Vol 4 | Issue 4 | October - December | 2024

ORIGINAL ARTICLE

ADJUNCTIVE TRIMETAZIDINE THERAPY IMPROVES LEFT VENTRICULAR EJECTION FRACTION BY REGULATING PLASMA ASYMMETRIC DIMETHYLARGININE AND VISFATIN IN HEART FAILURE WITH REDUCED EJECTION FRACTION: A CROSS SECTIONAL OBSERVATIONAL STUDY, TAMIL NADU, 2021-2022

Uma Maheswari G [(1)], Yamini B [(1)], Gokul Sudhakaran [(2)], Mohan Kumar R [(3)], Cecily Mary Majella Jayaraj [(4)], Kannan Radhakrishnan [(5)], Dhandapani V E [(1)], Kanchana Mala Karuppiah [(1)]

(1) Department of Cardiology, SRM Medical College Hospital and Research Center, Kattankulathur
(2) Department of Biotechnology, SRM Institute of Science and Technology, Kattankulathur
(3) Interdisciplinary Institute of Indian System of Medicine, SRM Institute of Science and Technology, Kattankulathur
(4) Department of Cardiology, Tamil Nadu Government Multi Super Speciality Hospital, Omandurar
(5) Department of Cardiology, Government Chengalpattu Medical College & Hospital, Chennai
(6) Department of Medical Research, SRM Medical College Hospital and Research Center, Kattankulathur

ABSTRACT

INTRODUCTION : Adjunctive Trimetazidine (TMZ) is known to improve symptoms of heart failure (HF), but its mechanistic effects on cardioprotection are not fully understood. This study evaluated whether adjunctive TMZ therapy improves left ventricular ejection fraction (LVEF) and alters plasma biomarkers in patients with heart failure with reduced ejection fraction (HFrEF) compared to standard HF therapy (STD).

METHODS : In this single-center observational study, 60 HFrEF patients were divided into two groups: TMZ + STD (n = 30) and STD alone (n = 30). LVEF was assessed by echocardiography, and biomarkers including visfatin, asymmetric dimethylarginine (ADMA), nicotinamide adenine dinucleotide (NAD+), reduced and oxidized glutathione (GSH and GSSG), and sirtuin1 (sirt1) mRNA were measured. Correlations between LVEF and biomarkers were analyzed.

RESULTS : LVEF was significantly higher in the TMZ + STD group compared to STD alone. Plasma levels of visfatin, ADMA, and GSSG decreased, while NAD+, GSH, and sirt1 mRNA levels increased in the TMZ + STD group. Negative correlations were observed between LVEF and visfatin, ADMA, and GSSG, while positive correlations were found with NAD+, GSH, and sirt1 mRNA.

CONCLUSION : Adjunctive TMZ therapy enhances cardiac function by improving LVEF and modulating biomarkers associated with oxidative stress, inflammation, and myocardial remodeling. These findings suggest TMZ's potential to optimize biomarker profiles and provide cardioprotection in HFrEF patients.

KEYWORDS : Heart failure; LVEF; Oxidative stress; Asymmetric Dimethylarginine; Visfatin; Echocardiography

INTRODUCTION

Excess oxidative stress (OS), inflammation and cellular senescence (CS), which are key contributors to Heart Failure (HF) pathogenesis, reinforce each other to form a vicious circle, to drive disease progression.[1] Biomarkers that contribute to HF progression include circulating levels of visfatin (adipocytokine/extracellular-visfatin/extracellular-nicotinamide-phosphoribosyltransferase, eNAMPT), asymmetric-dimethylarginine (ADMA), nicotinamide-adenine-dinucleotide (NAD+), sirtuin1 (sirt1), and glutathione (GSH and GSSG).[2-7]

In HF, plasma ADMA is elevated. Excess ADMA, via impaired nitric oxide and up-regulated free-radical production, reduces intracellular functions of cardiomyocytes and endothelial cells, leading to cardiac remodeling, fibrosis and HF progression.[8] Up-regulated circulating visfatin, a biomarker of OS, inflammation and endothelial damage, triggers CS in HF.[9,10] Circulating and intracellular NAD+ are decreased in HF11, due to impaired NAD+-biosynthesis/salvage of NAD+ or over-activation of NAD+-consuming enzymes. Sirt1, a NAD+-dependent deacetylase-enzyme, regulates pathophysiological mechanisms of myocardial

