6.9)	19 (18.3)	
Malignant cancer (%)			0.07
No	1,134 (93.6)	92 (88.5)	
Yes	77 (6.4)	12 (11.5)	
Vital signs			
MBP (mmHg)	75.48 (69.88, 82.59)	71.87 (64.26, 79.29)	<0.001
Heart rate (bpm)	81.08 (72.04, 90.19)	87.00 (78.01, 100.84)	<0.001
Respiratory rate (insp/min)	18.85 (16.88, 21.00)	20.73 (17.59, 23.68)	<0.001
Temperature (°C)	36.77 (36.60, 36.94)	36.68 (36.50, 37.02)	0.109
SpO_2 (%)	97.09 (95.84, 98.27)	96.59 (95.24, 98.61)	0.242
Laboratory tests			
Troponin T (ng/ml)	1.21 (0.31, 3.47)	0.65 (0.16, 2.43)	0.036
CKMB (ng/ml)	11.00 (4.00, 36.50)	14.50 (4.00, 51.25)	0.367
WBC (k/ul)	9.60 (7.50, 13.10)	11.40 (7.50, 17.70)	0.013
Hemoglobin (g/dl)	11.20 (9.50, 12.80)	10.00 (7.97, 11.70)	<0.001
Platelet (k/ul)	203.00 (158.00, 255.75)	182.50 (126.25, 229.75)	0.003
RDW (%)	14.10 (13.20, 15.50)	15.60 (14.10, 17.10)	<0.001
Anion Gap (mEq/l)	16.00 (13.00, 19.00)	19.00 (16.00, 23.50)	<0.001
Lactate (mmol/l)	1.60 (1.20, 2.30)	3.65 (2.28, 8.12)	<0.001
Potassium (mEq/l)	4.20 (3.90, 4.60)	4.60 (4.05, 5.20)	<0.001
Calcium Total (mg/dL)	8.70 (8.20, 9.10)	8.20 (7.70, 8.90)	<0.001
Glucose (mg/dl)	174.00 (130.00, 237.50)	195.00 (124.50, 315.00)	0.075
INR	1.20 (1.10, 1.30)	1.55 (1.20, 2.30)	<0.001
Creatinine (md/dl)	1.20 (0.90, 1.90)	1.90 (1.17, 2.90)	<0.001
Urea Nitrogen (mg/dl)	25.00 (17.00, 40.00)	37.50 (23.00, 56.25)	<0.001
Urine output (ml)	1535.00 (940.00, 2225.00)	785.00 (231.50, 1448.00)	<0.001
Bilirubin Total (mg/dl)	0.50 (0.30, 0.80)	0.80 (0.50, 1.50)	<0.001
ICU mortality (%)			
No	1,098 (90.7)	51 (49.0)	<0.001
Yes	113 (9.3)	53 (51.0)	

(Continued)

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TABLE 1 Continued

	Statin group $n = 1,211$	Non-statin group $n = 104$	p
In-hospital mortality (%)			
No	1,043 (86.1)	46 (44.2)	<0.001
Yes	168 (13.9)	58 (55.8)	

APSIII, acute physiology score-III; CCU, cardiac care unit; CRRT, continuous renal replacement therapy; PCI, percutaneous coronary intervention; CABG, coronary artery bypass grafting; MBP, mean blood pressure; SpO_2, peripheral capillary oxygen saturation; CKMB, creatine kinase isoenzyme; WBC, white blood cell; RDW, red cell distribution width; INR, international normalized ratio.

Clinical outcomes

Kaplan-Meier survival curves demonstrated that the survival probability was significantly higher in the statin group than in the non-statin group ($p < 0.0001$, Figure 2). Two Cox models were constructed: model 1 had no adjustments, and in model 2 we adjusted for age, gender, weight, ethnicity, APSIII, ventilator use, vasopressor use, CRRT use, PCI use, CABG use, diabetes, hyperlipidemia, hypertension, congestive heart failure, peripheral vascular disease, cerebrovascular disease, chronic pulmonary disease, renal disease, malignant cancer, liver disease, MBP, heart rate, respiratory rate, temperature, SpO_2, WBC, hemoglobin, platelet, RDW, anion gap, potassium, calcium total, creatinine, urea nitrogen, glucose, INR, urine output, total bilirubin, lactate, troponin T and CKMB. After adjusting for all of the above-mentioned covariates using Cox proportional-hazards models, the risks of ICU and in-hospital mortality were significantly lower in the statin than the non-statin group, with HRs of 0.14 (95% CI = 0.08–0.27, Table 2) and 0.28 (95% CI = 0.17–0.47, Table 2), respectively.

Subgroup analyses

Statins are most commonly used to reduce LDL cholesterol, and so a subgroup analysis was applied to the effect of statins on clinical outcomes in subgroups with hyperlipidemia. There were 854 patients with and 461 without hyperlipidemia. In the cohort with hyperlipidemia, the risks of ICU and in-hospital death were lower in the statin group than in the non-statin group, with HRs of 0.07 (95% CI = 0.02–0.20, Table 3) and 0.16 (95% CI = 0.07–0.41, Table 3), respectively; the corresponding values in the cohort without hyperlipidemia were 0.12 (95% CI = 0.04–0.40, Table 3) and 0.36 (95% CI = 0.16–0.84, Table 3), respectively.

Discussion

By collecting the statin use data of hospitalized patients with AMI complicated with diabetes, and comparing them with patients who did not use statins or other lipid-lowering drugs, this retrospective study found that statins had significant clinical benefits on the prognosis of hospitalized patients with diabetes and AMI. Compared with non-statins and other types of lipid-lowering drugs, statins can significantly reduce ICU and in-hospital mortality rates in patients with AMI and diabetes. In the population without hyperlipidemia, statins can still reduce the mortality in patients with AMI and diabetes. Statins can reduce serum LDL cholesterol levels. Current guidelines strongly recommend administering statins at high concentrations or at the maximum tolerance level of patients with AMI without contraindications (15, 16). Some previous studies have demonstrated that the benefits of statins far outweigh their potential risks (17, 18). Statin use is related to difficulty in controlling blood glucose in diabetes and pre-diabetes, but they greatly reduce the risk of cardiovascular events (19). In the current study, patients who took statins had significantly lower ICU mortality and in-hospital mortality risks than those who did not, with HRs of 0.16 (95% CI = 0.12–0.22) and 0.17 (95% CI = 0.13–0.24), respectively. After adjusting for some possible confounders, the advantage of statins in reducing the risk of death remained. In our study, adjusted ICU and in-hospital mortality rates were also significantly reduced, with HRs of 0.14 (95% CI = 0.08–0.27) and 0.28 (95% CI = 0.17–0.47), respectively.

While the present patients in the non-statin group did not use statins to control blood lipids, they may have used other types of lipid-lowering drugs such as fibrates, ezetimibe, and niacin. Several past meta-analyses have found that although fibrates can reduce the risk of cardiovascular events (20–22), they will not reduce all-cause or CVD mortality. Similarly, a meta-analysis found that a combination therapy of statins and fibrates had no more clinical benefits than statins alone (23). A previous study also found no difference in cardiac or all-cause or myocardial infarction mortality between simvastatin-ezetimibe and high-intensity statins in a population with AMI, although a significant reduction in the repeated revascularization rate was observed (24). A previous meta-analysis compared the effects of statins, ezetimibe, and PCSK9 inhibitors, and found that statins had the greatest probability of reducing all-cause and cardiovascular mortality (25). In another study on atherosclerotic vascular disease, compared with statins alone, the combination of niacin-laropiprant and statins not only failed to reduce cardiovascular event risk, but also increased the risks of bleeding, infection, and new-onset diabetes (26). These findings consistently suggest that statins have more benefits than other types of lipid-lowering drugs in patients at higher risks of

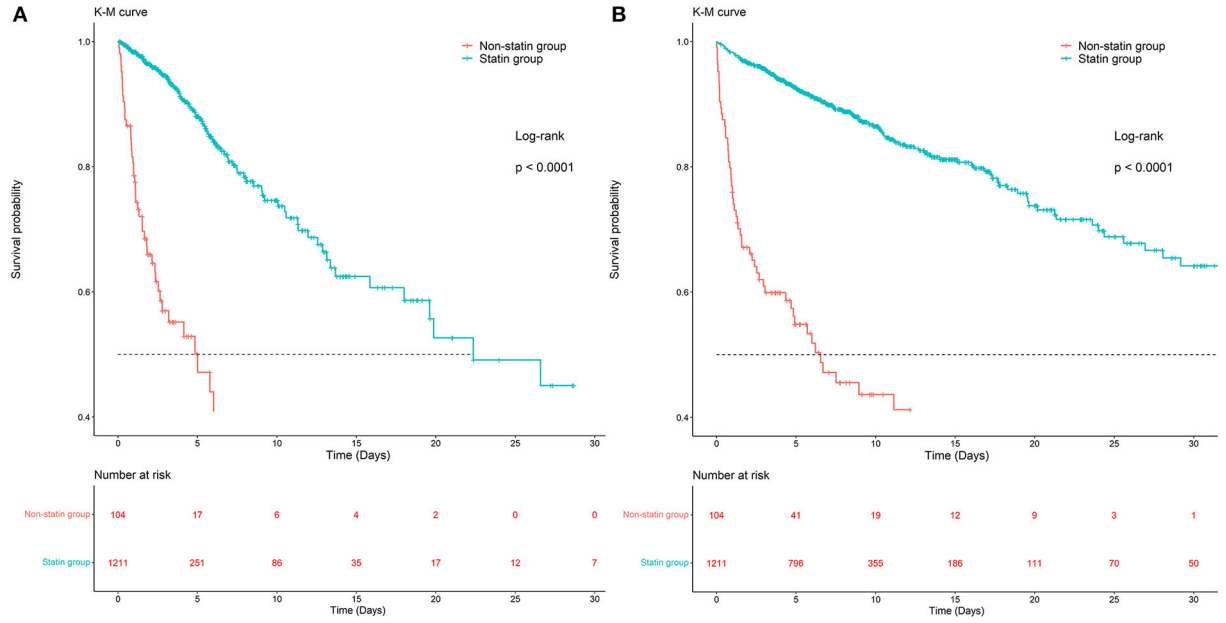

FIGURE 2
Kaplan-Meier survival curves of ICU and in-hospital mortality in the statin group and non-statin group. **(A)** Kaplan-Meier survival curves of ICU mortality in the statin group and non-statin group. **(B)** Kaplan-Meier survival curves of in-hospital mortality in the statin group and non-statin group.

TABLE 2 Clinical outcomes between statin group and non-statin group.

	Non-statin group	Statin group	
	HR (95%CI)	HR (95%CI)	*p*-value
ICU Mortality			
Unadjusted	Reference	0.16 (0.12,0.22)	<0.001
Adjusted	Reference	0.14 (0.08,0.27)	<0.001
In-hospital Mortality			
Unadjusted	Reference	0.17 (0.13,0.24)	<0.001
Adjusted	Reference	0.28 (0.17,0.47)	<0.001

HR, Hazard Ratio; ICU, intensive care unit.

cardiovascular events, and statins remain the most effective way to reduce mortality from these events.

Some novel conclusions can be drawn from this retrospective cohort study. Since statins are the most commonly used treatment for blood lipid control, we performed a subgroup analysis of whether patients were complicated with hyperlipidemia. In patients with hyperlipidemia, statins could significantly reduce ICU and in-hospital death risks, with HRs of 0.07 (95% CI = 0.02–0.20, $P < 0.001$) and 0.16 (95% CI = 0.07–0.41, $P < 0.001$), which is consistent with many guidelines (15, 16). The current study also demonstrated that statins can reduce ICU and in-hospital mortality rates in patients without hyperlipidemia, with HRs of 0.12 (95% CI = 0.04–0.40, $P = 0.001$) and 0.36 (95% CI = 0.16–0.84, $P = 0.018$), respectively. This suggests that statins act via other mechanisms to improve the prognosis of patients with AMI and diabetes. Some previous studies have found that in addition to reducing LDL cholesterol, statins also exert fascinating pleiotropic effects, including anti-inflammatory, inhibiting oxidative stress, antiplatelet aggregation, antithrombosis, and improving vascular tension (27). These effects are essential to inhibiting atherosclerotic plaque progression and thus contribute to an overall reduction of the CVD death risk. However, the exact underlying molecular mechanism has not been determined, and so further research is still needed to clarify it.

Our study had some limitations. First, this study is a single center regression study, which questions the universality of conclusion. Secondly, this study lacked data related to new-onset diabetes, such as fasting blood glucose and glycosylated hemoglobin before and after statins, so it was not able to explain the direct relationship between statins and new-onset diabetes. Third, LDL cholesterol is very important for the population of this study, but due to the limitations of the database, we failed to obtain these data. Finally, because most patients in the statin group in this study were treated with atorvastatin, we cannot provide the results of different statins separately. Notwithstanding these limitations, this study demonstrated

TABLE 3 The effect of statins on clinical outcomes in subgroups with hyperlipidemia.

	ICU mortality			In-hospital mortality		
	HR (95%CI)	*p*-value	p-interaction	HR (95%CI)	*p*-value	p-interaction
Hyperlipidemia			0.595			0.086
No ($n = 461$)	0.12 (0.04,0.40)	0.001		0.36 (0.16,0.84)	0.018	
Yes ($n = 854$)	0.07 (0.02,0.20)	<0.001		0.16 (0.07,0.41)	<0.001	

that statins have protective effects on patients with AMI and diabetes.

Conclusions

Compared with non-statins and other types of lipid-lowering drugs, statins can significantly reduce ICU and in-hospital mortality rates in patients with AMI and diabetes. Even in the population without hyperlipidemia, statins can still reduce the mortality in patients with AMI and diabetes. Although prospective randomized trials are needed to confirm the current results, they strongly suggest that statins have a protective effect on patients with AMI and diabetes.

Data availability statement

Publicly available datasets were analyzed in this study. This data can be found here: The data were available on the MIMIC-IV website at https://mimic.physionet.org/, https://doi.org/10.13026/a3wn-hq05.

Author contributions

XL and LZ created the study protocol, performed the statistical analyses, and wrote the first manuscript draft. SL conceived the study and critically revised the manuscript. DH assisted with data collection and manuscript editing. TH and HL assisted the analysis and explain of statistical methods. HY assisted with manuscript revision and data confirmation. JL contributed to data interpretation and manuscript revision. All authors read and approved the final manuscript.

Funding

This study received financial support from the National Natural Science Foundation of China (Nos. 82072232 and 81871585), the Natural Science Foundation of Guangdong Province (No. 2018A030313058), Technology and Innovation Commission of Guangzhou Science, China (No. 201804010308), Guangdong Provincial Key Laboratory of Traditional Chinese Medicine Informatization (2021B1212040007).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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frontiers | Frontiers in Cardiovascular Medicine

TYPE Original Research
PUBLISHED 09 August 2022
DOI 10.3389/fcvm.2022.975920

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Cardiovascular Therapeutics, a section of the journal Frontiers in Cardiovascular Medicine

RECEIVED 22 June 2022
ACCEPTED 18 July 2022
PUBLISHED 09 August 2022

CITATION
Li B, Yuan Z, Zhang Y, Li F, Huang L, Yang Z, Liu H and Liu Z (2022) Exploring the role of uterine fibroids in promotion of cardiovascular diseases by diabetes exposure: Findings from national health and nutrition examination survey 1999–2006.
Front. Cardiovasc. Med. 9:975920.
doi: 10.3389/fcvm.2022.975920

Exploring the role of uterine fibroids in promotion of cardiovascular diseases by diabetes exposure: Findings from national health and nutrition examination survey 1999–2006

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Objective: The relationship between uterine fibroids (UF) and cardiovascular diseases (CVDs) in the diabetes population seemed to remain undetermined in previous studies. This study aims to explore the association between UF and CVDs by using the database from the National Health and Nutrition Examination Survey (NHANES). To further evaluate the connection between UF and CVDs we also tested the potential differences due to diabetes exposure.

Materials and methods: National Health and Nutrition Examination Survey data (1999–2006) were collected and used in this study. A total of 5,509 individuals were included and analyzed. The student's *t*-test and the chi-squared test were used to explore the demographic characteristic between UF and non-UF groups. Logistic regression analysis was performed to determine the odds ratios of UF and covariates.

Results: Female participants were divided into UF (n = 694, 12.60%) and non-UF (n = 4,815, 87.40%) groups. The incidence of CVDs in UF patients (n = 245, 35.30%) were higher than non-UF individuals (n = 776, 16.12%) ($p < 0.001$). In addition, each subtype of CVDs were also different, which contains hypertension (33.29 vs. 15.31%, $p < 0.001$), heart failure (1.59 vs. 0.52%, $p < 0.01$), angina (2.59 vs. 0.62%, $p < 0.001$), heart attack (1.73 vs. 0.58%, $p < 0.01$) and coronary heart disease (1.44 vs. 0.54%, $p < 0.01$). The odds ratios

of CVDs according to logistic regression were 2.840 (95% CI: 2.387–3.379) for UF patients ($p < 0.001$), while the odds ratios (ORs) were 1.438 (95% CI: 1.175–1.760) after taking account for the age, body mass index (BMI), diabetes, race, education, and annual family income ($p < 0.001$). In addition, secondary analysis indicated more adverse effects in by UF exposure on CVDs risk among non-diabetes individuals (OR = 1.389, 95% CI = 1.124–1.718, $p < 0.01$) than diabetes patients ($p = 0.063$).

Conclusion: Overall, UFs were positively associated with CVDs, and this effect seems blunted by diabetes exposure.

KEYWORDS

cardiovascular diseases, uterine fibroids, diabetes, NHANES, hypertension, heart failure, coronary artery disease

Introduction

Cardiovascular diseases (CVDs) is one of the leading cause of death throughout the world. As a significant public health problem, the underlying factors that are linked to the incidence and progress of CVDs need to be identified. Uterine fibroid (UF), also known as uterine leiomyomas, is a tumor derived from the muscle layer of the uterus and affects millions of women (1). It is still controversial whether UFs contribute to the occurrence of CVDs. A previous study from Northern Finland indicated that increased blood lipids or metabolic syndrome are associated with a higher risk of UF (2). Thus, it is plausible that diabetes might be essential in UF, while a population study with 3,789 subjects from America indicated that the presence of type 2 diabetes has a protective effect on UF regardless of the medication intervention (3). Although diabetes is a traditional risk factor for CVDs, the connection between UF and CVDs needs more investigation. In addition, another study on symptomatic UF Dutch women did not find any correlation between UF and cardiovascular risks except for hypertension (4). This discrepancy in conclusions might be due to the difference in regions. In addition, elevated blood pressure is positively associated with UF in a prospective study (5). Thus, UF and hypertension might influence and make cause and affect each other, which possibly suggests a similar clinical risk in these two diseases.

Generally, asymptomatic patients do not need medical treatment, as UF belongs to a benign tumor. However, the etiology of UF is obscured, which contains genetic inheritance, hormones, and abnormal stem cells. Indeed, the hereditary susceptibility of UF is influenced by various hormones (6). Estrogen is one of the recognized factors that promote the growth of fibroids (7), while some studies indicated that growth hormone and prolactin collaborated with estrogen to promote mitosis in UF (8). In addition, these hormones are regulated by the hypothalamic-pituitary axis which probably plays a crucial role in its pathogenesis.

Recent studies have indicated that the renin-angiotensin-aldosterone system (RAAS) participated in the progress of UF and hypertension (9). Angiotensin II induces vasoconstriction and promotes the proliferation of leiomyoma cells (4). In addition, Hana et al. proved that the risks of hypertension were increased in UF patients (4). Although they only reveal the connection between UF and hypertension risks, hypertension is indeed a risk factor for various CVDs. Importantly, some studies demonstrated that Angiotensin-converting enzyme inhibitors reduced the incidence of UF (10). Additionally, Angiotensin II is a robust factor in inducing inflammation response (11), while inflammation seems to be a common factor that influences both CVDs and UF. The inflammatory cells not only invaded the uterus and increased the number of fibrotic cells (12), but also increased vascular constriction.

Here, we investigated the connection between CVDs and UF under the stimulation of diabetes by exploring the data from the National Health and Nutrition Examination Survey (NHANES).

Materials and methods

Data source and participants

Data from NHANES 1999 to 2006 was used in the analysis. NHANES provides a national estimation of health information in America by conducting interviews, and medical and laboratory examinations on each participant. UF individuals were verified by asking 'Told by doctor had UFs' from the questionnaire of RHQ380. The diagnosis of diabetes was verified from the questionnaire of DIQ010. CVDs patients contained hypertension, heart failure, coronary artery disease, angina, or heart attack. The verification of hypertension is based on the questionnaire from BPQ020, while the verification of heart failure, coronary artery disease, angina, and heart attack was according to the questionnaire of MCQ160B, MCQ160C, MCQ160D, and MCQ160E, respectively.

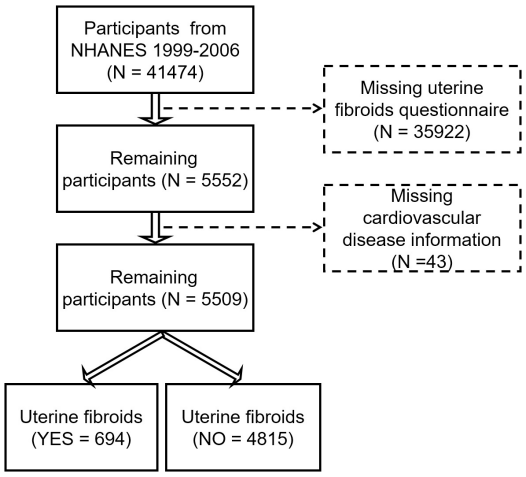

FIGURE 1
The flowchart of sample selection criteria for the association between cardiovascular diseases (CVDs) and uterine fibroids.

Statistical analysis

The data analyses were performed by R version 2.1.1 and SPSS statistical package version 20.0. The quantitative data are exhibited as the mean ± standard deviation (ME ± SD), while the qualitative data are shown as numbers (n) and percentages (%). A chi-squared test was performed to assess the differences between each subtype of CVDs. Logistic regression analysis was used to identify the factors that were independently associated with CVDs. The odds ratios (ORs) were used to calculate the risk factors for CVDs. A p-value of < 0.05 was considered statistically significant.

Results

Descriptive statistics

In the present study, we enrolled 41,474 participants in NHANES 1999–2006. The definition of CVDs was individuals with hypertension, heart failure, coronary artery disease, angina, or heart attack. After excluding participants without the information of UF (n = 35,922) and CVD (n = 43), a total of 5,509 females aged from 18 to 54 years old were enrolled (Figure 1). The Venn diagram showed the distribution and overlay of each subtype of CVDs that are enrolled. There are 968 individuals with hypertension, 36 individuals with hypertension, 48 individuals with angina, and 40 individuals with a heart attack in this study (Figure 2). Any individuals without the exact information of the above-described diseases were excluded. Of these included individuals, 2.55% suffered from at least 3 kinds of diseases,

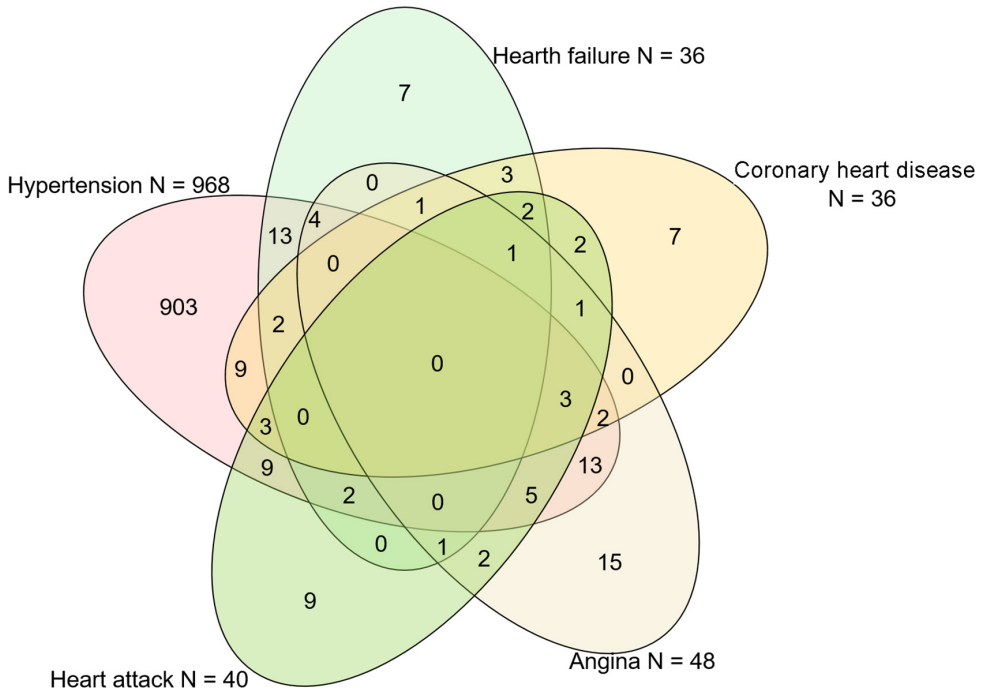

FIGURE 2
Venn diagram of individuals' distribution in each subgroup of cardiovascular diseases (CVDs).

TABLE 1 Demographics and clinical characteristics of participants [National Health and Nutrition Examination Survey (NHANES) 1999–2006].

Characteristic	Uterine fibroids (n = 694, 12.60%)	Non-uterine fibroids (n = 4815, 87.40%)	*P-value*
Age, years, *N* (%)			
<30	43 (6.20%)	1998 (41.50%)	<0.001[a]
30–40	164 (23.63%)	1361 (28.27%)	
> = 40	487 (70.17%)	1456 (30.24%)	
Mean ± SD	43.65 ± 7.35	34.53 ± 9.90	<0.001[b]
BMI, kg/m^2, *N* (%)			
<20	30 (4.34%)	331 (6.95%)	<0.001[a]
20–25	166 (24.02%)	1367 (28.69%)	
25–30	178 (25.76%)	1370 (28.75%)	
> = 30	316 (45.73%)	1694 (35.55%)	
Mean ± SD	30.50 ± 7.95	28.62 ± 7.24	<0.001[b]
Missing	1 (0.14%)	3 (0.06%)	
Diabetes, *N* (%)	63 (9.08%)	187 (3.88%)	<0.001[a]
Race, *N* (%)			
Mexican American	86 (12.39%)	1205 (25.03%)	<0.001[a]
Other Hispanic	23 (3.31%)	244 (5.07%)	
Non-Hispanic White	297 (42.80%)	2262 (46.98%)	
Non-Hispanic black	264 (38.04%)	879 (18.26%)	
Other race or multi-racial	24 (3.46%)	225 (4.67%)	
Education, *N* (%)			
<High school	117 (16.86%)	1201 (24.97%)	<0.001[a]
High school	149 (21.47%)	1082 (22.49%)	
>High school	428 (61.67%)	2527 (52.54%)	
Missing	0	5 (0.01%)	
Annual family income, *N* (%)			
<20,000 USD	140 (20.17%)	1358 (28.20%)	<0.001[a]
≥20,000 USD	532 (76.66%)	3317 (68.89%)	
Missing	22 (3.17%)	140 (2.91%)	
Current smoking status, *N* (%)			
Smoking	158 (22.77%)	1048 (21.77%)	0.126[a]
Non-smoking	138 (19.88%)	755 (15.68%)	
Missing	398 (57.35%)	3012 62.55%)	
Cardiovascular disease, *N* (%)	245 (35.30%)	776 (16.12%)	<0.001[a]
Hypertension, *N* (%)	231 (33.29%)	737 (15.31%)	<0.001[a]
Hearth failure, *N* (%)	11 (1.59%)	25 (0.52%)	<0.01[a]
Angina, *N* (%)	18 (2.59%)	30 (0.62%)	<0.001[a]
Heart attack, *N* (%)	12 (1.73%)	28 (0.58%)	<0.01[a]
Coronary heart disease, *N* (%)	10 (1.44%)	26 (0.54%)	<0.01[a]
SBP, Mean ± SD	121.66 ± 18.22	113.56 ± 14.59	<0.001[b]
DBP, Mean ± SD	74.27 ± 11.13	67.85 ± 12.14	<0.001[b]

Data are presented as *N*% (χ2 test) and mean ± standard deviation (SD) (independent *t*-test), which are denoted by [a] and [b], respectively.

7.65% suffered from at least 2 kinds of diseases, while the percent of hypertension patients suffering from heart failure, coronary artery disease, angina, or heart attack at the same time were 58.33, 55.56, 58.33, and 52.50%, respectively.

Demographics of participants

Our study included a total of 5,509 participants (**Figure 1**), in which we identified UF in 694 participants. The clinical characteristics of the participants from NHANES 1999–2006

are reported in Table 1. Approximately 12% (n = 694) of the included population are individuals with UF. They were older than the non-UF group, with 70.17% (n = 487) of individuals older than 40 years old. In addition, UF individuals have a higher level of body mass index (BMI) (30.50 ± 7.95 vs. 28.62 ± 7.24 kg/m^2, $p < 0.001$), with almost 45.73% of UF individuals having a BMI larger than 30 kg/m^2. Compared with non-UF individuals, UF patients that were more likely to suffer from diabetes, had higher education levels, higher family incomes, and higher blood pressure. However, they were no significant differences between smoking status. The morbidity of CVDs in UF patients (35.30%, 245/694) is higher than in non-UF individuals (16.12%, 776/4815). The incidence and subtype of CVDs are shown in Table 1 as numbers and percentages. Then we also explored their difference in each subtype of CVDs, in which, UF individuals have a higher proportion of hypertension, heart failure, angina, heart attack, and coronary heart disease.

Association between uterine fibroids and cardiovascular diseases

Table 2 shows the crude and adjusted odds ratios for the association between UF and CVDs. Compared to individuals without UF, these UF patients had higher odds ratios for CVDs (OR = 2.840, 95% CI = 2.387–3.379, $p < 0.001$). In addition, this relationship persisted after adjusting for age, BMI, diabetes, race, education, annual family income (OR = 1.438, 95% CI = 1.175–1.760, $p < 0.001$). In the adjusted model, except for education level, age, BMI, race, and family income were significantly associated with CVDs. It is no surprise that diabetes patients have the highest odds ratio for CVDs in the adjudged model (OR = 3.308, 95% CI = 2.459–4.452). Then we explored the relationship between UF and CVDs and presented the results stratified by the history of diabetes.

TABLE 2 Logistic regression analysis of uterine fibroids and cardiovascular diseases (CVDs) in National Health and Nutrition Examination Survey (NHANES) 1999–2006.

	OR	95% CI	*P-value*
Crude model			
Uterine fibroids			<0.001
Non-uterine fibroids	Ref.	Ref.	
Uterine fibroids	2.840	2.387–3.379	
Adjudged model			
Uterine fibroids			<0.001
Non-uterine fibroids	Ref.	Ref.	
Uterine fibroids	1.438	1.175–1.760	
Age, years	1.074	1.065–1.084	<0.001
BMI, kg/m^2	1.066	1.056–1.077	<0.001
Diabetes			<0.001
Yes	3.308	2.459–4.452	
No	Ref.	Ref.	
Race			<0.001
Mexican American	0.930	0.595–1.453	0.749
Other Hispanic	0.979	0.563–1.702	0.939
Non-Hispanic white	1.272	0.834–1.939	0.264
Non-Hispanic black	1.793	1.162–2.767	<0.01
Other race or multi-racial	Ref.	Ref.	
Education			0.064
<High school	Ref.	Ref.	
High school	1.081	0.858–1.361	0.510
>High school	0.866	0.702–1.070	0.183
Annual family income			<0.01
<20,000 USD	Ref.	Ref.	
≥20,000 USD	0.785	0.656–0.938	

Association analysis of unadjusted and adjusted models of uterine fibroids and cardiovascular diseases in patients with or without diabetes

Association analysis results between UF and CVDs in diabetes individuals were modeled crude and adjusted for age, BMI, race, education, and family income (Table 3). In diabetes patients, UF patients had a higher odds ratio for CVDs (OR = 2.785, 95% CI = 1.475–5.258, $p < 0.01$). However, in the adjusted model, there is no significance with p-values of 0.063. Then, we conducted a logistic regression analysis in patients without diabetes (Table 4). We found that UF patients had a higher odds ratio for CVDs (OR = 2.655, 95% CI = 2.203–3.200, $p < 0.001$). Of note, in the adjudged model, the odds ratio for CVDs in UF patients without diabetes (adjudged for age, BMI, race, education, and income) still has significance (OR = 1.389, 95% CI = 1.124–1.718, $p < 0.01$).

Discussion

In this cross-sectional study, we investigated the association between CVDs and UF in the general population and diabetes population by using the NHANES 1999–2006 data. We found that UF patients had a higher risk of CVDs, and this association still remained significant after taking into account age, BMI, diabetes, race, education, and family incomes. In addition, we evaluated models limited to women with or without diabetes in the secondary analysis. These models indicated the role of UF in promoting CVDs seems more significant in non-diabetes individuals.

Various evidence suggested similar risk factors or biological mechanisms between fibroids and CVDs, which contain atherosclerosis, hypertension, and hyperlipidemia (13).

TABLE 3 Logistic regression analysis of uterine fibroids and cardiovascular diseases (CVDs) in diabetes individuals: National Health and Nutrition Examination Survey (NHANES) 1999–2006.

	OR	95% CI	*P-value*
Crude model			
Uterine fibroids			<0.01
Non-uterine fibroids	Ref.	Ref.	
Uterine fibroids	2.785	1.475–5.258	
Adjudged model			
Uterine fibroids			0.063
Non-uterine fibroids	Ref.	Ref.	
Uterine fibroids	1.983	0.964–4.077	
Age, years	1.091	1.050–1.133	<0.001
BMI, kg/m^2	1.059	1.020–1.099	<0.01
Race			0.128
Mexican American	0.494	0.115–2.121	0.342
Other Hispanic	0.331	0.055–1.990	0.227
Non-Hispanic white	0.981	0.236–4.083	0.979
Non-Hispanic black	1.285	0.310–5.327	0.729
Other race or multi-racial	Ref.	Ref.	
Education			0.303
<High school	Ref.	Ref.	
High school	0.516	0.210–1.267	0.149
>High school	0.596	0.272–1.308	0.197
Annual family income			0.422
<20,000 USD	Ref.	Ref.	
≥20,000 USD	0.771	0.409–1.454	

TABLE 4 Logistic regression analysis of uterine fibroids and cardiovascular diseases (CVDs) in patients without diabetes: National Health and Nutrition Examination Survey (NHANES) 1999–2006.

	OR	95% CI	*P-value*
Crude model			
Uterine fibroids			<0.001
Non-uterine fibroids	Ref.	Ref.	
Uterine fibroids	2.655	2.203–3.200	
Adjudged model			
Uterine fibroids			<0.01
Non-uterine fibroids	Ref.	Ref.	
Uterine fibroids	1.389	1.124–1.718	
Age, years	1.074	1.065–1.083	<0.001
BMI, kg/m^2	1.067	1.056–1.078	<0.001
Race			<0.001
Mexican American	0.996	0.617–1.607	0.987
Other Hispanic	1.102	0.613–1.981	0.745
Non-Hispanic white	1.333	0.849–2.092	0.212
Non-Hispanic black	1.882	1.182–2.996	<0.01
Other race or multi-racial	Ref.	Ref.	
Education			0.053
<High school	Ref.	Ref.	
High school	1.134	0.892–1.441	0.305
>High school	0.890	0.714–1.110	0.302
Annual family income			<0.05
<20,000 USD	Ref.	Ref.	
≥20,000 USD	0.795	0.659–0.959	

Previous studies indicated that a high-fat diet is associated with levels of endogenous estradiol, a risk factor for UF (14). However, another study in Japan suggests no significant association between fat intake and UF, while alcohol intake increased the risk of UF (15). The dietary patterns have been proved to be linked with the occurrence of CVDs (16); therefore, it is plausible that they shared similar risk factors in dietary composition. In addition, our previous study suggested a relationship between the dietary inflammatory index and heart failure (17). Another interesting finding is that Simvastatin, a traditional cardiovascular drug, inhibits the Wnt/β-catenin pathway to delay the progress of UF (18). Statin is a kind of drug that is applied in lowing blood lipids, increasing the stability of atherosclerotic plaque and playing a role in anti-inflammation (19). Korkmaz1 et al. reported that uterine leiomyoma was connected with blood lipid profile, insulin resistance, and carotid intima-media thickness (CIMT) in reproductive-aged women in a small sample size study (20). Indeed, hyperlipemia, insulin resistance, and atherosclerosis are essential risk factors for various CVDs. On the contrary, in a cohort with 972 participants, Shannon et al. suggested that the presence of UF was not associated with CIMT and left ventricular mass (21). Nevertheless, our results partly support Korkmaz1's findings, as the incidence of coronary heart disease, angina, and heart attack are higher in UF patients in our study.

Another issue that should not be ignored is the side effects of the treatment of UF. A Norwegian cross-sectional study indicated a larger cumulative probability of CVDs after hysterectomy (22). Thus, this type of operation might affect the occurrence of CVDs. Androgen is also applied in UF for its role in inhibiting the growth of tumors, however, the roles of androgen in CVDs are still controversial (23). In addition, mifepristone as a glucocorticoid antagonist, used in inhibiting the growth of UF, reduced the high density lipoprotein-cholesterol (HDL-C) and high density lipoprotein (HDL) particle concentration (24). However, mifepristone did not affect corticosterone-induced hypertension (25). Indeed, these confounding factors need further basic and clinical perspective epidemiology investigation to clarify their relationships.

On the other hand, diabetes is one of the recognized cardiovascular risk factors, while various antidiabetics have been proved to be beneficial for improving cardiovascular outcomes (26). As expected, the factor of diabetes occupied the highest odds ratio for CVDs in the adjusted model in our study. Thus, we further performed secondary analysis according to the diagnosis of diabetes. Amazingly, in non-diabetes individuals, UF significantly has an OR other than in diabetes individuals.

A previous population study from America demonstrated that diabetes is a protective effect of UF regardless of medication type (3); therefore, it might blunt the effect of UF on CVDs. However, this interesting finding still needs further prospective studies to verify.

There are still some limitations in the present study. First, this is an observational study that obtain diagnosis information through interviews. Second, considering the censoring data on smoking in this study, we do not analyze the influence of smoking in the logistic model. Perhaps due to the societal attribute of female, more than 50% of the female participants refused to answer the question about smoking. Nevertheless, there was no significant difference in smoking status between UF patients and non-UF individuals in this study. In addition, a previous study showed no significant difference in smoking between UF and healthy individuals (2). Third, hypertension patients account for the highest proportion of CVDs in the study, which might contribute to a bias.

Conclusion

UF might increase the risk of CVDs, while this role seems more harmful in non-diabetes individuals rather than in diabetes. However, further prospective study or animal research is required to confirm their relationship and unveil the underlying mechanism.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://wwwn.cdc.gov/nchs/nhanes/Default.aspx.

Ethics statement

The studies involving human participants were reviewed and approved by the Participants provided written informed consent and the process were approved by the National Center for Health Statistic's Research Ethics Review Board. The present study is based on a secondary date analysis which lacked personal identifiers and does not need institutional reviewing. The patients/participants provided their written informed consent to participate in this study.

Author contributions

ZL, HL, and BL designed the experiments and wrote the manuscript. BL, ZL, YZ, FL, and ZYu collected and analyzed the data. BL, ZL, ZYu, LH, and ZYa organized the figures and tables. HL, ZL, LH, and ZYa helped with data interpretation. BL and ZL critically reviewed the manuscript. All authors contributed to the article and approved the submitted version.

Funding

This study was partly supported by a grant from the Natural Science Foundation of Fujian Province (2021J05287 and 2021J05283), the National Natural Science Foundation of China (82100385), the Health Technology Project of Fujian Province (2021QNB016), and the grant of Xiamen High-Level Health Talents.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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frontiers | Frontiers in Cardiovascular Medicine

TYPE Original Research
PUBLISHED 11 August 2022
DOI 10.3389/fcvm.2022.974802

OPEN ACCESS

EDITED BY
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SPECIALTY SECTION
This article was submitted to Cardiovascular Therapeutics, a section of the journal Frontiers in Cardiovascular Medicine

RECEIVED 21 June 2022
ACCEPTED 25 July 2022
PUBLISHED 11 August 2022

CITATION
Chen J, Wang X, Dong B, Liu C, Zhao J, Dong Y, Liang W and Huang H (2022) Cardiac function and exercise capacity in patients with metabolic syndrome: A cross-sectional study. *Front. Cardiovasc. Med.* 9:974802. doi: 10.3389/fcvm.2022.974802

Cardiac function and exercise capacity in patients with metabolic syndrome: A cross-sectional study

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Background: Metabolic syndrome is a pre-diabetes condition that is associated with increased cardiovascular morbidity and mortality. We aimed to explore how exercise capacity, cardiac structure, and function were affected in patients with metabolic syndrome.

Methods: Outpatients with echocardiography and exercise stress test combined with impedance cardiography (ETT + ICGG) results available from Nov 2018 to Oct 2020 were retrospectively enrolled. Echocardiographic, ETT + ICG profiles, and exercise performance were compared between patients with metabolic syndrome and the ones without. Sensitivity analyses were performed excluding patients without established coronary heart disease and further 1:1 paired for age and gender, respectively. Multiple linear regression was used to find out related predictors for maximal metabolic equivalents (METs).

