Original Article

The Role of Gliclazide on Galectin-3 Expression in Individuals with Left Ventricular Diastolic Dysfunction and Type 2 Diabetes Mellitus

Tharaa Thaer A. Alaziz and Bassim I. Mohammad

ABSTRACT

Objective: To investigate the role of the antidiabetic medication gliclazide on the galectin-3 expression, a biomarker associated with cardiac fibrosis and diastolic dysfunction, in those suffering from diastolic dysfunction of the left ventricle and type 2 diabetes.

Study Design: Cross-section study

Place and Duration of Study: This study was conducted at the Al-Diwaniyah Teaching Hospital in Diwaniyah, Iraq from 1st February 2023 to 31st December 2023.

Methods: A total of 62 patients with type 2 diabetes mellitus and left ventricular diastolic dysfunction were enrolled. Two groups were divided; one for gliclazide therapy and the other for control. Gliclazide group was given standard treatment for type 2 diabetes mellitus plus gliclazide while the control group received standard treatment alone.

Results: Galectin-3 expression was measured at baseline of treatment using quantitative real-time polymerase chain reaction (qRT-PCR) .The results showed that gliclazide treatment significantly increase in galectin-3 expression relative to the group under control. Galectin-3 levels have decreased levels correlated with improvements in left ventricular diastolic dysfunction parameters, including left ventricular filling pressure and diastolic function. Additionally, gliclazide treatment was associated with improved glycemic control, as evidenced by reduced HbA1c levels.

Conclusion: Gliclazide may have no beneficial effect on left ventricular diastolic dysfunction in patients with type 2 diabetes mellitus by modulating galectin-3 expression. The upregulation of galectin-3 may contribute to the deterioration in diastolic function observed with gliclazide treatment. It is necessary to do additional research to clarify the underlying mechanisms and validate these findings in a larger patient group.

Key Words: Gliclazide, Metformin, Left ventricular diastolic dysfunction, Type 2 diabetes mellitus, Galectin-3

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INTRODUCTION

Myocardial fibrosis (MF) is highly correlated with increased left ventricular (LV) chamber stiffness and reduced LV relaxation, which result in left ventricular diastolic dysfunction (LVDD). Heart failure with preserved ejection fraction, or HFpEF, is thought to have its precursor in LVDD.

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Adverse myocardial remodeling brought on by MF at the late stage of LVDD results in HFpEF. Reversing the poor left ventricular diastolic function (LVDF) in patients with HFpEF is challenging. Adverse cardiac events, such as newly acquired myocardial ischemia, arrhythmia, myocardial infarction (MI), angina pectoris, and heart failure (HF), are linked to higher all-cause mortality when LVDF is low.[1]

Chronic metabolic disease known as diabetes mellitus (DM) is typified by ongoing hyperglycemia. It could be brought on by decreased insulin secretion, resistance to insulin's peripheral effects, or both. Together with other metabolic abnormalities in individuals with diabetes mellitus, persistent hyperglycemia can harm multiple organ systems, resulting in the development of debilitating and potentially fatal health complications. Microvascular complications, such as retinopathy, nephropathy, and neuropathy, are the most common, while macrovascular complications raise the risk of cardiovascular diseases by two to four times.[2]

Type 1 diabetes, Type 2 diabetes, and gestational diabetes are the most prevalent types of the disease. The hallmarks of type 2 diabetes (T2DM) are insulin resistance and a relative lack of insulin production.[3] Secondary diabetes and monogenic diabetes are two more, less prevalent forms of the disease.[2]

A1c ≥6.5%, fasting glucose ≥126 mg/dL, glucose of ≥200 mg/dL two hours after a 75 gm glucose load, or random glucose ≥200 mg/dL with symptoms, all support the diagnosis and can be verified by a follow-up or second test.[4]