Please Scan this QR Code to View this Article Online
Article ID: 2024:04:04:04
Corresponding Author: Kanchana Mala Karuppiah
e-mail : mkarupiya@gmail.com

remodeling and HF, including, inflammation and energy metabolism. Intracellular sirt1-deficiency occurs under excess OS and/or inflammation. Hence, depending on the extent of free radicals and/or stress triggers, sirt1 can positively or negatively regulate myocardial remodeling through de-acetylation and redox-sensitive molecules.[12] Reduced antioxidants, due to down-regulated enzymatic and non-enzymatic antioxidants and diminished redox state (reduced to oxidized glutathione – GSH:GSSG ratio), drive HF pathogenesis.[6] The prognosis of HF is poor despite advances in prevention and therapeutic strategies. Because, although it's clear that OS and inflammation drive HF, current HF treatments are not mechanism-based.[1] As an adjuvant to conventional HF therapy, the anti-anginal drug trimetazidine (TMZ) improves LV function.[13] The purpose of the study is to characterize the additive effects of TMZ (TMZ+STD) on circulating biomarkers and LVEF, in HFrEF, in comparison with standard-HF-therapy (STD).

METHODS

Study Design and Participants : This was a single-center, observational, cross-sectional study conducted at the outpatient facility of the Department of Cardiology, SRM Medical College Hospital and Research Center, Kattankulathur, India. Patients were enrolled between June 2021 and June 2022 using convenience sampling.

Participant's inclusion and exclusion criteria: The study included a total of 60 patients diagnosed with heart failure (HF) with reduced ejection fraction (HFrEF, EF <45%) through echocardiography with a documented history of consuming standard HF therapy, with trimetazidine (TMZ) (n=30) and without adjunctive TMZ (n=30). Patients were excluded if they had recent acute coronary syndrome (<6 months), significant valvular heart disease, chronic kidney disease, or any active systemic illness. Baseline demographic and clinical characteristics are summarized in Table 1.

Ethical approval was obtained from the institutional ethics committee at SRM Institute of Science and Technology (SRMIST), Kattankulathur. Written informed consent was obtained from all participants before enrolment. The study adhered to the ethical principles outlined in the Declaration of Helsinki.

Study Procedures:

Echocardiography

Echocardiographic assessments were performed at enrollment using the Epiq 7c ultrasound system. Left ventricular ejection fraction (LVEF) was determined using the Biplane Simpson's method. Additional echocardiographic indices, including global longitudinal strain (GLS) and left ventricular mass index (LVMI), were evaluated. A detailed list of echocardiographic parameters is provided in Table 2.

Table 1. Baseline demographic and clinical characteristics of enrolled patients, Department of Cardiology, SRM Medical College Hospital and Research Center, June 2021 and June 2022, Tamil Nadu

Parameters	TMZ + STD (n = 30)	STD (n = 30)	P Value
Gender, Male (%)$	67 %	50 %	NS
Age (years)*	55.96 ± 8.72	55.96 ± 8.72	NS
Height (cm)*	160.1 ± 7.74	164.27 ± 7.73	NS
Weight (Kg)*	67.2 ± 12.88	75.93 ± 12.8	NS
Heart Rate (bpm)*	78.13 ± 9.19	77.8 ± 8.26	NS
Systolic BP (mmHg)*	124.33 ± 17.55	133.67 ± 15.42	NS
Diastolic BP (mmHg)*	75.67 ± 11.94	76.17 ± 10.96	NS
CAG – Normal (%)$	70 %	46 %	NS
CAG – SVD (%)$	30 %	26 %	NS
CAG – DVD (%)$	0 %	26 %	NS
Antiplatelet$	73 %	50 %	NS
Statins$	70 %	53 %	NS
ACE inhibitors$	26 %	30 %	NS
Beta blockers$	63 %	40 %	NS
Diuretics$	56 %	40 %	NS
Trimetazidine$	100 %	0 %	NS

P values for differences between the groups (TMZ + STD and STD) were calculated for categorical variables ($) [by using Chi-square tests] and for continuous variables (*). Data are presented as mean ± standard deviation.

ACE inhibitor – angiotensin converting enzyme inhibitor, BP – blood pressure, CAG – coronary angiogram, DVD – double vessel disease, NS – non-significant, SVD – single vessel disease, STD – standard heart failure therapy, TMZ + STD – trimetazidine and standard heart failure therapy

Biomarker Analysis

Peripheral blood samples were collected under standardized conditions. Plasma and blood cells were separated for further analysis.

ADMA and NAD+ Quantification

Plasma samples were ultrafiltered using 3-kDa filters. Asymmetric dimethylarginine (ADMA) and nicotinamide adenine dinucleotide (NAD+) were quantified simultaneously using reverse-phase high-performance liquid chromatography (HPLC) with dual wavelengths14. Data acquisition and analysis were performed using Lab Solution Software.