Results: Three hundred and twenty-third patients were included, among whom 97 were diagnosed as metabolic syndrome. Compared to patients without metabolic syndrome, echocardiography showed that patients with metabolic syndrome had a significantly lower E/A ratio ($p < 0.001$). Besides, they have larger left atrium, larger right ventricle, and thicker interventricular septum (all $p < 0.001$), but similar left ventricular ejection fraction ($P = 0.443$). ICG showed that patients with metabolic syndrome had significantly higher stroke volume at rest and maximum ($p < 0.001$), higher left cardiac work index at rest and maximum ($p = 0.005$), higher systemic vascular resistance (SVR) at rest ($p < 0.001$), but similar SVI ($p = 0.888$). During exercise, patients with metabolic syndrome had lower maximal METs ($p < 0.001$), and a higher proportion suffering from ST-segment depression during exercise ($p = 0.009$). Sensitivity analyses yielded similar results. As for the linear regression model, 6 independent variables (systolic blood pressure, BMI, E/A ratio, the height of O wave, the peak value of LCWi, and the baseline of SVR) had statistically significant effects on the maximal METs tested in exercise ($R = 0.525$, $R^2 = 0.246$, $P < 0.001$).

Conclusion: Patients with metabolic syndrome had significant structural alteration, apparent overburden of left ventricular work index, pre-and afterload, which may be the main cause of impaired exercise tolerance.

KEYWORDS

metabolic syndrome, cardiac function, exercise capacity, impedance electrocardiogram, exercise tolerance test

Introduction

Since the conception of metabolic syndrome (MS) was described thoroughly in 2001 (1), MS has become a challenging problem due to its increasingly higher prevalence and cardiovascular disease-related morbidity (2, 3). MS is a state in which multiple metabolic risk factors of cardiovascular disease, including systemic hypertension, hyperlipidemia, and impaired fasting glucose, gather in individuals. As has been researched for years, MS is associated with many cardiovascular diseases, such as coronary heart disease, myocardial infarction, acute heart failure, cardiogenic shock, and so on (3).

In a previous study, clinical and subclinical systolic and diastolic dysfunction have been demonstrated by echocardiography in MS patients without coronary artery disease (4, 5). Furthermore, LV diastolic dysfunction, instead of systolic dysfunction, has been associated with limited exercise capacity independent of ischemia (6). However, some studies have come to different conclusions. Chung et al. indicated that the MS group had relatively high physical activity levels compared to the normal group of elderly women (7). So further studies are still needed for the evaluation of the exercise capacity and cardiac function of MS patients. In a previous study, the exercise capacity was assessed using echocardiography, exercise tolerance test, or cardiopulmonary exercise test, all of which were imaging or metabolic evaluation indicators (8).

Nowadays, impedance cardiography (ICG) can be used to record cardiac output continuously during exercise by combing with an exercise tolerance test, which provides us with another tool to evaluate the exercise capacity dynamically. High-definition impedance cardiography (HD-ICG), also known as signal morphology impedance cardiography (SM-ICG), is a radical improvement of ICG technology. Different from the conventional ICG, HD-ICG doesn't need to measure chest impedance (Z0) and chest geometric volume to evaluate baseline chest fluid. Accordingly, Obese, edematous, and moving patients can be measured much more accurately (9). In this study, we aim to explore how cardiac function and exercise capacity are affected in patients with MS, using echocardiography and high-definition impedance cardiography combined with exercise tolerance test (HD-ICG + ETT).

Methods

Study populations

In the present study, we retrospectively recruited non-heart failure patients who underwent HD-ICG+ ETT as well as echocardiography examination in Sun Yat-sen University's first affiliated hospital from January 2019 to October 2020. A total of 323 patients were recruited. Next, we excluded people who had been previously diagnosed with coronary heart disease and then matched MS patients and non-MS patients with gender and age as matching factors in remanent non-coronary heart disease patients (Specific matching methods were described later in statistical analysis).

Diagnostic of metabolic syndrome

Metabolic syndrome (MS) was diagnosed following the National Cholesterol Education Program's Adult Treatment Panel III (NCEP: ATP III) criteria (1). Briefly, patients with at least 3 of the 5 conditions were diagnosed as MS: body mass index (BMI) $\geq$ 25 kg/m^2, high blood pressure ($\geq$130/85 mmHg), high triglyceride ($\geq$150 mg/dL), low high intensity lipoprotein-cholesterol (HDL-c, <40 mg/dL for men and <50 mg/dL for women), and high glucose ($\geq$110 mg/dL).

Clinical measurement

At the beginning of the inspection, patients were required to undergo an evaluation of height and body weight, which were used for the calculation of BMI. Resting blood pressure, including systolic blood pressure (SBP) and diastolic blood pressure (DBP), were measured three times with an electronic sphygmomanometer, taking down systolic and diastolic values respectively. Patients would be recorded as "increased blood pressure" if they were recorded with resting systolic blood pressure $\geq$130 mmHg or diastolic blood pressure $\geq$85 mmHg. Besides, the history of hypertension was also recorded.

Measurements included an M-mode echocardiogram performed after the selection of the measurement section by the B-mode scan. This allowed the assessment of left ventricular

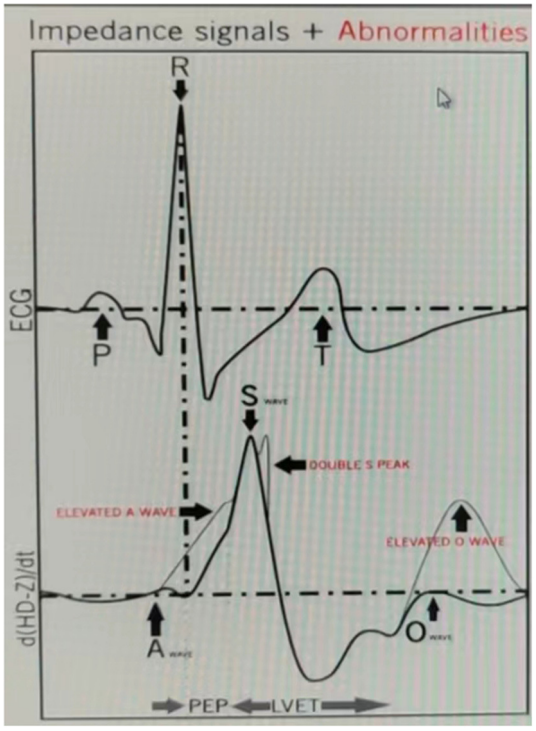

FIGURE 1
Impedance signals and the position of the A, S, and O wave.

diastolic and systolic diameters, left ventricular ejection fraction (LVEF), left atrial diameter (LAD), right ventricular diameter (RVD), E/A ratio, E/E' ratio, and the thickness of each left ventricular wall, and stroke volume (SV) calculated by Simpson's method. The details about the acquisition of the A, S, and O waves are as follow in Figure 1. After a slight power delay, the P wave on ECG coincides with the first wave peak, that is A wave, in the second derivative waveform (dHD-Z/dt) which describes fluid acceleration, the A point. The A point marks the onset of late diastolic filling. The A wave appears only when there is atrial contraction, and its peak corresponds to the A wave of the Doppler echocardiogram. The second wave in the second derivative waveform (dHD-Z/dt) is the S wave, which helps evaluate cardiac contractility. The O wave represents the last wave in the second derivative waveform (dHD-Z/dt) and is associated with the opening of the mitral valve. The peak of O wave corresponds to the peak of the trans-mitral E wave detected by Doppler echocardiography.

Moreover, patients were able to undergo high-definition impedance cardiography with exercise tolerance test. Cyclometer was used to perform the exercise test. Wattage increments were determined by patient's age, gender, weight, and height. The test would be ceased until patients exhaust or ischemia-related symptoms occur. It could provide the height of A, S, O wave, stroke volume, stroke volume index (SVI), cardiac output (CO), cardiac contractility (CTI), left cardiac work index (LCWi), systemic vascular resistance (SVR), systemic vascular resistance index (SVRi), and other indicators at rest and exercise peak.

In addition, the type of SV change would be taken down and sorted into three types: (1) normal response meant that SV was elevated consistently during the exercise; (2) SV flat meant that there is a plateau during the exercise; (3) SV descent was described as a decline of stroke volume after a stable rise during the exercise. Meanwhile, the exercise tolerance test was assessed following the universal method, taking down the depth of ST-segment depression. ST-segment depression was also divided into three categories: (1) ST-segment depression within 0–0.1 mV was defined as no depression; (2) ST-segment depression within 0.1–0.2 mV; (3) ST-segment depression more than 0.2 mV was divided into two groups. In our study, we only extracted the details of each patient from our database while all the inspections were required in the diagnostic routine. Since it was an observational retrospective study, informed consent from participants was not needed.

Statistical analysis

Continuous variables were presented as mean ± SD, and independent sample *t*-tests were used for comparisons between them. Categorical variables were presented as percentages and compared by chi-square tests. To further validate the differences in impedance cardiography and echocardiography between MS patients and non-MS patients, one sensitivity analysis was performed: (i) excluding those with coronary heart disease history (169 patients), (ii) further 1:1 paired with age (within 2 years old) and gender, in those without coronary heart disease history (154 patients). What's more, we used multiple linear regression to explore the relationship between metabolic equivalences and other indicators from echocardiography and impedance cardiography measured before. Besides, we used automatic linear modeling to explore the optimum combination of independent variables toward maximal METs. All statistical analyses were performed with SPSS version 26.0. $P < 0.05$ was considered statistically significant.

Results

Clinical, demographic, echocardiography, and HD-ICG + ETT variables of the included patients and 1:1 paired patients were shown in Tables 1–4.

Among patients with and without metabolic syndrome

We found significantly higher BMI ($p < 0.001$), and systolic and diastolic blood pressure ($p < 0.001$) at rest in patients

TABLE 1 The baseline characteristics of included patients and non-CHD one-one paired.

	Included patients			Non-CHD one-one paired		
	Non-MS	MS	*P*	Non-MS	MS	*P*
Age (years)	43.91 ± 15.83	51.37 ± 11.61	<0.001	48.78 ± 13.04	49.82 ± 11.38	0.599
Male	88, 38.94%	42, 43.30%	0.536	29, 37.7%	29, 37.7%	>0.999
BMI (kg/m^2)	22.36 ± 2.91	26.00 ± 0.30	<0.001	22.60 ± 2.84	26.13 ± 3.08	<0.001
SBP base (mmHg)	118.42 ± 13.61	131.20 ± 16.18	<0.001	121.58 ± 14.61	132.03 ± 15.59	<0.001
DBP base (mmHg)	74.91 ± 9.34	83.99 ± 10.27	<0.001	75.96 ± 9.92	85.26 ± 10.56	<0.001
Hypertension/increased blood pressure	–	–	–	12(17.9%)	65(74.7%)	<0.001

CHD, coronary heart disease; MS, metabolic syndrome; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure.

TABLE 2 The figures of ultrasound of included patients and non-CHD one-one paired.

	Included patients			Non-CHD one-one paired		
	Non-MS	MS	*P*	Non-MS	MS	*P*
LAD (mm)	30.89 ± 4.08	34.87 ± 3.83	<0.001	31.25 ± 4.14	34.60 ± 3.69	<0.001
RVD (mm)	20.66 ± 2.47	22.33 ± 2.45	<0.001	20.75 ± 2.38	22.40 ± 2.38	<0.001
IVS (mm)	8.72 ± 1.61	10.35 ± 1.66	<0.001	8.70 ± 1.57	10.34 ± 1.65	<0.001
LVEDD (mm)	45.58 ± 4.22	47.95 ± 4.83	<0.001	45.51 ± 3.56	47.71 ± 4.40	0.001
LVESD (mm)	27.53 ± 3.15	28.57 ± 4.02	0.026	27.22 ± 2.92	28.28 ± 3.76	0.052
LVPW (mm)	7.78 ± 1.25	9.23 ± 1.40	<0.001	7.81 ± 1.11	9.22 ± 1.43	<0.001
EF (%)	69.82 ± 5.48	70.67 ± 5.90	0.211	70.40 ± 5.28	71.05 ± 5.75	0.466
SV (ml)	66.96 ± 14.55	74.11 ± 16.14	<0.001	67.59 ± 12.40	74.03 ± 14.80	0.004
E	79.05 ± 20.05	69.42 ± 18.20	<0.001	76.29 ± 19.53	69.90 ± 19.15	0.042
A	66.02 ± 16.19	77.52 ± 18.08	<0.001	67.81 ± 15.83	76.91 ± 19.49	0.002
E/A	1.30 ± 0.46	0.96 ± 0.31	<0.001	1.20 ± 0.38	0.98 ± 0.32	<0.001
Average E/E'	7.57 ± 2.19	8.70 ± 2.73	<0.001	7.51 ± 1.76	8.58 ± 2.88	0.006
PASP (mmHg)	26.37 ± 5.18	26.79 ± 3.01	0.369	26.97 ± 6.45	26.88 ± 3.26	0.919

CHD, coronary heart disease; MS, metabolic syndrome; LAD, left atrium diameter; RVD, right ventricular diameter; IVS, interventricular septum thickness; LVEDD, left ventricular end-diastolic diameter; LVESD, left ventricular end-systolic diameter; LVPW, left ventricular posterior wall thickness; EF, ejection fraction; SV, stroke volume; PASP, pulmonary artery systolic pressure.

with MS. We conducted the comparison between MS and non-MS patients in echocardiography, which showed larger atrial and ventricular diameters and thicker ventricular thickness in patients with MS, but no difference in EF ($p = 0.211$), and pulmonary artery systolic pressure (PASP) ($p = 0.369$). As for behaviors in Impedance cardiography, some systolic variables were significantly different, including the height of S wave ($p < 0.001$), SV base and peak ($p < 0.001$), CO base ($p = 0.005$), CTI base and peak ($p < 0.001$), LCWi base ($p < 0.001$), and ventricular ejection time (VET) base ($p = 0.021$). Other systolic variables were not significantly different. In terms of diastolic variables, early diastolic filling rate (EDFR) base ($p = 0.004$), SVR base ($p < 0.001$), and SVR peak ($p < 0.001$) in patients with MS were significantly higher than in patients without. In addition, the EDFR peak ($p = 0.046$) in patients with MS seemed to be higher than in patients without. There is no difference in SV change between the two groups. The proportion of ST-segment depression seemed to be lower in patients without MS, while maximal METs were higher in patients without MS.

Among patients without coronary heart disease with 1:1 paired

For studying the differences further, we did a sensitivity analysis. As shown in Table 2, compared with non-MS patients, patients with MS were not significantly older ($p = 0.599$), but had a higher BMI ($p < 0.001$) as well as higher blood pressure ($p < 0.001$) at rest. For the echocardiographic parameters, MS patients showed noticeable myocardial hypertrophy [thicker LVPW ($P < 0.001$) and IVS ($P < 0.001$)] and cardiac enlargement [LAD ($P < 0.001$), larger RVD ($P < 0.001$) and larger LVEDD ($P = 0.001$)], as well as significantly worse diastolic function [lower E/A ratio ($P < 0.001$) and higher E/E'

TABLE 3 The figures of high-definition impedance cardiography of included patients and non-CHD one-one paired.

	Included patients			Non-CHD one-one paired		
	Non-MS	MS	*P*	Non-MS	MS	*P*
Height of A wave	45.62 ± 25.75	46.47 ± 26.20	0.787	45.30 ± 26.37	48.40 ± 25.63	0.460
Height of S wave	219.88 ± 86.86	163.63 ± 71.04	< 0.001	209.84 ± 87.32	164.97 ± 75.06	0.001
Height of O wave	21.38 ± 21.41	24.320 ± 18.17	0.238	21.95 ± 20.96	22.88 ± 17.86	0.767
SV base (ml)	65.96 ± 12.78	72.36 ± 11.27	< 0.001	65.57 ± 12.73	71.40 ± 11.27	0.003
SV peak (ml)	93.75 ± 19.89	103.23 ± 16.99	< 0.001	94.36 ± 20.49	102.88 ± 17.27	0.006
SV delta (ml)	27.79 ± 11.69	30.87 ± 10.44	0.026	28.79 ± 12.64	31.48 ± 10.40	0.151
SV delta percent	0.43 ± 0.17	0.43 ± 0.16	0.749	0.44 ± 0.19	0.45 ± 0.16	0.928
SVI base (ml/m^2)	39.95 ± 7.27	40.73 ± 6.28	0.358	40.03 ± 7.69	39.99 ± 6.25	0.973
SVI peak (ml/m^2)	58.704 ± 33.79	57.94 ± 9.95	0.827	56.70 ± 12.23	57.46 ± 9.94	0.672
SVI delta (ml/m^2)	18.76 ± 32.15	17.21 ± 6.37	0.639	16.67 ± 7.25	17.47 ± 6.22	0.464
SVI delta percent	0.29 ± 0.09	0.29 ± 0.07	0.915	0.42 ± 0.17	0.44 ± 0.16	0.430
CO base (ml/)	5.18 ± 1.00	5.52 ± 0.98	0.005	5.11 ± 0.98	5.58 ± 0.98	0.004
CO peak (ml/)	13.92 ± 3.24	14.00 ± 3.03	0.835	13.73 ± 3.43	14.24 ± 3.15	0.335
CO delta (ml/)	8.73 ± 2.96	8.47 ± 2.68	0.457	8.62 ± 3.29	8.67 ± 2.78	0.924
CO delta percent (%)	0.62 ± 0.08	0.59 ± 0.09	0.038	1.75 ± 0.78	1.59 ± 0.52	0.128
EDFR base (%)	48.16 ± 8.95	51.17 ± 7.88	0.004	48.94 ± 8.13	51.09 ± 8.30	0.107
EDFR peak (%)	58.48 ± 17.28	62.78 ± 18.64	0.046	62.84 ± 18.94	63.30 ± 17.95	0.876
CTI base	223.65 ± 88.20	165.03 ± 69.44	< 0.001	215.80 ± 92.38	166.65 ± 73.34	< 0.001
CTI peak	377.54 ± 143.57	309.97 ± 127.45	< 0.001	366.15 ± 156.76	308.87 ± 131.26	0.015
CTI delta	155.79 ± 104.14	146.56 ± 89.70	0.448	150.35 ± 112.39	142.22 ± 91.57	0.623
CTI delta percent (%)	0.23 ± 2.39	0.44 ± 0.19	0.421	0.78 ± 0.59	0.95 ± 0.69	0.096
LCWi base ($kg{\cdot}m/m^2$)	3.82 ± 0.92	4.27 ± 1.06	< 0.001	3.95 ± 0.96	4.35 ± 1.05	0.016
LCWi peak ($kg{\cdot}m/m^2$)	10.824 ± 3.09	11.52 ± 3.76	0.085	10.73 ± 2.96	11.90 ± 3.81	0.035
LCWi delta ($kg{\cdot}m/m^2$)	7.01 ± 2.86	7.24 ± 3.31	0.512	6.78 ± 2.75	7.55 ± 3.40	0.121
LCWi delta percent (%)	0.63 ± 0.11	0.60 ± 0.13	0.106	1.79 ± 0.80	1.79 ± 0.80	0.981
VET base (ms)	395.61 ± 48.65	382.17 ± 45.79	0.021	399.21 ± 51.76	379.44 ± 45.13	0.013
VET peak (ms)	265.39 ± 51.50	258.01 ± 47.94	0.229	265.36 ± 51.35	255.67 ± 45.39	0.217
VET delta (ms)	130.22 ± 66.06	124.15 ± 63.73	0.445	133.85 ± 65.41	123.77 ± 60.47	0.322
VET delta percent (%)	0.32 ± 0.16	0.32 ± 0.14	0.863	0.33 ± 0.15	0.32 ± 0.14	0.753
SVR base ($dyn{\cdot}s/cm^5$)	2,271.95 ± 438.78	2,538.69 ± 411.82	< 0.001	2,320.47 ± 514.95	2,547.09 ± 433.29	0.004
SVR peak ($dyn{\cdot}s/cm^5$)	1,054.92 ± 270.01	1,192.91 ± 272.89	< 0.001	1,098.26 ± 310.21	1,180.99 ± 253.22	0.072
SVRi base ($dyn{\cdot}s/cm^5{\cdot}m^2$)	1,387.98 ± 272.27	1,418.46 ± 211.69	0.327	1,417.56 ± 310.40	1,416.03 ± 206.64	0.971
SVRi peak ($dyn{\cdot}s/cm^5{\cdot}m^2$)	641.84 ± 169.26	669.69 ± 143.03	0.157	670.86 ± 190.89	659.96 ± 136.55	0.684

CHD, coronary heart disease; MS, metabolic syndrome; SV, stroke volume; SVI, stroke volume index; CO, cardiac output; EDFR, end-diastolic filling rate; CTI, cardiac contractility index; LCWi, left cardiac work index; VET, ventricular ejection time; SVRi, systemic vascular resistance index; SVR, systemic vascular resistance.

ratio ($P = 0.006$)], but similar LVESD ($p = 0.052$) and EF ($p = 0.466$).

Functional data were measured by impedance cardiography between patients with and without MS. It could be found that SV ($P = 0.003$) and CO ($P = 0.003$) in patients with MS were significantly higher than in those without MS under rest state. Moreover, lower height of S wave ($P = 0.003$), shorter left ventricular ejection time ($P = 0.013$), lower cardiac contractility index (CTI) ($P < 0.001$), higher left cardiac work index (LCWi) ($P < 0.001$) and higher systemic vascular resistance (SVR) ($P = 0.004$) were found in MS patients at rest. No significant difference was found in SVI ($p = 0.430$), EDFR ($P = 0.107$), and systemic vascular resistance index ($P = 0.971$). While comparing the indicators at peak exercise, patients with MS seemed to have higher SV ($P = 0.006$), lower CTI ($P = 0.015$), and higher LCWi ($P = 0.035$). No difference was found in other indicators when patients were at peak exercise. Besides, we also used the difference, which equaled the value at peak exercise minus the value at rest, to compare the trends of all the indicators between MS and non-MS patients from rest to

TABLE 4 The distribution of SV change types and ST-segment depression of included patients and non-CHD one-one paired.

	Included patients			Non-CHD one-one paired		
	Non-MS	MS	*P*	Non-MS	MS	*P*
METs max	7.83 ± 1.64	6.81 ± 1.54	<0.001	7.52 ± 1.43	6.83 ± 1.41	0.003
SV change			0.894			0.344
Normal response	92, 40.7%	39, 40.2%		37, 48.1%	33, 42.9%	
SV flat	63, 27.9%	27, 27.8%		17, 22.1%	25, 32.5%	
SV descent	71, 31.4%	31, 32.0%		23, 29.9%	19, 24.7%	
ST-segment depression			0.022			0.012
No depression	188, 83.2%	81, 83.5%		72, 93.5%	59, 76.6%	
0.1–0.2 mV	22, 9.7%	10, 10.3%		3, 3.9%	13, 16.9%	
>0.2 mV	16, 7.1%	7, 7.2%		2, 2.6%	5, 6.5%	

CHD, coronary heart disease; MS, metabolic syndrome; METs max, maximal metabolic equivalences; SV, stroke volume.

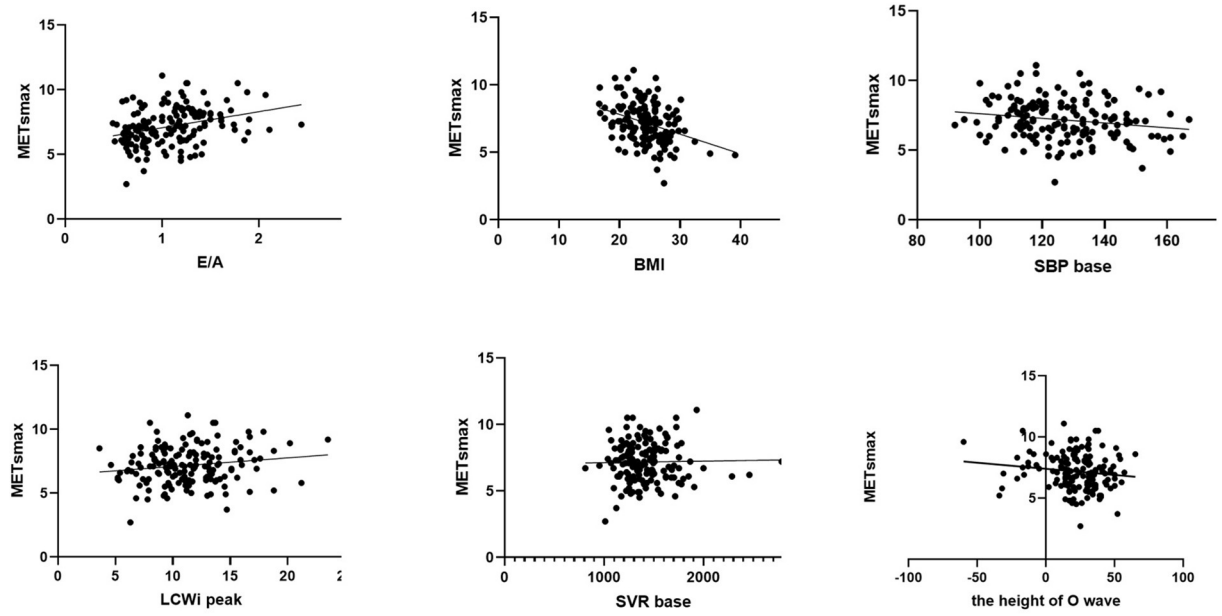

FIGURE 2
Scatterplot of simple linear regression equation of E/A ratio, BMI, SBP at baseline, LCWi at peak, SVR at baseline, the height of O wave, and Maximal METs, respectively.

peak exercise. As for the result shown in Table 3, it seemed that patients with and without MS have the same trends in the indicators included.

During ETT, we didn't find any difference in the frequency of SV change ($P = 0.344$) between the patients with and without MS. However, we noticed that the proportion of ST-segment depression ($P = 0.012$) was higher in patients with MS. Besides, patients with MS showed significantly lower maximal METs ($P = 0.001$) during ETT.

Afterward, we used automatic linear modeling to find out the optimum assembly of independent variables toward maximal Mets. It showed that among the indicators included, the best subset was the pattern of hypertension/increased blood pressure, BMI, E/A, LCWi peak, and SVR base. In Figure 2, the linear regression of each independent variable with METs was displayed. Meanwhile, we performed scatterplots to recognize the relationship between each variable and METs shown in Figure 2. It showed that the E/A ratio and LCWi peak have a positive correlation with METs while BMI, SBP base, and the height of the O wave were negatively correlated with METs. Perhaps oddly, it seemed that the correlation between SVR base and METs was too weak to be displayed in the figure. Then we made a linear regression model to explain the relationship between the pattern above and METs. Thereafter we found a

TABLE 5 The multiple linear regression results between METs max and the independent variables below.

	β	Standardized coefficient β	*P*	*R*	R^2	Adjusted R^2
Equation			<0.001	0.525	0.275	0.246
Constant	6.922	–	<0.001			
Hypertension/pre-hypertension	−0.707	−0.242	0.006			
BMI	−0.105	−0.248	0.002			
E/A ratios	0.697	0.178	0.028			
The height of O wave	−0.014	−0.184	0.012			
LCWi peak	0.102	0.242	0.002			
SVR base	0.001	0.221	0.004			

BMI, body mass index; LCWi peak, the peak value of left cardiac work index; SVRi base, systemic vascular resistance index at rest; METs max, maximal metabolic equivalents.

formula as follows:

$$\begin{aligned} \text{METs} &= 6.922 - 0.707\text{H} - 0.105\text{B} + 0.697\text{E} - 0.014\text{O} \\ &+ 0.102\text{L} + 0.001\text{S} \end{aligned}$$

(H means hypertension/increased blood pressure; B means BMI, E means E/A ratio; O means the height of O wave; L means LCWi peak; S means SVR base.)

According to Table 5, the formula is of significance ($P < 0.001$) while its explanation for METs was a little weaker ($R = 0.525$, $R^2 = 0.275$).

Discussion

The main results of this study, in which we have assessed the differences in cardiography and impedance cardiology with exercise tolerance test between patients with and without MS after excluding coronary heart disease, were: (1) to find subclinical cardiac hypertrophy, left ventricular diastolic dysfunction (LVDD). (2) to discover higher cardiac work and systemic vascular resistance. (3) to explore possible equations to predict the metabolic equivalents.

Structural differences in echocardiography

We used echocardiography to evaluate the structural changes in patients with MS but without CHD. Compared to the group without MS, patients with MS would show subclinical ventricular hypertrophy and chambers enlargement while the result was consistent with previous observational studies (10, 11). The ventricular hypertrophy seemed to be the product of MS, being associated with higher SV and cardiac work at rest. Even though LVEDD in MS patients was apparently higher, the left ventricular ejection fraction (LVEF) between the two groups was similar. It can partly be explained that the increment of LVEDD in MS patients was negligible as it was proved that clinical systolic dysfunction usually happens after diastolic dysfunction (12) while Nir Ayalon's research suggested that subclinical left ventricular diastolic dysfunction may be associated with MS but not left ventricular hypertrophy (10). Left atrial enlargement, which marks the structural change of the left heart, has been proved as an independent predictor of exercise capacity in patients with isolated diastolic dysfunction presented with exertional dyspnea (13).

Functional differences in echocardiography and impedance cardiography with exercise tolerance test

While routine echocardiography can only measure hemodynamic at rest, HD-ICG is a technique with kinetic consistent hemodynamic measurement, which helps analyse the change of cardiac function during exercise. More importantly, bioelectric impedance has been proved to have prognostic value in patients with heart failure (14), further research had been done to report that ICG responses during exercise offer important reclassification for predicting risk for adverse outcomes in heart failure (15, 16). As is known, the E/A ratio and E/E' ratio are reliable indicators for diastolic dysfunction. Differences in these two parameters, which presented subclinical diastolic dysfunction of LV in patients with MS, were in line with previous studies (7, 17, 18). The deterioration of diastolic function was also consistent with left heart structure alterations in the research. Moreover, it was reported that diastolic dysfunction increased the incidence of cardiovascular events or death by 2.53 times (19). Therefore, detection of subclinical diastolic dysfunction in MS patients seems reasonable.

In terms of systolic function, higher stroke volume and similar LVEF were caught in MS patients through echocardiography. Similarly, Grandi et al. (20) reported that only left ventricular diastolic dysfunction was detected in metabolic syndrome except for left ventricular systolic function (LVSF), which was also parallel to the research of Masugata et al. (12). All these parameters above were measured at

rest. Besides, we also used HD-ICG + ETT to evaluate hemodynamics and exercise capacity during incremental exercise, in which higher output status was recorded both at rest and exercise peak. Even though SV at both time points was significantly higher in MS patients, the stroke volume index was similar to the control group at both time points. On the one hand, it can partly attribute to higher body surface area (BSA) and BMI. On the other hand, cardiac contractility (CTI) in MS patients was recorded as lower than in the controlled. It seemingly implied that though MS patients showed higher SV, their SVI resembled the normal. Nevertheless, the worse CTI discovered in MS patients suggested that this group may have dysfunction in cardiomyocyte contractility. Hence, we considered that it indicates the contractility deterioration at the cardiomyocyte level, which may be associated with myocardial hypertrophy. In addition, it complied with the opinion of van Heerebeek et al. that insisted that myocyte stiffness instead of increased fibrosis was the main explanation for cardiovascular characteristics in MS (21). Meanwhile, the S wave was lower in MS patients than in non-MS patients. As is known, the S wave indicates cardiac contractility. We also noticed a decreased CTI in MS patients. The change of S wave corresponded to CTI, which also coincides with the identified order of cardiac dysfunction in patients with MS (12).

Exercise capacity and cardiac dysfunction

In our study, we evaluated exercise capacity using metabolic equivalents in exercise tolerance test. Our results were in line with previous studies that showed lower exercise capacity in patients with arterial hypertension, especially in those females with dyspnea (22, 23). A previous study evaluated the exercise capacity between the pre-hypertension group and the normal-blood pressure group, however, the result showed that no difference was found between them though subclinical cardiac structural and functional changes truly existed, such as increased left ventricular mass index and diastolic dysfunction (24). Lower exercise capacity was proved to be associated with MS in patients with coronary heart disease while using heart rate recovery (HRR) to assess the sympathetic balance index (25). In our study, it was found that compared to NMS patients, MS patients without CHD were still along with lower exercise capacity, which implied that MS directly influences the exercise capacity of individuals. SVI is an SV index that excludes the effect of height and weight, which was comparable between the two groups. It seems as if the similar SVI in two groups can lead to the conclusion that the systolic function of the LV is intact. Nevertheless, patients with MS had lower maximal metabolic equivalents during the exercise, which may deduce that diastolic dysfunction may be involved in the impairment of the exercise capacity. Besides, patients with MS had significantly higher systemic vascular resistance index, which might be related to the high prevalence of hypertension in MS.

HD-ICG with ETT and cardiac dysfunction

Impedance Cardiography (ICG) is a non-invasive and unobtrusive technique for measuring cardiac output. It assesses instant changes in thoracic electrical impedance to calculate hemodynamic variables and provides a way to dynamically evaluate SV changes. What's more, the sensitivity, specificity, and positive and negative predictive values for ICG were 100, 50, 79, and 100%, respectively, with coronarography as a gold standard for comparison (26). It is known that in the myocardial ischemia model, the reduction or loss of localized ventricular wall motion occurs firstly, followed by abnormal ST-segment changes and later pain and related symptoms. That is to say, there is an asymptomatic period in the process of myocardial ischemia, from the onset of imbalance between oxygen supply and oxygen demand to the clinical appearance of painful symptoms, which is called the "ischemic gap." In the included patients with metabolic syndrome, myocardial ischemic-related painful symptoms were not reported. However, it was noteworthy that recorded ST-segment depression in the metabolic syndrome group was more than in the non-metabolic syndrome group during submaximal exercise using ICG + ETT. The above phenomenon indicated that it is counterbalanced between myocardial oxygen supply and oxygen demand in patients with metabolic syndrome at rest while oxygen demand is far beyond the oxygen supply during strenuous exercise. It may imply that though no apparent stenosis was found in the main vessels, there may exist stenosis in micro vessels. Therefore, it is more direct to observe whether coronary microcirculation ischemia exists by dynamically measuring ICG + ETT-related data. Further research is needed to prove the hypothesis. Besides, the proportion of ST-segment depression was significantly higher in patients with MS, while there was no significant difference in SV increment between patients with and without MS. Pre-hypertension and central obesity may involve in the progression of myocardial ischemia since hypertension can accelerate arteriosclerosis by forcing endothelial cells and arterial smooth muscle cells to be chronically exposed to increased dilatability of the arterial wall (11). Whereby SV increment didn't change alongside with ST-segment, we speculate that the impairment of the exercise capacity might have nothing to do with obstructive coronary artery disease, which may result from functional myocardial ischemia, caused by increased end-diastolic pressure of LV as a result of diastolic dysfunction. The speculation needs further investigation to be proved. Furthermore, a large proportion of SV plateaus or decreases were noticed. However, the presence of SV plateaus or decreases in the normal population may also be a physiological response. A previous study has shown that echocardiography immediately after endurance exercise reveals a mild decrease in diastolic function with or without a decrease in systolic function, which some scholars refer to as "cardiac fatigue." This indicated that in the non-MS population,

transient cardiac dysfunction occurs when the exercise load during HD-ICG + ETT exceeds its extreme limits. Of course, comparative studies need to be refined to further confirm the hypothesis.

As for the multiple linear regression model, six independent variables were involved in the equation to determine individuals' maximal metabolic equivalents (METs). METS is an index reflecting relative energy metabolism level and exercise intensity. For patients with neither lung diseases nor musculoskeletal diseases, the connection between E/A ratio, systolic blood pressure, diastolic blood pressure, and METS can be explained as the impairment of the exercise capacity caused by diastolic dysfunction and hypertension. This is consistent with previous studies (6, 27–30). Notwithstanding, the R^2, which is used to analyze the explanatory power of the equation, showed that there was a slightly weak correlation between the two sides of the equation. Hence, the formula may not explain the maximal METs well, implying that its prediction was not strong enough. More research should be done to figure out better equations for METs.

Limitation

This study has some limitations. Firstly, since our research is a cross-sectional study, longitudinal and/or interventional studies are needed to further confirm our hypotheses. Secondly, our study tried to rule out patients with coronary heart disease, but not all the patients had coronary angiography examination, and the existence of coronary heart disease can't be totally ruled out by symptoms, Holter, and transthoracic echocardiography. Thirdly, although previous articles comparing ICG with ultrasound have demonstrated that there is no significant difference between the data obtained by ICG and ultrasound, and that the measurement error of HD-ICG is minimal due to its high accuracy, there may still be some difference with the actual SV value based on the algorithm problem of ICG itself for the SV value. Therefore, the calculation method of SV value should be continuously improved. Fourthly, more comparative analyses, which take age, gender, hypertension, smoking status, diabetes, and other risk factors of coronary diseases into account, should be done to find more details. Last but not least, we interpret the ST-segment depression as functional myocardial ischemia, caused by diastolic dysfunction due to a pre-hypertension state. This speculation needs further examination to be confirmed.

Conclusion

From the results we've got above, Patients with Mets showed significant structural alterations. Besides, noteworthy diastolic dysfunction was observed in patients with MS. The structural alterations and diastolic dysfunction may be the main cause of impaired exercise tolerance. Therefore, further research needs to be done to verify the relationship between exercise capacity and metabolic syndrome, which may help formulate the management of MS.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding authors.

Ethics statement

The studies involving human participants were reviewed and approved by Ethics Committee of the Department of Scientific Research, The First Hospital of Sun Yat-sen University, Guangzhou, China. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

HH and XW conceived and designed the review. JC, BD, and WL collected the data and contributed to the analysis of literature data. WL and JC performed the analysis of all data. XW, JC, BD, CL, JZ, YD, WL, and HH all participated in the discussion of the results. XW and JC wrote the paper. All authors contributed to the article and approved the submitted version.

Funding

This study was funded by the Guangdong Province Natural Science Foundation (2021A1515010114) and State Key Laboratory of Organ Failure Research, Southern Medical University, Guangzhou, China (Item No. 202002).

Acknowledgments

We thank Professor Fengjuan Yao, Dr. Yao Tong, and all the physicians involved in the examination of echocardiography and impedance electrocardiography of the study subjects.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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frontiers | Frontiers in Cardiovascular Medicine

TYPE Brief Research Report
PUBLISHED 12 August 2022
DOI 10.3389/fcvm.2022.966537

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Cardiovascular Therapeutics, a section of the journal Frontiers in Cardiovascular Medicine

RECEIVED 11 June 2022
ACCEPTED 27 July 2022
PUBLISHED 12 August 2022

CITATION
Li P, Luo X, Hou C, Wu S, Wang L, Sun N, Wang Z, Wang Z, Jin J, Wang J and Qin Z (2022) Maintenance of recovered dilated cardiomyopathy patients with half-dose neurohumoral blockades (MED-CHARM): A protocol for an open-label, pilot, randomized trial. *Front. Cardiovasc. Med.* 9:966537. doi: 10.3389/fcvm.2022.966537

Maintenance of recovered dilated cardiomyopathy patients with half-dose neurohumoral blockades (MED-CHARM): A protocol for an open-label, pilot, randomized trial

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Dilated cardiomyopathy (DCM) has brought great damage to the patients' health and social economy. The number of patients with recovered dilated cardiomyopathy (recDCM) has increased over the years as treatment progresses. However, there is a lack of relevant evidence to support the clinical management of patients with recDCM, thereby, the recommendations in guidelines remains sparse. Accordingly, the exploration of recDCM is important to improve patient prognosis and reduce societal burden. This is an open-label, randomized controlled, prospective study that will compare the safety and efficacy of original dose and halved dose of neurohumoral blockades for patients with recDCM.

Methods: An open-label, randomized controlled, prospective study will be conducted among eligible patients with recDCM. During the pilot study phase, we will recruit 50 patients. The primary endpoint is hospitalization for heart failure or heart failure relapse within 12 months. Secondary endpoint is major adverse cardiovascular events, including cardiovascular mortality, myocardial infarction, stroke, sustained atrial tachycardia, or ventricular tachycardia. The results will be analyzed using intention-to-treatment analysis.

Discussion: The study will provide important evidence of whether it is safe and effective to halve the dosage of neurohumoral blockades in recDCM patients.

Trial registration number: ChiCTR2100054051 (www.chictr.org.cn)

KEYWORDS

recovered dilated cardiomyopathy, neurohumoral blockades, heart failure, dosage adjustment, randomized controlled trail

Background and rationale

Patients with dilated cardiomyopathy (DCM) have a poor prognosis, manifested by persistent left ventricular enlargement and progressive deterioration of systolic function, ultimately leading to heart transplantation or death (1). On the other hand, the disease burden of DCM is increasing globally, including in China. In 2015, the global burden of disease study estimated that the global number of cardiomyopathy is 2.5 million, a figure that increased by 27% from 10 years ago (2). A study in China in 2014 showed a mortality of 42.24% among 767 patients with DCM in 52 months (3). Furthermore, to the best of our knowledge, DCM often affects young patients with fewer comorbidities and a theoretically high life expectancy (4). Consequently, the exploration of the progress and outcome in patients with DCM is important to improve the prognosis and reduce societal burden.

DCM patients with heart failure have improved survival with the benefits of angiotensin-converting enzyme inhibitors (ACEI) or angiotensin receptor blockers (ARB), beta-blockers, and spironolactone (5). The complete normalization of left ventricular ejection fraction (LVEF) in some heart failure patients has been termed heart failure with recovered ejection fraction (HFrecEF) (6). Among patients with HFrecEF, DCM occupies a high proportion, just second to heart failure resulting from energy and metabolic disorders (e.g., thyroid disease-related cardiomyopathy) (6). Due to the lack of a standardized definition and the current data from observational studies and clinical trials, the proportion of patients with improved ejection fraction ranges from 10% to 40% (6). In some of the patients with DCM, the ejection fraction and cardiac chamber size was observed to completely return to normal, which has been termed recovered dilated cardiomyopathy (recDCM) in some studies (7). However, there is a lack of relevant evidence to support the clinical management of patients with recDCM, thereby, the recommendations in guidelines remains sparse.