Atherosclerotic cardiovascular disease (ASCVD) is linked to diabetes mellitus (DM), and reducing risk requires statin use, blood pressure medication, regular exercise, and quitting smoking. Although it varies greatly, people with T2DM have an overall 15% higher excess death rate. About 4.4% of adult diabetics in the US have vision-threatening diabetic retinopathy, compared to 1% of those with end-stage renal disease. These days, vascular complications can be effectively managed to reduce morbidity and mortality. Pharmacotherapy for hyperglycemia, as well as ACE/ARB therapy for controlling blood pressure, lowering LDL cholesterol, and secondary prevention with aspirin, cans all help.[2,6]

The β-galactoside-binding lectins are known as galectins. Nearly all forms of cardiovascular illness have been linked to elevated serum galectin-3 levels, and patient predictive research on this protein's significance for various clinical outcomes has been done in great detail. The fibrosis and inflammatory biomarker galectin-3 has been linked to the onset and development of heart failure (HF) and has the potential to predict higher rates of morbidity and death. Increased expression levels of galectin-3 have been linked to mortality in both acute and chronic heart failure.[5]

This paper aims to investigate the effect of gliclazide, an oral sulfonylurea, on LVDD in T2DM patients by targeting galectin-3 and compare it with the standard treatment metformin.

METHODS

The Al-Diwaniyah Teaching Hospital in Diwaniyah, Iraq, served as the site of this cross-sectional study for the period 1st February 2023 to 31st December 2023. The patients diagnosed with T2DM and LVDD, aged between45 and 65 years, 36 of the participants were female and 26 of them male who are receiving standard care for diabetes management. The patients were divided into two groups at random: the gliclazide group and the metformin group. The gliclazide group received gliclazide as an add-on therapy to their existing antidiabetic regimen, while the metformin group continued their standard treatment taking gliclazide 90mg and gliclazide + metformine (90/1000) for at least six months. All patients have T2DM, HbA1C (6.5-9), age 45-65 years, onset of diseases >3 years and onset of current therapy at least 6month were included. Patients having HbA1C >9, renal impairment (eGFR <30 ml/min/1.73m^2 of body surface area) or diaylasis, hepatic impairment, pregnancy, overt heart failure, EF which is less than 50 and psychiatric patients were excluded.

Baseline demographic characteristics, clinical data, assessment of body mass index (weight of the patients included in the study is measured by electronic scale in kg. The height is measured by height scale in m²). The blood samples with 1 ml were collected from the patients that were aspirated from antecubital vein was placed in 1 ml EDTA tube, which is then kept cold until the moment of DNA extraction.Galectin-3 levels were measured at baseline in the beginning RNA extraction then RNA concentration measurement by Quantus™ Fluorometer (Promega, USA) Subsequently, the whole RNA was converted to cDNA by utilizing the ADDBio (Korea) kit. by adding H2O (6µl) to Reverse transcriptase (RT) Add two times as much cDNA (20µl), dNTPs (4µl), random oligos hexamer (2µl), and RNA (8µl) to the script; the total amount is 40µl. The expression of Galectin-3 gene was measured using the comparative Ct method (ΔΔCt), normalized to the control group level when GAPDH mRNA transcript levels were present. This was accomplished in accordance with the suggestion made by Schmidtgen and Livak.[7] Increasing the strength of the Galectin-3 gene was carried out using the following primers employed by Papaspyridonos et al.[8]

Gene of Interest (Galectin-3) Sequence (5'->3') Gal3-Forward Template strand (5'……3') TGCAGT-GAATGATGCTCACTTG and Gal3-Reverse the template strand CAGAAATTCCCAGTTTGCTGATT . Housekeeping gene (HKG) or internal reference gene; human glyceraldehyde 3-phosphate dehydrogenase Sequence (5'->3') GAPDH-F template strand CAGAACATCATCCCTGCCTCTA and GAPDH-R Template strand (5'……3') CCAGTGAGCTTCCC-GTTCA. Preparing Reverse Transcriptase PCR Quantitatively (RT-qPCR) The RT-qPCR amplification was first accomplished with AddScript RT-qPCR Syber master (AddBio, Korea). The reaction consisted of adding H2O (4 µl) to AddScript RT-qPCR (10 µl), as well as Forward and Reverse primers (0.05 pmol/20 µl and 2 µl, respectively), cDNA (2 µl), and a total of 20 µl. Normalization of RT-qPCR data: Transcript levels were adjusted to those of using the delta-delta Ct technique. GAPDHmRNA[7], wherein the subsequent formula was utilized: 2-ΔΔCT = [((internal control-CT gene of interest) sample A-(internal control-CT gene of interest) sample B)]. Note that sample A refers to a single group and sample B refers to a different specific group. The graph pad prizim software-8.4.3 was applied to the analysis of data. Each set of data was displayed as mean #standard deviation (SD).and a difference with

P values less than 0.05 were regarded as statistically significant.