GSH and GSSG Quantification

Glutathione (GSH) and oxidized glutathione (GSSG) levels were quantified using HPLC equipped with a photodiode array detector15.

Table 2. Echocardiographic characteristics of enrolled patients, Department of Cardiology, SRM Medical College Hospital and Research Center, June 2021 and June 2022, Tamil Nadu

Parameters	TMZ + STD (n = 30)	STD (n = 30)	P Value
LVEF (%) – Simpson	36.7 ± 4.19	33 ± 4.03	P < 0.05
LV mass (g)	228.63 ± 43.05	230 ± 26.81	P < 0.05
EDV (mL)	167.47 ± 43.75	125 ± 33.19	NS
ESV (mL)	109.67 ± 29.33	73 ± 30.74	NS
SV (L/min)	57.8 ± 24.87	52 ± 26.44	NS
CO (L/min)	4.41 ± 1.87	3.2 ± 1.55	NS
LVIDD (cm)	5.64 ± 0.27	5.7 ± 0.31	P < 0.05
LVIDS (cm)	4.57 ± 0.36	4.9 ± 0.33	P < 0.05
E (cm/s)	0.69 ± 0.19	0.6 ± 0.22	NS
A (cm/s)	0.55 ± 0.25	0.8 ± 0.2	NS
E/A	1.73 ± 1.27	0.75 ± 0.61	P < 0.05
e' Septal (cm/s)	0.05 ± 0.01	0.05 ± 0.01	NS
E/e' Septal	14.43 ± 5.15	12 ± 5.5	NS
e' Lateral (cm/s)	0.06 ± 0.01	0.06 ± 0.01	NS
E/e' Lateral	10.76 ± 3.06	10 ± 4.21	NS
GLS (%)	-9.26 ± 5.23	-11.27 ± 2.21	P < 0.05

Data are presented as mean ± standard deviation.
A – atrial systole, CO – cardiac output , E – early rapid filling in diastole, e' – early diastolic filling velocity, E/e' - ratio of transmitral blood flow velocity to tissue doppler velocity, EDV – end-diastolic volume, ESV – end-systolic volume, LVEF – left ventricular ejection fraction, LVIDD – left ventricular internal dimension in diastole, LVIDS – left ventricular internal dimension in systole, LV mass – left ventricular mass, NS – non-significant, SV – stroke volume, STD – standard heart failure therapy, TMZ + STD – trimetazidine and standard heart failure therapy.

Visfatin Quantification

Plasma visfatin concentrations were measured using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Catalog #E-EL-H1763). Concentrations were calculated by interpolating optical density (OD) values against the standard curve.

RNA Isolation and qRT-PCR

Total RNA was extracted from blood cells using the TRIzol reagent protocol. RNA purity and concentration were assessed with a NanoDrop-2000c spectrophotometer. Complementary DNA (cDNA) was synthesized using the PrimeScript RT reagent kit.

Quantitative reverse transcription polymerase chain reaction (qRT-PCR) was performed on a LightCycler-480 system with TB Green Premix Ex Taq II. Relative gene expression was calculated using the 2-ΔΔCt method, normalized to the expression of GAPDH as a housekeeping gene.

Primer Sequences:

Sirt1:

Forward: 5'-TCAGCTCTGGGATGACCTT-3'

Reverse: 5'-ACCATCAAGCCGCCTACTAATCTG-3'

GAPDH:

Forward: 5'-TTCAGCTCTGGGATGACCTT-3'

Reverse: 5'-CTCATGACCACAGTCCATGC-3'

Statistical analysis : Continuous variables were expressed as mean ± standard deviation (SD), and categorical variables as frequencies and percentages. Group comparisons were conducted using unpaired t-tests for continuous variables and the chi-square test for categorical variables. Relationships between variables were evaluated using Pearson's correlation coefficient. A p-value <0.05 was considered statistically significant. Statistical analyses and graphical data representations were performed using GraphPad Prism 5.0 software.

RESULTS

Changes in the association between LVEF versus circulating concentrations of biomarkers were assessed between those who received TMZ as an add-on-therapy for STD and those who received STD alone, in HFrEF.

Sixty patients were included in the study; 30 each, in STD or TMZ+STD. Patients' history showed both groups were taking the respective drugs for at least 6 months before recruitment. LVEF was lower in STD than TMZ+STD (Table 2). Global longitudinal strain (GLS) was lower in STD than in TMZ+STD. In STD, LV mass-index was increased than in TMZ+STD.