The fact that there has only been one prior prospective, randomized, controlled study in patients with recDCM is a mixed blessing. The TRED-HF study, withdrawal of pharmacological treatment for heart failure in patients with recovered dilated cardiomyopathy, published in *Lancet* in 2018, explored the safety and efficacy of withdrawing all drugs in patients with recDCM. That study found a significantly higher proportion of heart failure recurrence in the withdrawn group, while in the control group, there was no relapse at half-year follow-up, indicating that such patients were not suitable for total withdrawal treatment (7). However, all the patients in the control group took the guideline-recommended dosage, and it is not clear whether the cardiac function could be maintained normal if the drug class is not changed while the dose is halved.

In addition, many previous studies point out that in the actual clinical practice, many heart failure patients often fail to reach the target doses of guideline-directed medical therapy (GDMT) because of complicated multiple comorbidities (8–12), which is a great challenge to successfully up-titrate classes of HF medications (13). Moreover, a previous study showed that the best outcomes among heart failure patients were observed in those with a combination of ACEI/ARB and beta-blockers therapy, and unfortunately, this was rarely reached. Patients with the combination therapy of the two drugs reaching more than half of the target dose have better prognosis than those with a single drug therapy titrating to the target dose (14). Accordingly, we assume that the above speculation may apply to the clinical practice of recDCM.

Insights into the pathophysiological mechanisms of heart failure in recovered DCM patients, as well as advances in drugs for heart failure, have enabled the possibility of drug reduction in such patients. First, factors promoting adverse cardiac remodeling in patients with heart failure with recovered ejection fraction have been already suppressed with guideline-directed medications, and one study suggests that there may be a strong attenuation of adverse remodeling factors, such as sympathetic activation and renin angiotensin aldosterone system (RAAS) activation (6). In such circumstance, it may be possible for recDCM patients to reduce the doses of medications for heart failure. Second, with the advent of angiotensin receptor-neprilysin inhibitor (ARNI) and sodium-glucose cotransporter 2 inhibitors (SGLT2-i), patients have experienced significant improvement in the treatment of heart failure compared with previous ones (15). As a consequence, with the application of current novel class of drugs inhibiting cardiac remodeling, the original "ACEI/ARB/ARNI + beta-blockers" doses may have room for diminution. Actually, there are instances in which cardiac function is normalized in a subset of patients with low doses of GDMT for heart failure (14). These indicate that there may be a subset of patients who do not require a target dose to maintain cardiac function, and lower doses may be adequate. Thus, we reasoned that the "ACEI/ARB/ARNI + beta-blockers" halving dose therapy may be non-inferior to original target dose in maintaining cardiac function in recDCM patients.

This is an open-label, randomized controlled, prospective study that will be conducted in patients with recDCM, comparing the safety and efficacy of original target dose and halved dose of "ACEI/ARB/ARNI + beta-blockers." This will provide essential evidence of whether it is safe and effective to reduce doses of neurohumoral blockades in recDCM patients.

Methods and analysis

This is an open-label, randomized controlled, prospective study. Following the guidance of the ethical committee at our institution, we will recruit 50 patients in the pilot study phase.

Patients' recruitment

All patients with recDCM referred to Xinqiao Hospital (Army Medical University, Chongqing, China) will be screened by a senior cardiologist. Patients and their guardians are required to sign written informed consent. A special staff member will carefully describe the study to the patients and their guardians.

Inclusion criteria

1. Age≥18 years old.
2. Previous diagnosis of dilated cardiomyopathy with LVEF<40%.
3. Current therapeutic drugs with at least one of ACEI/ARB/ARNI and beta-blockers. Both ACEI/ARB/ARNI and beta-blockers doses are not up-titrated within 6 months; Other GDMT include a mineralocorticoid receptor antagonist (MRA), a SGLT2-i or diuretics.
4. Without symptoms of heart failure, and no hospitalization for heart failure for more than 6 months.
5. With at least two independent echocardiography (with interval ≥6 months) show that LVEF ≥50%, and left ventricular end-diastolic volume index (LVEDVi) <97ml/m^2.
6. With written informed consent.

Exclusion criteria

One of the following conditions is met:

1. Uncontrolled hypertension (blood pressure≥160/100 mmHg).
2. Valvular heart disease with moderate or greater severity.
3. Severe renal insufficiency (estimated glomerular filtration rate (eGFR) <30 ml/min/1.73 m^2, estimated according to Cockcroft-Gault formula).
4. Atrial, supra-ventricular, or ventricular arrhythmia requiring beta-blockers.
5. With the implantation of intra-cardiac defibrillator or cardiac resynchronization therapy.
6. Ischemic heart disease.
7. With severe systemic diseases.
8. Diagnosed with secondary cardiomyopathies.

Randomization

The enrolled patients will be randomly assigned into two groups. The randomization list will be automatically generated by the computer system. Before the start of the trial, the randomization list will be configured into the interactive network response system (IWRS, Jinling Rat mini-apps, Nanjing Jihu Network Technology Co., Ltd, Jiangsu, China), and IWRS will assign random numbers to these finally screened patients. Finally, the patients will be randomly assigned to either the withdraw group or control group with a ratio of 1:1 using IWRS.

Primary endpoint

The patients will be followed up for 12 months. The primary endpoint is hospitalization for heart failure or heart failure relapse. Heart failure relapse is defined if one of the following criteria is met: (1) LVEF reduction≥10% with LVEF<50%; (2) LVEDVi increased by more than 10%, and exceeded the normal values; (3) 2-fold elevation of NT-proBNP and more than 400 pg/ml; (4) Clinical evidence of heart failure (judged by symptoms, signs and supplementary examination).

Secondary endpoint

Secondary endpoints are major adverse cardiovascular events, including cardiovascular mortality, myocardial infarction, stroke, sustained atrial tachycardia, or ventricular tachycardia.

Sample size

Regarding the results from clinical trials in heart failure patients, the incidence of cardiac death or heart failure readmission within 12 months is 15–30% with guideline-directed medical therapy. The patients with DCM enrolled in this study have recovered cardiac function and ejection fraction, and TRED-HF study showed a primary endpoint event rate of 0% in such patients with medication doses unchanged in 6 months. Thus, in this study, it is estimated that the primary endpoint rate in control group and in withdraw group is 5 and 7% over 12 months of follow-up. It is estimated that 218 patients would provide the trial with 80% power to show the non-inferiority of halved doses to original doses, a two-sided alpha of 0.05 and a non-inferiority margin of 10% is applied. Considering a loss to follow up rate of 10%, the final sample size is 240. According to the suggestion of the ethical committee, the current study is a pilot study and 50 patients are intended to be included initially, 25 patients per group. After the initial analysis, more patients might be recruited in a further stage of the study.

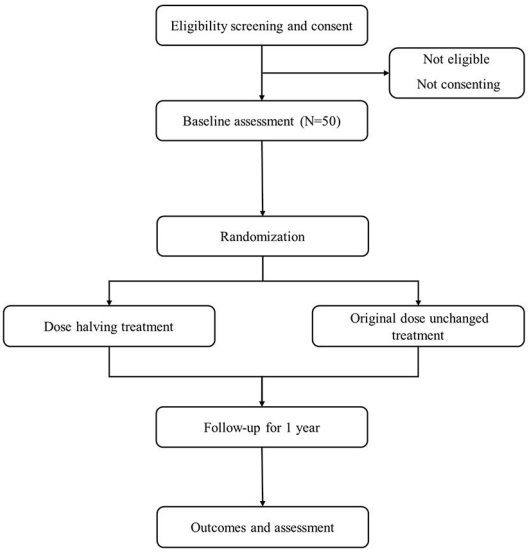

FIGURE 1
Study flow diagram.

Statistical analysis

Studies will be analyzed using intention-to-treatment and included all patients randomized, regardless of treatment received. Continuous data are expressed as mean ± SD or interquartile range (IQR 25–75). Categorical variables are expressed as a percentage. Continuous variables are compared by an independent sample *t*-test or rank-sum test. The chi-square or Fisher's exact test is used in the analysis of categorical variables. The Cox proportional hazards model was used to analyze and compare the differences in the occurrence of primary endpoint events and key secondary endpoint events within 12 months of follow-up between the trial group and the control group, and the hazard ratio (HR) and 95 % confidence interval will be calculated.

Intervention

According to ICH-GCP and local regulations, no study procedure can be started until the patient signs the written informed consent. The screening will be carried out after accomplishing the patient's written informed consent. The flow chart is shown in Figure 1. The patients who only meet research criteria will be randomized, otherwise, they will be not.

In the withdraw group, the doses of ACEI/ARB/ARNI will be reduced by 50% firstly. The symptoms and signs will be assessed in the following 1 month. The echocardiography and NT-proBNP will be performed to see if they could tolerate a further dose reduction of beta-blockers. Then, the dose of beta-blockers will also be reduced by 50% if the cardiac function of participants is stable. The other drugs of the participants will keep unchanged during the follow-up. In the control group, ACEI/ARB/ARNI, beta-blockers, spironolactone, SGLT2-i, and other drugs will be taken with original doses during the follow up.

Follow-up and measurement

The participants in this study will be followed up by face-to-face interviews and telephone. First, the demographic data, medical history, physical examination, laboratory tests will be documented as baseline at the beginning of the study. Then, the cardiac function (NYHA classification), 6-min walking test, and Kansas City Cardiomyopathy Questionnaire (KCCQ) score will be evaluated. Patients will be followed up at 1st, 3rd, 6th, and 12th month. Hospitalization for heart failure, heart failure relapse, and other adverse events will be documented. In the last follow-up, the participants will accomplish the examinations such as genes and metabolites and cardiac MRI as appropriate according to the evaluation.

Patient and public involvement

Patients, their guardians, and public representatives will be informed about the study, and yet they were neither involved development of study questions nor the planning of research design. In the same way, they also neither took part in the recruitment nor the conduct of this study. Results of the study will be published only in peer-reviewed journals, it is no other information on the results of the study that are provided to patients and their guardians.

Ethics and dissemination

The study protocol has been approved by an ethical committee of Xinqiao hospital, The Army Medical University, Chongqing, China, and was registered at the Chinese Clinical Trial Register (www.chictr.org.cn, ChiCTR2100054051). Patients and their guardians are required to sign written informed consent if the inclusion criteria are met before the beginning of the study, and meanwhile, researchers are supposed to be sure of participants' voluntariness strictly. When there are clear and ongoing contraindications or the patient requests that the study be terminated, the study should be terminated as soon as possible. Participants are free to withdraw at any time. Important modifications to the protocol that may affect the study's progress will be reported to the above-mentioned committee. Results of the study will be disseminated as published articles in peer-reviewed journals.

Schedule

The participants will be registered from December 1, 2021, to December 31, 2023. The end date of follow-up is December 31, 2024.

Discussion

The main aim of the present study is to evaluate the efficacy and safety of patients with recDCM who received half-dose neurohumoral blockades and received original dose maintenance therapy, and then by comparing the two doses of treatment, it may be concluded that "ACEI/ARB/ARNI + beta-blockers" halving dose was non-inferior to patients' maintenance dose in maintaining recovered cardiac function in DCM patients. This will provide a significant basis for the choice of whether to reduce dosage or to adhere to original dose maintenance therapy in DCM patients who recovered, to reduce the economic and psychological burden on patients.

Furthermore, this study is only the pilot study of the original research we want to do because the ethics committee needs to protect the interests of patients. Therefore, one of the limitations of this study is the small sample size, and if this study is effective, we will conduct more research.

Data availability statement

The datasets during analyzed and/or during the current study are available from the corresponding author upon reasonable request.

Ethics statement

The studies involving human participants were reviewed and approved by Medical Ethics Committee Office, Pharmacology Building, No. 183 Xinqiao zheng Street, Shapinba District, Chongqing. The patients/participants provided their written informed consent to participate in this study.

Author contributions

ZQ, XL, JW, and JJ designed the study. PL and XL drafted the manuscript. PL, CH, SW, LW, and NS helped to critically revised the drafts of the manuscript. ZebW and ZelW read and approved the final manuscript. All authors contributed to the article and approved the submitted version.

Funding

This study was supported by the Chongqing Medical Scientific Research Project (Joint Project of Chongqing Health Commission and Science and Technology Bureau) (No. 2022MSXM115).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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frontiers | Frontiers in Cardiovascular Medicine

TYPE Mini Review
PUBLISHED 18 August 2022
DOI 10.3389/fcvm.2022.985020

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Cardiovascular Therapeutics, a section of the journal Frontiers in Cardiovascular Medicine

RECEIVED 03 July 2022
ACCEPTED 01 August 2022
PUBLISHED 18 August 2022

CITATION
Jia Y, Li D, Yu J, Jiang W, Liao X and Zhao Q (2022) Potential diabetic cardiomyopathy therapies targeting pyroptosis: A mini review. *Front. Cardiovasc. Med.* 9:985020. doi: 10.3389/fcvm.2022.985020

Potential diabetic cardiomyopathy therapies targeting pyroptosis: A mini review

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Pyroptosis is primarily considered a pro-inflammatory class of caspase-1- and gasdermin D (GSDMD)-dependent programmed cell death. Inflammasome activation promotes the maturation and release of interleukin (IL)-1β and IL-18, cleavage of GSDMD, and development of pyroptosis. Recent studies have reported that NLRP3 inflammasome activation-mediated pyroptosis aggravates the formation and development of diabetes cardiomyopathy (DCM). These studies provide theoretical mechanisms for exploring a novel approach to treat DCM-associated cardiac dysfunction. Accordingly, this review aims to summarize studies that investigated possible DCM therapies targeting pyroptosis and elucidate the molecular mechanisms underlying NLRP3 inflammasome-mediated pyroptosis, and its potential association with the pathogenesis of DCM. This review may serve as a basis for the development of potential pharmacological agents as novel and effective treatments for managing and treating DCM.

KEYWORDS

diabetic cardiomyopathy, pyroptosis, NLRP3, caspase-1, pharmacology

Introduction

Diabetes and heart failure have a bidirectional link. The prevalence of diabetes in patients with heart failure caused by cardiomyopathy ranges from 10 to 40% (1). Meanwhile, heart failure is a common and serious cardiovascular complication in patients with diabetes. The Framingham Heart Study showed that the incidence of heart failure is two- to fivefold higher in patients with diabetes compared with that in healthy individuals (2). In addition, pre-diabetes is also related to an elevated risk of heart failure, and the relative risks is 1.09–1.40 according to different diagnosis criteria (3). Notably, both pre-diabetes and diabetes are associated with an increasing risk of cardiac events

and mortality in patients with heart failure (4, 5). Therefore, glycometabolism disorder is an important hazard factor for heart failure, and the two potential mechanisms are as follows: promoting the development of coronary atherosclerotic stenosis, which leads to ischemic heart disease characterized by systolic dysfunction; and more importantly, the classic presentation of diabetes, namely, diabetes cardiomyopathy (DCM) (1).

Diabetes cardiomyopathy is characterized by cardiac changes in function, metabolism, and structure without typical chronic cardiovascular complications, such as valvular heart disease, hypertension, and ischemic heart disease (6). DCM is the most frequent complication of diabetes and causes myocardial fibrosis, ventricular enlargement, and cardiac dysfunction, ultimately leading to clinical heart failure (7–9). Owing to its substantial impact on individuals cardiovascular health and lack of relevant targeted therapy, the pathogenesis of DCM has been a trending theme of research.

The abnormal metabolism of DCM is primarily due to myocardial tissue insulin resistance, compensatory hyperinsulinemia, and hyperglycemia, resulting in several conditions, including glycolipid metabolic disorders, oxidative stress, and advanced glycation end product deposition (1, 10). Previous review had well summarized the mechanisms of DCM, such as mitochondrial dysfunction, endoplasmic reticulum stress, and inflammation (11–13). Among the multiple mechanisms of DCM, cardiomyocyte death is a terminal pathway during the development of DCM, following by systolic dysfunction, myocardial compensatory hypertrophy, cardiac fibrosis, and electrocardiographic conduction disorder (14). Previous studies have analyzed that development of DCM caused by cardiomyocyte death, involving apoptosis, autophagy, necrosis, and entosis, and recent evidence obtained using electron microscopy has shown that pyroptosis-regulated cell death (pyroptosis) is a key pathogenetic factor in diabetes and DCM (15–17). Subsequently, an increasing number of pre-clinical studies have investigated the association between pyroptosis and DCM. Several molecular mechanisms have been elucidated, however, further related research is warranted.

Mechanisms of pyroptosis

Pyroptosis presented as programmed and inflammatory cell death and characterized by caspase-1- and gasdermin D (GSDMD)-mediated formation of plasma membrane pores, following by cell lysis and the secretion of proinflammatory cytokines, such as IL-1β and IL-18, and cellular component (18). Pathogen associated molecular patterns (PAMPs) and damage associated molecular patterns (DAMPs) are identified by pattern recognition receptors (PRRs) to activate the intrinsic immune reaction (19–22). PRRs is divided into cell membrane PRR and cytoplasmic PRR according to receptors site. The former is expressed on the membrane of immunocyte, commonly known as Toll-like receptors (TLRs), which can identify the exogenous infection signals of the intracellular environment (20). The latter expressed in cytoplasm, it can identify invasive pathogens; The most common are retinoic acid-inducible gene I-like receptors, absent in melanoma 2 (AIM2)-like receptors (ALRs) and nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs) (22–25).

When the ALRs and NLRs recognize DAMPs and PAMPs, the caspase-1 activated complex initiates assembly, this process is called formation of inflammasome (26). Further, it was regard as a processor of pro-caspases-1 to active caspase-1, which subsequently promoting maturation and release of IL-1β and IL-18 from precursor (26). Thus, inflammasome mainly contain three components: caspase-1, apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC), and receptors. According to different receptors, inflammasomes mainly classified as AIM2, NOD-like receptor protein 1 (NLRP1), NLRP3, NLR family CARD-domain containing protein 4 (NLRC4), and NLRP6 inflammasomes. Although multiple kinds of inflammasomes are being intensively studied, NLRP3 inflammasome is currently the most studied, with the most abundant relevant evidence, and the most widely involved in inflammatory and immune diseases. Importantly, accumulating studies have revealed that NLRP3 inflammasome-mediated pyroptosis plays a significant role in inducing the formation and development of DCM. Thus, our current review aims to elucidate the molecular mechanisms of NLRP3 inflammasome-mediated pyroptosis and its potential association with the pathogenesis of DCM (**Figure 1**). Finally, we summarized the progress of clinical drug research for DCM targeting pyroptosis (**Table 1**).

Pre-clinical diabetes cardiomyopathy treatments targeting nucleotide-binding oligomerization domain-like receptor protein 3 inflammasome-mediated pyroptosis

Natural extracts

Cardiac pumping dysfunction in DCM is mainly due to cardiomyocyte injury and death. Thus, suppressing pyroptosis in cardiomyocytes is important. Gypenosides,

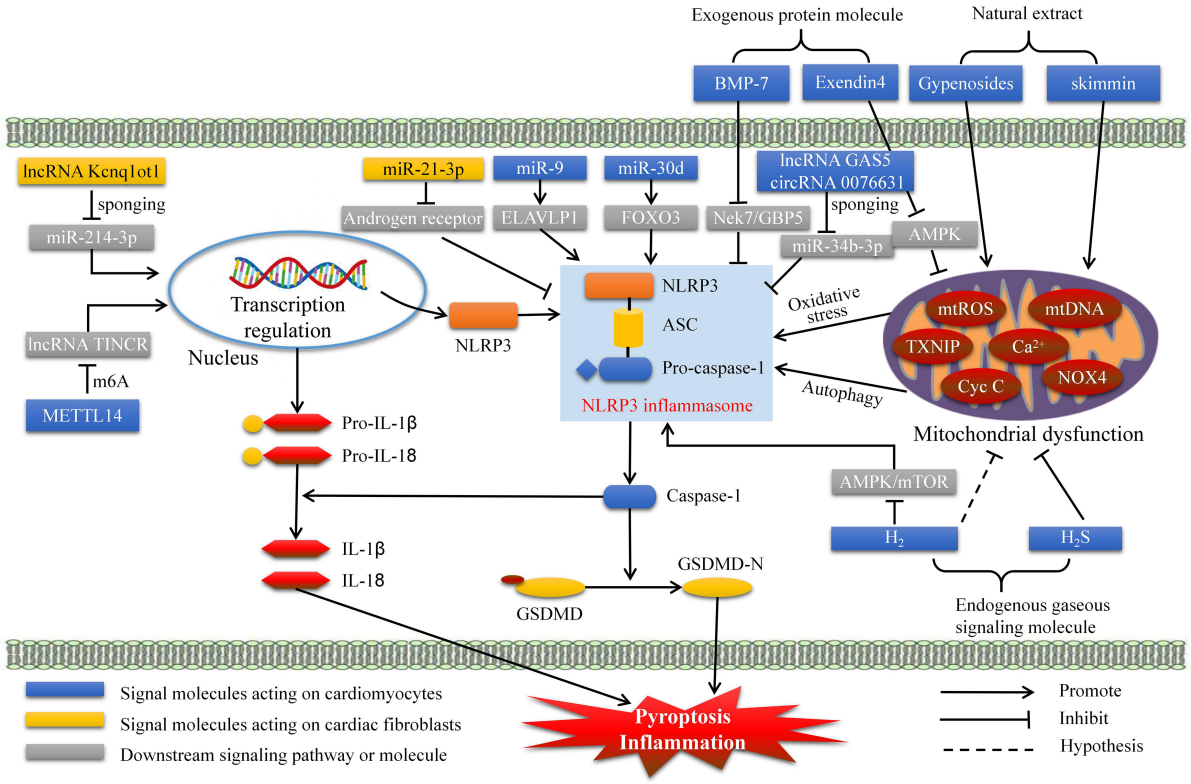

FIGURE 1
Pre-clinical treatment for DCM targeting NLRP3 inflammasome mediated pyroptosis. DCM, diabetes cardiomyopathy; GSDMD, gasdermin D; H2, hydrogen; H2S, hydrogen sulfide; METTL14, methyltransferase-like 14; BMP-7, bone morphogenetic protein-7; ELAVLP1, ELAV like protein 1; mtROS, mitochondrial reactive oxygen species.

the principal component of *Gynostemma*, exert various cardiovascular protective effects, such as reducing blood pressure, improving lipid and glucose metabolism, and inhibiting inflammation (27, 28). Zhang et al. reported that gypenosides can ameliorate high glucose-induced DCM by inhibiting reactive oxygen species (ROS)- and cytochrome c-mediated NLRP3 inflammasome activation and pyroptosis (29). Skimmin is a coumarin and glycoside with several biological activities, including antifibrosis, antioxidation, and anti-inflammation (30, 31). A recent study has shown that skimmin protects against streptozotocin (STZ)-induced DCM by improving autophagy and inhibiting NLRP3 inflammasome-mediated pyroptosis in rat cardiac tissues. Therefore, ginsenosides and skimmin are promising therapeutic drugs for DCM treatment.

Non-coding RNAs

Studies in recent years have recognized the important physiological roles of non-coding RNAs (ncRNAs), including circular RNAs (circRNAs), long ncRNAs (lncRNAs), and microRNAs (miRNAs) (32). These ncRNAs are related to DCM through transcriptional and post-transcriptional regulation but not directly involved in protein translation. For example, miRNA-9 expression is downregulated in the cardiac tissue of patients with diabetes and in cardiomyocytes treated with high glucose (33). Furthermore, miRNA-9 mimics inhibit pyroptosis (determined from the caspase-1 and IL-1β levels) in cardiomyocytes by targeting ELAV-like protein 1 to ameliorate hyperglycemia-induced DCM (33). Moreover, Li et al. observed that miR-30d level was increased and in STZ-treated diabetic rat hearts and high glucose-induced cardiac cell. Next, miR-30d was proved to inhibit Forkhead box O3 activities (apoptosis inhibitor) and exacerbates pyroptosis in DCM (34).

Xu et al. confirmed that GAS5 sponges miR-34b-3p to promote aryl hydrocarbon receptor expression and subsequently suppresses NLRP3 inflammasome-mediated pyroptosis in cardiomyocytes to alleviate DCM (35). Interestingly, Yang et al. reported that caspase-1-associated circRNA (hsa_circ_0076631) also sponges miR-214-3p (endogenous) to enhance high glucose-treated NLRP3 inflammasome activation and pyroptosis in cardiomyocytes (36). In addition, epigenetic regulation of lncRNAs can be modified by N6-methyladenosine (m6A), whose level and activity impact cell pathophysiology (37). Recent research

TABLE 1 Clinical registered studies for diabetic cardiomyopathy targeting pyroptosis.

Registration time	Identifier	Phase	Study title	Conditions	Interventions	Status	Primary outcome measures
2012	NCT01752842	Unknown	Lipid Biomarkers for Diabetic Heart Disease	. Type 2 diabetes mellitus . Diabetes complications	Drug: fenofibrate Drug: placebo	Completed	Change in cardiac diastolic function as measured by E′ and fractional shortening percent
2019	NCT04200586	IV	The Effects of SGLTi on Diabetic Cardiomyopathy (SGLTi)	. Type 2 diabetes . Heart failure with reduced ejection fraction	Drug: dapagliflozin Drug: placebo	Active, not recruiting	Rate of change in myocardial T1 values with manganese enhanced cardiac MRI
2019	NCT01803828	IV	REmodelling in Diabetic CardiOmyopathy: Gender Response to PDE5i InhibiTOrs (RECOGITO)	. Diabetic cardiomyopathy . Diabetes mellitus type 2	Drug: tadalafil Drug: placebo	Completed	Change from baseline in left ventricular torsion
2019	NCT04141475	Unknown	Evaluation of Alpha-Lipoic Acid in Diabetic Cardiomyopathy (CARDIALA)	. Diabetic cardiomyopathies	Drug: physiomance acide lipoïque gold Drug: placebo	Recruiting	Change of LVEF between before and after 12 weeks of treatment
2020	NCT04591639	IV	The DAPA-MEMRI Trial (DAPA-MEMRI)	. Heart failure . Diabetic cardiomyopathies	Drug: dapagliflozin Drug: placebo	Recruiting	Change in myocardial perfusion reserve index
2022	NCT04083339	III	Safety and Efficacy of AT-001 in Patients With Diabetic Cardiomyopathy	. Diabetic cardiomyopathy	Drug: AT-001 Drug: placebo	Recruiting	Peak VO2 during cardio-pulmonary exercise test

10.3389/fcvm.2022.985020

has reported that the overexpression of the lncRNA TINCR enhances cardiomyocyte NLRP3 inflammasome activities, pyroptosis, and DCM, and the epigenetic regulation of TINCR is controlled by methyltransferase-like 14-mediated m6A methylation.

In addition to cardiomyocytes, cardiac fibroblasts are also vulnerable to high glucose levels and exacerbate fibrosis and DCM. Yang et al. reported that lncRNA Kcnq1ot1 activates the caspase-1 and TGF-β1 pathways to aggravate fibrosis and DCM by sponging miR-214-3p in cardiac fibroblasts. Moreover, miR-21-3p expression in cardiac fibroblasts is upregulated under STZ treatment, whereas functional inhibition of miR-21-3p improves pyroptosis and collagen deposition by elevating the androgen receptor. These studies demonstrate that ncRNAs play crucial roles in DCM pathogenesis.

Endogenous gaseous signaling molecules

Endogenous gas signaling molecules serve important physiological and pharmacological functions and are associated with diabetes and related complications. Kar et al. found that hydrogen sulfide can be regulated by physical exercise and serves as a cardioprotective antioxidant that suppresses the activation of NLRP3, IL-1β, IL-18, and caspase-1 (38). Another recent study has demonstrated that hydrogen inhibits cardiomyocyte pyroptosis in cardiac fibroblasts by blocking the AMPK/mTOR/NLRP3 signaling pathway and improves fibrosis by inhibiting the TGF-β1/Smad signaling pathway (39). Moreover, hydrogen, as a therapeutic antioxidant, can reduce intracellular oxygen free radicals and inhibit ROS production (40). Thus, hydrogen inhibits the pathogenesis of DCM through multiple pathways. Hydrogen sulfide and hydrogen have been validated as gaseous signaling molecules that prevent DCM by alleviating pyroptosis.

Exogenous protein molecules

Bone morphogenetic protein-7 (BMP-7), also known as osteogenic protein-1, is used in clinical medicine to treat osteoporosis and fracture (41). BMP-7 inhibits inflammation and improves neovascularization (42, 43). Furthermore, BMP-7 inhibits NLRP3 inflammasome-mediated pyroptosis by blocking Nek7/GBP5 signaling to improve deleterious cardiac function and remodeling (44). Exendin-4, a glucagon-like peptide-1 analog, has an extended half-life because it avoids the clearance of dipeptidyl peptidase IV (45). Numerous studies have emphasized its protective effects on glucose metabolism and cardiac function (46). Additionally, exendin-4 inhibits pyroptosis *via* the ROS/AMPK/TXNIP/NLPR3 pathway, indicating that exendin-4 is a potential therapeutic drug for DCM (47). Secreted frizzled-related proteins (SFRPs) are a family of secreted proteins, and they were characterized by negative regulation of pyroptosis through Wnt/β-catenin and Notch signaling pathways in cardiovascular disease and inflammatory disease (48, 49). Recent study demonstrated that SFRP5 is a powerful prognostic assessment factor of heart failure for patients with type 2 diabetes (T2D). Thus, SFRPs may be a novel and potential exogenous inhibitory molecules of DCM by targeting pyroptosis (50). It is innovative and significant to carry out related research.

Clinical diabetes cardiomyopathy therapies targeting pyroptosis

Sodium-glucose cotransporter-2 inhibitors

Sodium-glucose cotransporter-2 (SGLT2) inhibitors are a relatively new type of hypoglycemic drug that increases urinary glucose excretion for the treatment of T2D (51, 52). Several clinical trials have revealed that SGLT2 inhibitors exert powerful cardiovascular protective effects, such as empagliflozin, canagliflozin, and dapagliflozin, on patients with T2D (53–55). Furthermore, the EMPRISE trial verified that empagliflozin decreases the risk of hospitalization for heart failure in patients with T2D (56). However, these cardiovascular protective effects of SGLT2 inhibitors cannot be attributed merely to their hypoglycemic and natriuretic effects. Pre-clinical studies revealed that SGLT2 inhibitors attenuate myocardial oxidative stress, fibrosis, and DCM by inhibiting NLRP3 inflammasome-mediated pyroptosis in diabetic mouse heart (57–59). To date, clinical studies confirming that SGLT2 inhibitors can improve DCM are lacking, although two related clinical trials are in progress. The DAPA-MEMRI trial (Identifier: NCT04591639) enrolled heart failure patients with T2D from October 2020 to explore the protective effects of SGLT2 inhibitors on cardiac function and remodeling by using cardiac magnetic resonance imaging (MRI) and echocardiography (**Table 1**). The results of this study provide direct evidence that SGLT2 inhibitors can improve DCM in humans. However, the other study (Identifier: NCT04200586) has not recruited participants yet (**Table 1**). These results suggest that SGLT2 is a promising drug for DCM treatment.

Phosphodiesterase type 5 inhibitors

Cyclic guanosine monophosphate-phosphodiesterase type 5 (PDE5) inhibitors have gained attention because they can

alleviate cardiac stress responses and improve hypertrophy and cardiac damage from multiple adverse stimuli in clinical and pre-clinical studies (60, 61). Early studies in men with heart failure and preserved ejection fraction showed that PDE5 inhibitors improved pulmonary pressure, cardiac geometry, and pump function (62). A recent RECOGITO study (Identifier: NCT01803828) has enrolled 122 men and women with well-controlled T2D and revealed that treatment with 20 mg tadalafil for 20 weeks can significantly mitigate DCM in men but not in women (63) (**Table 1**).

Mechanistically, PDE5 inhibitors exert protective effects on the cardiovascular system by activating protein kinase G (PKG), PKG-dependent hydrogen sulfide generation, nitric oxide expression, and glycogen synthase kinase-3β phosphorylation (64–66). In addition to hydrogen sulfide-mediated NLRP3 activation (38, 39), the PDE-5 inhibitor TPN171H, an icariin derivative, displays significant anti-inflammatory activities *via* suppressing NLRP3 inflammasome-mediated pyroptosis *via* cathepsin B (67–69). These results indicate that PDE5 inhibitors provide cardioprotection against DCM by inhibiting NLRP3 inflammasome-mediated pyroptosis; however, direct evidence is still lacking.

Aldose reductase inhibitors

Aldose reductase, as a polyol pathway enzyme, is significant upregulated in the conditions of oxidative stress and is the important inducer of the ROS related inflammatory response in diabetes (70). Pal et al. demonstrated that aldose reductase inhibitors prevent NLRP3 inflammasome-mediated pyroptosis and cytokine release in monocytes and STZ-induced diabetic mouse heart (71). Thus, aldose reductase inhibitors targeting NLRP3 inflammasome-mediated pyroptosis may be potential agents for DCM treatment. To the best of our knowledge, only one phase III trial (Identifier: NCT04083339) has been conducted to test the safety and efficacy of AT-001 (aldose reductase inhibitor) in patients with DCM. Although this study was started on 10 September 2019, the anticipated results have not been published yet (**Table 1**).

Fenofibrate

Fenofibrate is a peroxisome proliferator-activated receptor α agonist that has been widely used in the clinic for several decades because of its remarkable effect of reducing triglycerides (72). It can ameliorate diabetic retinopathy and stimulate angiogenesis by deregulating the activity of the NLRP3 inflammasome in STZ-induced diabetic mice (73, 74). Fenofibrate exerts a considerable protective effect on the heart, but whether it can ameliorate DCM remains unclear. A randomized controlled study (Identifier: NCT01752842) tested whether 160 mg fenofibrate per day for 12 weeks can improve heart muscle function in patients with T2D (**Table 1**). However, results of this study revealed no significant difference in cardiac diastolic function as measured by E′ (cm/s) and fractional shortening percentage between the placebo and fenofibrate groups.

Alpha-lipoic acid

Alpha-lipoic acid (ALA), also known as thioctic acid, is a vitamin-like sulfur-containing organic compound abundant in human organs and tissues (75). Early studies demonstrated that ALA is involved in improving hyperglycemia and deregulating inflammation (76–78). Recent studies have reported that ALA alleviates dyslipidemia and inflammation by modulating NLRP3 inflammasome activation in rats with high-fat diet- and STZ-induced T2D (79, 80). A randomized controlled study (Identifier: NCT04141475) involving patients diagnosed with diabetes from October 2019 evaluated the effect of ALA (Physiomance Acide Lipoïque Gold) in DCM by measuring the left ventricular ejection fraction (**Table 1**). The results of this study are worth investigating further.

Conclusion and perspectives

Pyroptosis is primarily considered a pro-inflammatory class of caspase-1- and GSDMD-dependent programmed cell death *via* the NLRP3 inflammasome. An increasing number of preclinical studies have emphasized that pyroptosis, which is different from apoptosis and necrosis, is involved in the pathogenesis of DCM. For example, natural extracts (derivatives), ncRNAs, endogenous gaseous molecules, and exogenous proteins have been explored and recognized for their key roles in pyroptosis and DCM. These studies offer theoretical mechanisms for developing new drugs to treat DCM-related cardiac dysfunction in the future. In addition, some clinical studies are actively exploring marketed drugs that may treat DCM, such as SGLT2 inhibitors, PDE5 inhibitors, aldose reductase inhibitors, fenofibrate, and ALA. The pharmacology of these drugs involves the inhibition of NLRP3 inflammasome-mediated pyroptosis. Thus, they may be the earliest evidence-based medicine for clinical use. However, basic and clinical investigations are still warranted to establish novel and effective treatments targeting pyroptosis for managing and treating DCM.

Author contributions

YJ, DL, and QZ designed the research. YJ and DL wrote the first draft of the manuscript. JY, QZ, WJ, and XL reviewed the

manuscript and provided critical scientific input. QZ had main responsibility for the final content of the manuscript. All authors approved the final draft of the manuscript.

Funding

This work was financially supported by grants from National Key Research and Development Program of China (Nos. 2020AAA0105000 and 2020AAA0105005), Sichuan Science and Technology Program (Nos. 2022YFS0279, 2021YFQ0062, and 2022JDRC0148), Health Commission of Sichuan Province (Nos. ZH2022-101 and ZH2020-104), Sichuan University West China Nursing Discipline Development Special Fund Project (Nos. HXHL20017, HXHL20046, and HXHL21016).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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frontiers | Frontiers in Cardiovascular Medicine

TYPE Original Research
PUBLISHED 06 September 2022
DOI 10.3389/fcvm.2022.908037

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Cardiovascular Therapeutics, a section of the journal Frontiers in Cardiovascular Medicine

RECEIVED 30 March 2022
ACCEPTED 05 August 2022
PUBLISHED 06 September 2022

CITATION
Zhan X, Cheng L, Huo N, Yu L, Liu C, Liu T, Li G and Fu H (2022) Sodium–glucose cotransporter-2 inhibitor alleviated atrial remodeling in STZ-induced diabetic rats by targeting TLR4 pathway.
Front. Cardiovasc. Med. 9:908037.
doi: 10.3389/fcvm.2022.908037

Sodium–glucose cotransporter-2 inhibitor alleviated atrial remodeling in STZ-induced diabetic rats by targeting TLR4 pathway

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Purpose: The mechanism of sodium–glucose cotransporter-2 inhibitor (SGLT-2i) reducing the incidence of atrial fibrillation remains unclear. We hypothesize that sodium–glucose cotransporter-2 inhibitor alleviated atrial remodeling in STZ-induced diabetic rats by targeting TLR4 pathway.

Methods: A total of 42 rats were randomly assigned into three groups: control group (CON group); diabetes group (DM group): diabetes mellitus rats were established by 65 mg/kg streptozotocin (STZ) intraperitoneal injection; and diabetes + dapagliflozin group (DM + DAPA group): diabetic rats were given DAPA gavage administration (DAPA 2mg/kg/d for 4 weeks by gavage administration), 14 rats in each group. Epicardial multiple-lead recording and intracardiac electrophysiology studies were performed to investigate the electrical remodeling in the heart and the atrial fibrillation inducibility in each group. Western blot analysis and real-time PCR were used to determine the protein and mRNA expression of toll-like receptor 4 (TLR4), interleukin receptor-associated kinase 1 (IRAK1), tumor necrosis factor receptor-associated factor 6 (TRAF6), nuclear factor-kappa B (NF-κB), and type I collagen (collagen I).

Results: Compared with rats in CON group, rats in DM group showed marked myocardial fibrosis, ectopic pacing excitement, reduced conduction velocity, decreased cardiac function. TLR4/IRAK1/TRAF6/NF-κB, collagen I proteins expressions and incidence of atrial fibrillation (27.3%) were increased in DM group. Parts of these changes were reversed by treatment of DAPA. Incidence of atrial fibrillation was decreased in DM + DAPA group (2.8%).

Conclusions: SGLT-2i dapagliflozin may prevent diabetic rats' atrial remodeling and reduce the inducibility of atrial fibrillation partly by targeting TLR4/IRAK1/TRAF6/NF-κB inflammatory pathway.

KEYWORDS

atrial remodeling, atrial fibrillation, dapagliflozin, diabetes mellitus, TLR4

Introduction

Atrial fibrillation (AF), one of the most common arrhythmias, is prone to major adverse cardiovascular events (1). Diabetes mellitus (DM) is an independent risk factor for atrial fibrillation (2). Sodium–glucose cotransporter-2 inhibitor (SGLT-2i) is applied in the treatment of symptomatic chronic heart failure (HFrEF) in adults with reduced ejection fraction, with or without type 2 diabetes mellitus (3). As a classical antidiabetic medication, it has become one of the novel cornerstones in the treatment of heart failure. There are clinical evidences that SGLT-2i reduces 32% hospitalization of heart failure and incidence of atrial fibrillation (4, 5). SGLT-2i can reduce the risk of cardiovascular events and all-cause mortality in patients with type 2 diabetes (6). However, the mechanism of SGLT-2i reducing the incidence of atrial fibrillation remains unclear. Recent research revealed that toll-like receptor 4 (TLR4) expression was upregulated under hyperglycemia (7). When activated TLR4 was silenced, it could inhibit atrial fibrosis and susceptibility to AF by regulating NLRP3-TGF-β in hypertensive rats (8).

Abbreviations: SGLT-2i, sodium–glucose cotransporter-2 inhibitor; DAPA, dapagliflozin; STZ, streptozotocin; CON, control; DM, diabetes mellitus; TLR4, toll-like receptor 4; IRAK1, interleukin receptor-associated kinase 1; TRAF6, tumor necrosis factor receptor-associated factor 6; NF-κB, nuclear factor-kappa B; AF, atrial fibrillation; HFrEF, heart failure with reduced ejection fraction; T2DM, type 2 diabetes mellitus; FS, fractional shortening; EF, ejection fraction; BCA, bicinchoninic acid; CV, conduction velocity; SCL, sinus cycle length; WCL, Wenckebach cycle length; SNRT, sinus node recovery time; ERP, effective refractory period; LVEF, left ventricular ejection fraction; LVFS, left ventricular fractional shortening; HW/BW, heart weight to body weight; HW/TL, heart weight to tibia length; SBP, systolic blood pressure; DBP, diastolic blood pressure; LAD, anteroposterior diameter of left atrium; PAT, pulmonary artery acceleration time; IVS, interventricular septum; LVID;d, left ventricular internal diameter at diastolic period; LVID;s, left ventricular internal diameter at systolic period; LVPW, left ventricular posterior wall; H&E, hematoxylin and eosin; ROS, reactive oxygen species; APD, action potential duration; ZDF, Zucker diabetic fatty; HSP60, heat shock protein 60; I/R, ischemia/reperfusion; ATP, adenosine 5′-triphosphate; EPO, erythropoietin; NLRP3, NOD-like receptor 3; GSDMD, gasdermin-D-N; AMPK, adenosine mono-phosphate kinase; CACNA1C, voltage-dependent L-type calcium channel; NCX, sodium–calcium exchanger; NHE1, sodium–hydrogen exchanger 1; NHE, sodium-hydrogen exchanger; MMPs, metalloproteinases; TIMPs, tissue inhibitors of metalloproteinases; AT, adipose tissue; ICIs, immune checkpoint inhibitors; DOXO, doxorubicin; NOXs, nicotinamide adenine dinucleotide phosphate oxidase; TGF-β1, transforming growth factor-β1; βOHB, β-hydroxybutyrate, d-β-hydroxybutyrate; HDACs, histone deacetylases.