RESULTS

After the intervention period, there is a statistical difference between the two groups, The gliclazide group exhibited a significant rise in galectin-3 levels when compared to the metformin group (p less than 0.0001) .Significantly different ($P < 0.05$), as shown in table 1 and 2. Mean for metformin 1.541 and 13.96 for Gliclazide with Standard deviation for metformin is 1.596 and 2.788 for gliclazide. As shown the successful amplification curves with corresponding crossing threshold (CT) as the number of cycles with the round forming unit (RFU) (Figs.1-2).

This demonstrates a notable increase in Galectin-3 expression in the gliclazide treated group ($P<0.0001$) in comparison with metformine treatment. This was analyzed by Graph pad prizim software (version 8.4.3). Descriptive statistics in the galectin-3 expression in gliclazide and metformine treated groups (Fig. 3). Patients taking metformin has lower effect on heart grading than those taking Gliclazide (Table 1). The results show variations in patients taking metformin has higher effect in relation to RBS, sex and age, than those taking Gliclazide while Gliclazide patients grouping has higher effect in relation to BMI (Table 2).

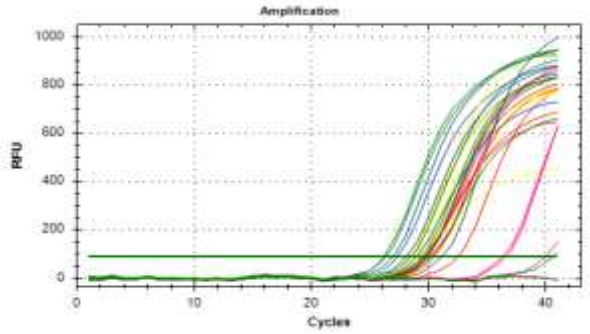

Figure No. 1: Amplification curve of the tested samples for expression of gene of interest (Galectin-3) in the gliclazide group

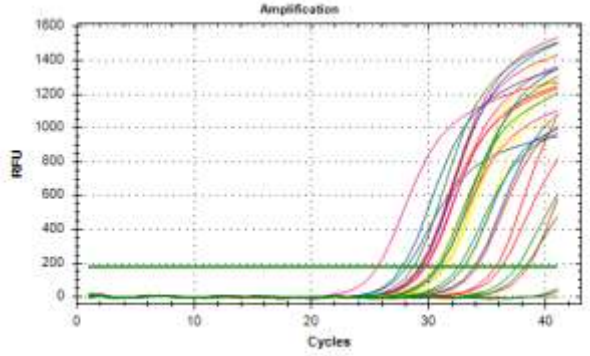

Figure No. 2: Amplification curve of the tested samples for expression of gene of interest (Galectin-3) in the metformine group

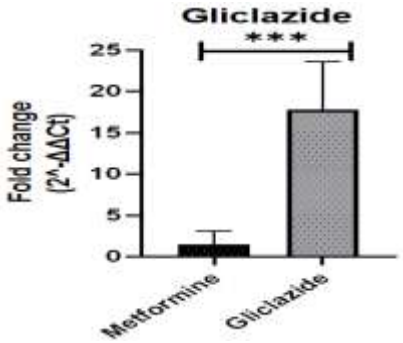

Figure No. 3: Gene expression of Galectin-3 in the Gliclazide

Table No.1: Demonstrate Ranks of metformin and gliclazide (n=62)

Drug	No.	Mean Rank	Sum of Ranks
Metformin	30	37.00	1110.0
Gliclazide	32	26.34	843.0