In TMZ+STD, NAD+, GSH and sirt1 were higher, in contrast to decreased visfatin, ADMA and GSSG (Fig.1). As in Table 3, the correlation of ADMA, GSH, NAD+, visfatin and sirt1 with EF, in STD, was significant. In TMZ+STD, a significant correlation exists between ADMA and visfatin, with EF. There was a strong negative correlation between ADMA and visfatin with EF in both groups. In STD, a strong positive correlation is observed between NAD+, GSH and sirt1, however, a moderate positive correlation exists in TMZ+STD.

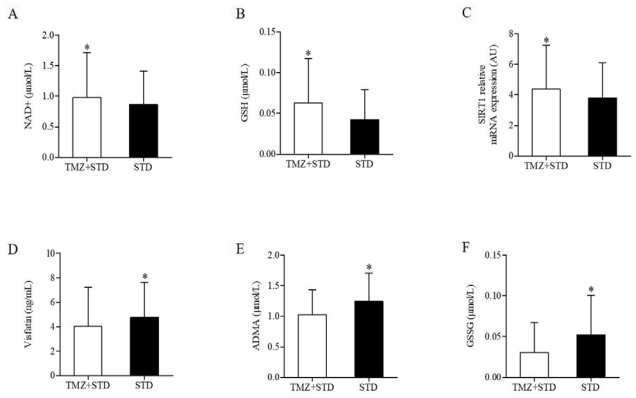


*Figure 1. Changes in the (A) circulating levels of nicotinamide adenine dinucleotide (NAD+), (B) plasma levels of reduced glutathione (GSH), (C) relative sirt1 mRNA expression in peripheral blood cells, (D) circulating levels of visfatin, (E) plasma levels of asymmetric dimethylarginine (ADMA) and (F) plasma levels of oxidised glutathione (GSSG), between the two groups (TMZ+STD and STD). Data are expressed as mean ± SD (*P<). TMZ + STD – trimetazidine and standard heart failure therapy; STD – standard heart failure therapy, sirt1 – sirtuin1.*

Table 3. The correlation of circulating biomarkers in patients with HFrEF, Department of Cardiology, SRM Medical College Hospital and Research Center, June 2021 and June 2022, Tamil Nadu

Biomarkers	STD (n=30) EF%	TMZ + STD (n=30) EF%
Sirt1	r = 0.7539 p < 0.00001	r = 0.6175 p = 0.0002
NAD+	r = 0.7294 p < 0.00001	r = 0.6062 p = 0.0003
ADMA	r = -0.8979 p < 0.00001	r = -0.8667 p < 0.00001
GSH	r = 0.8419 p < 0.00001	r = 0.6852 p = 0.00002
GSSG	r = -0.3756 p = 0.04	r = -0.6607 p = 0.00007
Visfatin	r = -0.7471 p < 0.00001	r = -0.7614 p < 0.00001

Asymmetric dimethylarginine (ADMA), nicotinamide adenine dinucleotide (NAD+), oxidized glutathione (GSSG), reduced glutathione (GSH), sirt1 – sirtuin1, STD – standard heart failure therapy, TMZ + STD – trimetazidine and standard heart failure therapy.

DISCUSSION

Our study finds that TMZ+STD-mediated changes elicit additive effects to STD and enhance LVEF by regulating specific biomarkers associated with disease-driving mechanisms. In line with our findings, reports[2-7] show altered levels of visfatin, ADMA, NAD+, sirt1, and glutathione (GSH and GSSG) in HF. How could TMZ+STD influence circulating biomarkers to render cardioprotection? Potentially through TMZ-mediated sirt1-activation and sirt1-mediated events. TMZ is a sirt1 activator with anti-inflammatory and anti-oxidant effects[16]; selected biomarkers are associated with inflammation and OS, hence the observed TMZ+STD-mediated effects.

Following TMZ+STD-mediated sirt1-activation, multiple sirt1-mediated mechanisms/molecules, under excess OS5 and/or inflammation[17] could have influenced HF-associated pathophysiological events, such as myocardial remodelling[12], levels of ADMA14, oxidants and antioxidants. Among the biomarkers, visfatin and ADMA are negatively correlated with EF. ADMA is a well-studied molecule and studies show the direct influence of ADMA on cardiac structure and function.[8] However, visfatin is a complicated molecule18 with a disease-altering capacity. Reports on visfatin are controversial, be it pathophysiological or therapeutic mechanisms.[19] So far, under excess OS, cells secrete multi-fold levels of visfatin and plasma visfatin is associated with disease-driving mechanisms.[2] Hence, normalizing plasma visfatin concentration is considered for therapeutic benefits. As our observations on TMZ+STD show a significant negative correlation between visfatin and EF, we wanted to understand how TMZ could alter visfatin concentration, which in turn improves EF. We propose three pathophysiological events (Fig.2) that connect TMZ–circulating visfatin–EF.