Therefore, we hypothesize that SGLT-2i can alleviate atrial remodeling in STZ-induced diabetic rats by targeting TLR4 pathway.

Materials and methods

Rat model and study design

All procedures in this experiment were approved by the Animal Regional Ethics Committee of the Tianjin Medical University. A total of 42 healthy adult male Wistar rats (Beijing Huayu Kang Biotechnology, China), weighing 150–200 g, were housed in standard environmental conditions with food and water *ad libitum*. Rats were randomly assigned into three groups: control group (CON group), diabetes group (DM group) rats received a single intraperitoneal injection of 65 mg/kg streptozotocin (STZ) (STZ was dissolved in citrate buffer), and diabetes+dapagliflozin group (DM+DAPA group) diabetic rats received dapagliflozin (AstraZeneca Pharmaceuticals LP, United States) 2 mg/kg daily for 4 weeks by gavage. Diabetes mellitus models were validated by measuring blood glucose taken from the tail vein and defined as the blood glucose level higher than 11 mmol/L twice or 20 mmol/L once. Three groups of rats were kept together to ensure the same external environment such as temperature, humidity, and feeding as far as possible. Four weeks later, the animals in the three groups were subjected to blood glucose, blood pressure, epicardial multiple-lead recording, and intracardiac electrophysiological study, and molecular biology research was carried out after obtaining tissues.

Blood pressure measurement

Rat was fixed with net and bag and then placed in a heating preservation tube to keep warm. Blood pressure was measured by using the tail-cuff method (BP98AL, Softron, Japan) as previously described (9). The measurements were repeated five times for each rat, and the average value was included.

Histopathological studies

Histopathological studies were performed as described previously. Briefly, hearts were harvested and fixed in 10% formalin for at least 3 days. Tissues were cut transversely into 4–5 μm slices. Hematoxylin and eosin staining (Solarbio, Beijing, China) and modified Masson's trichrome staining (Solarbio, Beijing, China) were performed according to instructions to observe cell morphology and fibrotic area. Image Pro 6.0 was used for the analysis of the results.

Echocardiography

The rats were anesthetized with 1.5% isoflurane and assessed by a Vevo 2100 system (VisualSonics Vevo 2100, SONICS, Newtown, CT, United States). Parasternal LV long-axis view, short axis at the mid-papillary muscle level, and four-chamber view were recorded during three consecutive cardiac cycles. Fractional shortening (FS %) and ejection fraction (EF %) were calculated. All the results were repeated three times for subsequent analysis.

Intracardiac electrophysiology study

The intracardiac electrophysiology study was performed as described previously (10). The rats were fixed on the operating table, and the neck skin was exposed. The surface ECG was connected. A 1.6F catheter (EPR-802, Millar Instruments, United States) was inserted into right jugular vein. Surface ECG and intracardiac ECG were displayed and recorded using a PowerLab data acquisition system. Baseline ECG waveform was recorded before a series of subsequent stimulations. Exogenous stimulations were performed by an external stimulator (STG-3008, AD instruments, Australia) and applied at the electrode where atrial waveform was most pronounced. Atrial burst stimulation was performed at S1S1 stimulation cycle lengths starting from 40 ms with 2 ms stepwise reduction down to 20 ms. The stimulation was repeated five times, and the interval of each recovery period was 1 min. Sinus cycle length (SCL), Wenckebach cycle length (WCL), sinus node recovery time (SNRT), and effective refractory period (ERP) were defined as previously described and recorded to analyze the changes in cardiac electrical function. Atrial fibrillation was defined as a rapid irregular atrial rhythm with irregular R-R intervals lasting at least 1 sec. The corrected SNRT (CSNRT) was expressed according to the variation of sinus cycle length [CSNRT = SNRT–sinus cycle length (SCL)].

Epicardial mapping technique

The epicardial electrical conduction characteristics were performed by epicardial mapping technique as described previously (11). Rats were anesthetized, artificially ventilated, and subjected to middle thoracotomy. A 36-electrode microelectrode array (MEA, Multichannel Systems, Britain) was put on epicardial surface in the left and right atrium. The moment of the fastest decline on the descending branch of the single heartbeat waveform is defined as the exciting point. The atrial waves with uniform atrial conduction were selected to measure the atrial conduction velocity, and at least three consecutive atrial waves were selected and recorded to calculate the atrial conduction heterogeneity and conduction heterogeneity index. Each recording lasted 5 sec. All the measurements were analyzed by EMapScope 4.0 software (MappingLab Ltd., United Kingdom).

Western blot analysis

The heart tissues were quickly collected and frozen in liquid nitrogen for further research. Then, heart tissues were lysed in ice-cold RIPA buffer with a 1% protease and phosphatase inhibitor cocktail. The protein concentration in the lysis buffer was determined by bicinchoninic acid (BCA) protein assay reagent kit (Thermo Scientific, United States), and 20 μg proteins were separated by SDS-PAGE (8% or 10%) and transferred to a polyvinylidene fluoride microporous membrane (Millipore, Burlington, MA). Subsequently, membranes were blocked with 5% skim milk or 1% BSA and incubated with specific primary antibodies for toll-like receptor 4 (TLR4) (Abcam, rabbit Ab, 1:300), interleukin receptor-associated kinase 1 (IRAK1) (Abcam, rabbit Ab, 1:1,000), tumor necrosis factor receptor-associated factor 6 (TRAF6) (Santa Cruz, mouse Ab, 1:500), nuclear factor-kappa B (NF-κB) (Cell Signaling Technology, rabbit mAb, 1:1,000), type I collagen (collagen I) (Bioss, rabbit Ab, 1:1,000), β-actin (Proteintech, mouse Ab, 1:4,000–5,000) at 4°C overnight and followed by incubation with appropriate peroxidase-conjugated secondary antibodies. ImageJ software (NIH) was used for quantitative analysis.

Real-time PCR

Total RNA was isolated with Eastep® Super Total RNA Extraction Kit (Promega, Shanghai, China) from heart and quantified using NanoDrop (Thermo Fisher Scientific, United States). Then, 1 μg RNA was reverse transcribed using the Reverse Transcription Kit (GenePharma, Shanghai, China). The primers for targets are listed in Table 3. Subsequently, cDNA was applied to the ABI 7500 Real-Time PCR System (Applied Biosystems, United States). β-actin was used as an internal control. The obtained amplification data were analyzed by using the $2^{-\Delta\Delta Ct}$ method.

Statistical analysis

All data were expressed as mean ± SD or median with an interquartile range. ANOVA was used to make comparisons between multiple groups, followed by Tukey's *post–hoc* analysis for comparisons between two groups. Non–parametric Kruskal–Wallis test was used to analyze the data that did not conform to normal distribution. Fisher's exact test was used for evaluating the incidence of AF. All data were analyzed using SPSS 19.0 and GraphPad Prism 8. $P < 0.05$ was considered statistically significant. All figures were completed by GraphPad Prism 8.

Results

Effects of DAPA on basic parameters and cardiac function in STZ-induced diabetic rats

Compared with CON group, the DM group showed remarkably an increase of blood glucose, and the blood glucose level was decreased in DM + DAPA group (6.838 ± 0.6567, 29.28 ± 4.074, and 13.23 ± 4.210, respectively, $P < 0.0001$, Figure 1A).

There was no significant difference in blood pressure among groups (shown in Figure 1B). Furthermore, the DM group rats tended to develop heart hypertrophy and decreased in DM + DAPA group as shown in Figures 1C,D.

Typical echocardiographic images are shown in Figures 1E–H. Left atrial diameter in DM group and DM + DAPA group was larger than in CON group, but there was no statistical difference ($P > 0.05$). Interventricular septum in DM rats was thinner than CON rats ($P < 0.05$), and these changes did not reverse in DM + DAPA group ($P > 0.05$). Left ventricular ejection fraction (LVEF) and left ventricular fractional shortening (LVFS) were decreased in DM rats ($P < 0.05$), and these changes were reversed by the administration of DAPA (shown in Table 1).

DAPA alleviates atrial pathological structure in diabetic rats

H&E staining is shown in Figures 2A–C. The cell arrangement was disorder, and the cross-sectional areas of atrial cardiomyocytes were increased in DM rats and reversed in DM + DAPA group. Fibrotic area was higher in DM group compared with CON group, and fibrotic area was decreased in DM + DAPA group (2.933 ± 0.7480 vs. 5.502 ± 1.174 vs. 3.923 ± 0.8100, $P = 0.0008$, respectively, Figures 2D–G).

Effects of DAPA on epicardial electrical conduction characteristics in STZ-induced diabetic rats

Epicardial electrical conductivity was measured by epicardial mapping technique. The representative epicardial mapping images are presented in Figures 3A–C. In the CON group, electrical conduction of atrium was uniform and spread away into the surrounding area. There were uneven conductions in DM rats, which were partly reversed in DM + DAPA group. Left atrial conduction velocity was decreased in the DM group compared with the CON group and increased in DM + DAPA rats (0.6274 ± 0.1342, 0.4030 ± 0.08665, and 0.5775 ± 0.07739, $P = 0.0029$, Figure 3D). Compared with CON group, left atrial conduction dispersion was greater in DM group but there was no significance, and DPAP administration could not change the higher dispersion (absolute inhomogeneity: 3.069 ± 0.9607 vs. 3.687 ± 1.237 vs. 3.213 ± 0.8930, $P > 0.05$; index: 2.015 ± 0.9049 vs. 2.880 ± 1.166 vs. 2.143 ± 0.9149, $P > 0.05$, Figures 3E,F). Right atrial conduction velocity was decreased in DM group and reversed in DM + DAPA rats (Figure 3G). Higher right atrial conduction dispersion (Figures 3H,I) was observed in DM rats and DM + DAPA group compared with CON group.

DAPA could inhibit atrial electrical remodeling and the occurrence of atrial fibrillation in diabetic rats

In order to further confirm the effect of DAPA on atrial electrical remodeling in diabetic rats, we performed intracardiac electrophysiology study. During intracardiac stimulation, large stimulation artifacts are recorded on the surface ECG, following atrial wave and ventricular wave as shown in Figure 4A. Figure 4B represents atrial fibrillation after a series of burst stimulations and subsequently spontaneous converting to sinus rhythm. As shown in Figure 4C, the same exogenous stimulations were applied in three groups, and the inducibility of atrial fibrillation in DM group was 27.3%, compared with the control group 0% ($P < 0.0001$). The occurrence of atrial fibrillation was lower in DM + DAPA rats (2.8%) than in diabetic rats ($P < 0.0001$). Meanwhile, atrial duration was counted, and the duration of atrial fibrillation in DM rats was 1.05 s to 114.045 s, while the duration of AF in DM + DAPA rats varied from 5.18 s to 18.07 s ($P < 0.05$, Figure 4D). As shown in Table 2, SCL in DM rat was significantly prolonged compared with that in the control group ($P < 0.0001$, Table 2). There was marked prolongation in WCL, SNRT, CSNRT, and ERP in DM rats than in CON rats as shown in Table 2 ($P < 0.05$). Meanwhile, SCL, SNRT, CSNRT, and ERP could be abbreviated in DM + DAPA group ($P < 0.05$, Table 2), and there is no difference in WCL between DM rats and DM + DAPA rats ($P > 0.05$, Table 2).

DAPA inhibits TLR4/IRAK1/TRAF6/NF-κB pathways and collagen I expression in atrium

Finally, in order to reveal the possible mechanism, we verified the role of TLR4/IRAK1/TRAF6/NF-κB in the inhibition of atrial remodeling and atrial fibrillation by DAPA. Compared with the CON group, TLR4, IRAK1, TRAF6, NF-κB, and collagen I were upregulated in diabetic rats, while suppressed in DM+DAPA group (TLR4: 1.008 ± 0.3615 vs.

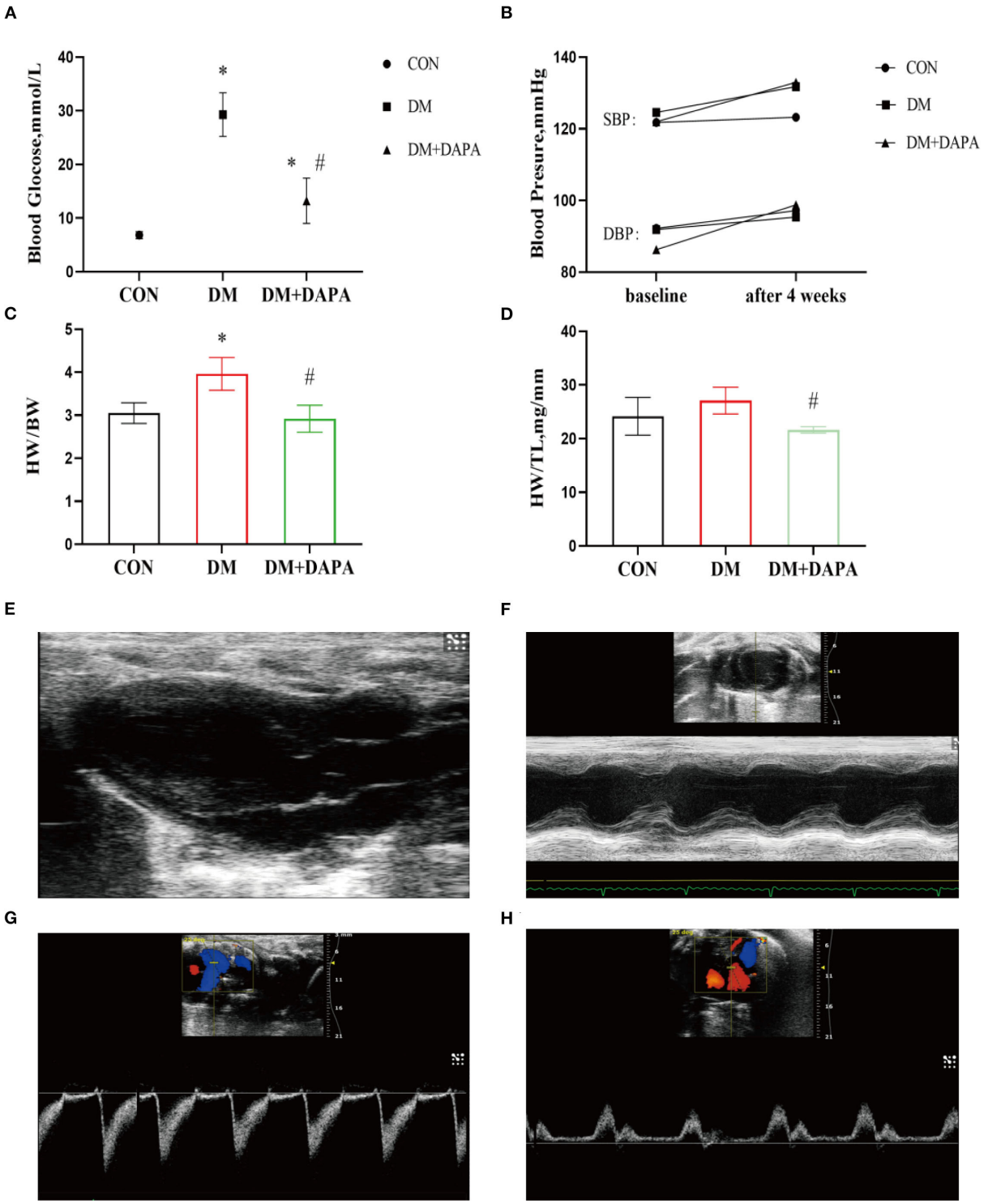

FIGURE 1
Effects of DAPA on the basic parameters and cardiac function in STZ-induced diabetic rats. **(A)** Random blood glucose after 4 weeks among three groups ($n = 8$); **(B)** systolic and diastolic blood pressure in baseline and after 4 weeks ($n = 7$); **(C)** heart weight to body weight (HW/BW) ratio in different groups ($n = 8$); **(D)** heart weight to tibia length (HW/TL) ratio in different groups ($n = 8$); **(E)** parasternal left ventricle long-axis view; **(F)** M-type echocardiogram of short-axis view; **(G)** Doppler flow imaging of short-axis view of the heart base; **(H)** Doppler flow imaging of four-chamber view. CON, control group; DM, diabetes group; DM + DAPA, diabetes + dapagliflozin group; SBP, systolic blood pressure; DBP, diastolic blood pressure. Data are expressed as the mean ± SEM. $^{*}P < 0.05$ vs. CON group. $^{\#}P < 0.05$ vs. DM group.

TABLE 1 Echocardiographic parameters.

	CON ($n = 8$)	DM ($n = 8$)	DM + DAPA ($n = 8$)	*P* values
LAD	4.011 ± 0.3483	4.571 ± 0.9002	4.723 ± 0.4867	0.1107
PAT	32.96 ± 8.505	33.11 ± 6.505	29.28 ± 7.753	0.5608
IVS	2.026 ± 0.2486	1.689 ± 0.2380*	1.689 ± 0.1020*	0.0155
LVID;d	6.439 ± 0.3950	7.185 ± 0.7655	6.508 ± 0.6074	0.0521
LVID;s	3.455 ± 0.6751	4.202 ± 0.5355*	3.348 ± 0.5044#	0.0152
LVPW	2.189 ± 0.3152	1.804 ± 0.1974	2.086 ± 0.5004	0.0864
EF (%)	78.19 ± 7.996	69.55 ± 3.389*	78.30 ± 6.495#	0.0260
FS (%)	50.21 ± 7.617	40.36 ± 2.755*	48.47 ± 6.918#	0.0171

LAD, anteroposterior diameter of left atrium; PAT, pulmonary artery acceleration time; IVS, interventricular septum; LVID;d, left ventricular internal diameter at diastolic period; LVID;s, left ventricular internal diameter at systolic period; LVPW, left ventricular posterior wall; EF, ejection fraction; FS, fractional shortening; CON, control group; DM, diabetes group; DM + DAPA, diabetes + dapagliflozin group. Data are expressed as the mean ± SEM. *$P < 0.05$ vs. CON group. #$P < 0.05$ vs. DM group.

1.519 ± 0.2976 vs. 0.9314 ± 0.2746, $P = 0.0140$; IRAK1: 1.141 ± 0.4182 vs. 1.712 ± 0.5146 vs. 0.8939 ± 0.3516, $P = 0.0030$; TRAF6: 1.083 ± 0.2963 vs. 2.033 ± 0.6714 vs. 1.169 ± 0.4642, $P = 0.0029$; NF-κB: 0.6789 ± 0.2403 vs. 1.552 ± 0.5397 vs. 1.099 ± 0.2456, $P = 0.0003$; collagen I: 0.5637 ± 0.2079 vs. 1.387 ± 0.3925 vs. 0.6279 ± 0.1973, $P = 0.0015$) (shown as in Figures 5A–E). As shown in Figures 5F–J, the mRNA expression of TLR4, IRAK1, TRAF6, NF-κB, and collagen I was increased in DM rats and downregulated in DM + DAPA group (TLR4: 0.8798 ± 0.1681 vs. 1.829 ± 0.4619 vs. 0.3664 ± 0.1717, $P < 0.0001$; IRAK1: 0.9381 ± 0.2163 vs. 1.472 ± 0.3509 vs. 0.4934 ± 0.1929, $P = 0.0003$; TRAF6: 1.032 ± 0.2877 vs. 2.071 ± 0.5725 vs. 1.407 ± 0.4928, $P = 0.0151$; NF-κB: 1.014 ± 0.1856 vs. 1.580 ± 0.5045 vs. 0.6292 ± 0.1899, $P = 0.0017$; collagen I: 0.6919 ± 0.2063 vs. 2.144 ± 0.6320 vs. 1.394 ± 0.3283, $P = 0.0013$).

Discussion

This study indicated that there were structural remodeling and dysfunctions of the heart in diabetic rats, which let the DM rats prone to atrial fibrillation. Part of these changes can be alleviated by the administration of DAPA. DAPA may have cardiac protective benefits *via* inhibiting TLR4/IRAK1/TRAF6/NF-κB pathway.

Atrial remodeling and diabetes mellitus

AF is a major arrhythmia in clinic, and its basic therapy includes rate control, rhythm control, and cerebral stroke. As is known to us, atrial fibrillation predisposes to worse prognosis in the context of diabetes mellitus (12). A persistent chronic inflammatory state is a hallmark of DM. Previous studies have indicated that the interaction between diabetes mellitus and atrial fibrillation is related to structural, electrical, electromechanical, and autonomic remodeling (12). Abnormal deposition and distribution of fibrosis are often closely linked to disruption of myocardial architecture (13). Evidences suggest that inflammation (14), oxidative stress (15), and mitochondrial dysfunction (16) were attributed to the fibrosis. The deposition of cardiac collagen fibers depends on the dynamic regulation process between metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) in cardiac tissue (17). However, in diabetes, the balance between MMP and TIMP is broken, resulting in excessive accumulation of collagen fibers (17). Suffering from ischemia, inflammation, and toxic insult, the normal myocardium was replaced with fibrous tissue, leading to structural remodeling (13). Echocardiography showed that interventricular septum in DM rats was thinner than CON rats. The situation may be due to ventricular dilatation and ventricular volume overload or the large body size difference between diabetic and normal rats (18). Therefore, we introduced standardized indexes of cardiac hypertrophy (e.g., HW/BW, HW/TL), excluding body size differences, and the results showed that the phenomenon of cardiac hypertrophy occurred in diabetic rats. The formation of fibrosis can interfere with the normal function of intercellular gap junctions and ion channels, decrease atrial effective refractory period (ERP) and action potential duration (APD), slower atrial conduction, and dysfunction of ion channels (19). Similar changes in electrical properties were verified in diabetes-induced atrial fibrillation (20). Therefore, the deposition of cardiac collagen fibers is regarded as the vitally important histological substrate of arrhythmias.

TLR4/IRAK1/TRAF6/NF-κB pathway in atrial fibrillation

The TLR family, primarily recognized as a receptor that initiates innate immunity, is involved in the progression of tumorigenesis (21). Recent research found that compared with myocarditis, pericarditis may be a cause of atrial arrhythmias (22). TLR4 signaling participated in the pericardium–myocardium interactions inducing atrial arrhythmogenesis (23). Classically, lipopolysaccharide-induced activation of TLR4 results in the activation of a series of downstream inflammatory molecules, including IRAK1, TRAF6, NF-κB (24). Excessive activation of TLR4/IRAK1/TRAF6/NF-κB pathway commonly exists in myocardial inflammation (25). For example, IRAK1 participates in the heat shock protein 60 (HSP60)/TLR4 signaling, mediating myocardial apoptosis and inflammation after ischemia/reperfusion (I/R) shocking (24). Milano et al. (25) reported that TRAF6 is a contributor to the doxorubicin/trastuzumab-induced cardiac toxicity.

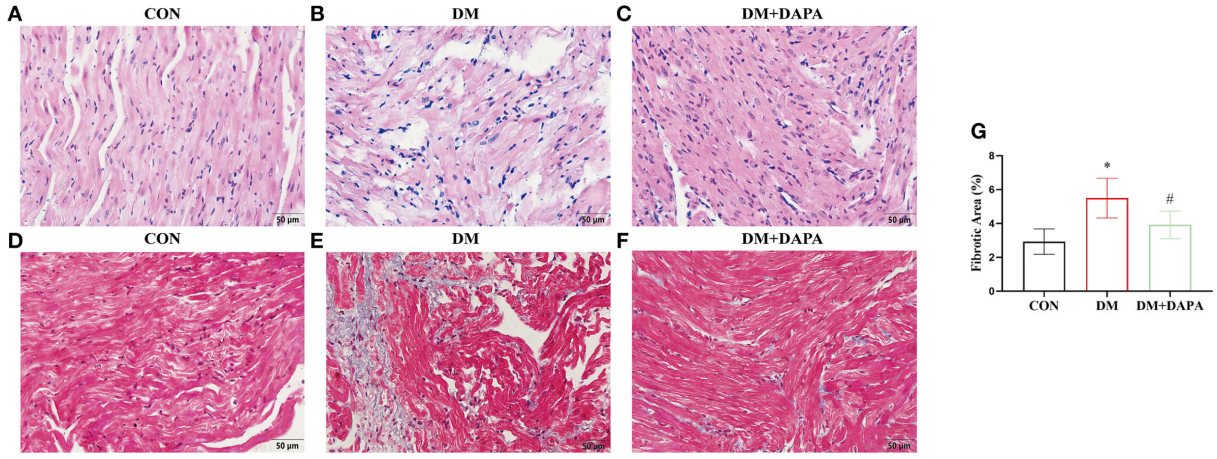

FIGURE 2
DAPA alleviates atrial remodeling in diabetic rats. **(A—C)** Typical images of H&E staining (n = 5); **(D–F)** representative pictures of Masson staining; **(G)** statistical analysis of Masson staining (n = 8). **(A,D)** CON group; **(B,E)** DM group; **(C,F)** DM + DAPA group; CON, control group; DM, diabetes group; DM + DAPA, diabetes + dapagliflozin group. Data are expressed as the mean ± SEM. $^{*}P < 0.05$ vs. CON group. $\#P < 0.05$ vs. DM group.

NF-κB, composed of p50 (NF-κB1) or p52 (NF-κB2) usually associated with members of the Rel family (p65, c-Rel, Rel B), is reported to improve mitochondrial morphology and function (26) and directly affect the cardiomyocytes function (27). Previous researches are in line with our results, and TLR4/IRAK1/TRAF6/NF-κB pathway was markedly activated under hyperglycemia.

SGLT-2i and atrial remodeling

SGLT-2 receptors are mainly distributed in the proximal convoluted tubules and are responsible for nearly 90% of glucose reabsorption (28). SGLT-2i is a new class of hypoglycemic agents that demonstrated excellent cardioprotective effects, reducing atherosclerotic events and protecting renal function (29). It significantly improved both cardiac diastolic and strain function, slightly lower body weight, blood pressure, and waist circumference (30). The cardioprotective effect associated with SGLT-2i was previously thought to be related to their diuretic and antihypertensive effects, which ameliorate ventricular loading (28). Unlike the loop diuretic bumetanide, SGLT-2i primarily reduced interstitial fluid without affecting blood volume (31). A meta-analysis showed that SGLT-2 inhibitors could not only reduce body weight and hematocrit but also significantly reduce blood pressure in patients with type 2 diabetes (32). The DAPA is a kind of SGLT-2i, which have little effect on SBP in patients with HFrEF (33). The results of this paper show that there is no significant difference between diabetic group and diabetic+DAPA group in SBP and DBP. These results suggest that the cardioprotective effect of DAPA may rely on other mechanisms. Under normoglycemic situation, DAPA attenuates reactive oxygen species (ROS) production and connexin 43 phosphorylation, reducing the occurrence of arrhythmia in infarcted rat (33). The mechanism of cardiac benefits of SGLT-2i may be independent of their hypoglycemic effects (34). Current studies support the use of SGLT-2i in the treatment of diseases other than diabetes mellitus. The results of this paper show that in addition to hypoglycemia, dapagliflozin may prevent diabetic rats' atrial remodeling and reduce the inducibility of atrial fibrillation. This is consistent with the results of previous studies that SGLT-2i can reduce the occurrence of atrial fibrillation (35).

DAPA treatment can attenuate electrical remodeling in AT II-stressed diabetic mice, an effect that was associated with inhibition of voltage-dependent L-type calcium channel (CACNA1C), the sodium–calcium exchanger (NCX), the sodium–hydrogen exchanger 1 (NHE) membrane transporters and fibrosis as well as inflammation (36). Cytoplasmic Na^{+} and Ca^{2+} concentration upregulates mitochondrial Ca^{2+} concentration, through affecting the activity of cardiac sodium-hydrogen exchanger (NHE), without concerning SGLT-2 receptor (36). In cardiomyocytes, NHE1 is the major exchanger isoform modulating sodium–proton exchange. The activity of NHE has been proved to be elevated in patients with severe heart failure and atrial fibrillation, suggesting that it may be associated with the pathogenesis of atrial fibrillation and heart failure (37). Uthman et al. revealed that SGLT-2i modulated myocardial fibrosis by inhibiting NHE1 activity, which reduced calcium influx into the myocardium and, consequently, mitochondrial damage (38). Moreover, SGLT-2i might modulate nutrient availability in cardiomyocytes and might influence the cardioprotective effect (39). The evidence

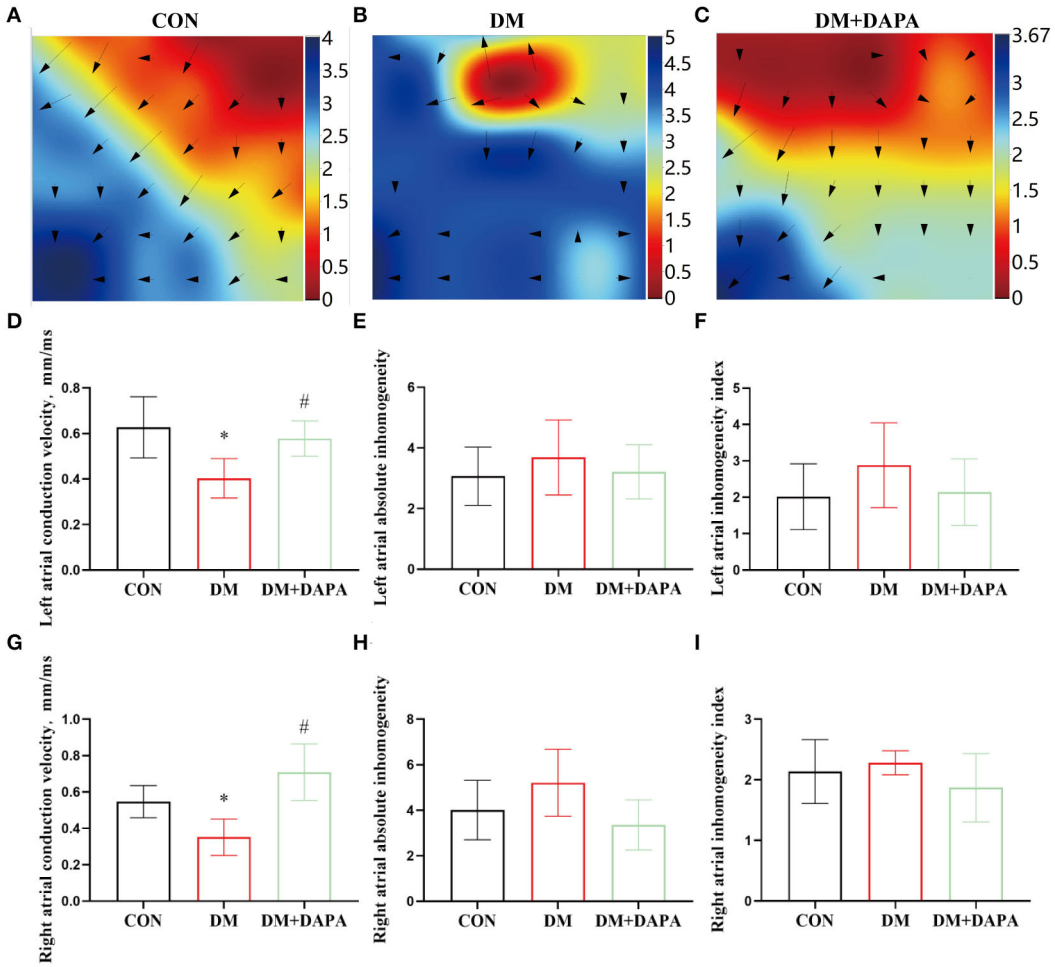

FIGURE 3
Effects of DAPA on epicardial electrical conduction characteristics in STZ-induced diabetic rats. **(A–C)** Typical images of electrical mapping; The arrow represents the direction of the excitement; **(D)** statistical analysis of conduction velocity in left atrium ($n = 8$); **(E)** statistical analysis of absolute inhomogeneity in left atrium ($n = 8$); **(F)** statistical analysis of inhomogeneity index in left atrium ($n = 8$); **(G)** statistical analysis of conduction velocity in right atrium ($n = 8$); **(H)** statistical analysis of absolute inhomogeneity in right atrium ($n = 8$); **(I)** statistical analysis of inhomogeneity index in right atrium ($n = 8$). CON, control group; DM, diabetes group; DM + DAPA, diabetes + dapagliflozin group. Data are expressed as the mean ± SEM. $^{*}P < 0.05$ vs. CON group. $^{\#}P < 0.05$ vs. DM group.

also indicated that SGLT-2i exerts a cardioprotective effect by regulating energy metabolism and by activating autophagy when cells are in the starvation state following a decrease in the body glucose burden (39). SGLT-2 inhibitor can alleviate cardiac inflammation by regulating the macrophage polarization *via* STAT3 signaling and interfering with oxidative stress and glucotoxicity (40, 41).

SGLT-2i application in anticancer-induced cardiotoxicity

The disorder of apoptotic mechanism is a pathogenic mechanism to inspire the onset of cancer. ped/pea-15, as a widely recognized antiapoptotic protein, has been found to be overexpressed in T2DM (42). At the same time, the overexpression of ped/pea-15 is associated with the increase of the susceptibility in chemically induced skin tumor development (43). Recent studies also have shown that diabetes is an important risk factor for colorectal cancer. Common systemic metabolic diseases, including obesity and diabetes, further modify the interplay between adipose tissue (AT) and breast cancer. Indeed, metabolic perturbations are accompanied by well-known alterations of AT functions, which might contribute to worsen cancer phenotype (44). As a common concomitant disease of diabetes, obesity can not only activate an inflammatory response, but also actively produce free fatty acids, adipokines, angiogenic factors, and extracellular matrix components as an endocrine organ, and ultimately build a microenvironment-supporting tumor.

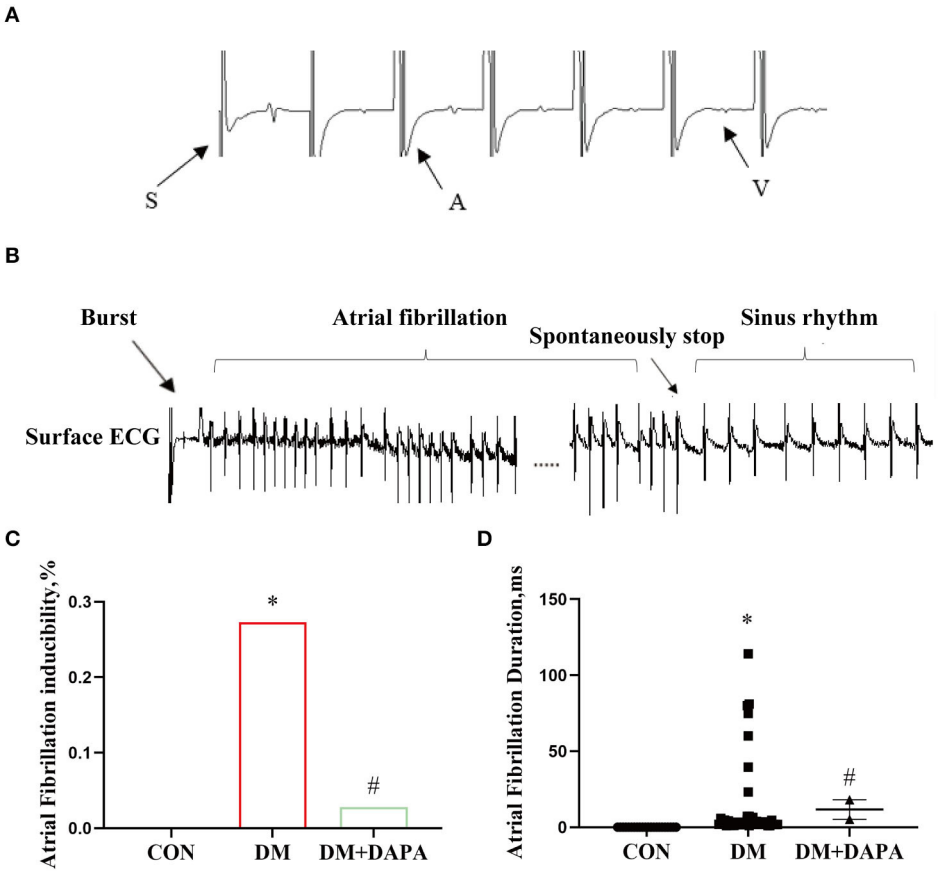

FIGURE 4
DAPA could inhibit atrial electrical remodeling and the occurrence of atrial fibrillation in diabetic rats. **(A)** Large stimulation artifacts are recorded on the surface ECG, following atrial wave and ventricular; **(B)** atrial fibrillation spontaneously stops after a series of burst; **(C)** atrial fibrillation inducibility; **(D)** statistical analysis of atrial fibrillation duration. CON, control group; DM, diabetes group; DM + DAPA, diabetes + dapagliflozin group. Data are expressed as the mean ± SEM. $^{*}P < 0.05$ vs. CON group. $^{\#}P < 0.05$ vs. DM group. The arrow represents the direction of the excitement.

Immune checkpoint inhibitors (ICIs) have revolutionized cancer treatment, achieving unprecedented efficacy in multiple malignancies (45). However, ICIs are associated with immune-related adverse events involving cardiotoxicity (46). Quagliariello V et al. took the first evidence that hyperglycemia exacerbates ipilimumab-induced cardiotoxicity and decreases its anticancer efficacy in MCF-7 and MDA-MB-231 cells. The study also sets the stage for further tests on other breast cancer cell lines and primary cardiomyocytes and for preclinical trials in mice aimed to decrease glucose through nutritional interventions or administration of gliflozines during treatment with ipilimumab (47).

Quagliariello V et al. aimed to evaluate the effects of SGLT-2i on myocardial strain of nondiabetic mice treated with doxorubicin (DOXO). They concluded that EMPA reduced ferroptosis, fibrosis, apoptosis, and inflammation in doxorubicin-treated mice through the involvement of NLRP3- and MyD88-related pathways, resulting in significant improvements in cardiac functions (48). The protective effects of SGLT-2i may also be due to its potent antioxidant properties, which protect cardiac tissue from oxidative damage and help to maintain myocardial cell membrane integrity and function (48). SGLT-2i can also ameliorate sunitinib-induced cardiac dysfunction by regulating AMPK–mTOR signaling pathway-mediated cardiomyocyte autophagy (49). Tian et al. found that DAPA could mitigate the cardiac fibrosis and inhibit the endothelial-tomesenchymal transition via AMPKα/TGF-β/Smad signaling (50).

SGLT-2i and its renoprotection

A previous study reported that SGLT-2i provided renoprotection by lowering the intraglomerular hypertension by modulating the pre- and post-glomerular vascular tone (39). Diabetic rats show an upregulation of renal fibroblasts, mesangial cells, and podocytes as well as nicotinamide adenine dinucleotide phosphate oxidase (NOXs) and increased production of reactive oxygen species in renal tissues. Inhibition of NOXs significantly protects the kidney from structural

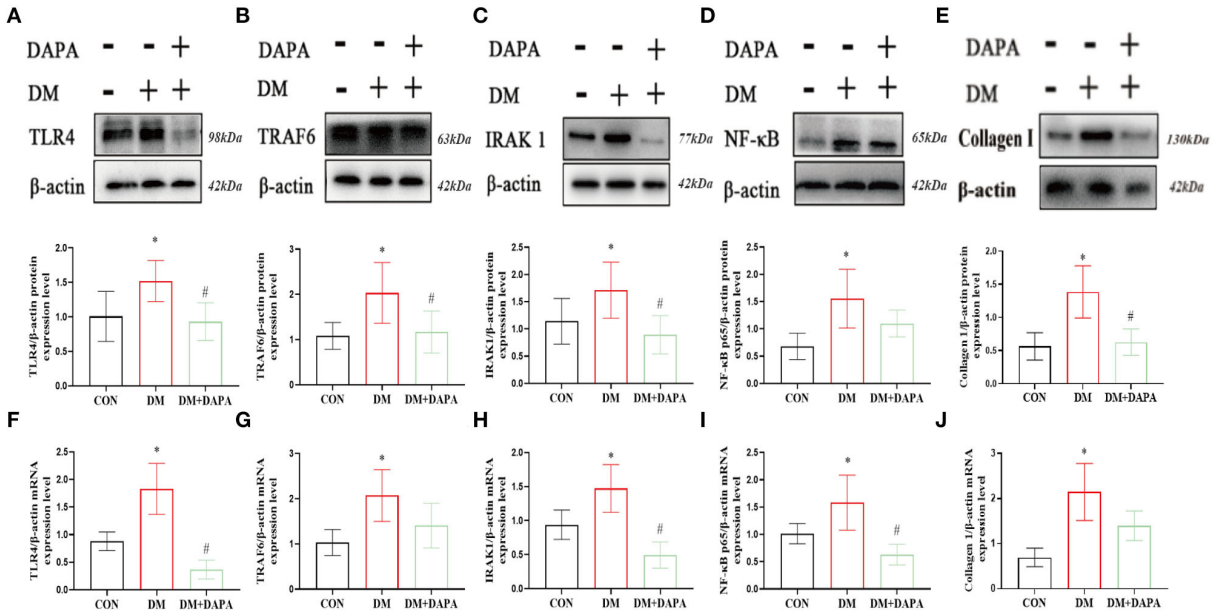

FIGURE 5
DAPA inhibits TLR4/IRAK1/TRAF6/NF-κB inflammatory pathways and Collagen I in atrium. **(A–E)** Protein expression of TLR4, TRAF6, IRAK1, NF-κB and Collagen I ($n = 5$-8). **(F–J)** mRNA expression of TLR4, TRAF6, IRAK1, NF-κB, and Collagen I ($n = 5$). CON, control group; DM, diabetes group; DM + DAPA, diabetes + dapagliflozin group. Data are expressed as the mean ± SEM. *$P < 0.05$ vs. CON group. #$P < 0.05$ vs. DM group.

TABLE 2 Electrophysiological parameters.

	CON ($n = 9$)	DM ($n = 10$)	DM+DAPA ($n = 7$)	*P* values
Weight, g	397.9 ± 59.50	211.5 ± 34.55*	298.1 ± 27.26*	< 0.0001
SCL, ms	149.6 ± 16.58	190.8 ± 15.30*	152.0 ± 14.34#	<0.0001
Wenckebach cycle length, ms	87.78 ± 10.34	104.5 ± 14.50*	92.57 ± 12.70	0.0230
SNRT, ms	184.0 ± 19.54	232.4 ± 28.00*	180.9 ± 19.73#	0.0004
CSNRT, ms	36.00 ± 6.414	47.33 ± 21.79	30.33 ± 7.840	0.0948
ERP, ms	74.22 ± 8.342	89.00 ± 6.728*	70.57 ± 5.893#	< 0.0001

SCL, sinus cycle length; AV, atrioventricular; WCL, Wenckebach cycle length; SNRT sinus node recovery time; CSNRT, corrected sinus node recovery time; ERP, the effective refractory period. CON, control group; DM, diabetes group; DM + DAPA, diabetes + dapagliflozin group. Data are expressed as the mean ± SEM. *$P < 0.05$ vs. CON group. #$P < 0.05$ vs. DM group.

TABLE 3 Primer sequences.

Primer sequences Gene		Primer sequence(5′–3′)
TLR4	forward	CTGGCCTCATCTTCATTGT
	reverse	GGGCTTCTTGAGTCTTCT
IRAK1	forward	CTCTGCCTCCACCTTCCTC
	reverse	AACCACCCTCTCCAATCCT
TRAF6	forward	AAAGCGAGAGATTCTTTCCCTG
	reverse	ACTGGGGACAATTCACTAGAGC
NF- κb	forward	TCTGTTTCCCCTCATCTTT
	reverse	TGGTATCTGTGCTTCTCTC
Collagen I	forward	CCCAGCGGTGGTTATGACTT
	reverse	TCGATCCAGTACTCTCCGCT
β-actin	forward	CCGCCTTGGAGTCCATCTAC
	reverse	GCGGCTTGTACCACTCTCG

and functional renal damage. This may be the molecular mechanism of Canagliflozin's effect on renal protection (51). SGLT-2i plays a renoprotective role in rats with acute kidney injury after myocardial infarction by increasing the circulating level of ketone body d-β-hydroxybutyrate (βOHB), and βOHB upregulates antioxidant molecules by inhibiting histone deacetylases (HDACs) (52). Panchapakesan et al. reported that empagliflozin could reduce the expression of TLR4 and the secretion of IL-6 and NF-κB in human renal proximal convoluted tubular epithelial cells. Thus, it reduces the expression of inflammatory factors and fibrotic markers induced by high glucose toxicity (53).

SGLT-2i reduces the inducibility of AF *via* TLR4 pathway

The specific mechanism by which SGLT-2i can reduce the incidence of atrial fibrillation after DM is unclear. These changes

may be related to the abnormal distribution of intercellular gap junction proteins (54), activation of reactive inflammatory signaling pathways (55), and ion channel dysfunction disorder (56). The protective effect of drugs on the heart is related to a variety of signal pathways. Previous studies have suggested that the cardioprotective effect of SGLT-2i may be related to the effect of inhibiting inflammatory pathways. SGLT-2i not only inhibits the inflammatory responses, reducing oxidative stress productions and mitochondrial stress, but also changes the electrical characteristics of the heart, affecting the function of ion channels, the disordering of electrical conduction. As mentioned earlier, the immune inflammatory response, especially TLR4, is consistently involved throughout diabetic vascular disease (56). TLR4 can induce and amplify the inflammatory response, and it plays an important role in cell proliferation, differentiation, and apoptosis (57). Previous studies have demonstrated that TLR4 can be involved in hyperinsulinemia, insulin resistance, lipid metabolism disorder, endothelial cell dysfunction, and blood coagulation (58). TLR4 could mediate the inflammatory response via activating the NF-κB pathway and downstream inflammatory factors to aggravate the damage of the inflammatory response (59). In this study, TLR4 and NF-κB proteins and collagen I protein expressions were significantly increased in DM rats, and they were downregulated in DAPA treatment group. The activation of NF-κB pathway and the subsequent overexpression of its downstream targets such as transforming growth factor-β1 (TGF-β1) are a critical pathway in progressive diabetic nephropathy (60).

In a diabetic rabbit model, we have previously shown that probucol prevented atrial remodeling and suppresses AF development effected on oxidative stress, NF-κB, TGF-β, and TNF-α overexpression (61). So, in this study, the DAPA treatment prevented atrial remodeling and suppresses AF development partly by suppressing the overexpression of the TLR4 and NF-κB involved in an immune inflammatory response.

Elaheh Abdollahi and his colleagues revealed that DAPA exerted direct anti-inflammatory effects, at least partly, by inhibiting the expression of TLR4 and activation of NF-κB along with the secretion of pro-inflammatory (62). However, experimental results in cell model should be proved in real organism. It is of significance to use animal model for the clinical transformation of drugs. Secondly, our manuscript revealed that SGLT-2i produces a marked cardioprotective effect through the TLR4/NF-κB pathway in diabetic animal model and SGLT-2i could suppress atrial structural remodeling and electrical remodeling and reduce the incidence of AF in diabetic rats. In conclusion, our present results show that the cardiac protection of DAPA may rely on the inhibition of TLR4/IRAK1/TRAF6/NF-κB pathway. However, the mechanism of DAPA for atrial fibrillation still needs validation in the future work.

Study limitations

There exist several limitations that should be noted. Firstly, we did not verify the relationship between DAPA and TLR4/IRAK1/TRAF6/NF-κB pathway *in vitro*, and we could not further clarify that what mediators DAPA interacts with the TLR4 pathway. Secondly, we cannot rule out the effect of DAPA on control heart, which may cause disturbance of ion channel function and possibly have adverse effects on heart function. Thirdly, ion channel currents of DAPA administration were not studied in this study.

Conclusions

SGLT-2i dapagliflozin may prevent diabetic rats' atrial remodeling and reduces the inducibility of atrial fibrillation. TLR4/IRAK1/TRAF6/NF-κB pathway is involved in this process.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The animal study was reviewed and approved by Animal Regional Ethics Committee of the Tianjin Medical University.

Author contributions

HF conceived the work and designed the experiments. XZ and LC recorded the data and wrote the manuscript. NH and LY performed the statistical analysis. CL, TL, GL, and HF participated in the critical manuscript revision. All authors read and approved the final manuscript.

Funding

This study was funded by Tianjin Natural Science Foundation (16JCYBJC25000, 21JCYBJC01740, and 21JCYBJC01460), Key Laboratory Scientific Research Foundation of Second Hospital of Tianjin Medical University (2018ZDSYS03 and 2019ZDSYS03), Clinical Study of Second Hospital of Tianjin Medical University (2019LC03), Tianjin Key Medical Discipline (Specialty) Construction Project, Tianjin Key Medical Discipline(Specialty) Construction Project

(TJYXZDXK-029A), and National Natural Science Foundation of China (No. 82100342).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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frontiers | Frontiers in Cardiovascular Medicine

TYPE Original Research
PUBLISHED 08 September 2022
DOI 10.3389/fcvm.2022.1002030

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Cardiovascular Therapeutics, a section of the journal Frontiers in Cardiovascular Medicine

RECEIVED 24 July 2022
ACCEPTED 11 August 2022
PUBLISHED 08 September 2022

CITATION
Yu B, Mo Y, Hu X, Wang W, Liu J, Jin J, Lun Z, Luo Bu CR, Dong H and Zhou Y (2022) Triglyceride-glucose index is associated with quantitative flow ratio in patients with acute ST-elevation myocardial infarction after percutaneous coronary intervention. *Front. Cardiovasc. Med.* 9:1002030. doi: 10.3389/fcvm.2022.1002030

Triglyceride-glucose index is associated with quantitative flow ratio in patients with acute ST-elevation myocardial infarction after percutaneous coronary intervention

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Background: The triglyceride-glucose (TyG) index is a novel marker representing the degree of insulin resistance (IR) and is closely related to cardiovascular diseases. However, the association between the TyG index and vascular function in patients with acute ST-elevation myocardial infarction (STEMI) after percutaneous coronary intervention (PCI) remains unknown.

Materials and methods: This study was a *post hoc* analysis of a multicenter, prospective cohort study. In this study, patients with STEMI who underwent PCI were included, and coronary angiography data were analyzed by Quantitative coronary angiography (QCA) and quantitative flow ratio (QFR). In addition, the TyG index was calculated as follows: Ln [fasting triglyceride (mg/dl) × fasting blood glucose (mg/dl) × 1/2]. According to the post-PCI QFR, patients were divided into two groups: post-PCI QFR ≤ 0.92 group and post-PCI QFR > 0.92 group. Construction of logistic regression model to explore the relationship between the TyG index and post-PCI QFR.

Results: A total of 241 STEMI patients were included in this study. Compared with patients in the post-PCI QFR > 0.92 group, the TyG index was higher in the post-PCI QFR ≤ 0.92 group. Logistic regression model showed that after adjusting for other confounding factors, the TyG index was positively correlated with the risk of post-PCI QFR ≤ 0.92 (OR = 1.697, 95% CI 1.171–2.460, P = 0.005). Restricted cubic

splines showed the cutoff value of TyG index associated with post-PCI QFR $\leq$ 0.92 risk was 9.75.

Conclusion: The TyG index was associated with the risk of post-PCI QFR $\leq$ 0.92 in STEMI patients. The risk of post-PCI QFR $\leq$ 0.92 increased when the TyG index exceeded 9.75.

KEYWORDS

triglyceride-glucose index, quantitative flow ratio, ST-elevation myocardial infarction, percutaneous coronary intervention, fasting blood glucose

Introduction

Acute ST-elevation myocardial infarction (STEMI) remains one of the causes of high mortality. With the development of percutaneous coronary intervention (PCI) technology and secondary prevention treatment strategies, the prognosis of STEMI patients has improved, but some patients still have adverse events after PCI, such as in-stent restenosis and unintended revascularization, partly explained by residual ischemia of the coronary arteries (1–3). Physiological assessment after PCI, such as quantitative flow ratio (QFR) analysis, can be used as an effective means to quantify residual coronary ischemia (4–6). Studies have confirmed that poor post-PCI QFR is associated with a poorer prognosis, but the susceptibility factors affecting poor postoperative QFR remain unclear (7–9). Therefore, identifying risk factors for poor postoperative QFR has important clinical significance for reducing the risk of coronary residual ischemia.

Insulin resistance (IR) is a recognized indicator of systemic inflammation and metabolic disorders, is closely related to atherosclerotic cardiovascular disease, and is a high-risk factor for diabetes mellitus (DM) and cardiovascular disease (10, 11). The current methods for assessing IR include the hyperinsulinemia-euglycemic clamp and homeostasis model assessment-estimated IR (HOMA-IR), but their clinical use is limited due to time-consuming and expensive (12, 13). The triglyceride-glucose (TyG) index based on fasting blood glucose (FBG) and triglyceride (TG) has become an effective surrogate index for evaluating IR because of its rapidity and simplicity (14–16). More and more studies have found that the TyG index is not only significantly associated with the risk of atherosclerosis, DM, and coronary artery disease (CAD), but its elevated levels increase the poor prognosis of cardiovascular disease such as in-stent restenosis, atrial fibrillation (17–20).

However, to date, the association between the TyG index and residual coronary ischemia after PCI in STEMI patients has not been explored. Therefore, the aim of this study was to investigate the relationship between the TyG index and post-PCI QFR in STEMI patients.

Materials and methods

Study population

This study is a *post hoc* analysis of a multicenter, prospective cohort study (the outcomes in patients with STEMI with high thrombus burden treated by deferred versus immediate stent implantation in primary percutaneous coronary intervention: a prospective cohort study, which was registered at www.chictr.org.cn, ChiCTR1800019923), which was conducted in three cardiovascular centers (Guangdong Provincial People's Hospital, Guangzhou City; Guangdong Provincial People's Hospital Zhuhai Hospital, Zhuhai City; and Jiexi County People's Hospital, Jiexi City) from January 2018 to April 2021. STEMI patients who successfully underwent PCI were included in this study. The main exclusion criteria were: (1) age $<$ 18 years; (2) culprit vessel treated with underwent balloon angioplasty without stents implantation; (3) no fasting blood glucose measurement; (4) history of coronary artery bypass grafting. This study complies with the Declaration of Helsinki and was approved by the Ethics Committee of Guangdong Provincial People's Hospital. Written consent was obtained from all participants for this study.

Data collection and definition

Data regarding patient demographics, clinical characteristics, echocardiography, coronary angiography and laboratory results were obtained from electronic medical records. Blood samples for analysis were drawn after an overnight fast ($>$ 10 h). High-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), total cholesterol (TC), and TG levels were detected using AU5800 spectrophotometer (Beckman Coulter, United States) *via* colorimetry or immunoturbidimetry. Triglyceride-glycemic index was calculated by the formula Ln [fasting TG (mg/dL) $\times$ FBG (mg/dL)/2].

Hypertension was defined as systolic blood pressure $\geq$ 140 mmHg and/or diastolic blood

TABLE 1 Baseline characteristics.

	Total (N = 241)	Post-PCI QFR ≤ 0.92 (n = 90)	Post-PCI QFR > 0.92 (n = 151)	P-value
Age, years	62 ± 12	61 ± 11	63 ± 12	0.184
Male	203 (84.2)	78 (86.7)	125 (82.8)	0.469
BMI, kg/m^2	24.18 ± 3.53	24.28 ± 3.78	24.11 ± 3.38	0.721
LVEF, %	49.3 ± 11.4	46.7 ± 12.0	50.9 ± 10.8	**0.006***
Medical history, n (%)				
Smoking	110 (45.6)	38 (42.2)	72 (47.7)	0.426
Hypertension	106 (44.0)	42 (46.7)	64 (42.2)	0.592
DM	33 (13.7)	13 (14.4)	20 (13.2)	0.847
Family history of CAD	4 (1.7)	1 (1.1)	3 (2.0)	1.000
Previous myocardial infarction	27 (11.2)	10 (11.1)	17 (11.3)	1.000
Peripheral vascular disease	5 (2.1)	0 (0)	5 (3.3)	0.160
O to D, hours	9.23 ± 11.58	9.25 ± 13.11	9.23 ± 10.61	0.990
STEMI types				**0.002***
Anterior	124 (51.5)	58 (64.4)	66 (43.7)	
Non-anterior	117 (48.5)	32 (35.6)	85 (56.3)	
Laboratory tests				
FBG, mmol/L	8.78 ± 3.98	9.62 ± 4.49	8.27 ± 3.57	**0.016***
Creatine, mmol/L	86.91 ± 72.92	80.91 ± 34.01	90.49 ± 88.25	0.325
TG, mmol/L	2.20 ± 1.75	2.42 ± 1.94	2.07 ± 1.62	0.139
TC, mmol/L	4.58 ± 1.74	4.56 ± 1.91	4.59 ± 1.63	0.889
HDL-C, mmol/L	1.07 ± 0.28	1.04 ± 0.22	1.09 ± 0.30	0.182
LDL-C, mmol/L	3.37 ± 0.93	3.46 ± 1.07	3.31 ± 0.84	0.250
Peak CK-MB, U/L	284.56 ± 388.97	249.60 ± 216.61	305.39 ± 461.59	0.282
Peak TnT, pg/mL	8919.4 ± 45512.1	5258.7 ± 3403.3	10129.3 ± 57432.2	0.303
Peak NT-proBNP, pg/mL	3201.0 ± 8043.9	2916.1 ± 4866.0	3370.8 ± 9455.2	0.672
TyG index	9.31 ± 0.79	9.50 ± 0.82	9.21 ± 0.75	**0.005***

PCI, percutaneous coronary intervention; QFR, quantitative flow ratio; BMI, body mass index; LVEF, left ventricular ejection fraction; DM, diabetes mellitus; CAD, coronary artery disease; O to D, time from onset to door; STEMI, ST-elevation myocardial infarction; FBG, fasting blood glucose; TG, triglyceride; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; CK-MB, peak creatine kinase isoenzyme MB; TnT, troponin T; NT-proBNP, N terminal pro B type natriuretic peptide; TyG, triglyceride-glucose.
Bold term and * indicates statistically significant.

pressure ≥ 90 mmHg (21). The diagnostic criteria for DM were FBG ≥ 7.0 mmol/L or random blood glucose > 11.1 mmol/L, and glycosylated hemoglobin ≥ 6.5% (22).

Quantitative coronary angiography and quantitative flow ratio analysis

Quantitative coronary angiography (QCA) and QFR analyses were performed before and after PCI in culprit vessel. QCA and QFR before PCI referred to QCA and QFR after pretreatment with balloon. The measurement of QCA needs to select two parts with an end-diastolic projection angle > 25° and no shortening or overlapping part for coronary 3D reconstruction. The measurement contents include reference vessel diameter, lesion length, minimum lumen diameter, and diameter stenosis rate. QFR assessment was performed on all criminal vessels that underwent PCI. The measurement of QFR is based on three-dimensional QCA analysis and frame technique analysis without drug congestion. QFR calculations were performed by two experienced technicians (Independent Core Laboratory, Shanghai, China) using prototype software (AngioPlus Core, Pulse Medical Imaging Technology, Shanghai, China).

Statistical analysis

Continuous variables were described as mean ± standard deviation, and categorical variables were described as counts and percentages. For continuous variables, use the T-test or the Mann–Whitney U test, and for categorical variables, use the Chi-square test or Fisher's exact test to compare the differences between the two groups. Logistic regression analysis explored the relationship between the TyG index and post-PCI QFR by odds ratio (OR) with a 95% confidence interval (CI).

TABLE 2 Coronary characteristics.

	Total (N = 241)	Post-PCI QFR ≤ 0.92 (n = 90)	Post-PCI QFR > 0.92 (n = 151)	P-value
Culprit vessel				**0.008***
LAD	124 (51.5)	58 (64.4)	66 (43.7)	
LCX	14 (5.8)	4 (4.4)	10 (6.6)	
RCA	103 (42.7)	28 (31.1)	75 (49.7)	
Pre-PCI QCA				
Reference vessel diameter, mm	2.94 ± 0.86	2.89 ± 0.84	2.97 ± 0.88	0.463
Minimal lumen diameter, mm	1.07 ± 0.59	1.05 ± 0.57	1.08 ± 0.61	0.788
Diameter stenosis, %	63.1 ± 17.1	63.2 ± 16.0	63.0 ± 17.7	0.939
lesion length, mm	14.3 ± 6.5	13.4 ± 5.4	14.8 ± 7.0	0.103
Post-PCI QCA				
In-stent reference vessel diameter, mm	3.12 ± 0.61	3.04 ± 0.51	3.17 ± 0.67	0.125
In-stent minimal lumen diameter, mm	2.54 ± 0.59	2.40 ± 0.55	2.63 ± 0.60	**0.002***
In-stent diameter stenosis, %	18.6 ± 11.3	21.2 ± 13.5	17.1 ± 9.4	**0.012***
Stent length, mm	24.4 ± 16.1	28.0 ± 16.1	30.3 ± 16.1	0.277
Pre-PCI QFR	0.40 ± 0.40	0.37 ± 0.37	0.41 ± 0.41	0.418
Post-PCI QFR	0.93 ± 0.08	0.87 ± 0.11	0.96 ± 0.02	< **0.001***

LAD, left anterior descending artery; LCX, Left circumflex artery; RCA, right coronary artery; QCA, quantitative coronary angiography; PCI, percutaneous coronary intervention; QFR, quantitative flow ratio.
Bold term and * indicates statistically significant.

Factors that might affect post-PCI QFR in baseline data were included in the regression equation to control for the influence of confounding factors. Model 1 adjusted for age, sex, body mass index (BMI), and model 2 adjusted for left ventricular ejection fraction (LVEF), smoking, hypertension, DM, previous myocardial infarction, and creatinine based on model 1. Model 3 continued to adjust the culprit vessel, stent length, in-stent minimum lumen diameter, and in-stent diameter stenosis rate based on model 2. Restricted cubic splines were used to explore the association between the TyG index and the risk of post-PCI QFR ≤ 0.92 on a continuous scale. Sensitivity analysis was performed to evaluate the association between the TyG index and the risk of post-PCI QFR ≤ 0.92 in the non-chronic total occlusion (CTO) subgroup, and the results are presented in the **Supplementary material**. All statistical analyses were performed on IBM SPSS Statistics 26 and R language 4.1.2. Two-sided $P < 0.05$ was considered statistically significant.

TABLE 3 Association of TyG index with the risk of post-PCI QFR ≤ 0.92 in logistic regression models.

	OR	95% CI	P-value
Unadjusted model			
TyG, per 1-unit increase	1.611	1.142–2.273	**0.007***
Model 1			
TyG, per 1-unit increase	1.611	1.142–2.273	**0.007***
Model 2			
TyG, per 1-unit increase	1.672	1.177–2.374	**0.004***
Model 3			
TyG, per 1-unit increase	1.697	1.171–2.460	**0.005***

Model 1: adjusted for age, sex, and BMI.
Model 2: adjusted for age, sex, BMI, LVEF, smoking, hypertension, DM, previous myocardial infarction, and creatine.
Model 3: adjusted for age, sex, BMI, LVEF, smoking, hypertension, DM, previous myocardial infarction, creatine, culprit vessel, length of stents, in-stent minimal lumen diameter and in-stent diameter stenosis.
TyG, triglyceride-glucose; OR, odds ratio; CI, confidence interval; BMI, body mass index; LVEF, left ventricular ejection fraction; DM, diabetes mellitus; PCI, percutaneous coronary intervention; QFR, quantitative flow ratio.
Bold term and * indicates statistically significant.

Results

Baseline characteristics

A total of 241 STEMI patients were included in this study. As shown in **Table 1**, among the included patients, 151 patients had optimal PCI results (post-PCI QFR > 0.92), while 90 patients had suboptimal PCI results (post-PCI QFR ≤ 0.92). Of the total population, 84.2% were male, and the mean age was 62 years. The prevalence of smoking, hypertension, DM, and family history of CAD, previous myocardial infarction, and peripheral vascular disease were 45.6, 44.0, 13.7, 1.7, 11.2, and 2.1%, respectively. The distribution of STEMI types differed between the two groups, with patients in the post-PCI QFR ≤ 0.92 group having a higher proportion of anterior STEMI, whereas patients in the post-PCI QFR > 0.92 group had a higher proportion of non-anterior STEMI. Compared with the Post-PCI QFR > 0.92 group, the patients in the post-PCI

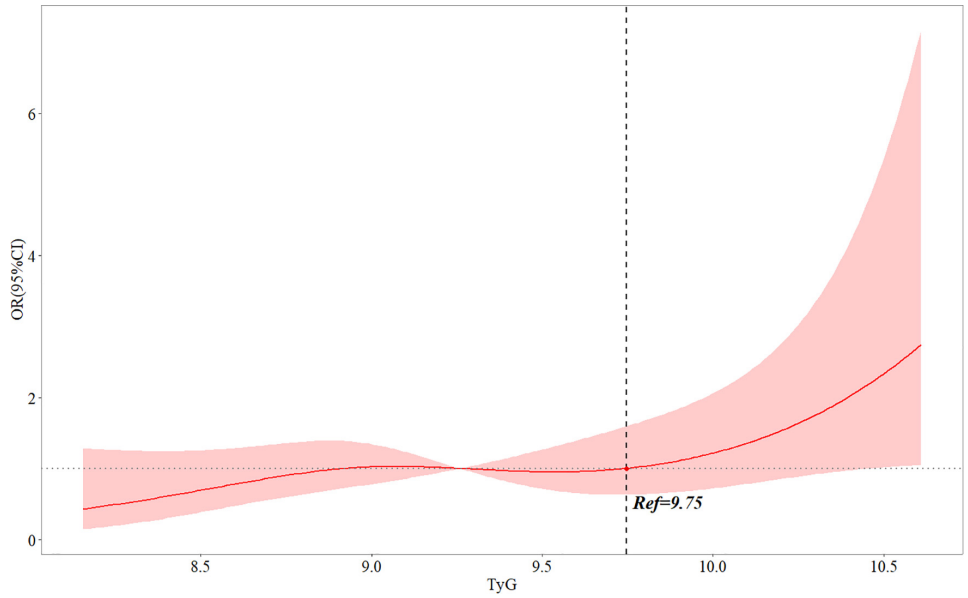

FIGURE 1
Multivariable adjusted OR for the risk of post-PCI QFR ≤ 0.92 according to levels of TyG index on a continuous scale. Odds ratios and 95% CIs derived from restricted cubic spline regression, with knots placed at the 5th, 35th, 65th, and 95th percentiles of the distribution of TyG index. The reference point for TyG index is located at OR = 1. Ref represents the level of TyG index at increased risk of post-PCI QFR ≤ 0.92. Analyses were adjusted for age, sex, BMI, LVEF, smoking, hypertension, DM, previous myocardial infarction, creatine, culprit vessel, length of stents, in-stent minimal lumen diameter and in-stent diameter stenosis. OR, odds ratio; QFR, quantitative flow ratio; TyG, triglyceride-glucose; BMI, body mass index; LVEF, left ventricular ejection fraction; DM, diabetes mellitus; PCI, percutaneous coronary intervention; CI: confidence interval.

QFR ≤ 0.92 group showed a higher FBG and TyG index, but a lower LVEF. Age, male ratio, BMI, smoking, hypertension, DM, family history of CAD, previous myocardial infarction, incidence of peripheral vascular disease, time from onset to door, TG, TC, HDL-C, LDL-C, peak creatine kinase isoenzyme MB (CK-MB), peak troponin T (TnT), and Peak NTpro-brain natriuretic peptide (BNP) were not different between the two groups. The QCA and QFR analysis for all patients was listed in **Table 2**. Post-PCI QFR ≤ 0.92 and post-PCI QFR > 0.92 groups had mean post-PCI QFR of 0.87 and 0.96, respectively. Patients in the suboptimal PCI result group had a higher proportion of the culprit vessel in the left anterior descending artery, a smaller in-stent minimum lumen diameter, and a greater in-stent diameter stenosis rate.

Association between the triglyceride-glucose index and post-percutaneous coronary intervention quantitative flow ratio

In an unadjusted logistic regression model, the TyG index was positively associated with the risk of post-PCI QFR ≤ 0.92. After adjusting for other factors, the risk of TyG index and post-PCI QFR ≤ 0.92 in model 1 (OR = 1.611, 95% CI 1.142–2.273, $P = 0.007$), model 2 (OR = 1.672, 95% CI 1.177- 2.374, $P = 0{,}004$), and model 3 (OR = 1.697, 95% CI 1.171–2.460, $P = 0.005$) were still independently associated (**Table 3**). Furthermore, the relationship between the TyG index and the risk of post-PCI QFR ≤ 0.92 remained significantly associated in the non-CTO population (OR = 1.687, 95% CI 1.162–2.448, $P = 0.006$) (**Supplementary Table 3**). The relationship between the TyG index and the risk of post-PCI QFR ≤ 0.92 was non-linear in the continuous range of the TyG index (**Figure 1**). The cutoff value of the TyG index associated with risk of post-PCI QFR ≤ 0.92 was 9.75 (**Figure 1**).

Association between the triglyceride-glucose index and post-percutaneous coronary intervention quantitative flow ratio in different subgroups

The association of the TyG index with post-PCI QFR ≤ 0.92 was assessed in different subgroups (**Figure 2**). After adjusting for other factors, the positive association of the TyG index with the risk of post-PCI QFR ≤ 0.92 was more significant in the subgroups of women, age ≥ 65 years, BMI ≤ 24 kg/m^2, non-smokers, no DM, and LVEF > 50%. In addition, there may be a slight interaction between the TyG index and male gender and age (**Figure 2**).

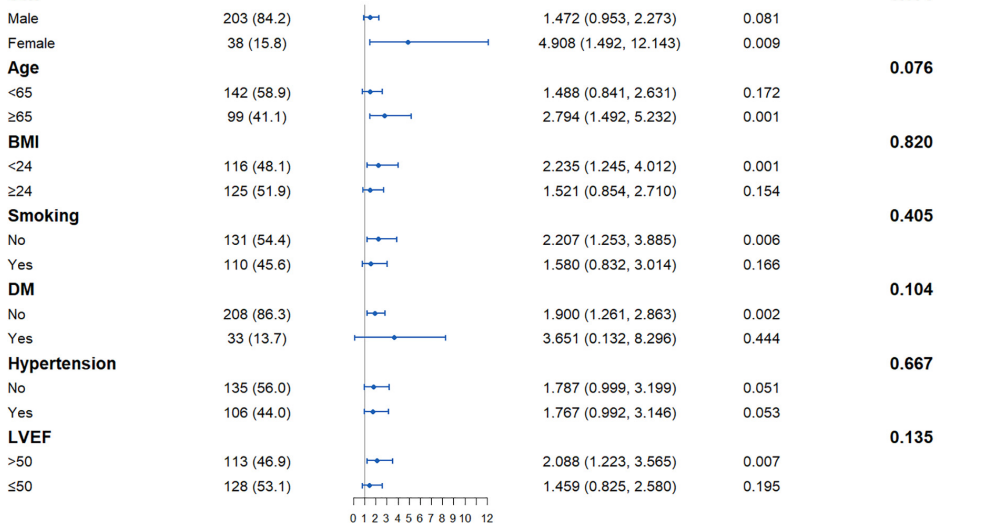

FIGURE 2
Forest plot investigating the association between the TyG index and with the risk of post-PCI QFR ≤ 0.92 in different subgroups. TyG, triglyceride-glucose; QFR, quantitative flow ratio; BMI, body mass index; LVEF, left ventricular ejection fraction; DM, diabetes mellitus; PCI, percutaneous coronary intervention; OR, odds ratio; CI, confidence interval.

Discussion

In the present study, we investigated the relationship between the TyG index and post-PCI QFR in STEMI patients undergoing PCI. The main findings of this study were as follows: (1) the TyG index was significantly correlated with post-PCI QFR ≤ 0.92 in STEMI patients. After adjusting for other confounding factors, the TyG index was still an independent risk factor for post-PCI QFR ≤ 0.92; (2) When the TyG index exceeds 9.75, the risk of post-PCI QFR ≤ 0.92 increases; (3) The positive association between the TyG index and the risk of post-PCI QFR ≤ 0.92 in STEMI patients was more significant in women, the older, non-smoking, no DM, and people with good cardiac function.

STEMI is a dangerous disease that seriously affects the life and quality of life of patients. With the development of interventional treatment technology, the prognosis of patients with STEMI has been improved, but one-quarter of patients still have adverse events during follow-up, which was related to residual coronary artery ischemia (1–3). Current coronary interventions aim to relieve anatomical stenosis, but coronary function after stenting has not been evaluated. A large number of recent studies have shown that QFR can well evaluate the physiological function of coronary arteries, and post-PCI QFR was significantly associated with future adverse cardiovascular events (7, 23). In the PANDA III trial, QFR ≤ 0.92 was the best cutoff value for predicting adverse cardiovascular events within 2 years, and after the QFR exceeded 0.92, increasing QFR had no effect on prognosis (8). Therefore, identifying the risk factors that affect the risk of QFR ≤ 0.92 after PCI has clinical value and can be used as an early intervention treatment method.

Insulin resistance refers to the lower-than-expected biological effect of insulin, manifested as a disturbance in the uptake and utilization of glucose. IR can not only induce chronic hyperglycemia, but also affect lipid metabolism and increase TG levels (24). The TyG index is a composite index composed of TG and FBG. It has been confirmed that the TyG index can be used as a substitute index for IR, which is a simpler and faster assessment of the body's IR status (13, 25–27). Several studies have found that the TyG index is significantly associated with the occurrence of atherosclerotic cardiovascular disease (28–31). A cohort study found that, in long-term follow-up, the TyG index can identify people at high risk for cardiovascular events (28). At the same time, a meta-analysis summarizing multiple cohort studies found that after adjusting for the effects of age, gender, and DM, the TyG index was still independently associated with the risk of cardiovascular disease (29). In a RCSCD-TCM study, Su et al. conducted a retrospective analysis of 731 patients with CAD and found that the TyG index was associated with the severity of CAD, and an elevated TyG index could increase the risk of coronary multivessel disease (30). Alessandra et al. analyzed baseline data from patients in secondary cardiac care and found that the TyG index was associated with metabolic risk factors for the heart, and that a high level of the TyG index was more likely to develop symptomatic CAD (31). Moreover, the TyG index is considered to be a marker for identifying the risk of subclinical arteriosclerosis and is closely related to the degree of

coronary artery calcification, carotid intima-media thickness, and brachial-ankle pulse wave velocity, which is not affected by traditional risk factors (32–36). In addition, recent evidence has also shown that the TyG index is not only an independent risk factor for stable CAD, but also has a positive correlation with poor prognosis in patients with acute myocardial infarction (18, 19). A study of 1,092 STEMI patients who underwent successful PCI for 1 year of follow-up found that high levels of TyG index increased the risk of cardiovascular adverse events during follow-up (18). Zhu et al. investigated the occurrence of in-stent stenosis in 1,574 patients with acute coronary syndrome during 1-year follow-up after stenting and found that the increase in the TyG index level was independently associated with in-stent stenosis (19). However, no study has investigated the relationship between TyG index and post-PCI QFR in STEMI patients. In the present study, we found that the TyG index was associated with residual coronary ischemia, as the higher the TyG index level, the greater the risk of post-PCI QFR $\leq$ 0.92. Therapeutic measures to lower the TyG index may be beneficial in reducing residual coronary ischemia in the future. Meanwhile, we also found that the risk of post-PCI QFR $\leq$ 0.92 was increased when the TyG index level exceeded 9.75, which may serve as a threshold for assessing residual coronary ischemia. In addition, the results of subgroup analysis showed that the TyG index and post-PCI QFR were also stable in different subgroups. Unexpectedly, this relationship was more significant in women, non-smoking patients, and non-DM patients. Although the exact mechanism is unclear, it is also a factor that we need to consider together.

The exact mechanism between the TyG index and post-PCI QFR in STEMI patients remains unclear, but this association may be based on IR status as assessed by the TyG index. First, IR can damage coronary endothelial function through oxidative stress and inducing inflammation (10). Second, DM is related to coronary vascular dysfunction, which may damage microcirculatory vasodilation and reduce coronary blood flow. IR is an important pathophysiological pathway leading to DM, and the two may have commonalities in the physiological and structural damage of coronary arteries (37–40). Third, IR in patients with acute myocardial infarction promotes local platelet activation and thrombin generation, increasing coronary thrombus burden, which may explain this relationship (41, 42). There may be more studies in the future to clarify the relationship between TyG index and post-PCI QFR.

This study has important clinical value to explore the relationship between the TyG index and the risk of post-PCI QFR $\leq$ 0.92. First, TyG index is a risk factor for post-PCI QFR $\leq$ 0.92, and controlling the level of TyG index may reduce residual ischemia in coronary arteries after PCI. Second, the TyG index is a conveniently measurable, easily accessible, and reproducible blood index that can assess patient coronary physiological function in real time during follow-up. Finally, TyG index above 9.75 increases the risk of post-PCI QFR $\leq$ 0.92, and 9.75 may be used as a threshold for the need for intensive drug therapy to improve coronary ischemia after PCI.

At the same time, there are some limitations in our study. First, this was a single-center study with limited sample size and limited generalization of the results. Second, the present study lacked a comparison of the HOMA-IR and the TyG index. Finally, the TyG index during hospitalization was only assessed once, and the dynamic changes of the TyG index were lacking.

Conclusion

The TyG index was independently associated with the risk of post-PCI QFR $\leq$ 0.92 in STEMI patients. Meanwhile, when the TyG index exceeded 9.75, the risk of post-PCI QFR $\leq$ 0.92 increased.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by the Ethics Committee of Guangdong Provincial People's Hospital. The patients/participants provided their written informed consent to participate in this study.

Author contributions

BY: manuscript preparation and writing—original draft. XH, WW, JL, JJ, and ZL: data collection and collation. BY, YM, and WW: data analysis. CRLB and HD: writing—critical revisions. HD and YZ: conceptualization and approval of the final version of the manuscript for submission. All authors read and approved the final manuscript.

Funding

This work was supported by the Department of Science and Technology of Guangdong Province (2020B1111170011), the Department of Science and Technology of Guangdong Province (No. 202102080466), and the National Key Research and Development Program of China (No. 2016YFC1301202).

Acknowledgments

We thank LetPub (www.letpub.com) for its linguistic assistance during the preparation of this manuscript.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2022.1002030/full#supplementary-material

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CLINICAL TRIAL
published: 15 September 2022
doi: 10.3389/fcvm.2022.928695

IntraCoronary Artery Retrograde Thrombolysis vs. Thrombus Aspiration in ST-Segment Elevation Myocardial Infarction: Study Protocol for a Randomized Controlled Trial

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OPEN ACCESS

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Specialty section:
This article was submitted to Cardiovascular Therapeutics, a section of the journal Frontiers in Cardiovascular Medicine

Received: 26 April 2022
Accepted: 18 May 2022
Published: 15 September 2022

Citation:
Shen M, Wang J, Li D, Zhou X, Guo Y, Zhang W, Guo Y, Wang J, Liu J, Zhao G, Zhao S and Tian J (2022) IntraCoronary Artery Retrograde Thrombolysis vs. Thrombus Aspiration in ST-Segment Elevation Myocardial Infarction: Study Protocol for a Randomized Controlled Trial. Front. Cardiovasc. Med. 9:928695. doi: 10.3389/fcvm.2022.928695

Background: Type 2 diabetes (T2DM) is a major risk factor for myocardial infarction. Thrombus aspiration was considered a good way to deal with coronary thrombus in the treatment of acute myocardial infarction. However, recent studies have found that routine thrombus aspiration is not beneficial. This study is designed to investigate whether intracoronary artery retrograde thrombolysis (ICART) is more effective than thrombus aspiration or percutaneous transluminal coronary angioplasty (PTCA) in improving myocardial perfusion in patients with ST-segment elevation myocardial infarction (STEMI) undergoing primary percutaneous coronary intervention (PPCI).

Methods/Design: IntraCoronary Artery Retrograde Thrombolysis (ICART) vs. thrombus aspiration or PTCA in STEMI trial is a single-center, prospective, randomized open-label trial with blinded evaluation of endpoints. A total of 286 patients with STEMI undergoing PPCI are randomly assigned to two groups: ICART and thrombus aspiration or PTCA. The primary endpoint is the incidence of >70% ST-segment elevation resolution. Secondary outcomes include distal embolization, myocardial blush grade, thrombolysis in myocardial infarction (TIMI) flow grade, and in-hospital bleeding.

Discussion: The ICART trial is the first randomized clinical trial (RCT) to date to verify the effect of ICART vs. thrombus aspiration or PTCA on myocardial perfusion in patients with STEMI undergoing PPCI.

Clinical Trial Registration: [https://www.chictr.org.cn/], identifier [ChiCTR1900023849].

Keywords: ST-segment elevation myocardial infarction, intracoronary artery retrograde thrombolysis, thrombus aspiration, reperfusion preconditioning, percutaneous coronary intervention (PCI)

BACKGROUND

Patients with type 2 diabetes (T2DM) are at high risk and have a poor prognosis of myocardial infarction, especially after ST-segment elevation myocardial infarction (STEMI). STEMI is due to plaque rupture leading to intracoronary thrombosis, thus blocking the coronary artery (1, 2). The strategy to treat myocardial infarction is to open the infarct-related coronary artery to achieve reperfusion as soon as possible (3). However, in the process of opening the occluded vessels, it leads to myocardial damage. This phenomenon is called reperfusion injury (4). Myocardial death caused by reperfusion accounts for 25% of the total death area. Animal experiments show that the proportion is even as high as 50% (5). Reperfusion injury can cause reperfusion arrhythmia, myocardial stunning, microvascular occlusion, intramyocardial hemorrhage, and lethal myocardial reperfusion injury (6).

At present, methods of opening occluded vessels include intravenous thrombolysis, primary percutaneous coronary intervention (PPCI), and emergency coronary artery bypass grafting (CABG) (7, 8). The advantage of intravenous thrombolysis is that it can be implemented in general hospitals and even primary hospitals, and can be carried out quickly before hospitalization or even in ambulances (9–11). However, the success rate of intravenous thrombolysis is relatively low (12, 13). Even after intravenous thrombolysis success, emergency percutaneous coronary intervention (PCI) should be performed within 24 h, which increases the risk of hemorrhagic events or even fatal hemorrhage. Therefore, intravenous thrombolysis was gradually reduced or even stopped in the hospitals that could perform primary PCI in time or quickly transfer to primary PCI hospitals (7). Because of the complexity of CABG and the high requirement of patients' own conditions, it is relatively difficult to carry out emergency CABG (8).