Protein-protein interaction network

To identify visfatin (NAMPT) interacting partners, an in-silico prediction analysis was carried out using STRING-database.[20] Predicted protein-protein-interaction (PPI)-network (Fig.3) shows that sirt1 and NAD+-biosynthesis-associated proteins are binding partners of visfatin/NAMPT.

Excess OS alters PPI-association,[21] which causes protein structure modification (oligomer to dimer/monomer) and changes in the composition of PPI assembly. Further, changes in structure and PPI assembly in alpha-B-crystalline[22] promote the formation of dimers and monomers from oligomers and increase its activity. In HF, excess OS generates more oxidants and less sirt1,[5] which could modulate the visfatin/NAMPT-PPI network to facilitate

more monomer formation. Besides, sirt1 and visfatin are functionally interrelated[23] and sirt1-deficiency exists in HF.[5] Thus, visfatin/NAMPT-PPI-network allows us to suggest that in HF, excess OS shifts the catalytically-active dimeric visfatin to catalytically-inactive, pro-inflammatory monomer,[24] which alters enzyme activity/function of visfatin/NAMPT; modifies the affinity between interacting-proteins and perturbed composition of visfatin/NAMPT-PPI-network.

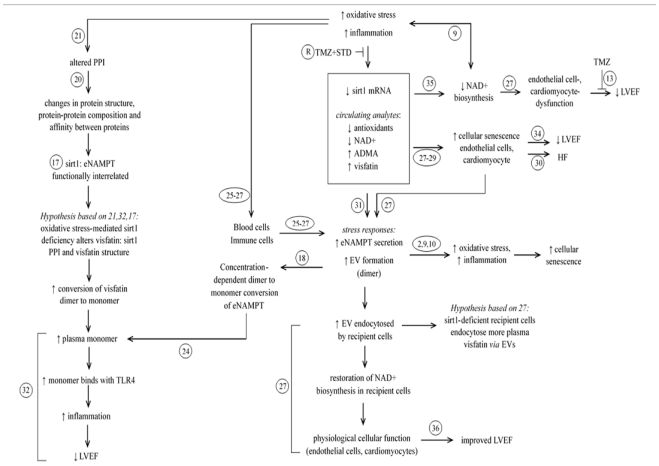
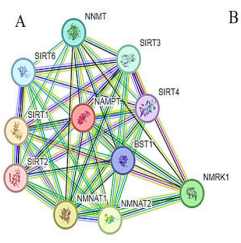


Figure 2. Potential pathophysiological mechanisms that link trimetazidine (TMZ) – circulating visfatin (eNAMPT) – left ventricular ejection fraction (LVEF) in heart failure. ADMA – asymmetric dimethylarginine, EV – extracellular vesicles, NAD+ - nicotinamide adenine dinucleotide, NAMPT – nicotinamide phosphoribosyltransferase, PPI – protein-protein-interaction, R – results from this study, sirt1 – sirtuin1, STD – standard HF therapy, TMZ – trimetazidine, TLR4 – toll-like receptor4, Numbers indicate the respective references.



B

	Predicted Functional Partners	Score
SIRT1	Sirtuin 1	0.993
NMNAT1	Nicotinamide/nicotinic acid mononucleotide adenylyltransferase 1	0.992
NMNAT2	Nicotinamide/nicotinic acid mononucleotide adenylyltransferase 2	0.976
NMRK1	Nicotinamide riboside kinase 1	0.971
SIRT3	Sirtuin 3	0.971
NNMT	Nicotinamide N-methyltransferase	0.966
SIRT6	Sirtuin 6	0.964
BST1	ADP-ribosyl cyclase/cyclic ADP-ribose hydrolase 2	0.960
SIRT4	Sirtuin 4	0.959
SIRT2	Sirtuin 2	0.958

Figure 3. In silico results show (A) human NAMPT (visfatin) protein-protein interaction network and (B) the confidence scores of predicted functional partners indicating the strength of true interaction, based on STRING database 2023.

If excess OS alters PPI assembly, could TMZ(+STD) counter such modifications? Our observation demonstrates that TMZ-mediated sirt1 up-regulation optimizes visfatin and sirt1. This could facilitate the formation of physiological visfatin/NAMPT-PPI-network and normal NAD+-biosynthesis, to improve EF.