Primary percutaneous coronary intervention is still the best way to treat acute myocardial infarction because of its simple operation and exact effect (14). Thrombus load is an independent risk factor for PPCI. Slow-flow and no-reflow after stent implantation affect the prognosis (15). There are two ways to deal with thrombus before PPCI. The first is thrombus aspiration (16). However, routine thrombus aspiration before PPCI does not reduce the rate of all-cause mortality, rehospitalization of myocardial infarction, or stent thrombosis, and even increase the risk of stroke (17). Even in patients with high thrombus burden, routine thrombus aspiration does not improve outcomes (18). The second is to give antithrombotic or thrombolytic agents through the transcatheter, such as glycoprotein (GP) IIb/IIIa inhibitors, alteplase, et al. (19). However, due to the blood flow erosion, antithrombotic agents, and thrombolytic agents cannot stay for a long time at the thrombus site, affecting the drug effect; if the dosage is increased, the hemorrhagic risk will increase at the same time (20). It has also been studied that thrombolytic drugs are given after thrombus aspiration (21), the efficacy is similarly affected for the above reasons. There is still a long way to go to solve the problem of intracoronary thrombus, especially in patients with large thrombus burdens.

The sequence of continuous thrombosis is white thrombus, mixed thrombus, and red thrombus. The white thrombus in the head is mainly composed of platelets. The red thrombus at the tail end is mainly composed of red cells within the fibrin network (22). According to the characteristics of thrombus, we put forward the method of ICART, applied it to a small sample population, and achieved a certain effect (23). Only 10% of intravenous thrombolysis is needed for the ICART procedure. Before the newly formed thrombus is destroyed, a very high concentration of thrombolytic agent is produced in the red thrombus of the occluded vessel, which could stay for a long time, resulting in a very high thrombolytic efficiency, and reducing the incidence of no-reflow and slow-flow. At the same time, due to the slow opening of occluded vessels, there is a reperfusion preadaptation, which can reduce the reperfusion injury in theory. At the same time, low-dose thrombolysis does not increase the risk of hemorrhage and stroke. ICART may provide a new plan to solve thrombus burden in STEMI. However, there were only eight cases in the previous study (23). Therefore, we intend to verify the effect of ICART vs. thrombus aspiration or PTCA on myocardial perfusion in patients with STEMI undergoing PPCI.

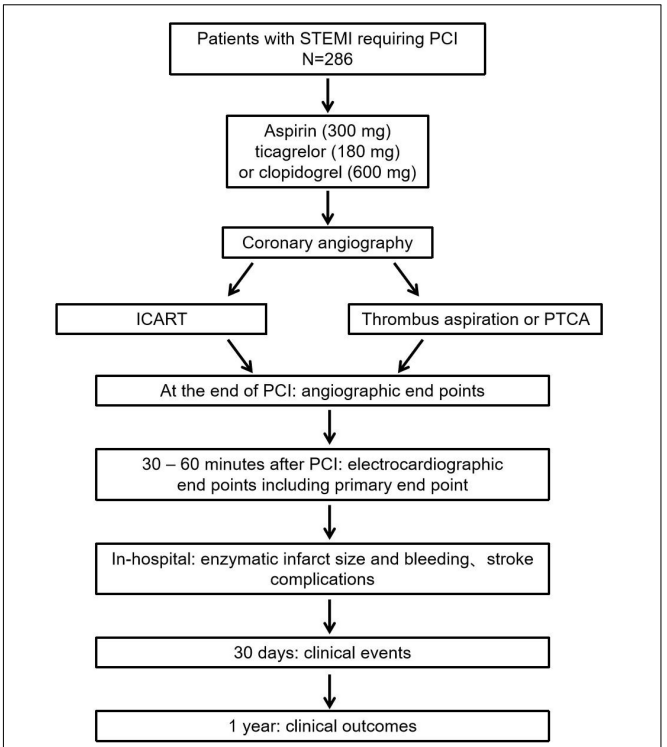

FIGURE 1 | The ICART trial flow chart. ICART, intracoronary artery retrograde thrombolysis; STEMI, ST-segment elevation myocardial infarction; PCI, percutaneous coronary intervention; PTCA, percutaneous transluminal coronary angioplasty.

STUDY DESIGN

The ICART trial is a single-center, prospective, randomized controlled trial with a blinded evaluation of endpoints (**Figure 1**). A total of 286 patients with STEMI undergoing PPCI were randomly divided into ICART or thrombus aspiration or PTCA

groups. Randomization is performed by the Empower network random system when deciding to perform PCI. The study was approved by the institutional committee on human research of the Chinese People's Liberation Army General Hospital and complies with the declaration of Helsinki. The protocol of this trial has been registered at chictr.org.cn (ChiCTR1900023849).

STUDY POPULATION

All consecutive STEMI patients who are ready to receive primary PCI are suitable for inclusion. The inclusion criterion includes: (1) STEMI with chest pain lasting 30 min to 12 h; (2) older than 18 years old; (3) volunteered and signed informed consent. The exclusion criteria includes: (1) active peptic ulcer; (2) severe hepatic or renal insufficiency; (3) pregnancy; (4) uncontrolled hypertension; (5) rescue PCI after thrombolytic therapy; (6) unable to sign informed consent; (7) less than 18 years old; (8) left main coronary artery disease; (9) cardiogenic shock; (10) mechanical complications after myocardial infarction (e.g., interventricular septum perforation, papillary muscle rupture); (11) recent history of major surgery, trauma, hemorrhagic disease, cerebrovascular accident, or thrombocytopenia; (12) previous history of CABG; (13) other obvious abnormal signs, laboratory tests, and clinical diseases. According to the judgment of the clinician, the patients are not suitable for the study.

PREPARATION OF VISUALIZED THROMBOLYTIC AGENTS AND PROCESS OF INTRACORONARY ARTERY RETROGRADE THROMBOLYSIS

As we previously described (23), visualized thrombolytic agents are made by dissolving 100,000 units urokinase or 5 mg prourokinase with 15 ml physiological saline and 5 ml iopromide.

The flow diagram for the ICART system is shown in **Figure 2**. Coronary angiography identifies the occluded coronary artery. First, the guidewire is sent to the distal end of the culprit's vessel. A microcatheter or a cut balloon is sent to the occluded section through the guidewire. One milliliter of thrombolytic cocktail is bolus-injected through the microcatheter, which is repeated every 30 s. The effect of thrombolysis is observed by X-ray.

TREATMENT

Before PCI, ICART or thrombus aspiration is decided according to the grouping. Thrombus is aspirated by the Export aspiration catheter (Medtronic Inc., Santa Rosa, CA, United States) as previously described (24). Finally, a stent is implanted. In certain patients, pre- or postdilatation with a balloon may be necessary.

Patients are given a loading dose of aspirin (300 mg) and ticagrelor (180 mg) or clopidogrel (600 mg), and are diagnosed STEMI by electrocardiography. It's up to the operator to decide whether to use heparin or bevaludine for anticoagulation. In the setting of STEMI, radial access is preferred. In patients with radial artery pathways, sheaths are pulled out immediately following the PCI procedure. The femoral approach is reserved for patients without the radial approach. Sheaths are exchanged immediately at the end of the PCI procedure by the Angio-Seal device (St. Jude Medical, Inc., St. Paul, MN, United States). After PCI, tirofiban is used for 36 h. Then low molecular weight heparin is given for 1–3 days after tirofiban. According to the international guidelines, standard post PCI medication includes aspirin (100 mg), ticagrelor (90 mg bid), or clopidogrel (75 mg), beta-blockers, lipid-lowering drugs, and angiotensin converting enzyme inhibitors or angiotensin II receptor blockers (8).

ELECTROCARDIOGRAPHY

A standard 18-lead ECG is acquired at first medical contact. A standard 12 lead ECG is performed 30–60 min after PPCI. Times of symptom manifestation, arrival at the hospital, guidewire passing, thrombolysis, balloon dilatation, end of PCI, and ECG are recorded. The magnitude of ST-segment deviation is calculated 60 ms from the J-point. The ST segment of post-intervention ECG at 30–60 min is compared with that of the ECG at presentation. ST-segment elevation resolution is defined as complete ($>$70%), partial (30–70%), or absent ($<$30%) (Trials 2009, 10:90). Residual ST-segment deviation after PCI is counted as the sum of residual ST-segment depression and elevation in all leads (25). All ECG data are analyzed by a physician who is blinded to the clinical grouping and data.

CORONARY ANGIOGRAPHY

The baseline, peri-, and post-procedural angiographic features will be recorded: the presence of thrombus, treatment of a non-culprit vessel during the same procedure, myocardial blush grade (MBG), thrombolysis in myocardial infarction (TIMI) flow grades, side branch occlusion, the presence of angiographically visible distal embolization, minimum lumen diameter, stent diameter, stent length, no-reflow, and slow flow. TIMI flow grades are evaluated according to the previously described method (26). The evaluation of thrombus adopts the previous evaluation standard (27). The thrombus is assessed according to TIMI thrombus classification: 0 = none, 1 = suspected thrombus, 2 = thrombus, linear size $\leq$ 1/2 vessel diameter, 3 = thrombus, 1/2 $\leq$ linear size $<$ two times of vessel diameter, 4 = thrombus, linear size $\geq$ two times of vessel diameter, 5 = complete occlusion due to thrombosis. MBG's evaluation is based on the previous description (28): 0 = no myocardial staining, 1 = slight myocardial staining, or contrast density, 2 = moderate myocardial staining or contrast density, but less than that of a contra- or ipsilateral non-infarct-related area, and 3 = normal myocardial staining or contrast density, comparable with that of a contra- or ipsilateral non-infarct-related area. Continuous myocardial staining indicates that the contrast agent leaks into the extravascular space, which is defined as grade 0. Distal embolization is defined as filling defects and/or abrupt cutoff of distal vessels in the target lesion (29). The coronary angiograms are analyzed by a physician who does not know the treatment allocation and clinical data.

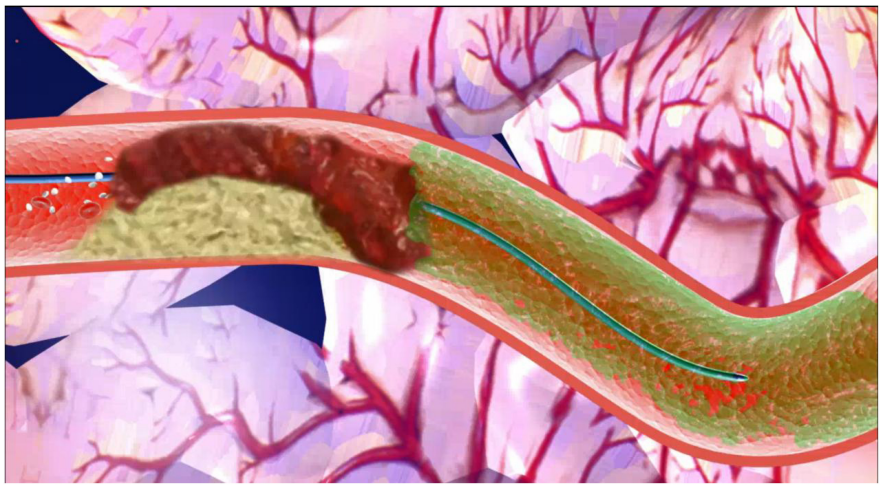

FIGURE 2 | Pattern diagram of intracoronary artery retrograde thrombolysis system. After the passing of the wire, a microcatheter or a cut balloon is sent to the occluded section through the guidewire. One milliliter of thrombolytic cocktail is bolus-injected through the microcatheter to the distal lumen of the vessel, which is repeated every 30 s.

INFARCT SIZE

The infarct size is estimated by serial monitoring of cardiac markers such as creatine kinase (CK), creatine kinase-MB (CK-MB), and troponin T. Blood samples are taken at baseline and 3, 6, 9, 12, 18, 24, and 48 h after PCI. Peak value, time to peak release as well as area under the curve are determined.

ENDPOINTS ASSESSMENT

The primary endpoint is to observe the probability of ST-segment resolution > 70% acquired before and 30–60 min after PPCI.

Secondary endpoints include:

1. Angiographic endpoints: TIMI flow grade, distal embolization, no reflow, slow flow, side branch occlusion, myocardial blush grade post-PPCI.
2. Electrocardiographic end points: residual ST-segment deviation 30–60 min after primary PCI.
3. Enzymatic infarct size.
4. Mortality and Major Adverse Cardiac Events: All-cause death, cardiogenic death, cardiogenic shock, reinfarction, revascularization of target vessels, malignant arrhythmia, NYHA class IV heart failure, stent thrombosis, and myocardial infarction rehospitalization are to be registered at 30 days and 1 year.

Safety endpoints consist of in-hospital hemorrhagic and stroke complications.

Furthermore, the primary and secondary endpoints will be analyzed in a preset subgroup, which is defined as:

1. Age (<65 vs. >65 years)
2. Gender
3. Smoker
4. Diabetes mellitus
5. ST-segment resolution
6. Number of diseased vessels (multi-vessel vs. single vessel)
7. Proximal lesions
8. Infarct-related artery [left anterior descending artery (LAD) vs. non-LAD]
9. Bivalirudin
10. Ischemic time (<3 vs. >3 h)
11. Initial TIMI thrombus grade
12. Angiographic presence of thrombus
13. Intra-aortic artery balloon pump
14. Left ventricular ejection fraction
15. Type of P2Y12 inhibitor
16. Killip class
17. Symptom onset
18. Pre-procedural TIMI flow
19. Post-procedural TIMI flow
20. Post-PCI myocardial blush grade
21. Pacing use.

CLINICAL FOLLOW-UP

All-cause death, cardiogenic death, cardiogenic shock, reinfarction, revascularization of target vessels, stroke, malignant arrhythmia, NYHA class IV heart failure, bleeding complications, stent thrombosis, and myocardial infarction rehospitalization are to be registered at 30 days and 1 year. The follow-up information will be obtained from hospital records as well as by telephone follow-up with the patients and/or their relatives.

DATA COLLECTION AND MANAGEMENT

Data and instrumental measurements will be collected from all subjects using an electronic data capture system (EmpowerEDC,

Shanghai, China). Data entry and management will be completed by an independent data administrator to guarantee data accuracy. Only the principal investigator could access the data. The final dataset can be acquired only by the principal investigator and the independent statistician. All procedures will comply with the confidentiality standards for medical data. All documents related to clinical trial implementation will be retained by the principal investigator. Important scheme modifications during this project will be communicated to the institutional review board, trial registry, investigators, trial participants, and the journal of publication. Management and analysis of data will be carried out by an independent expert statistician.

STATISTICAL CONSIDERATIONS

Sample Size Estimation

It has been reported that the incidence of ST-segment elevation resolution >70% has been reported to be 56.6% in STEMI patients treated with thrombus aspiration (24). We hypothesize that ICART administration during PCI increases the incidence of ST-segment elevation resolution >70–75%. To detect this difference between ICART and thrombus aspiration or PTCA groups, 286 patients are required to reach a 5% significance level (two-sided) with 90% power, which allows 5% of cases to fall off.

Statistical Analysis

Statistical analysis will be carried out using the SPSS statistical software package (version 18.0; SPSS, Inc., Chicago, IL, United States), and the level of significant difference will be established at $\alpha = 0.05$. The data analysis will be performed by an independent professional statistician who is blinded to allocation. Analysis of efficacy will include all participants who complete the whole study.

Descriptive statistics will be used to compare the baseline characters between ICART and thrombus aspiration or PTCA group. If the normality test is satisfied, the independent *t*-test will be used; otherwise, the Mann-Whitney U test will be carried out. Regarding the primary and secondary endpoint measures, multiple logistic regression will be used to compare the differences. The mean and standard deviation values of these parameters will be reported.

DISCUSSION

The ICART project is a single-center, prospective, randomized controlled trial to make clear whether ICART procedure is more effective than thrombus aspiration or PTCA in improving myocardial perfusion and reducing reperfusion injury in STEMI patients undergoing primary PCI. This is the first RCT trial to clarify the effect of ICART vs. thrombus aspiration or PTCA on myocardial perfusion in STEMI patients undergoing PPCI.

TRIAL STATUS

The trial is currently in the recruitment phase. The current protocol version number is V1.0 from 14 June 2019. The start recruitment date is January 2022. The expected end date is December 2023.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding authors.

ETHICS STATEMENT

This study has been approved by the Ethics Committee Board of the Chinese PLA General Hospital, Beijing, China on 25 March 2014. Written, informed consent to participate will be obtained from all participants.

AUTHOR CONTRIBUTIONS

MS designed this project in collaboration with JT, SZ, JW, and DL. MS also drafted this manuscript. DL, XZ, YG, WZ, YG, JW, JL, and GZ revised the manuscript critically. DL was also responsible for the study of some cases in another center. All authors read and approved the final manuscript.

FUNDING

This study was supported by the Hainan Science and Technology project (ZDYF2020123, LCYX202106, ZDYF2017096, ZDYF2020027, and ZDKJ2019012), Hainan Province Clinical Medical Center, National Key R&D plan (2020YFC2004706), and National Natural Science Foundation of China (fund numbers 81500202). Open subject of National Clinical Research Center of Geriatrics Disease NCRCG-PLAGH-2018014 (MS). All funders have no influence in the study design, data collection and analysis, or publishment.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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TYPE Original Research
PUBLISHED 23 September 2022
DOI 10.3389/fcvm.2022.947130

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EDITED BY
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SPECIALTY SECTION
This article was submitted to Cardiovascular Therapeutics, a section of the journal Frontiers in Cardiovascular Medicine

RECEIVED 18 May 2022
ACCEPTED 12 August 2022
PUBLISHED 23 September 2022

CITATION
Zhong J, Zhang H, Li Z, Qian D, Zhang Y, Li C, Song Y, Qin Z, Yu J, Bian S-z, Yu Y, Wang K and Li J-W (2022) Effect of social app-assisted education and support on glucose control in patients with coronary heart disease and diabetes mellitus. *Front. Cardiovasc. Med.* 9:947130. doi: 10.3389/fcvm.2022.947130

Effect of social app-assisted education and support on glucose control in patients with coronary heart disease and diabetes mellitus

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Background: Social app-assisted education and support may facilitate diabetes self-management. We aim to evaluate the effect of WeChat, a popular social app, on glycemic control in patients with coronary heart disease (CHD) and diabetes mellitus (DM).

Methods: We conducted a parallel-group, open-label, randomized clinical trial that included 160 patients with both CHD and diabetes mellitus from a tertiary hospital in China. The intervention group ($n = 80$) received educational materials (information on glucose monitoring, drug usage, medication, and lifestyle) and reminders in response to individual blood glucose values *via* WeChat. The control group ($n = 80$) received usual care. The primary outcome was a change in glycated hemoglobin (HbA1C) levels over 3 months. Secondary outcomes included fasting blood glucose (FBG), systolic blood pressure, and low-density lipoprotein (LDL) cholesterol from baseline to 3 months. Analysis was conducted using a linear mixed model.

Results: The intervention group had a greater reduction in HbA1C (−0.85 vs. 0.15%, between-group difference: −1.00%; 95% *CI* −1.31 to −0.69%; $p < 0.001$) compared with the control group. Change in fasting blood glucose was larger in the intervention group (−1.53 mmol/L; 95% *CI* −1.90 to −1.17; $p < 0.001$) and systolic blood pressure (−9.06 mmHg; 95% *CI* −12.38 to −5.73; $p < 0.001$), but not LDL (between-group difference, −0.08 mmol/L; 95% *CI* −0.22 to 0.05; $p = 0.227$).

Conclusion: The combination of social app with education and support resulted in better glycemic control in patients with CHD and DM. These results suggest that education and support interaction *via* social app may benefit self-management in CHD and DM.

KEYWORDS

coronary heart disease, diabetes mellitus, social APP, WeChat, education and support intervention

Introduction

Diabetes mellitus (DM) currently affects more than 440 million individuals worldwide and 92.4 million adults in China, accounting for about 10.9% of Chinese adults (1). Coronary heart disease (CHD) affects ∼126 million individuals and leads to 9 million deaths worldwide (2), and is the cause of ∼1.7 million deaths, which is the second cause of death in China (3). The prevalence of CHD in diabetes is high and ranges from 12 to 31% among middle-aged patients with diabetes (4). It is also the major contributory cause of death, responsible for 30% of all deaths in DM (5). On the other hand, diabetes has been recognized as a risk factor for CHD, conferring up to a 4-times increasing risk of cardiovascular mortality (6). These two diseases often coexist and have a more aggressive course and worse prognosis. As China and the world enter an aging society, the burden of the two diseases increases aggressively.

The complications of cardiovascular disease and diabetes can be prevented or delayed by glycemic control. Diabetes self-management education and support (DSMES) have been proven to be efficacious and cost-effective to improve health outcomes (7). Previously, face-to-face DSMES have been shown to bring better outcomes compared with technology (telephone or online) ones (8). Recently, mobile health (mHealth) has been applied to this area and shown significant reductions in hemoglobin A1c (Hb1AC) compared with the telephone-based or usual care method (9, 10). The BlueStar mobile diabetes coach is a type 2 diabetes app that provides real-time automated educational and behavioral messages sent in response to patient-report, resulting in a mean 1.2% decline in glycated hemoglobin over 1 year (11). WeChat is the most popular communication app in China, with a penetration rate of 93% in developed cities since 2015. A meta-analysis showed that WeChat-assisted DSMES in diabetics lead to a 1.07 decline in HbA1C compared with that of controls, with reduced adverse reactions and improved satisfaction (12). However, patients with CHD and DM need to manage both diseases, which require following more lifestyle and treatment recommendations than each of the diseases alone, and evidence of social app-assisted DSMES beyond usual care in this population is still lacking. We here investigated the effects of a culturally tailored social app-assisted education and support to improve glycemic control and cardiovascular risks among patients with CHD and DM.

Methods

Study design

This study was a parallel-design, open-label, randomized clinical trial that evaluated a social app for assisted education and support of glucose control with a follow-up of 3 months. Individuals with both CHD and DM were recruited from the department of cardiology, Xinqiao hospitals, Chongqing, China. The trial was conducted in accordance with the Consolidated Standards Of Reporting Trials (CONSORT) checklists. All study participants have provided written informed consent. Ethical approval was obtained from the Ethics Committee of the Xinqiao Hospital Review Board (No. 2021-021-01).

Participants

Potential participants were identified through the screening of inpatients from 2021 to 2022 with a preliminary diagnosis of CHD and DM. The inclusion criteria were ≥18 years of age, had documented diagnoses of CHD and type 2 DM, and had access to a smart mobile phone with WeChat installed and could read and send text messages through Wechat. The exclusion criteria were cognitive or communication disorders that prevented them from finishing this study.

Randomization and intervention

Participants were randomized to either the intervention or control group in a 1:1 ratio using a random number table. Participants were aware of their treatment group. The homogenization nursing process was conducted during the hospitalization of all participants. First, patients were informed and signed the informed consent and then randomized to each group. Later blood glucose was regularly monitored 6 times per day, and treatment adjustments were made by their doctors if necessary. Moreover, relevant knowledge and health education on CHD and DM were given face-to-face before discharge. Participants in the intervention were invited to join a special WeChat account and group. They were also gotten simple training from research staff to ensure they were capable of receiving, reading, and sending relevant messages through WeChat on mobile phones.

After participants were discharged from hospital. Participants in the intervention group used the WeChat Group to receive both educational materials and reminders. This includes information about CHD and DM, glucose monitoring and control, blood pressure control, medication usage, and lifestyle recommendations. A team of cardiologists, endocrinologists, and nurses developed or shared the message. All the information was selected from relevant topic areas. Messages were drafted based on existing evidence and guidelines. The messages were sent at least 3 days per week. The control group received outpatient follow-up, as well as standard treatment. The blood glucose was recorded at 1 and 3 months in all participants. Participants were informed that they could withdraw from the study. Cardiologists and endocrinologists were also in the group chat and answered any questions that were raised. To ensure the confidentiality of all

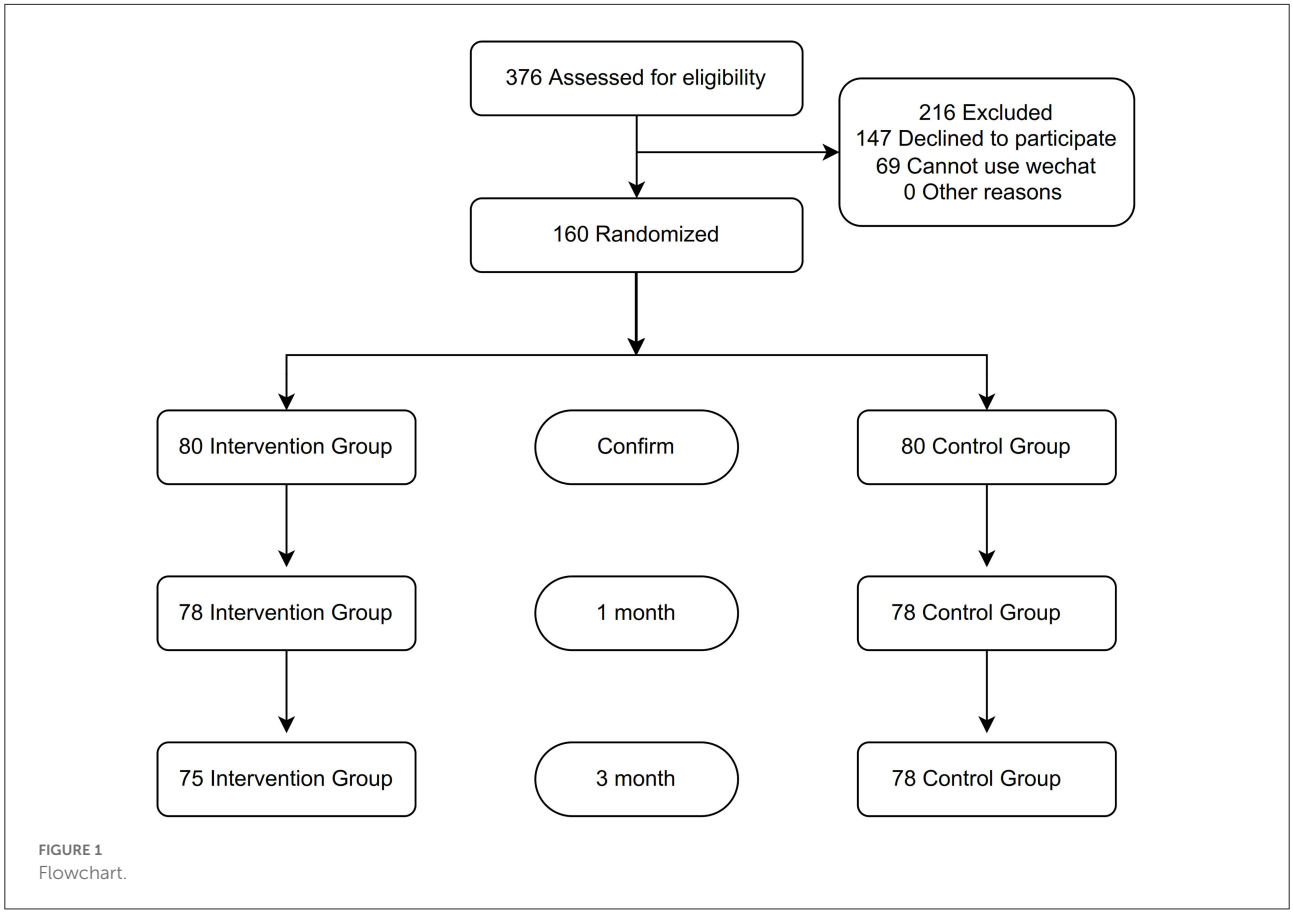

FIGURE 1
Flowchart.

personal information, the data confidentiality policies on data collection, storage, and analysis were strictly imposed.

Outcome measures and patient characteristics

The primary outcome was the change in glycated hemoglobin [HbA1C (hemoglobin A1C)] from baseline to 3 months. Secondary outcomes included plasma fasting blood glucose (FBG), low-density lipoprotein cholesterol (LDL-C), and systolic blood pressure (SBP) to 3 months. All blood biomarkers were measured at the Xinqiao laboratory.

Statistical analysis

Based on previous report, we estimated that a sample size of 150 would provide 80% power to detect a 1.0% absolute difference in HbA1C change at 3 months, compared with the control group, assuming a mean HbA1C level of 8.2% at baseline (SD, 1.6%) using PASS, version 11.0 (NCSS, Kaysville, UT), for sample size calculation.

Data were collected at baseline, 1-, and 3-months follow-up visit for all participants. Epidemiological and demographic data, insurance status, and in-hospital medications were collected through the electronic medical record system. Primary and secondary outcomes, like adverse cardiovascular events such as CV death, myocardial infarction, hospitalized heart failure, and revascularization, were collected at each visit.

All analyses were conducted according to the intention to treat principle. Categorical variables were described as frequencies (percentages) and continuous variables as means ± SDs unless skewed, as medians [interquartile ranges (IQRs)]. The change of continuous variable was determined using linear mixed models. For categorical secondary outcomes, a chi-square test was used. Additionally, we performed subgroup analyses of primary outcomes by age ($\leq$65 and $>$65 years), sex (male and female), insurance status (resident or other), hypertension status (yes and no), diabetes duration (yes and no), smoking status (current smoker or not), body mass index (BMI) ($<$25 and $\geq$25), and insulin usage (yes and no). Results are presented as mean differences with 95% *CI*s. All tests set significance with a two-tailed α of 0.05. Statistical analyses were performed using SAS, version 9.4 (SAS Institute, Cary, NC).

TABLE 1 Baseline participant characteristics.

	All ($n = 160$)	Intervention ($n = 80$)	Placebo ($n = 80$)	*P*
Age (years)	65.01 (10.05)	66.13 (10.24)	63.90 (9.80)	0.162
Female (%)	55 (34.38%)	27 (33.75%)	28 (35.00%)	0.868
High education (%)*	51 (31.88%)	25 (31.25%)	26 (32.50%)	0.865
Smoking (%)	62 (38.75%)	32 (40.00%)	30 (37.50%)	0.746
Resident insurance (%)	93 (58.13%)	44 (55.00%)	49 (61.25%)	0.423
Previous MI (%)	7 (4.38%)	4 (5.00%)	3 (3.75%)	0.699
Previous PCI (%)	30 (18.75%)	14 (17.50%)	16 (20.00%)	0.685
Hypertension (%)	108 (67.50%)	53 (66.25%)	55 (68.75%)	0.736
Renal disease (%)	25 (15.63%)	13 (16.25%)	12 (15.00%)	0.828
Diabetes duration (years)	8.56 (6.71)	8.09 (6.51)	9.03 (6.91)	0.379
Blood pressure (mm Hg)				
Systolic	129.29 (18.41)	130.13 (17.15)	128.46 (19.66)	0.570
Diastolic	75.32 (11.74)	76.48 (9.17)	74.16 (13.82)	0.214
Heart rate (bpm)	81.13 (14.40)	80.08 (15.67)	82.19 (13.02)	0.355
Body-mass index	24.13 (3.08)	24.00 (2.94)	24.26 (3.23)	0.599
LVEF (%)	58.38 (11.26)	59.27 (11.75)	57.57 (10.82)	0.377
Glycated hemoglobin (%)	8.13 (1.56)	8.25 (1.21)	8.01 (1.84)	0.327
Fasting blood glucose (mmol/l)	7.83 (2.39)	7.64 (1.93)	8.01 (2.77)	0.321
Creatinine (μmoI/L)	80.60 (65.55, 95.85)	80.45 (65.90, 99.20)	80.75 (64.75, 90.55)	0.540
LDL (mmol/L)	2.04 (0.77)	2.05 (0.65)	2.03 (0.87)	0.850
Triglycerides (mmol/L)	1.39 (0.54)	1.32 (0.43)	1.46 (0.63)	0.103
In-hospital medication (%)				
β-blocker (%)	124 (77.50%)	60 (75.00%)	64 (80.00%)	0.449
Oral hypoglycemic (%)	111 (69.38%)	52 (65.00%)	59 (73.75%)	0.230
Insulin (%)	69 (43.13%)	34 (42.50%)	35 (43.75%)	0.873
MI (%)	27 (16.88%)	11 (13.75%)	16 (20.00%)	0.291
Stent implantation (%)	63 (39.38%)	31 (38.75%)	32 (40.00%)	0.872

LDL, low-density lipoprotein; LVEF, left ventricular ejection fraction; MI, myocardial infarction; PCI, percutaneous coronary intervention.
*High education means junior high school or above.

Results

Between January 2020 and December 2021, 376 patients were screened for eligibility. After excluding 147 patients who declined to participate, and 69 who could not use WeChat, a total of 160 participants were included, randomly assigned to the intervention ($n = 80$) or control group ($n = 80$; Figure 1). After randomization, 6 (3.8%) patients were lost to follow-up in total and 1 patient (0.6%) died during the study period. The median duration of follow-up was 3 months. Participants had a mean age of 65.0 years, and 34.4% were women (Table 1). The mean HbA1C level was 8.1%, mean FBG was 7.8 mmol/L, mean blood pressure was 130/75 mm Hg, mean LDL-C was 2.0 mmol/L, and mean BMI was 24.1 kg/m^2. Baseline characteristics were similar between the intervention and control groups.

Primary outcome

The main effects of the intervention on outcomes are presented in Figure 2. A significantly greater reduction in HbA1C between baseline and 3 months was observed between the intervention group and the control group, with a mean absolute difference in the HbA1C level of −1.00% (−0.85 vs. 0.15%; 95% *CI* −1.31 to −0.69%; $p < 0.001$). A significant interaction between the intervention and the subgroup levels except baseline HbA1C level ≤8.1 vs. >8.1% years ($p < 0.001$) were observed, a marginal interaction was observed for (left ventricular ejection fraction) LVEF ($p = 0.027$), but not for other subgroup age ≤65 vs. >65 years ($p = 0.859$), men vs. women ($p = 0.362$), resident vs. other ($p = 0.372$), hypertension yes vs. no ($p = 0.072$), diabetes duration <10 vs. ≥10 years ($p = 0.685$), not smoking vs. current smoker ($p = 0.226$), BMI

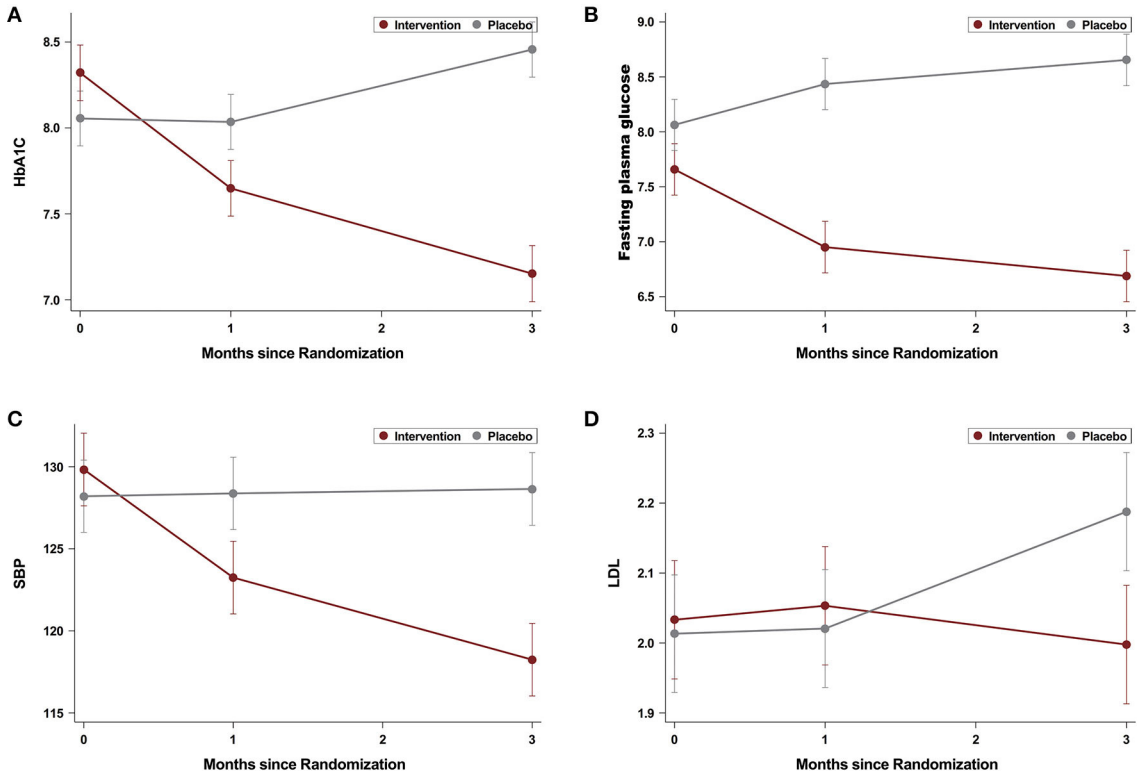

FIGURE 2
Changes in hemoglobin A1C (HbA1C) **(A)**, fasting blood glucose (FBG) **(B)**, systolic blood pressure (SBP) **(C)**, and low-density lipoprotein (LDL) **(D)** levels during 3 months of follow-up.

<25 vs. ≥25 ($p = 0.552$), and insulin usage vs. no insulin ($p = 0.666$), respectively (Table 2). The mean difference in change from baseline to 3 months between the intervention and control groups was −0.57% (95% *CI* −0.93 to −0.20%) for participants with baseline HbA1C level ≤8.1 and −1.68% (95% *CI* −2.18 to −1.17%) for participants with baseline HbA1C level >8.1%. The sensitivity analysis using the analysis of covariance (ANCOVA) method got similar results, with a mean absolute difference in the HbA1C level of −0.91% (−0.80 vs. 0.11%; 95% *CI* −1.22 to −0.60%; $p < 0.001$).

Secondary and other outcomes

Participants in the intervention group experienced a larger reduction in FBG from baseline to 3 months than the control group (−0.95 vs. 0.59 mmol/L, between-group difference, −1.53 mmol/L; 95% *CI* −1.90 to −1.17; $p < 0.001$), as well as SBP (−9.05 vs. 0.01 mmHg, between-group difference, −9.06 mmHg; 95% *CI* −12.38 to −5.73; $p < 0.001$) and DBP (−2.44 vs. 0.19, mean difference, −2.63 mmHg; 95% *CI* −4.85 to −0.42; $p = 0.020$). The LDL-C (−0.004 vs. 0.08 mmol/L, between-group difference, −0.08 mmol/L; 95% *CI* −0.22 to 0.05; $p = 0.227$) did not differ between the groups (Figure 2). A greater proportion of participants achieved HbA1C <7% (54.7% in the intervention group vs. 16.7% in the control group; $p < 0.001$). Hypoglycemia (defined as blood glucose ≤3.9 mmol/L), diabetic ketoacidosis, diabetic lactate acidosis, and hyperosmolar non-ketotic diabetic syndrome were not observed in all the participants. During a 3-months follow-up period, a marginal difference was observed in the adverse cardiovascular event (0 in the intervention group and 6 for the placebo group, $p = 0.028$, Table 3).

Discussion

Adequate glycemic control of diabetes is still lacking. The HbA1C target <7% is only achieved in about 30–50% of patients with diabetes (13, 14). The situation may be even worse in patients with both CHD and diabetes as they need to pay more attention to CHD as symptoms are more apparent rather than diabetes. From this analysis, we found that a social app-assisted education and support improved glycated hemoglobin by −1.0% over 3 months. The magnitude of improvement observed in this study is consistent with the average reduction 1.07% in a recent meta-analysis, which reported glucose level after self-management among patients with type 2 diabetes *via* the

10.3389/fcvm.2022.947130

TABLE 2 Subgroup analyses of the difference between the intervention and control groups in the mean change of hemoglobin A1C (HbA1C) from baseline to 3 months.

Subgroup	Difference (SE)	*P*	*P* interaction
Age (years)			
≤65	−0.96 (0.24)	<0.001	0.859
>65	−1.04 (0.19)	<0.001	
Sex			
Female	−1.34 (0.24)	<0.001	0.362
Male	−0.89 (0.20)	<0.001	
HbA_{1C} (%)			
≤8.1	−0.57 (0.18)	0.002	<0.001
>8.1	−1.68 (0.25)	<0.001	
Insurance			
Resident	−1.19 (0.17)	<0.001	0.372
Other	−0.94 (0.28)	0.001	
Stent implantation			
No	−1.07 (0.22)	<0.001	0.705
Yes	−0.89 (0.24)	<0.001	
MI			
No	−0.95 (0.17)	<0.001	0.594
Yes	−1.24 (0.42)	0.008	
Previous MI			
No	−1.00 (0.16)	<0.001	0.802
Yes	−1.56 (1.46)	0.364	
Previous revasculization			
No	−0.94 (0.18)	<0.001	0.600
Yes	−1.30 (0.21)	<0.001	
Hypertension			
No	−0.58 (0.33)	0.084	0.072
Yes	−1.24 (0.16)	<0.001	
Renal disease			
No	−1.04 (0.16)	<0.001	0.867
Yes	−1.09 (0.35)	0.005	
Diabetes duration (years)			
<10	−0.94 (0.24)	<0.001	0.685
≥10	−1.12 (0.21)	<0.001	
Smoking			
No	−0.86 (0.23)	<0.001	0.226
Yes	−1.27 (0.18)	<0.001	
Education			
Low	−1.19 (0.16)	<0.001	0.114
High	−0.63 (0.31)	0.052	
Heart rate (bpm)			
<85	−0.94 (0.21)	<0.001	0.798
≥85	−1.06 (0.23)	<0.001	
BMI			
<25	−0.96 (0.22)	<0.001	0.552
≥25	−1.29 (0.23)	<0.001	
LVEF (%)			
≤50	−1.18 (0.17)	<0.001	0.027
>50	−0.55 (0.48)	0.266	
Fasting blood glucose (mmol/l)			
≤7	−0.81 (0.19)	<0.001	0.352
>7	−1.09 (0.23)	<0.001	
Creatinine (μmoI/L)			
≤80	−1.10 (0.21)	<0.001	0.678
>80	−0.92 (0.23)	<0.001	
LDL (mmol/L)			
≤2.6	−0.94 (0.19)	<0.001	0.496
>2.6	−1.14 (0.26)	<0.001	
β-blocker			
No	−1.17 (0.22)	<0.001	0.694
Yes	−0.98 (0.19)	<0.001	
Oral hypoglycemic treatment			
No	−0.74 (0.36)	0.050	0.237
Yes	−1.15 (0.17)	<0.001	
Insulin			
No	−1.08 (0.15)	<0.001	0.666
Yes	−0.93 (0.28)	0.001	

BMI, body mass index; LDL, low density lipoprotein; LVEF, left ventricular ejection fraction; MI, myocardial infarction; PCI, percutaneous coronary intervention.

TABLE 3 Clinical outcome at 3 months after initial treatment.

	Intervention	Placebo	*p* value
Total event	0	6	0.0284
CV death	0	1	
Angina	0	2	
MI	0	1	
Hospitalized HF	0	1	
Revascularization	0	1	

CV, cardiovascular; HF, heart failure; MI, myocardial infarction.

WeChat application (12). Our result showed a larger reduction in population with poorly controlled glycated hemoglobin (>8.1%) than in people with less severe ones (≤8.1%). Fasting plasma glucose and blood pressure were also improved, but not LDL-C, and more adverse CV events were observed in the control group than in the treatment group.

Smart mobile phones are popular, according to Deloitte Global mobile consumer trends, more than 80% of the population in the world has a smartphone, and in China, this is higher (about 86%) (15). WeChat is the only instant messaging app in China to have over one billion active users, having

similar functions to Whatsapp and Facebook messenger. In our study, social app-assisted education and support could improve blood glucose control more than the control group. Although social app itself could not benefit blood glucose control, it helps patients and medical staff to establish a connection out of hospital. The improvement could be explained with the following reasons. First patients could receive relevant education and support *via* not only texts and voices, but also pictures and videos. The addition facilitates patients' understanding of these materials (16). Second, the group chat function support one medical staff share material and corresponds to multiple patients, and patients can discuss with each other. Third, this method could let patients communicate with their doctors in a real-time manner. This prevents patients from making the wrong choice when instant support is needed. Fourth, this is a simple, low-cost approach to augment existing public health services compared with other methods, such as adding wearable devices. Therefore, the combination of usual care and social app-assisted education and support would be more conducive to patient self-management.

The social app-assisted education and support also has some disadvantages. First, patients should have a smart phone and connected to the network, otherwise the information could not be obtained. Second, this would add extra work to already busy medical staff, limiting its use.

The between-group difference is a combination of the decrease in HbA1C in the intervention group and mild increase in the control group. Similar trend are shown for fasting plasma glucose and blood pressure but not LDL. The absence of LDL may be due to patients who could not measure their cholesterol frequently by themselves at home, and therefore do not pay enough attention.

The results showed that social app-assisted education and support could improve glycemic control in diabetic patients with CHD, similar to those without CHD. Moreover, expansion of such education and support to CHD but not only diabetes could lead to improved blood pressure control and the potential to reduce adverse cardiovascular events. In fact, smartphone and social media-based cardiac rehabilitation and secondary prevention in CHD could improve 6-min walk distance (17), help quitting drinking and smoking (18), improve medication adherence, and blood pressure control (19). Thus social app-assisted education and support could benefit two diseases at a single shot in such situation.

Our study has several limitations. First, although our intervention provided lifestyle guidance regarding food, exercises, emotions, and other risk management behaviors, there was no specific measurement indicator about the effectiveness on lifestyle changes, such as resting energy expenditure, physical activity levels, and dietary intake. Additional research is needed to answer such questions. Second, our primary outcomes are not hard endpoint, such as recurrent myocardial infarction or CV death. Third, we only follow participants for a time of 3 months, whether longer intervention could translate into larger benefits is still unknown. Fourth, self-reported measures are subjected to recall biases. Last, our sample size was relatively small and we only included Chinese participants. Future large samples and studies from other countries are needed to confirm the reliability of the results.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by Ethical approval was obtained from Ethics Committee of Xinqiao Hospital Review Board (No. 2021-021-01). The patients/participants provided their written informed consent to participate in this study.

Author contributions

JZ and J-WL conceived the concept of the study and drafted the manuscript. DHQ and J-WL performed the statistical analyses and drafted the manuscript. HMZ and YZL collected baseline and follow-up data. All authors contributed to the article and approved the submitted version.

Funding

This study was supported by the Chongqing overseas students returning home entrepreneurship and innovation support plan (41422145).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

All authors were employed by PATH.

Publisher's note

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frontiers | Frontiers in Cardiovascular Medicine

TYPE Original Research
PUBLISHED 05 October 2022
DOI 10.3389/fcvm.2022.999254

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Cardiovascular Therapeutics, a section of the journal Frontiers in Cardiovascular Medicine

RECEIVED 20 July 2022
ACCEPTED 05 September 2022
PUBLISHED 05 October 2022

CITATION
Xi Y, Chen D, Dong Z, Zhang J, Lam H, He J, Du K, Chen C, Guo J and Xiao J (2022) Multi-omics insights into potential mechanism of SGLT2 inhibitors cardiovascular benefit in diabetic cardiomyopathy. *Front. Cardiovasc. Med.* 9:999254. doi: 10.3389/fcvm.2022.999254

Multi-omics insights into potential mechanism of SGLT2 inhibitors cardiovascular benefit in diabetic cardiomyopathy

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Background: Metabolic and energy disorders are considered central to the etiology of diabetic cardiomyopathy (DCM). Sodium-glucose cotransporter-2 inhibitors (SGLT2i) can effectively reduce the risk of cardiovascular death and heart failure in patients with DCM. However, the underlying mechanism has not been elucidated.

Methods: We established a DCM rat model followed by treatment with empagliflozin (EMPA) for 12 weeks. Echocardiography, blood tests, histopathology, and transmission electron microscopy (TEM) were used to evaluate the phenotypic characteristics of the rats. The proteomics and metabolomics of the myocardium in the rat model were performed to identify the potential targets and signaling pathways associated with the cardiovascular benefit of SGLT2i.

Results: The diabetic rat showed pronounced DCM characterized by mitochondrial pleomorphic, impaired lipid metabolism, myocardial fibrosis, and associated diastolic and systolic functional impairments in the heart. To some extent, these changes were ameliorated after treatment with EMPA. A total of 43 proteins and 34 metabolites were identified as targets in the myocardium of diabetic rats treated with EMPA. The KEGG analysis showed that arachidonic acid is associated with the maximum number of related pathways and may be a potential target of EMPA treatment. Fatty acid (FA) metabolism was enhanced in diabetic hearts, and the perturbation of biosynthesis of unsaturated FAs and arachidonic acid metabolism was a potential enabler for the cardiovascular benefit of EMPA.

Conclusion: SGLT2i ameliorated lipid accumulation and mitochondrial damage in the myocardium of diabetic rats. The metabolomic and proteomic data revealed the potential targets and signaling pathways associated with the cardiovascular benefit of SGLT2i, which provides a valuable resource for the mechanism of SGLT2i.

KEYWORDS

diabetic cardiomyopathy, SGLT2 inhibitors, mechanisms, proteomics, metabolomics

Introduction

Diabetes is the most important comorbidity of cardiovascular disease (CVD), and CVD is the main cause of death and disability in patients with diabetes worldwide (1–4). At present, the global prevalence of diabetes is steadily increasing. An estimated 578 million people will be diagnosed with diabetes by 2,030, and the total number of patients will reach 700 million by 2,045 (2). Many studies have demonstrated that diabetes can affect cardiac structure and function independent of coronary artery disease, ischemia, hypertension, and other risk factors, which are recognized as a distinct clinical entity called diabetic cardiomyopathy (DCM) (5, 6). DCM is a common cardiovascular complication in diabetes patients characterized by myocardial fibrosis, ventricular remodeling, and cardiac dysfunction (7, 8). It is closely linked to the high incidence and mortality of heart failure in patients with diabetes (9, 10). Patients with diabetes and even prediabetes [defined as impaired fasting glucose (IFG), impaired glucose tolerance (IGT), or elevated HbA1c] were associated with an increased risk of HF, and DCM plays an important role in this progress (11). Although DCM was identified more than 40 years ago, and many pathogenic mechanisms have been discovered through extensive research, effective strategies for DCM prevention and treatment remain elusive. Sodium-glucose cotransporter-2 inhibitors (SGLT2i) are the first anti-diabetic medications to effectively reduce the risk of cardiovascular death and heart failure in patients with type 2 diabetes. Notably, increasing evidence has demonstrated the beneficial cardiovascular effects of SGLT2i, independent of its glycemic control effects (12, 13).

However, since the main mechanistic contributor to the cardiovascular benefit of SGLT2i has not been elucidated, further studies are required to explore the mechanisms underlying the clinical benefits of SGLT2 inhibitors. Recent research has shown that SGLT2i improves cardiac metabolism by shifting myocardial substrate utilization from glucose toward oxidation of FAs, ketone bodies, and branched-chain amino acids, thereby improving the energy-starved heart in diabetes and heart failure (14, 15). In diabetic hearts, insulin resistance and HG induce metabolic alterations that decrease glucose utilization while increasing FA uptake and β-oxidation (16). The mismatch between uptake and β-oxidation of FAs results in intracellular lipid accumulation and lipotoxicity that initiates a cascade of downstream pathophysiological changes and myocardial mechanics (17). In terms of the DCM mechanism, mitochondrial dysfunction, impairment of mitochondrial Ca^{2+} handling, oxidative stress, increased production of advanced glycation end products (AGEs), inflammation, activation of the renin-angiotensin-aldosterone system (RAAS), autonomic neuropathy, endoplasmic reticulum stress, microvascular dysfunction, cardiomyocyte death, and cardiac metabolic disorders participate in the pathophysiological process (7, 17–19). The interactions between these abnormal pathophysiological processes result in cardiac stiffness, fibrosis, and hypertrophy, leading to diastolic and systolic dysfunction and heart failure.

Although the mechanism of DCM appears to be multifactorial, the pathophysiological changes seem to be induced by cardiac metabolic alterations, which are distinct from other cardiomyopathies (17, 20). While metabolic and energy disorders are considered central to the etiology of DCM and the progression to failure, the metabolic changes associated with functional and eventually cardiac failure remain unclear, especially after being treated with SGLT2i. In the present study, we present an integrative multi-omics analysis, including proteomics and metabolomics, to systematically analyze the metabolic changes in the diabetic heart, through which we could evaluate the progression of metabolic disturbances in the diabetic heart and the potential mechanisms underlying the cardiovascular clinical benefits of SGLT2i.

Materials and methods

Animal models

A total of 36 7-week-old male rats were provided by the SPF Biotechnology company (SPF, Beijing, China). The rats were housed in the hygienic animal facility at 20–25°C temperature and 40–70% relative humidity with a 12/12 h light-dark cycle. All animal experiments were conducted per the procedures approved by the Medical Ethics Committee of the Dongguan Affiliated Hospital of Jinan University.

To induce diabetes, the rats were intraperitoneal (i.p.) injected with a single dose of streptozocin (STZ, Macklin, Shanghai, China, S817944, 70 mg/kg, diluted to 1% using fresh sodium citrate buffer, pH = 4.5) for 5 consecutive days. The rats in the control group (n = 6) were i.p. injected with equivalent doses of STZ solvent. Once a week, tail-vein blood was collected from the rats to test their fasting blood glucose levels. All animals were fed a normal diet for 18 weeks, and those with blood glucose levels of > 16.7 mmol/L for three consecutive weeks were presumed to be diabetic. The diabetic rats were randomly divided into the hyperglycemia (HG) group (n = 10) and the empagliflozin (EMPA) group (n = 8), matched for body weights and uniformity. The rats in the EMPA group were treated with empagliflozin (EMPA; Boehringer Ingelheim) for 12 weeks. EMPA was mixed in drinking water at an average dose of 30 mg/kg/day.

Echocardiography

Animals were intraperitoneally anesthetized with phenobarbital (2%, 50 mg/kg, i.p., H20057384, fujian mindongrejuenation, China). Two-dimensional (2D) guided M-mode echocardiography was performed at 18 and 30 weeks using an L15-7io probe (Ultrasound Transducer Bothell, WA 98021, USA). Left ventricle internal dimension at end-diastole/systole (LVID;d/s), left ventricle posterior wall thickness at end-diastole (LVPW'd), and interventricular septum thickness at end-diastole (IVS'd) were measured by M-mode tracing. The left-ventricular fractional shortening (FS) was calculated as [(LVIDD-LVIDS)/LVIDD] *100%, the ejection fraction (EF) percentage using the equation: [(EDV-ESV)/EDV] *100%, where EDV represents end-diastolic volume and ESV represents end-systolic volume.

Blood measurements and histopathology

Rats were anesthetized with an intraperitoneal injection of phenobarbital sodium (60–80 mg/kg, i.p.). Blood samples were collected from all the rats. Plasma samples were used for the measurement of the following parameters: blood insulin (CUSABIO, Wuhan, China, E05070r), glucagon (CUSABIO, CSB-E12800r), total cholesterol (TC, Nanjing Jiancheng, China, A111-2-1), triglyceride (TG, Nanjing Jiancheng, A110-1-1), high-density lipoprotein cholesterol (HDL-c, A112-1-1), low-density lipoprotein cholesterol (LDL-c, A113-1-1), brain natriuretic peptide (BNP, CUSABIO, CSB-E07972r), cardiac troponin I (cTn I, CUSABIO, CSB-E08594r), and creatinine (Cr, Nanjing Jiancheng, C011-2-1).

After euthanasia, the heart was harvested and processed for subsequent tissue and molecular analyses. Mid-ventricular heart sections were fixed in 4% paraformaldehyde, embedded in paraffin, sectioned at 4 μm thickness, and stained with hematoxylin and eosin (H&E, BBC biochemical) and Masson (Abcam, UK, ab150681) according to the manufacturer's protocol. The fibrotic content (three rats per group, 10 fields each) was quantified using ImagePro PLUS software (Media Cybernetics, USA).

Transmission electron microscopy

The mitochondrial structure was further examined by standard transmission electron microscopy (TEM) in the laboratory of Guangzhou Huiyuanyuan Pharmaceutical Technology Co., LTD. Fresh myocardium sample preparation for TEM was performed as previously described (21).

Proteomics

The heart sample was ground in liquid nitrogen, lysed with PASP buffer (100 mM NH_4HCO_3, 8 M Urea, pH 8), and crushed by ultrasonication on ice for 5 min. The raw data were obtained using the following steps: total protein extraction from the sample, protein quality test, trypsin treatment (22), DDA spectrum library construction, and liquid chromatography-mass spectrometry (LC-MS)/MS analysis-DIA mode (23). The raw file obtained from the DDA scanning was decomposed by Proteome Discoverer 2.2 (PD 2.2, Thermo Fisher Scientific), which converted the spectrum data into protein data. After quality control, the protein data was imported into Spectronaut (Version 14.0, Biognosys) to construct the DDA library. Moreover, the raw files obtained by DIA scanning were compared to the DDA library for protein identification by Spectronaut. The protein quantitation results were statistically analyzed using the t-test. Proteins with significantly different quantities between the experimental and control groups ($p < 0.05$ and $|\log2FC| > 1.5$) (fold change, FC) were defined as differentially expressed proteins.

Metabolomics

LC-MS technology based on the ACQUITY UPLC Xevo TQ-S platform was used to perform N300 metabolomics. The experimental process mainly included sample collection, target metabolite extraction, LC-MS/MS on-board, and data analysis (24, 25). The MS detection process relies on on-machine detection of blank samples (blank), quality control samples (QC), and experimental samples. To obtain absolute

quantitative results of metabolites in the samples, we performed chromatographic data analysis using MassLynx V4.1 software. Next, multivariate statistical analysis of metabolites, including principal component analysis (PCA), partial least squares discriminant analysis (PLS-DA), etc., revealed the differences in metabolic patterns of different groups. Hierarchical clustering (HCA) and metabolite correlation analysis were used to reveal relationships between sample metabolites. Finally, the biological significance, such as metabolic pathways of the metabolites, was explained by functional analysis. The proteomics and metabolomics were carried out at the laboratory of Beijing Novogene Co., Ltd. The schematic depiction of this study is shown in **Figure 1A**.

Statistical analysis

Data were expressed as mean ± SD. One-way ANOVA determined differences between groups with the SPSS 13.0 software and GraphPad Prism 6.0. For all the tests, a two-sided p-value of < 0.05 was considered significant.

Results

Phenotypic characteristics of the animals

Compared to the rats in the control group, STZ intraperitoneal injection led to marked HG (blood glucose levels > 16.7 mmol/L) and weight loss in rats. These significant weight losses and HG were observed at 1 week and persisted for up to 30 weeks (**Figures 1B,C**). During the follow-up, there was no significant weight gain in rats fed with EMPA. At 30 weeks (EMPA fed for 12 weeks), the blood glucose of rats was reduced ($p < 0.05$) but remained at a high level (> 30.0 mmol/L). The plasma parameters of TG, TC, and HDL-c were increased in the EMPA group compared with the control group ($p < 0.05$), but no significant changes were detected in LDL-c, BUN, Cr, BNP, insulin, and glucagon among the groups at 30 weeks (**Table 1**).

TEM imaging of myocardium in the HG group at 30 weeks revealed pleomorphic mitochondria, lipid deposition, and fewer cardiomyocytes, indicating mitochondrial damage and impaired lipid metabolism in cardiomyocytes (**Figure 1D**). Masson's staining showed significantly increased myocardial fibrosis in the HG group (**Figure 1E**). Notably, lipid deposition, mitochondrial changes, and myocardial fibrosis were ameliorated in the EMPA group. In addition, echocardiography showed abnormalities in cardiac structure and movement in the STZ-treated rats, which manifested as increased left ventricle internal dimension at end-diastole/systole (LVID; d/s), left ventricle posterior wall thickness at end-diastole (LVPW; d), and decreased EF (**Figure 2**).

Overview of proteome differences

We performed protein expression profiling of rat hearts from the three groups at 30 weeks (**Figure 3**). A total of 4,452 proteins were identified (**Supplementary Table 1**). Of these, 492 proteins exhibited differential expression between at least two groups ($p < 0.05$, fold change > 1.5 or < 0.67). The C-means cluster analysis revealed that treatment with EMPA could reverse some dysregulated proteins caused by STZ-induced diabetes in the rat's myocardium (**Figure 3B**). The HCA analysis (**Figure 3C**) revealed that the regulatory profiles of proteins differed significantly between the HG and EMPA groups (upregulated for 185 proteins, downregulated for 162 proteins). GO enrichment analysis showed that the upregulated proteins of the EMPA group (compared with the HG group) were mostly associated with the regulation of the glucocorticoid signaling pathway (GO: 0042921, GO: 43402, GO: 0004883) and lipid metabolic processes such as lipid transport (GO: 0006869), lipoprotein metabolic process (GO: 0042157), and lipid binding (GO: 0008289), while the downregulated proteins were associated with MF regulator (GO: 0098772), enzyme regulator activity (GO: 0030234), and peptide cross-linking (GO: 0018149) (**Figure 3D**). In the category of cellular component (CC), the upregulated and downregulated proteins were enriched in mitochondrial respiratory chain complex I (GO: 0005747) and protein phosphatase type 2A complex (GO: 0000159), respectively. Solute carrier family 25 member 34 (SLC25A34), serum amyloid P-component (APCS), and probable E3 ubiquitin-protein ligase HERC4 were the three most significantly upregulated proteins in the EMPA group compared with the HG group, which were associated with fatty acid (FA) metabolism, mitochondrial function, and apoptosis. Its upregulation may explain why EMPA could alter lipid deposition, mitochondrial changes, and myocardial fibrosis in the diabetic heart.

Among the differential proteins of the control group and the HG group, GO enrichment showed that most of the upregulated proteins were associated with FA and lipid metabolic processes such as monocarboxylic acid metabolic process (GO: 0032787), cellular lipid metabolic process (GO:0044255), lipoprotein metabolic process (GO: 0042157), FA beta-oxidation (GO: 0006635), and lipid transport (GO: 0006869), while the downregulated proteins were associated with oxidation-reduction process (GO: 0055114), GDP-mannose biosynthetic process (GO: 0009298), viral genome replication (GO: 0019079), negative regulation of cysteine-type endopeptidase activity involved in the apoptotic process (GO: 0043154), and regulation of hydrolase activity (GO: 0051336) at the biological process (BP) level. In the category of CC, the upregulated proteins were enriched in the extracellular region (GO: 0005576), an integral component of the membrane (GO: 0016021), while the downregulated proteins were enriched in the extracellular region (GO: 0005615, GO: 0005576, GO: 0044421). For

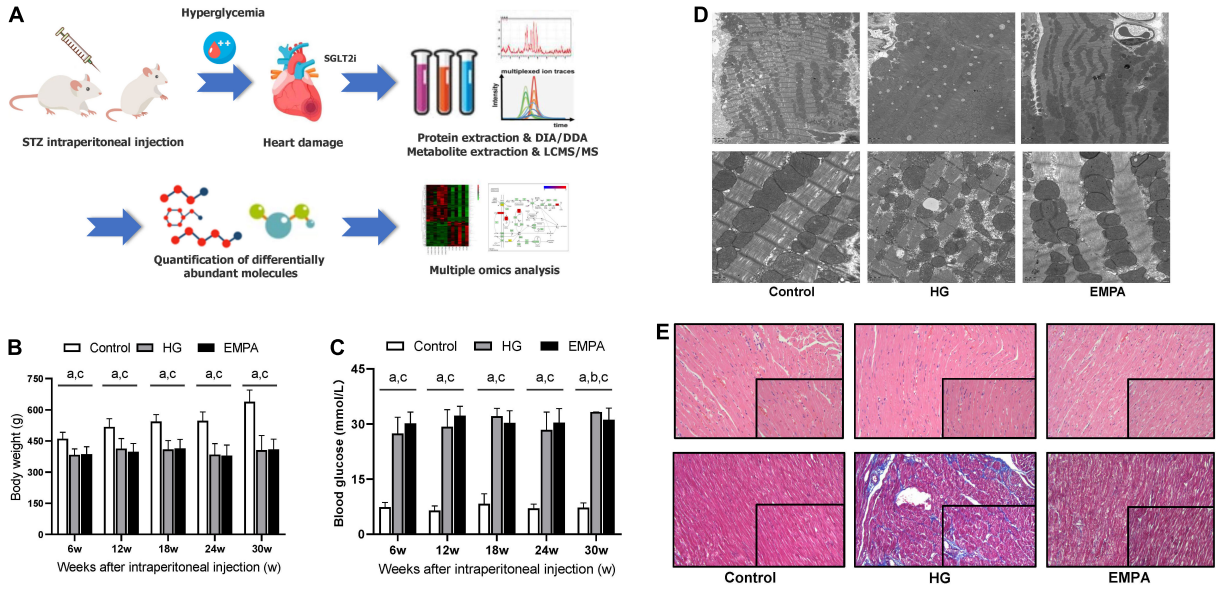

FIGURE 1
The study design and phenotypic characteristics of the rats after STZ intraperitoneal injection. **(A)** Schematic of the study design. **(B,C)** The body weight **(B)** and blood glucose **(C)** of rats in the control, high glucose (HG), and empagliflozin (EMPA) group. (a) comparison is significant for Control vs. HG, (b) comparisons are significant for HG vs. EMPA, and (c) is significant for Control vs. EMPA. **(D)** Transmission electron microscopy was observed on the rat heart of the three groups. 5,000x (upper part); 20,000x (lower part). **(E)** Representative photomicrograph of H and E staining (upper part) and Masson's staining (lower part) of the myocardium, 200x (insert 400x).

TABLE 1 Plasma parameters of animals.

	Control ($n = 6$)	HG ($n = 10$)	EMPA ($n = 8$)	P-value (ANOVA)
TG, mmol/L	1.42 ± 0.49^{a}	2.15 ± 0.85	2.58 ± 0.96	0.052
TC, mmol/L	1.57 ± 0.36^{a}	1.69 ± 0.55^{b}	2.33 ± 0.73	0.046
HDL-c, mmol/L	0.30 ± 0.12^{ac}	0.53 ± 0.24	0.58 ± 0.18	0.040
LDL-c, mmol/L	1.08 ± 0.53	0.87 ± 0.62	1.07 ± 0.58	0.707
BUN, pg/ml	0.46 ± 0.28	1.32 ± 0.83	1.69 ± 1.63	0.135
Cr, μmol/L	44.87 ± 10.18	42.62 ± 5.91	43.67 ± 10.53	0.882
BNP, ng/ml	<0.125*	0.31 ± 0.72	0.81 ± 0.97	0.166
Insulin, μUI/ml	0.65 ± 0.19	0.63 ± 0.24	0.58 ± 0.25	0.844
Glucagon, pg/ml	39.92 ± 11.81	36.16 ± 23.96	31.09 ± 12.97	0.667

HG, hyperglycemia; EMPA, empagliflozin; TG, triglyceride; TC, total cholesterol; HDL-c, high-density lipoprotein cholesterol; LDL-c, low-density lipoprotein cholesterol; BUN, blood urea nitrogen; Cr, creatinine; BNP, brain natriuretic peptide. With LSD multiple comparison tests, (a) comparison significant for control vs. EMPA, (b) comparisons significant for HG vs. EMPA, and (c) comparisons significant for control vs. HG. *Below the detection limit of the kit. Data were presented as mean ± standard deviation.

molecular function (MF), the upregulated proteins were related to ribose phosphate diphosphokinase activity (GO: 0004749), acyl-CoA dehydrogenase activity (GO: 0003995), magnesium ion binding (GO: 0000287), and oxidoreductase activity (GO: 0016616) (Supplementary Table 2).

Overview of metabolome differences

The results of the N300 metabolome differences are shown in Figure 4 and Supplementary Table 3. The N300 metabolome is a targeted metabolomic technology for high-throughput absolute quantification of small-molecule metabolites, which can perform absolute quantification of 300 + metabolites in myocardium samples. These metabolites cover multiple metabolic pathways, including the tricarboxylic acid (TCA) cycle, glycolysis/gluconeogenesis, amino acid metabolism, FA synthesis, and bile acid biosynthesis. The detailed detection list of N300 is shown in Supplementary Table 4. A total of 188 metabolites were identified. Of these, 49 metabolites (32 upregulated, 17 downregulated) exhibited differential expression between the control and HG groups, and 22 metabolons (two upregulated and 20 downregulated) exhibited differential expression between the HG and EMPA groups

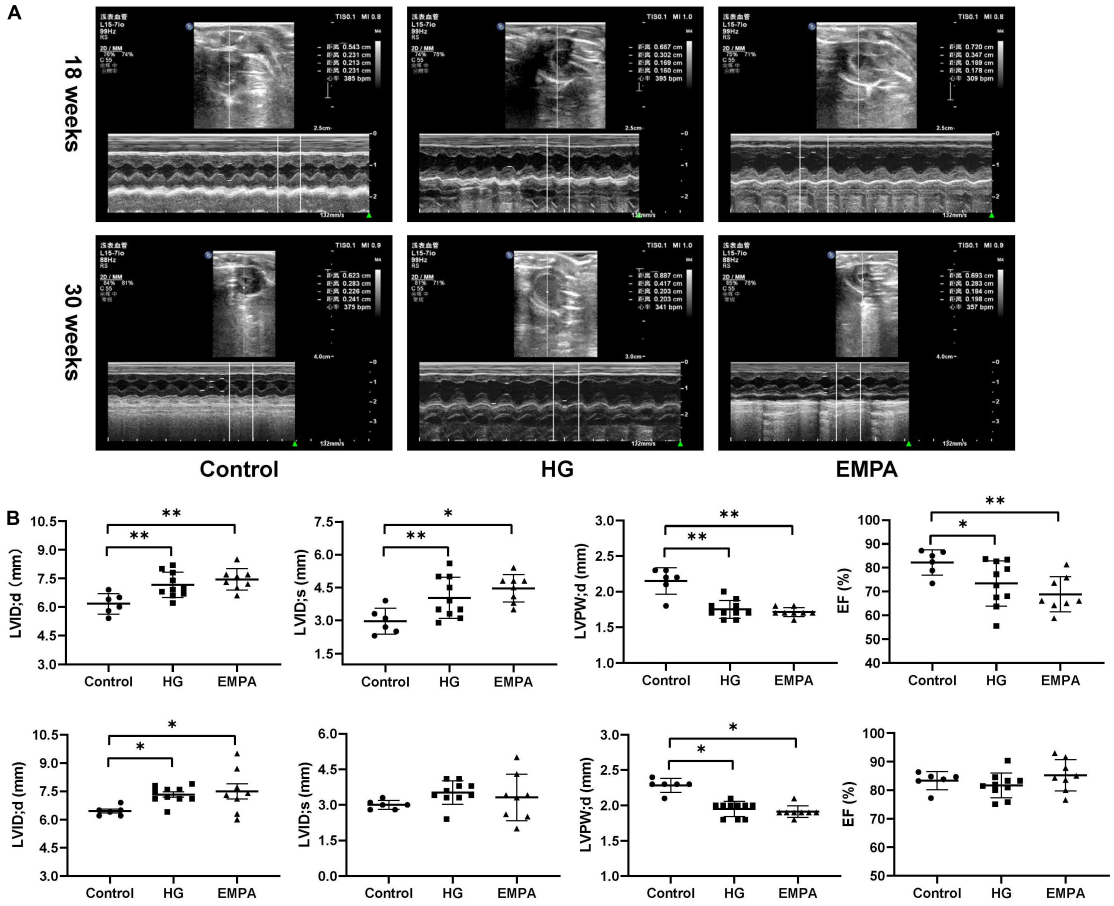

FIGURE 2
In three groups, echocardiographic measurements of the rats at 18 weeks and 30 weeks after STZ injection. **(A)** Representative M-mode tracings of rats in three groups (n = 6 for the control group, n = 10 for the high glucose (HG) group, and n = 8 for the empagliflozin (EMPA) group). **(B)** Plots of echocardiographic measurement results for left ventricle internal dimension at end-diastole/systole (LVID;d/s), left ventricle posterior wall thickness at end-diastole (LVPW'd), and ejection fraction (EF) percentage, upper part for 18 weeks, the lower part for 30 weeks. *$p < 0.05$, **$p < 0.01$.

($p < 0.05$, fold change > 1.2 or < 0.833) (Supplementary Table 3). The majority of differential metabolites exhibited a high correlation with other metabolites (Figure 4D), indicating that the screened-out metabolites may cooperate. The volcano plots and hierarchical clusters visually display the overall distribution of differential metabolites (Figures 4A–C).

The upstream TCA cycle metabolite citric acid was significantly increased, while the downstream metabolites isocitrate, oxoglutaric acid, and succinic acid showed no significant change, and the malic acid content was significantly decreased. This may reflect a relative reduction in TCA turnover, leading to the accumulation of metabolic intermediates and insufficient ATP production in the diabetic heart. Alpha-linolenic acid, gamma-linolenic acid, linoleic acid, and dihomogamma-linolenic acid were significantly increased in the rat's heart in the HG group, which was associated with biosynthesis of unsaturated FAs and linoleic acid metabolism. Beta-alanine, GABA, and anserine related to beta-alanine metabolism were downregulated in the HG group. In summary, the metabolome data of the control and HG groups suggested that FA metabolism was enhanced in the diabetic heart. Figure 4E shows the ROC curves of metabolites associated with EMPA treatment. Arachidonic acid, malic acid, GLCA, anserine, and EPA were the top five metabolites with the largest area under the curve (AUC), which may be important target metabolites for SGLT2i to exert cardio-protective effects.

Combined analysis of proteome and metabolome differences

To further investigate the mechanism of SGLT2 inhibitors in treating DCM, KEGG pathway enrichment of proteome and metabolome differences was performed in the EMPA and HG groups (Figures 5A,B). The results revealed that the Fc epsilon RI signaling pathway (map04664), Fc gamma

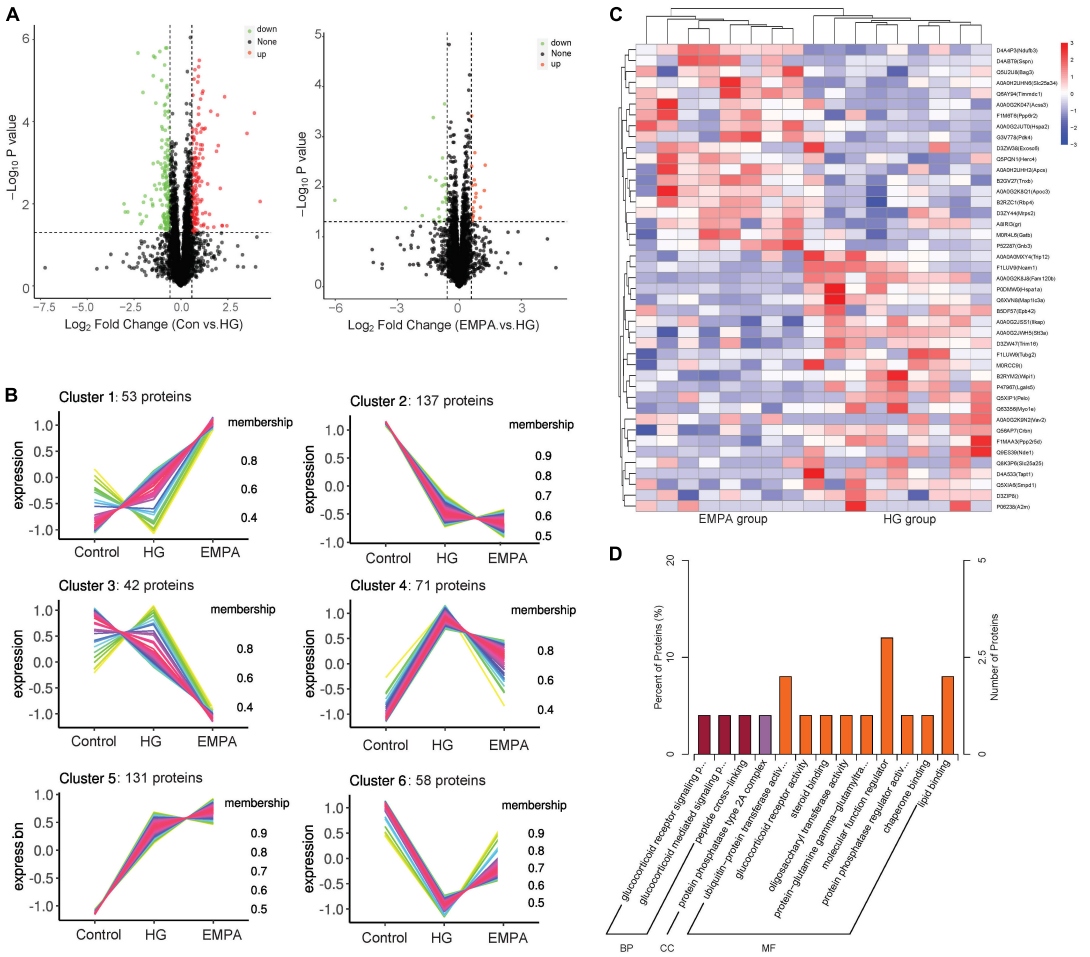

FIGURE 3
The protein expression changes in the myocardium. **(A)** Volcano plots of protein expression changes. Cut-off value: $p < 0.05$, fold change (FC) ≥ 1.5 (upregulation) or ≤ 0.67 (downregulation), the red and green point represents the upregulated and downregulated proteins, respectively. **(B)** C-means cluster diagram of differential proteins. The proteins are classified into 6 clusters according to their expression levels in each group, and the horizontal coordinate is the group, the vertical coordinate is the protein expression level (correction Z-value), each line represents one protein, larger membership values indicate that the protein is closer to the average level protein of this cluster. **(C)** Hierarchical clustering analysis of differential in empagliflozin (EMPA) and high glucose (HG) group. **(D)** GO enrichment of differential proteins in EMPA and HG groups. BP, biological process; CC, cellular component; and MF, molecular function.

R-mediated phagocytosis (map04666), ferroptosis (map04216), serotonergic synapse (map04726), necroptosis (map04217), and retrograde endocannabinoid signaling (map04723) were common enrichment pathways for differential metabolites and proteins (Figure 5D). The upregulated proteins Ndufb3 and Gnb3, the downregulated proteins Smpd1, Vav2, and Map1lc3a, and the downregulated metabolite arachidonic acid were repeatedly enriched in multiple related items. They could be associated with the pathogenesis of EMPA treatment (Figure 5D).

As shown in Figure 5C, Spearman's correlation analysis was conducted on the 20 most differentially expressed proteins and metabolites to identify the protein-metabolites relationship after EMPA treatment. Strong correlations ($p < 0.05$) were observed in 82 protein-metabolite pairs (Figure 5C). Various metabolites, such as arachidonic acid, were significantly decreased after EMPA treatment and showed a strong positive correlation with Trip12 and Fam120b and a strong negative correlation with Hspa2 and Acss3. The increased metabolites anserine and carnosine after EMPA treatment were negatively correlated with Ilkap, Stt3a, and Trim16 (Supplementary Table 5).

Discussion

As novel hypoglycemics, SGLT2 inhibitors (SGLT2i) have recently received tremendous attention. The cardiovascular benefits of SGLT2i may be multifactorial and beyond glycemic control (10). Significant reductions in major adverse cardiovascular events (MACEs) have been observed in various

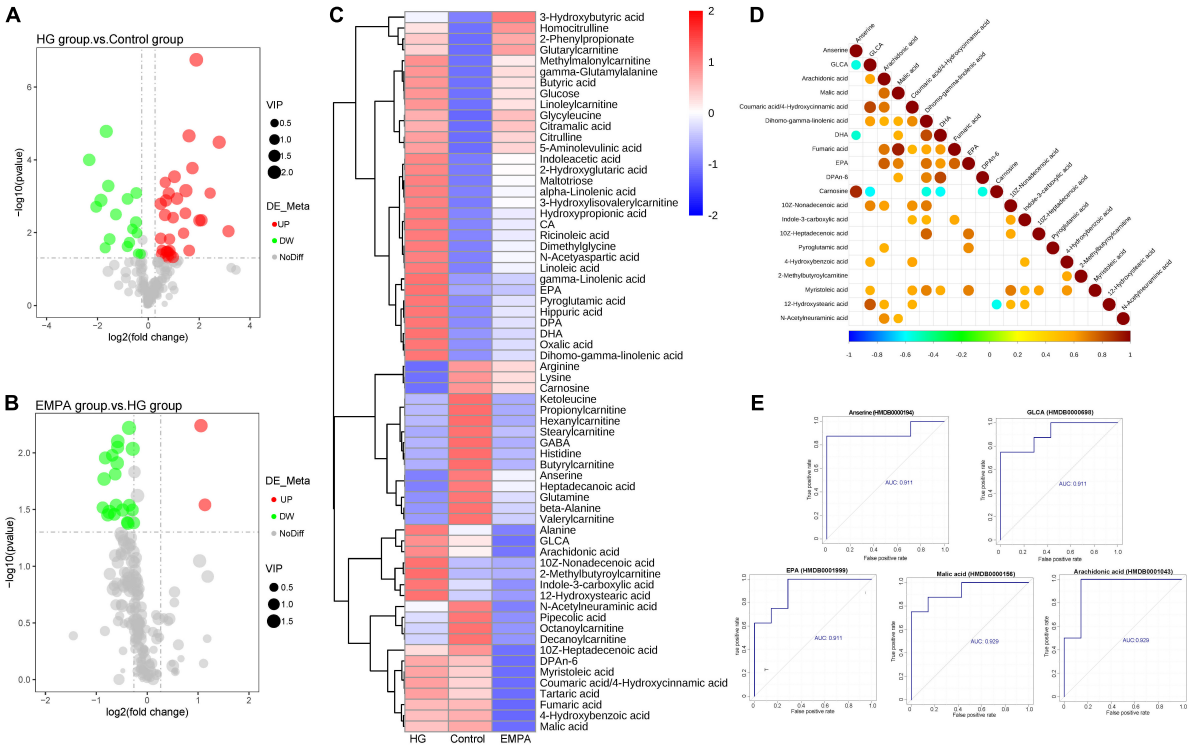

FIGURE 4
The differential metabolite of the myocardium. **(A,B)** Volcano plots of differential metabolite. **(A)** High glucose (HG) group vs. control group; **(B)** empagliflozin (EMPA) group vs. HG group. Cut-off value: $p < 0.05$, fold change (FC) > 1.2 (upregulation) or < 0.67 (downregulation), the red and green point represents the upregulated and downregulated proteins, respectively. **(C)** Hierarchical clustering analysis of differential metabolite of myocardium in three groups. **(D)** The correlation diagram of differential metabolites in groups EMPA and HG (top 20). The blue point represents a negative correlation (negative value), and the red represents a positive correlation (positive value). **(E)** Receiver operating characteristic (ROC) curves of top 5 metabolites [top 5 areas under the curve (AUC)]. The vertical coordinate is the true positive rate (sensitivity), and the horizontal coordinate is the false positive rate (1—specificity).

large-scale clinical trials (26–28), prompting investigators to continue exploring the mechanism of action of SGLT2i.