Endocytosis

Excess OS and inflammation, besides causing sirt1-deficiency, trigger more visfatin secretion from several cells[25] (endothelial-, blood- immune-cells and cardiomyocytes), as a stress-compensatory mechanism. Hence, excess plasma visfatin is a stress-response effect, which suggests plasma visfatin could have physiological and pathological functions.[26] Dimeric visfatin, packed within extracellular vesicles, gets endocytosed by recipient cells to increase NAD+ in other tissues,[26] to prevent diabetes and to extend lifespan. Based on these studies, we propose that, in HF, where OS is up-regulated, more plasma visfatin facilitates sirt1-deficient cells to endocytose plasma visfatin (as extracellular vesicles) to normalize intracellular sirt1. This, in turn, increases cellular functions and improves EF. Here, TMZ+STD could address sirt1 deficiency by optimizing intracellular and circulating sirt1, to facilitate physiological cellular functions in cardiomyocytes and endothelial cells, to improve EF.

Prevention of the formation of senescent cells

In HF, more oxidants degrade sirt1 and NAD+; NAD+-depletion accelerates OS and CS;[27-29] and excess cardiomyocyte-senescence contributes to pathogenesis.[30] Sirt1-deficient-senescent-cells secrete dimeric visfatin/eNAMPT, implying, pathologically senescent-cells are sources of plasma visfatin/eNAMPT.[31] Secretory molecules/extracellular vesicles from senescent cells (non-myocytes) accelerate cardiomyocyte-senescence.[32] These reports suggest that OS-mediated sirt1-deficiency could be a stress-stimuli that triggers extracellular-vesicle formation and thus, elevated circulating visfatin. Hence, in HF, plausibly, more senescent cells contribute to increased plasma visfatin. Results show that TMZ+STD up-regulates sirt1. Sirt1-restoration could have prevented normal cells from becoming senescent, thus, leading to reduced plasma visfatin.

The presence of healthier cells, rather than senescent cells, could have improved myocardial function. One report shows that the conversion of dimeric visfatin to monomer is concentration-dependent. Plasma visfatin exists as a dimer at low physiological concentrations (~1ng/ml); at pathological concentrations (~5ng/ml), the dimer converts to monomer.[33] Results show that TMZ+STD reduced visfatin than STD. Based on these reports and our results, TMZ+STD could influence the rate of visfatin conversion, dimer to monomer. Attenuated pro-inflammatory monomeric visfatin could have improved EF in TMZ+STD.

Results demonstrate cytoprotective effects;

TMZ+STD reduces ADMA and improves antioxidants. Mitigated ADMA improves cardiac function[34] while augmented GSH restores cell death mechanisms.[7] Thus, TMZ+STD could improve EF through ADMA and antioxidants. Together, cumulative effects of TMZ+STD on biomarkers have altered OS, and pro-inflammatory- and CS mechanisms to render cardioprotection. The cross-sectional design precluded establishing causal relationships between TMZ + STD-mediated biomarker alterations and improvements in LVEF. The study had a small sample size, limiting the generalizability of the findings.

Hence, it should be considered a pilot study. Direct enzyme activity of circulating visfatin or sirt1 in peripheral blood cells was not measured. Instead, NAD+ concentrations (a surrogate marker for sirt1 activity) and sirt1 mRNA expression levels were assessed. The lack of healthy controls limited the ability to assess the baseline influence of HF on the studied biomarkers. These limitations emphasize the need for future longitudinal studies with larger cohorts, including healthy controls and patients in earlier stages of HF, are essential to evaluate TMZ's role in preventing disease progression and its impact on biomarkers such as asymmetric dimethylarginine (ADMA) and visfatin.

CONCLUSION

Adjunctive therapy with trimetazidine (TMZ) significantly improved ejection fraction compared to standard heart failure (HF) therapy alone, indicating its potential to enhance cardiac function by modulating key biomarkers in patients with heart failure with reduced ejection fraction (HFrEF). These findings highlight the potential of TMZ to target mechanisms related to oxidative stress, inflammation, and myocardial remodeling.

CONFLICT OF INTEREST

None

REFERENCES

1. Aimo A, Castiglione V, Borrelli C, Saccaro LF, Franzini M, Masi S, et al. Oxidative stress and inflammation in the evolution of heart failure: From pathophysiology to therapeutic strategies. Eur J Prev Cardiol. 2020 Mar;27(5):494-510.