In this present study, we established the T1DM rat model by intraperitoneal injection of STZ and treatment with EMPA for 12 weeks to investigate changes in proteins and metabolites in the myocardium. The animal experiment results confirmed that diabetes contributed to a pronounced DCM characterized by mitochondrial dysfunction, impaired lipid metabolism, myocardial fibrosis, and associated diastolic and systolic functional impairments of the heart, which were consistent with the observations in previous studies (21, 29). Dysregulation of metabolism increased production of AGEs and apoptosis, inflammation, and impaired calcium were all suggested to explain the cardiac impairment in DCM (30). Mitochondria play a central role in energy metabolism. More than 95% of ATP is provided by mitochondrial oxidative phosphorylation, and its morphology is linked to energy metabolism. Numerous studies have shown that changes in mitochondrial morphology reflect alterations in energy metabolism. TEM in this study revealed that the mitochondria in the rat cardiomyocytes of the HG group were irregular and disordered. Studies in patients have demonstrated that mitochondrial dysfunction is linked to ventricular hypertrophy and fibrosis (31). A previous study suggested that SGLT2i may improve mitochondrial function and reduce oxygen-reactive stress (32). The present study found that the mitochondrial morphology changes and myocardial fibrosis were ameliorated in the rat cardiomyocytes of the EMPA group, which confirmed the cardiovascular benefit of SGLT2i. Moreover, the results of metabolomics and proteomics in myocardium uncovered numerous differential proteins and metabolites indicative of energy metabolism changes during the diabetic state and after the treatment of EMPA.

HF is preceded by abnormalities in myocardial energy metabolism (33). The present study found that the cardiometabolic alterations of diabetes are mainly independent of plasma FA/lipids, which indicates that only focusing on the lipid level in peripheral blood is insufficient to objectively reflect the actual metabolism of the myocardium. Our proteomics data suggest that FA metabolism is enhanced in the diabetic heart. Proteins involved in the lipid metabolic process [such as acyl-coenzyme A oxidase, peroxisomal bifunctional enzyme, trifunctional enzyme subunit alpha

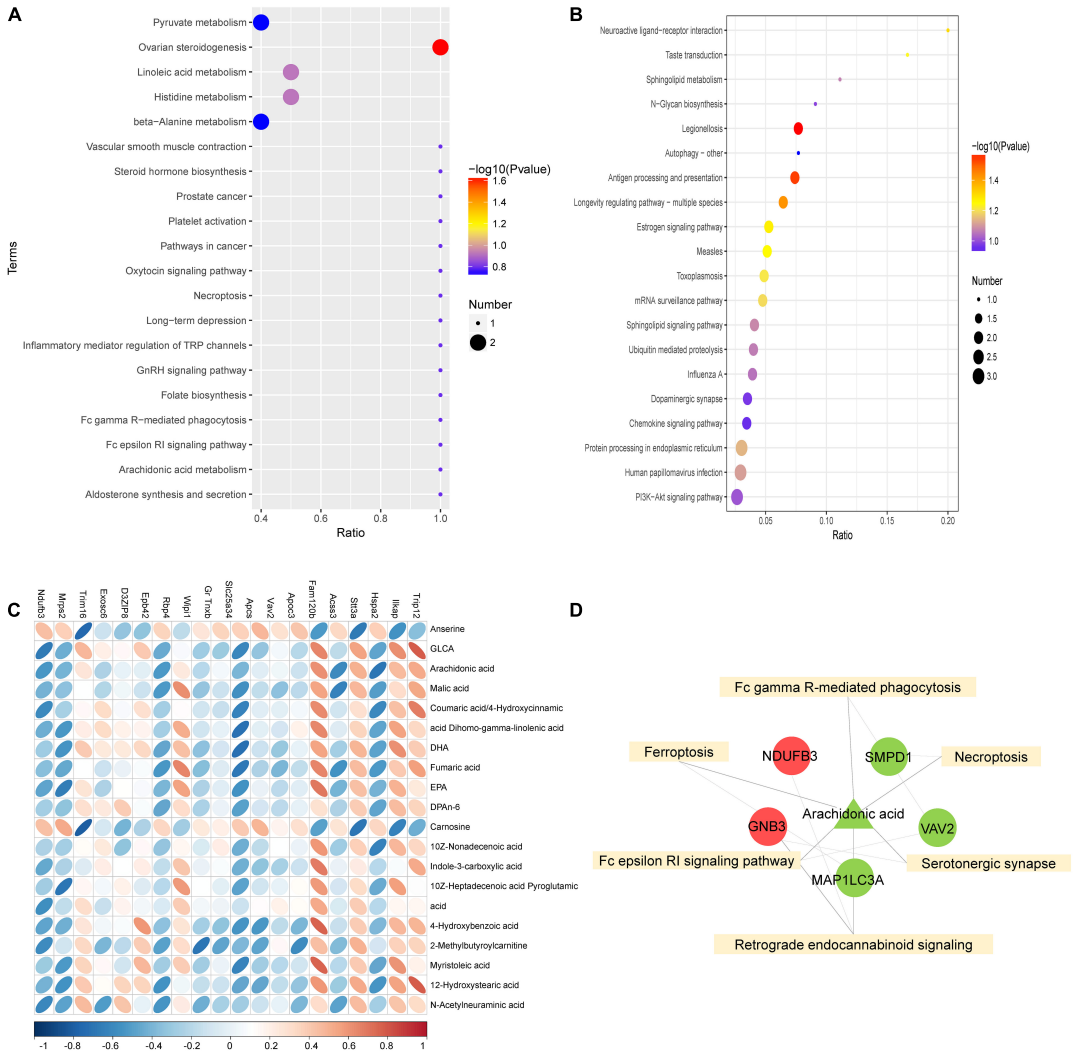

FIGURE 5
Combined analysis of differential proteins and differential metabolites. **(A,B)** KEGG enrichment bubble diagram of differential proteins **(A)** and differential metabolites **(B)** in groups EMPA and HG. **(C)** Heat map of correlation between differential protein and differential metabolite expression. The horizontal axis represents proteins, and the vertical axis represents metabolites. **(D)** Combined analysis of pathway enrichment of differential protein and metabolites. The yellow square represents the pathways shared by differential metabolites and differential proteins, and the circles and triangle represent the differential proteins and differential metabolites involved in the pathway, respectively. Circles represent differential proteins, triangles represent differential metabolites, red is represent up-regulated and green is down-regulated.

(mitochondrial), malonyl-CoA decarboxylase (mitochondrial), and inositol-1-monophosphatase] were upregulated in diabetic hearts. Under normal conditions, there is very little lipid storage in the myocardium, which depends on the optimal regulation of FA uptake and oxidation. However, in a diabetic heart, the imbalance of FA uptake and utilization promotes the accumulation of lipids and toxic intermediates (such as ceramide and diacylglycerol) in cardiomyocytes, which in turn affects cardiac function (34). Increased intracardiac triglyceride concentrations in diabetic patients are associated with concentric left ventricular remodeling (even in the absence of hypertension), reduced cardiac energy, and reduced peak systolic strain (35). SGLT2i produces unobtrusive changes in plasma lipid levels, typically increases LDL-C and HDL-C levels, and decreases triglyceride levels slightly, probably due to reduced LDL catabolism (36, 37). Similar plasma lipid changes were also observed in the present study. However, our results further showed that lipid accumulation in the cardiomyocytes of diabetic rats was significantly reduced after EMPA treatment, which predicted the improvement of myocardial energy metabolism.

Next, we examined the expression profiles at the protein and metabolic levels and identified 43 proteins and 34 metabolites regulated in the myocardium of diabetic rats by

EMPA treatment. The correlation and KEGG enrichment analyses were performed to better understand these proteins and metabolites. The perturbation of biosynthesis of unsaturated FAs and arachidonic acid metabolism were considered responsible for the EMPA treatment's effect. In the myocardium of diabetic rats treated with EMPA, the level of arachidonic acid was significantly decreased and correlated with many differential proteins, suggesting that it may play an important role in the cardiovascular benefit of EMPA. Arachidonic acid (AA) is an important FA that is metabolized into several bioactive compounds by cyclooxygenases, lipoxygenases, and P450 enzymes, which play an important role in the cardiovascular system (38). Studies have revealed that the metabolites of arachidonic acid play a role in enhancing cardiac dysfunction in diabetic rats following ischemia/reperfusion injury (39) and in the development and progression of cardiac hypertrophy (40). Moreover, the pathway analysis revealed that the pathways, such as the Fc epsilon RI signaling pathway, Fc gamma R-mediated phagocytosis, ferroptosis, serotonergic synapse, necroptosis, and retrograde endocannabinoid signaling, were involved in the mechanism of EMPA treatment. Previous studies have found that increased apoptosis is implicated in several diabetic complications and plays an important role in the progression of DCM (41, 42). Ferroptosis is an iron-dependent regulated cell death characterized by lipid peroxidation and iron overload, which is morphologically, biochemically, and genetically different from other types of programmed cell death (43). The occurrence and execution of ferroptosis are regulated by amino acids, lipids, and iron metabolism (44). Hence, we speculated that EMPA reversed high glucose-induced cardiomyocyte injury by ameliorating cardiomyocyte apoptosis, ferroptosis, and abnormal metabolism. In addition, enrichment analysis revealed some novel dysregulated signaling pathways, such as the Fc epsilon RI signaling pathway and the retrograde endocannabinoid signaling pathway, whose functions need further investigation.

In summary, metabolic disorders play a major role in the pathogenesis of DCM. The present study confirmed that SGLT2i treatment ameliorated lipid accumulation and mitochondrial damage in the myocardium of diabetic rats. Metabolomic and proteomic analyses in the myocardium of EMPA-treated diabetic rats identified the potential targets and signaling pathways related to the cardiovascular benefit of SGLT2i, which provides a valuable resource for comparative studies.

Data availability statement

The proteomics data presented in this study are deposited in the ProteomeXchange repository, accession number: PXD036090.

Ethics statement

This animal study was reviewed and approved by the Medical Ethics Committee of the Dongguan Affiliated Hospital of Jinan University.

Author contributions

YX, DC, JX, and JG conceived and designed the experiments. YX wrote the main manuscript text. ZD, HL, JH, JZ, YX, CC, and KD performed the experiments. YX and ZD analyzed the data. All authors reviewed the manuscript.

Funding

This study was supported by the Guangdong Basic and Applied Basic Research Foundation (No. 2021B1515140036), the Scientific Research Project of the Binhaiwan Central Hospital of Dongguan (Nos. 2021010 and 2022004), the Clinical Frontier Technology Program of the First Affiliated Hospital of Jinan University, China (No. JNU1AF-CFTP-2022-a01218), and the Guangzhou Science and Technology Project (No. 202103000010).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2022.999254/full#supplementary-material

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frontiers | Frontiers in Cardiovascular Medicine

TYPE Case Report
PUBLISHED 08 December 2022
DOI 10.3389/fcvm.2022.1019945

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Cardiovascular Therapeutics, a section of the journal Frontiers in Cardiovascular Medicine

RECEIVED 15 August 2022
ACCEPTED 14 November 2022
PUBLISHED 08 December 2022

CITATION
Zhu C, Zhou L, Gao H, Wang J, Li J, Chen H and Li H (2022) Case report: Oral anticoagulant combined with percutaneous coronary intervention for peripheral embolization of left ventricular thrombus caused by myocardial infarction in a patient with diabetes mellitus.
Front. Cardiovasc. Med. 9:1019945.
doi: 10.3389/fcvm.2022.1019945

Case report: Oral anticoagulant combined with percutaneous coronary intervention for peripheral embolization of left ventricular thrombus caused by myocardial infarction in a patient with diabetes mellitus

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Background: Left ventricular thrombus (LVT) is a well-recognized complication of myocardial infarction (MI) in patients with diabetes. An embolic complication caused by LVT is a key clinical problem and is associated with worsened long-term survival.

Case presentation: A 45-year-old man with persistent left abdominal pain for 1 week and left leg fatigue was admitted to the emergency department. The cause of abdominal pain was embolism of the renal artery, the splenic artery, and the superior mesenteric artery caused by cardiogenic thrombosis, which further led to splenic infarction and renal infarction. It was unclear when MI occurred because the patient had no typical critical chest pain, which may have been related to diabetic complications, such as diabetic peripheral neuropathy. Diabetes plays a pivotal role in MI and LVT formation. Because coronary angiography suggested triple vessel disease, percutaneous transluminal coronary angioplasty (PTCA) was conducted, and two drug-eluting stents were placed in the left anterior descending coronary artery (LAD). Due to a lack of randomized clinical control trials, the therapy of LVT and associated embolization has been actively debated. According to the present guidelines, this patient was treated with low-molecular-weight heparin and warfarin (oral anticoagulants) for 3 months in addition to aspirin (100 mg/day) and clopidogrel (75 mg/day) for 1 year. No serious bleeding complications were noted, and a follow-up examination showed no thrombus in the left ventricle or further peripheral thrombotic events.

Conclusion: Peripheral embolization of LVT caused by MI leading to multiple organ embolization remains a rare occurrence. Diabetes plays a pivotal role in MI and LVT formation. Successful revascularization of the

infarct-related coronary artery and anticoagulation therapy is important to minimize myocardial damage and prevent LVT. The present case will help clinicians recognize and manage LVT in patients with diabetes and related peripheral arterial thrombotic events with anticoagulation.

KEYWORDS

diabetes mellitus, myocardial infarction, left ventricular thrombus, peripheral embolization, oral anticoagulant

Introduction

Left ventricular thrombus (LVT) is a well-recognized complication of acute myocardial infarction (AMI), and it is more common (15–25%) in individuals with a large, transmural anterior Q-wave infarction (1). With the widespread use of percutaneous coronary intervention (PCI) therapy over the last decades, the incidence of LVT caused by myocardial infarction (MI) has decreased. However, LVT caused by MI remains a significant cause of morbidity and mortality (2). LVT is related to an increased risk of cerebral and peripheral ischemic events and to poor long-term survival (3, 4). Multiple peripheral embolization, including the splenic artery, the renal artery, the superior mesenteric artery, and the bilateral lower extremity arteries, is an extremely rare occurrence.

Some studies indicated that diabetes mellitus, higher wall motion score index, and hypercoagulation independently increase the risk for LVT formation in patients with acute anterior MI following thrombolytic therapy (5–8). In patients with diabetes, coagulation factors are increased, while inhibitors of coagulation are decreased (9). Diabetes mellitus has been demonstrated to be a potent, independent risk factor for MI with LVT formation.

Due to a lack of randomized clinical control trials, the therapy for LVT and associated embolization has been actively debated. Successful revascularization of the infarct-related coronary artery as soon as possible is important to minimize myocardial damage and prevent LVT. Current guidelines for LVT resolution recommend anticoagulant treatment with oral anticoagulants (OACs) and vitamin K antagonists (VKAs) for 3 months (10) or 6 months (11). Here, we present a rare case of splenic infarction and bilateral renal infarction resulting from multiple peripheral embolization of LVT in a patient with diabetes mellitus who was treated successfully with OAC and intervention therapy.

Case description

A 45-year-old man with persistent left abdominal pain for 1 week was admitted to the emergency department. He denied any chronic illness history and regular physical check-ups. For 3 days prior to admission, the patient felt fatigue and had numbness in his left leg below the ankle. The smoking history of the patient was more than 20 years with three to four cigarettes per day, and his history of drinking was more than 10 years with 1,000 ml of beer per day. His weight was 80 kg (BMI 24.07 kg/m^2), and his abdominal girth was 106 cm. The blood pressure of the patient on admission was 150/108 mmHg, with a pulse rate of 91 beats per minute and a pulse oxygen level of 99% (indoor air). Physical assessment revealed pressing pain in the middle of the left abdomen with no rebound pain as well as muscle tension, percussion pain in the left kidney area, and weak fluctuation of the bipedal dorsal artery pulse.

Laboratory tests provided the following results: a white blood cell (WBC) count of 7.68 $\times$ 10^9/L (normal range 3.5–9.5 $\times$ 10^9/L); a platelet (PLT) count of 397 $\times$ 10^9/L (normal range 125–350 $\times$ 10^9/L); hemochrome (HGB) of 154 g/L; and a weak positive fecal occult blood (OB) test, which turned negative after subsequent reexamination. The myocardial enzyme markers (creatine kinase isoenzyme index and troponin I or T) and NT-proBNP levels were within the normal range. Renal function showed mild impairment, with a creatinine (Cr) level of 105.0 μmol/L (normal range 41–111 μmol/L) and an estimated glomerular filtration rate (eGFR) of 60.54 ml/min/1.73 m^2. The D-dimer level was slightly elevated with a value of 1.80 mg/L (normal range 0–1.5 mg/L). The patient also had high blood sugar (Glu) and elevated blood lipids. The serum lipid profile of the patient was as follows: total cholesterol level (CHOL) of 8.13 mmol/L (normal range 3.9–5.2 mmol/L); triglyceride (TG) level of 2.10 mmol/L (normal range 0.57–1.7 mmol/L); and low-density lipoprotein cholesterol (LDL-c) level of 4.97 mmol/L (normal range 2.34–3.12 mmol/L). At admission, the blood sugar and glycosylated hemoglobin (HbA1c) levels of the patient were 23.33 mmol/L (normal range 3.92–6.16 mmol/L) and 11.70% (normal range 4.27–6.07), respectively, and an oral glucose tolerance test (OGTT) confirmed diabetes mellitus. Fundus examination (Supplementary Figures 1A,B) showed vitreous hemorrhage on the right eye, macular edema on the left eye, and skin pigmentation on both lower limbs (Supplementary Figure 1C), which indicated long-term diabetic lesions with retinopathy and dermopathy. Laboratory tests for hypercoagulability (proteins C and S) were performed with normal results.

Enhanced computed tomography (CT) scan of the abdomen and the pelvis of the patient revealed splenic infarction and splenic artery thrombosis (Figure 1A), as well as left renal artery embolization (Figure 1B), bilateral renal infarction (Figures 1C,D), and superior mesenteric artery thrombosis (Figure 1D). Ultrasonography of the lower limb artery revealed the bilateral popliteal artery and left posterior tibial artery thrombosis (Figures 1E,F). In a patient with multiple peripheral thromboembolic events, cardiogenic thrombosis should be taken into consideration. Furthermore, echocardiography showed abnormal ventricular wall motion with LVT (size of 1.13 × 1.77 cm and area of 1.72 cm^2) at the apex to the inferior part (Figures 1G,H; Supplementary Video 1). Wall motion was observed in the middle segment of the ventricular septum and anterior wall. Hypokinesis to akinesis in LV motion was observed lateral of the basal to apical segments of the LV inferior wall, with a left ventricular ejection fraction (LVEF) decrease of 48%.

The following electrocardiogram (ECG) findings were observed: sinus rhythm; Q waves in the II, III, and augmented vector foot (aVF) leads; the Q waves of the inspiratory phase became shallow but still existed; poor R-wave progression in chest leads; and T-wave inversion in leads V2 to V5 (Figure 2).

The patient was treated with low-molecular-weight heparin and warfarin, with a target international normalized ratio (INR) of 1.8–2.5. Invasive procedures could not be performed due to the presence of LVT. To evaluate coronary artery and myocardial necrosis, a coronary CT scan, myocardial magnetic resonance, and radionuclide imaging were performed. The coronary CT scan showed that the proximal anterior descending artery had severe calcification and stenosis (Figures 3A–C). Subsequent cardiac magnetic resonance imaging confirmed the presence of an apical aneurysm with mobile thrombus at the apex and transmural MI on the apical septal and inferior walls (Figures 3D,E). Resting myocardial radionuclides showed transmural MI in the apical segment of the left ventricle and apical septal segments [approximately 7% of the left ventricular (LV) wall], and blood perfusion was decreased in the inferior wall of the left ventricle (Figure 3F).

The patient was also treated with aspirin (100 mg/day), clopidogrel (75 mg/day), and proton-pump inhibitors (PPIs) to prevent gastrointestinal bleeding events. After 2 weeks of antithrombotic treatment with warfarin, aspirin, and clopidogrel, follow-up echocardiography revealed that the thrombus had almost disappeared. Because the coronary angiography suggested triple vessel disease, percutaneous transluminal coronary angioplasty (PTCA) was conducted, and two drug-eluting stents were placed in the left anterior descending coronary artery (LAD) (Figure 4).

After PCI, antithrombotic therapy with warfarin, aspirin, and clopidogrel was essential for the patient. A repeat echocardiogram revealed that the LV apical thrombus disappeared, and an enhanced CT scan showed peripheral embolism of multiple organs with no further aggravation compared to the previous scan. Diabetes was controlled with a combination of diet and insulin, acarbose, and dapagliflozin. The patient was discharged after a normal physical exam and was prescribed warfarin (3 mg/day), aspirin (100 mg/day), and clopidogrel (75 mg/day) for anticoagulation. With regular outpatient follow-ups, echocardiography showed no thrombus in the LV. Moreover, the patient was prescribed warfarin for 3 months and dual antiplatelet therapy (DAPT) for 1 year.

Discussion

Left ventricular thrombus is a well-recognized complication of AMI. Approximately 15% of LVT cases are caused by MI, and the occurrence rate of LVT in anterior MI is even higher (1). Adverse consequences of LVT include embolic events, especially stroke, and multiple embolization of peripheral arteries. Embolic events cause poor clinical prognoses and affect the quality of life of patients.

The following pathophysiology leading to LVT can be explained by Virchow's triad: hypercoagulable state, ventricular aneurysm stasis, ventricular endothelial injury, and ejection fraction decrease after MI (12). LVT increases the risk of major cardiovascular events and mortality, leading to poorer long-term survival. Short-term thrombus dissolution by anticoagulants can improve the prognosis of patients.

The underlying endomyocardial pathological changes and low regional intracardiac blood flow velocity caused by MI are the most important mechanisms of thrombus formation (13, 14). In most instances, a hypercoagulable state may also contribute to some extent (5, 6). In diabetes patients with increased plasma lipid levels, coagulation factors such as fibrinogen, factor VII, factor VIII, von Willebrand's factor, and factor X are increased, but inhibitors of coagulation such as antithrombin II, protein C, and protein S are decreased (9, 15). Diabetes plays a pivotal role in MI and LVT formation.

In the present case, the patient presented with abdominal pain as the first symptom, which was attributed to embolism of the renal artery, the splenic artery, and the superior mesenteric artery caused by cardiogenic thrombosis, further leading to splenic infarction and renal infarction. It was unclear when the MI occurred because the patient had no typical critical chest pain, which may have been due to diabetic complications, such as diabetic peripheral neuropathy. Changes in retinopathy and dermopathy indirectly indicate a long history of diabetes. Diabetic peripheral neuropathy is one of the most common long-term complications of diabetes and is associated with cardiovascular risk factors and mortality. The ECG and magnetic resonance imaging of the patient suggested an old anterior MI. The LVT can form within several days after the MI, and the hypokinesis to akinesis in LV motion throughout the entire myocardium may have enhanced thrombus formation (15).

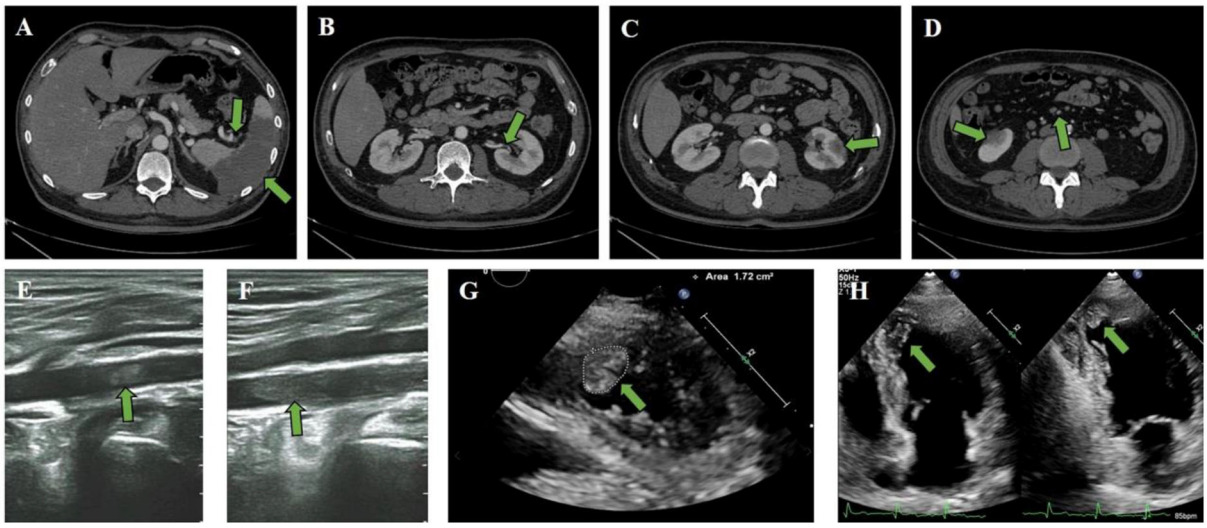

FIGURE 1
Clinical CT scan and ultrasound imaging. **(A–F)** Enhanced CT scan revealed **(A)** splenic infarction and splenic artery thrombosis, as well as **(B)** left renal artery embolization, **(C)** left renal infarction, and **(D)** superior mesenteric artery thrombosis and right renal infarction. Ultrasonography of the lower limb artery revealed **(E)** right popliteal artery thrombosis and **(F)** left posterior tibial artery thrombosis. **(G,H)** The attached thrombus (size 1.13 × 1.77 cm and area 1.72 cm^2) was observed in the apex part of the LV.

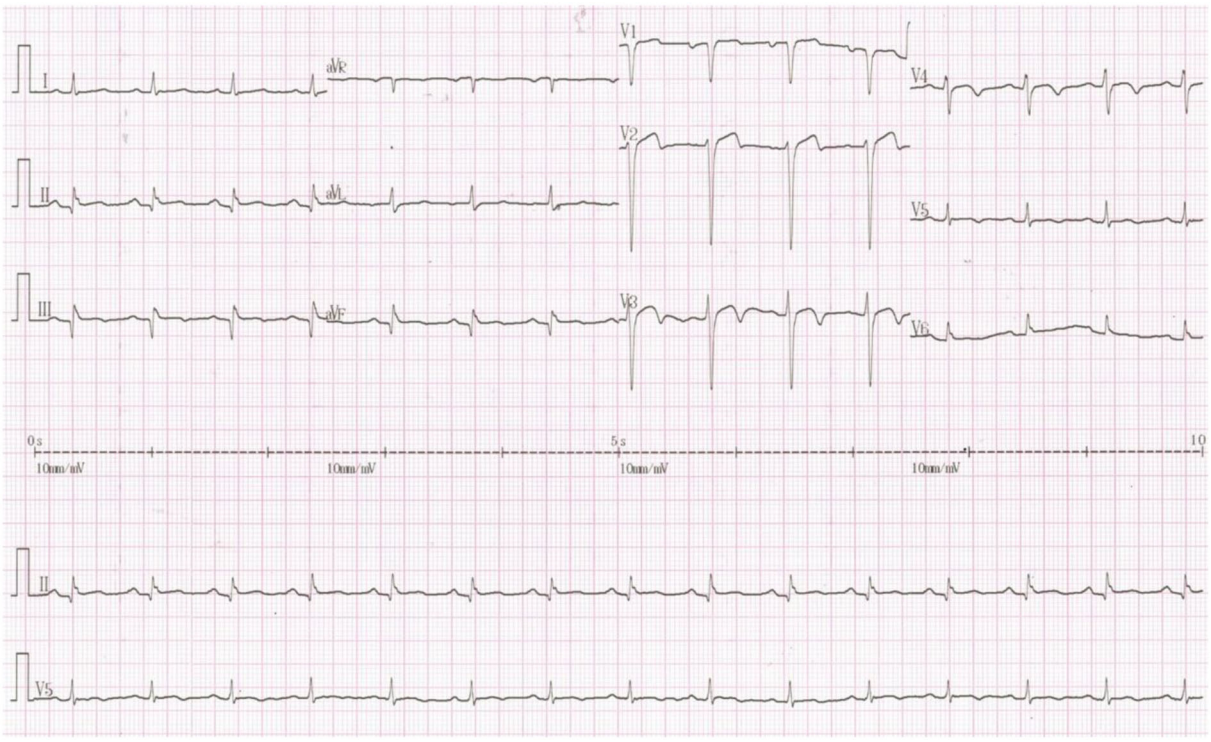

FIGURE 2
Twelve-lead ECG findings on admission. The following ECG findings were observed: sinus rhythm; Q waves in the II, III, and aVF leads; the Q waves of the inspiratory phase became shallow but still existed; poor R-wave progression in chest leads; and T-wave inversion in leads V2 to V5.

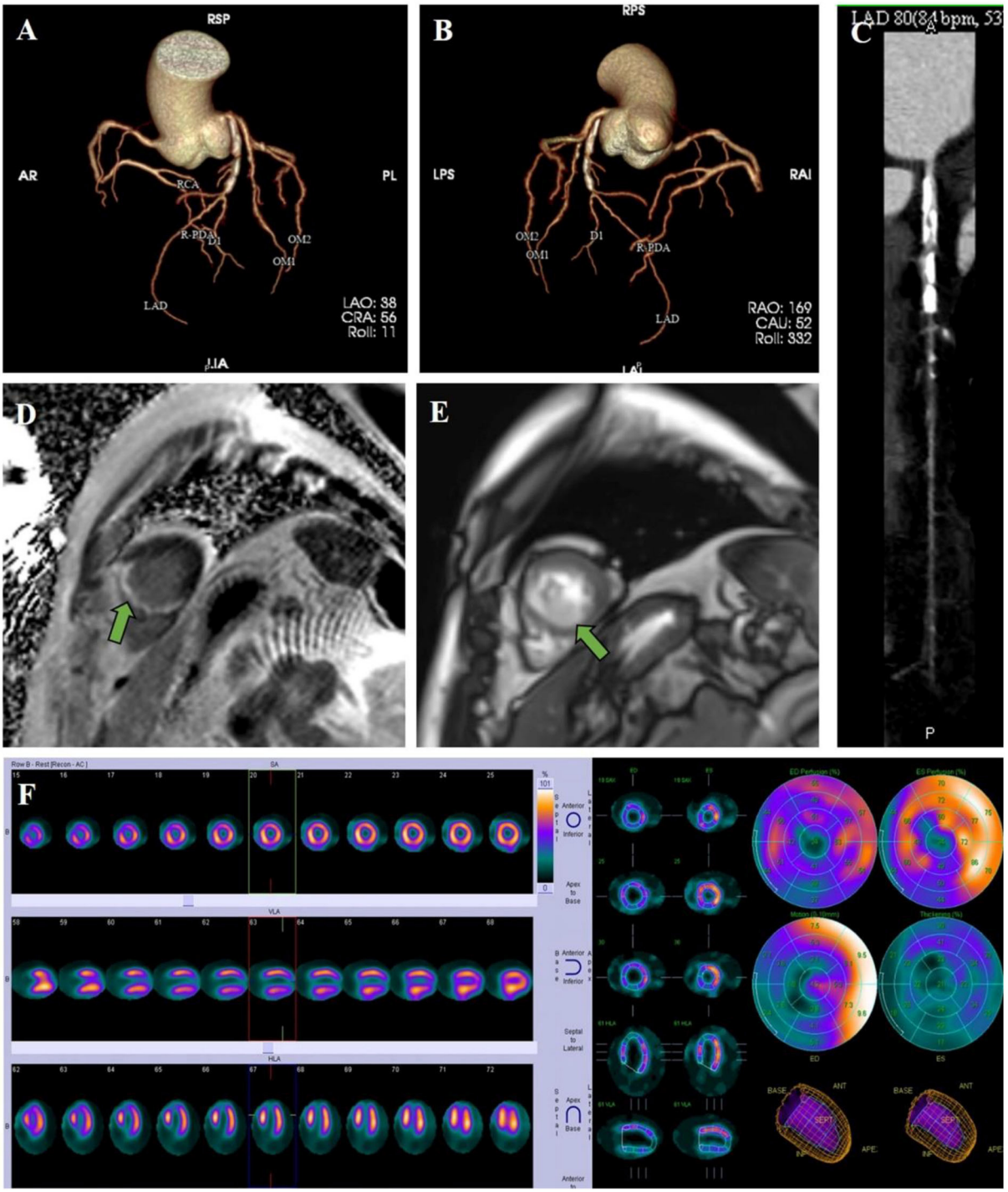

FIGURE 3
Clinical imaging. **(A–C)** A coronary CT scan showed that the proximal anterior descending artery had severe calcification and stenosis. **(D)** Cardiac magnetic resonance imaging revealed delayed enhancement on the apical septal and inferior walls, which presented transmural MI. **(E)** Cardiac magnetic resonance imaging confirmed the presence of a thrombus at the apex. **(F)** Resting myocardial radionuclides showed transmural MI and blood perfusion decrement.

Due to a lack of randomized clinical control trials, the management of LVT and associated embolization has been actively debated (12, 16). Anticoagulation therapy has been shown to reduce the risk of embolic complications in patients with LVT, but no prospective randomized study has been performed (17). Current guidelines for LVT resolution

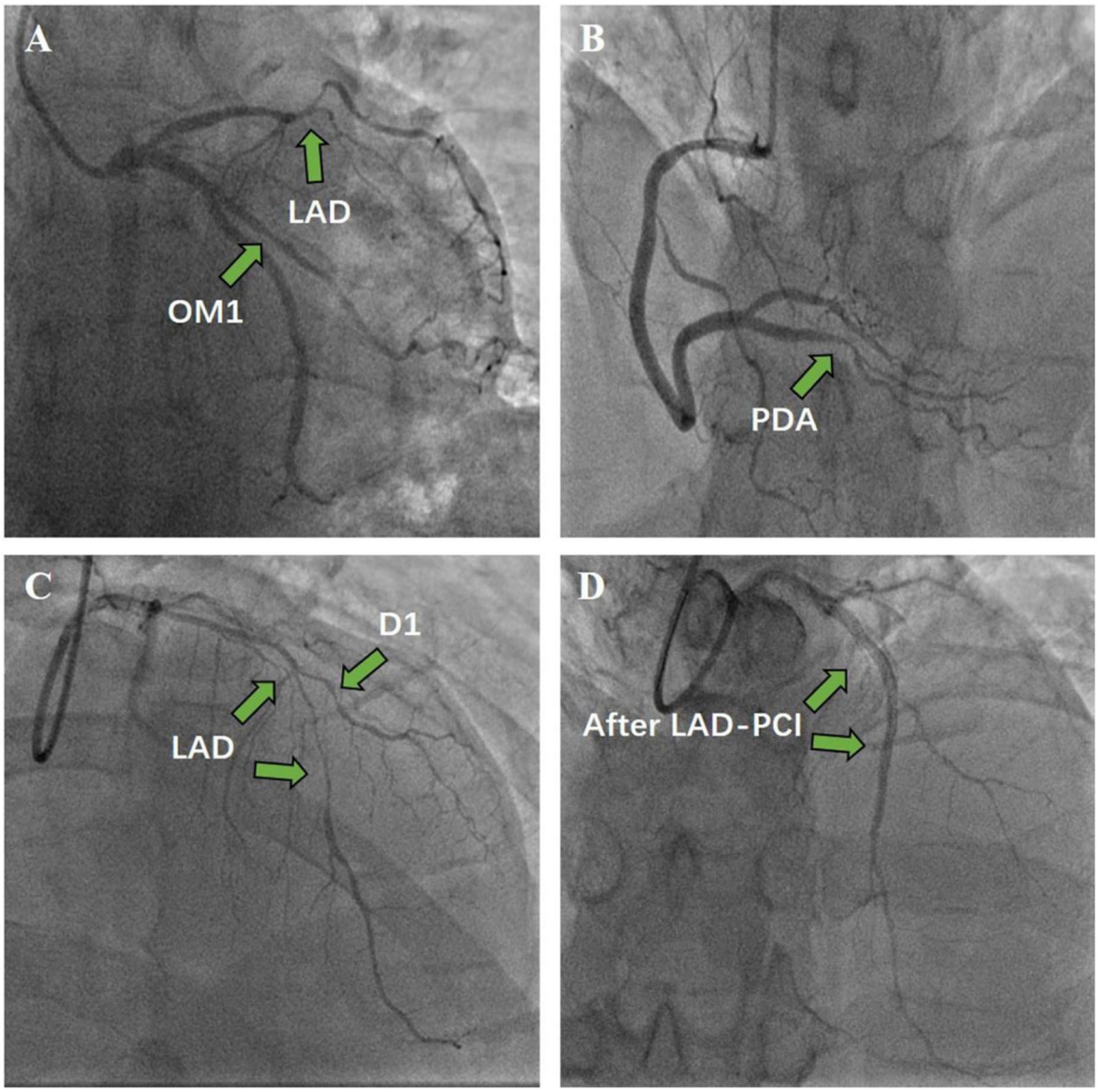

FIGURE 4
Coronary angiography and percutaneous coronary intervention. **(A)** Left coronary angiography. **(B)** Right coronary angiography. **(C)** Before stent. **(D)** Two drug-eluting stents were implanted in the LAD to achieve revascularization.

recommend anticoagulant treatment with VKAs for 3 or 6 months (8, 18). Larger infarct sizes have greater risks of forming into thrombosis. A larger infarction can lead to myocardial injury, inflammatory response, hypercoagulable state, and abnormal wall motion (7, 19). The incidence of LVT early after AMI is low if primary PCI with stenting is successfully performed to salvage the myocardium from dying. Thus, successful revascularization of the "criminal" coronary artery is important to minimize the MI area and prevent LVT. Importantly, dual antiplatelet treatment after PCI has no or only limited influence on thrombin generation, which plays a key role in the development of LVT (20).

Triple antithrombotic therapy increases the risk of bleeding, especially considering the patient's history of hypertension and positive fecal occult blood (OB) test in our case. Therefore, the anticoagulant therapy strategy and duration are important. However, the optimal duration of triple antithrombotic therapy is controversial, especially after PCI and DAPT. The current consensus suggests that patients with LVT should be prescribed OAC with VKA therapy for up to 6 months (18). However, this recommendation does not consider the need for DAPT after stenting. VKAs and DAPT may increase the risk of bleeding, and the optimal duration of triple antithrombotic therapy is decided by bleeding risk and stent thrombus. If echocardiography or

cardiac magnetic resonance imaging shows no thrombi after 3 months of antithrombotic therapy, OAC can be stopped earlier than 6 months, especially if the abnormal wall motion is improved (21). Some authors recommended that patients after PCI who have LVT or are at risk of LVT with apical akinesis or dyskinesis should be prescribed VKAs for up to 3 months and that the DAPT duration should be based on the type of stent (22). According to the present guidelines, the present case of LVT and peripheral embolization was treated with low-molecular-weight heparin and warfarin (OAC) in addition to aspirin and clopidogrel. The patient was rated as having a high risk for bleeding according to the CRUSADE score for bleeding risk. With a high bleeding risk and improved wall motion, the patient was treated with warfarin (OAC) for 3 months and DAPT for 1 year after PCI. The patient is currently only taking one antiplatelet drug (clopidogrel). The patient did not experience any serious bleeding complications after treatment with warfarin and DAPT. The anticoagulant therapy strategy and duration were important based on the risk of ischemia and bleeding. Here, we present successful anticoagulation therapy for LVT in a patient with diabetes and related peripheral arterial thrombotic event, providing a reference for similar cases.

Conclusion

Left ventricular thrombus formation is a serious complication of MI, and peripheral embolic events caused by LVT are related to poor long-term survival. Multiple embolization due to LVT leading to splenic infarction, bilateral renal infarction, and bilateral popliteal artery thrombus is rare. The presence of wall motion abnormalities, MI location, and MI size is the most powerful independent predictors of LVT formation. In the present case, the patient suffered from an asymptomatic MI, and the infarct-related coronary artery was successfully revascularized through PCI. Diabetes plays a pivotal role in asymptomatic MI and LVT formation. The present patient was treated with low-molecular-weight heparin followed by warfarin for 3 months in addition to aspirin and clopidogrel for 1 year. The anticoagulation treatment prevented further thromboembolic events and caused the LVT to disappear without any serious bleeding complications. The present case will help clinicians recognize and manage LVT in patients with diabetes and related peripheral arterial thrombotic events with anticoagulation. More studies on anticoagulation therapy in patients with LVT are needed to improve long-term quality of life and reduce morbidity and mortality.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding authors.

Ethics statement

The studies involving human participants were reviewed and approved by Ethics Committee Board of the Beijing Friendship Hospital, Capital Medical University. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

CZ, LZ, and HG performed patient management and data collection. CZ, JW, and JL drafted the manuscript and edited the figures. LZ and HC performed the angioplasty. HC and HL critically revised the manuscript for important intellectual content. All authors contributed to the article and approved the submitted manuscript.

Funding

The research was supported by the Youth Program of the National Natural Science Foundation of China (Grant No. 82000311) and the National Key R&D Program of China (Grant No. 2021ZD0111004).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2022.1019945/full#supplementary-material

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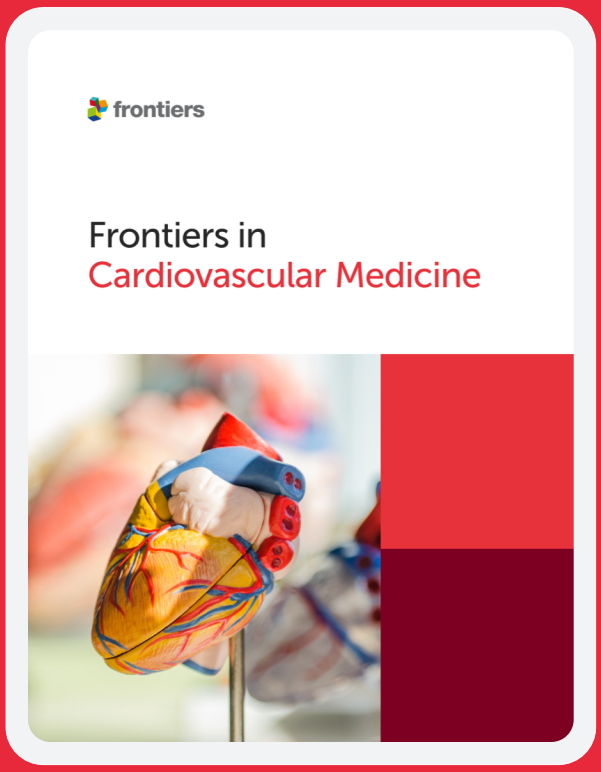

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