2. Beiras-Fernandez A, Reinwand S, Kreth S, A. Beiras, A. Moritz, I. Werner. Modulation of Nesfatin, Visfatin and Resistin in Patients Undergoing Heart Transplantation. J Hear Lung Transplant 2016 Apr;35:S189.

3. Dückelmann C, Mittermayer F, Haider DG, Altenberger J, Eichinger J, Wolzt M. Asymmetric-dimethylarginine enhances cardiovascular risk prediction in patients with chronic heart failure. Arterioscler Thromb Vasc Biol. 2007 Sep;27(9):2037-42.

4. Wang DD, Airhart SE, Zhou B, Shireman LM, Jiang S, Melendez-Rodriguez C, et al. Safety and Tolerability of Nicotinamide Riboside in Heart-Failure With Reduced Ejection Fraction. JACC Basic Transl Sci. 2022 Sep 14;7(12):1183-1196.

5. Akkafa F, Halil-Altiparmak I, Erkus ME, Aksoy N, Kaya C, Ozer A, et al. Reduced SIRT1 expression correlates with enhanced oxidative stress in compensated and decompensated heart failure. Redox Biol. 2015 Dec;6:169-173.

6. Campolo J, De-Maria R, Caruso R, Accinni R, Turazza F, Parolini M, et al. Blood glutathione as independent marker of lipid peroxidation in heart failure. Int J Cardiol. 2007 Apr 12;117(1):45-50.

7. Tan M, Yin Y, Ma X, Zhang J, Pan W, Tan M, et al. Glutathione system enhancement for cardiac protection: pharmacological options against oxidative stress and ferroptosis. Cell Death Dis. 2023 Feb 16;14(2):131.

8. Guo X, Xing Y, Jin W. Role of ADMA in the pathogenesis of microvascular complications in type-2-diabetes-mellitus. Front Endocrinol (Lausanne). 2023 Apr 21;14:1183586.

9. Romacho T, Valencia I, Ramos-González M, Vallejo S, López-Esteban M, Lorenzo O, et al. Visfatin/eNampt induces endothelial-dysfunction in vivo: a role for Toll-Like Receptor 4 and NLRP3 inflammasome. Sci Rep. 2020 Mar 25;10(1):5386.

10. Peiró C, Romacho T, Carraro R, Sánchez-Ferrer CF. Visfatin/PBEF/Nampt: A New Cardiovascular Target? Front Pharmacol. 2010 Nov 23;1:135.

11. Robson A. Restoration of NAD+ levels as a therapy for HFpEF. Nat Rev Cardiol. 2021 May;18(5):307.

12. Wang Y, Zhao R, Wu C, Liang X, He L, Wang L, et al. Activation of the sirtuin silent information regulator-1 pathway inhibits pathological myocardial remodeling. Front

Pharmacol. 2023 Feb 10;14:1111320.

13. Fragasso G, Palloshi A, Puccetti P, Silipigni C, Rossodivita A, Pala M, et al. A randomized clinical trial of trimetazidine, a partial free fatty-acid-oxidation-inhibitor, in patients with heart failure. J Am Coll Cardiol. 2006 Sep 5;48(5):992-8.

14. Raj V, Natarajan S, Marimuthu C, Chatterjee S, Ramasamy M, Ramanujam GM, et al. Cholecalciferol and metformin protect against lipopolysaccharide-induced endothelial dysfunction and senescence by modulating sirtuin-1 and protein-arginine-methyltransferase-1. Eur J Pharmacol. 2021 Dec 5;912:174531.

15. Yap LP, Sancheti H, Ybanez MD, Garcia J, Cadenas E, Han D. Determination of GSH, GSSG, and GSNO using HPLC with electrochemical detection. Methods Enzymol. 2010;473:137-47.

16. Chen J, Lai J, Yang L, Ruan G, Chaugai S, Ning Q, et al. Trimetazidine prevents macrophage-mediated septic myocardial dysfunction via activation of the histone deacetylase sirtuin-1. Br J Pharmacol. 2016 Feb;173(3):545-61.

17. Hwang JW, Yao H, Caito S, Sundar IK, Rahman I. Redox regulation of SIRT1 in inflammation and cellular senescence. Free Radic Biol Med. 2013 Aug;61:95-110.

18. Imai SI. The NAD World 2.0: the importance of the inter-tissue communication mediated by NAMPT/NAD+/SIRT1 in mammalian aging and longevity control. NPJ Syst Biol Appl. 2016 Aug 18;2:16018.

19. Uma-Maheswari G, Yamini B, Guru A, Velayutham M, Dhandapani VE, Karuppiah KM, et al. Trimetazidine with an adjuvant-therapy to normalize the circulating visfatin concentration: Future perspective and mechanistic strategies. J King Saud Univ - Sci 2023 Dec;35:102950.

20. Szklarczyk D, Kirsch R, Koutrouli M, Nastou K, Mehryary F, Hachilif R, et al. The STRING database in 2023: protein-protein-association networks and functional enrichment analyses for any sequenced genome of interest. Nucleic Acids Res. 2023 Jan 6;51(D1): D638-D646.

21. Sharma C, Kim SR. Linking Oxidative-Stress and Proteinopathy in Alzheimer's Disease. Antioxidants (Basel). 2021 Jul 30;10(8):1231.

22. Eronina TB, Mikhaylova VV, Chebotareva NA, Tugaeva KV, Kurganov BI. Effect of Betaine and Arginine on Interaction of αB-Crystallin with Glycogen Phosphorylase-b. Int J Mol Sci. 2022 Mar 30;23(7):3816.

23. Imai S, Yoshino J. The importance of NAMPT/NAD/SIRT1 in the systemic regulation of metabolism and ageing. Diabetes Obes Metab. 2013 Sep;15 Suppl 3(0 3):26-33.

24. Camp SM, Ceco E, Evenoski CL, Danilov SM, Zhou T, Chiang ET, et al. Unique Toll-Like Receptor 4 Activation by NAMPT/PBEF Induces NFκB-Signaling and Inflammatory Lung Injury. Sci Rep. 2015 Aug 14;5:13135.

25. Di-Stefano M, Galassi L, Magni G. Unique expression pattern of human nicotinamide-mononucleotide-adenylyltransferase isozymes in red blood cells. Blood Cells Mol Dis. 2010 Jun 15;45(1):33-9.

26. Grolla AA, Travelli C, Genazzani AA, Sethi JK. Extracellular nicotinamide-phosphoribosyltransferase, a new cancer metabokine. Br J Pharmacol. 2016 Jul;173(14):2182-94.

27. Kuehnemann C, Hu KQ, Butera K, Patel SK, Bons J, Schilling B, et al. Extracellular Nicotinamide-Phosphoribosyltransferase Is a Component of the Senescence-Associated-Secretory-Phenotype. Front Endocrinol. 2022 Jul 14;13:935106.

28. Massudi H, Grant R, Guillemin GJ, Braidy N. NAD+ metabolism and oxidative stress: the golden nucleotide on a crown of thorns. Redox Rep. 2012;17(1):28-46.

29. Alegre GFS, Pastore GM. NAD+ Precursors Nicotinamide-Mononucleotide (NMN) and Nicotinamide-Riboside (NR): Potential Dietary Contribution to Health. Curr Nutr Rep. 2023 Sep;12(3):445-464.

30. Evangelou K, Vasileiou PVS, Papaspyropoulos A, Hazapis O, Petty R, Demaria M, et al. Cellular-senescence and cardiovascular diseases: moving to the "heart" of the problem. Physiol Rev. 2023 Jan 1;103(1):609-647.

31. Han L, Long Q, Li S, Xu Q, Zhang B, Dou X, et al. Senescent

Stromal Cells Promote Cancer Resistance through SIRT1 Loss-Potentiated Overproduction of Small Extracellular-Vesicles. Cancer Res. 2020 Aug 15;80(16):3383-3398.

32. Sayers SR, Beavil RL, Fine NHF, Huang GC, Choudhary P, Pacholarz KJ, et al. Structure-functional changes in eNAMPT at high concentrations mediate mouse and human beta-cell dysfunction in type-2 diabetes. Diabetologia. 2020 Feb;63(2):313-323.

33. Achan V, Broadhead M, Malaki M, Whitley G, Leiper J, MacAllister R, et al. Asymmetric-dimethylarginine causes hypertension and cardiac dysfunction in humans and is actively metabolized by dimethylarginine-dimethylaminohydrolase. Arterioscler Thromb Vasc Biol. 2003 Aug 1;23(8):1455-9.

34. Redgrave RE, Dookun E, Booth LK, Camacho-Encina M, Folaranmi O, Tual-Chalot S, et al. Senescent cardiomyocytes contribute to cardiac dysfunction following myocardial infarction. NPJ Aging. 2023 Jun 14;9(1):15.

35. Imai SI, Guarente L. It takes two to tango: NAD+ and sirtuins in aging/longevity control. NPJ Aging Mech Dis. 2016 Aug 18;2:16017.

36. Chen MS, Lee RT, Garbern JC. Senescence mechanisms and targets in the heart. Cardiovasc Res. 2022 Mar 25;118(5):1173-1187.