Table No.2: Demonstrate Group statistics of age, sex,BMI and RBS

Variable	Drug	Mean±SD
Age	Metformin	59.70±10.87
	Gliclazide	50.56±13.48
Sex	Metformin	1.63±0.49
	Gliclazide	1.53±0.507
BMI	Metformin	29.58±4.97
	Gliclazide	31.67±5.94
RBS (md/dl)	Metformin	198.60±91.85
	Gliclazide	181.81±80.99

DISCUSSION

In the present study metformin treatment showed significantly higher reduction in Gal-3 concentration indicate its potential as an adjunct therapy targeting LVDD compared to Gliclazide therapy in addition to standard of care group. Other study reported same findings on other treatments done by Ates et al.[9] The use of simvastatin reduced TBARS levels. By suppressing the effects of ROS and upregulating antioxidant enzymes, which block free radicals, simvastatin may prevent the production of oxidants.[6]

The majority of inflammatory cells produce galectin-3, and the degree of this expression varies in response to both internal and external stimuli. The acute inflammatory response involves several processes that are significantly influenced by galectin-3, including neutrophil adhesion and activation[10], monocyte or macrophage chemoattraction[11], and opsonization of apoptotic neutrophils. Streptococcus pneumoniae membrane fraction treatment was demonstrated to cause alveolar macrophages to produce Galectin-3.[12] Henderson and Sethi[13] discovered that Galectin-3 was stimulated to be expressed and released upon macrophage activation via IL-4 and IL-13. The current

investigation found that rats treated with lipopolysaccharide (LPS) had higher levels of Galectin-3 expression in their alveolar macrophages. But in rats given LPS, injections of simvastatin reduced its expression. Their immune response patterns resembled those of the control group. Simvastatin thereby alleviated the acute inflammatory alterations that took place in the animals' lungs after receiving an LPS injection.

In several investigations, researchers demonstrated that Galectin-3 increased vascular inflammation by causing macrophages to express pro-inflammatory products.[11] Additionally, it may regulate inflammation through a variety of methods. The increased survival of inflammatory cells caused by the production of Galectin-3 may also worsen inflammation.[14] The lung tissue's bronchial epithelium and pneumocytes both showed an increase in Galectin-3 immuno re-activities in the LPS group. When statins were administered during mouse atherosclerosis, both the amount of plaque macrophages and plaque alectin-3 expression were decreased.[15] Prior simvastatin dosing reduced the densities and quantity of immunoreactive Galectin-3 in lung tissue's bronchial epithelium and pneumocytes.

This paper found that simvastatin administration protected against septic ALl In ALI, lowering the levels of galectin-3 might work as an internal compensatory anti-inflammatory strategy. Other study by Sygitowicz et al.[16] The sulphated or acetylated heparin derivatives known as heparin-based inhibitors represent a relatively novel and appealing class of galectin-3 inhibitors. Studies conducted in vitro have shown that they are selective for galectin-3 and non-cytotoxic (i.e., they do not block galectin-1, -4, or -8). Experimental in vivo experiments using nude mice showed that chemicals produced by galectin-3 markedly reduced human melanoma and colonic cancer cells' ability to metastasize to the lungs. The substances also appeared to be prospective therapeutic agents despite having no discernible anti-thrombotic action.[17]

Regarding gliclazide, its primary mechanism of action is to stimulate insulin secretion from pancreatic beta cells, thereby reducing blood glucose levels. It primarily acts by binding to the pancreatic beta cells' sulfonylurea receptors, which causes the ATP-sensitive potassium channels to close, the cell membrane to depolarize, and then calcium to influx, which causes the release of insulin. As for the specific interaction between gliclazide and galectin-3, the available literature is limited. While gliclazide's primary role is to control blood glucose levels in patients with type 2 DM, some studies have suggested that sulfonylureas may have pleiotropic effects beyond glycemic control. For example, sulfonylureas have been reported to have potential anti-inflammatory and antioxidant properties, which could have implications for cardiovascular diseases.[18]

In the context of LVDD, there is limited direct evidence on the effects of gliclazide specifically on galectin-3 levels or LVDD outcomes. The majority of studies on gliclazide have focused on its glycemic control effects and its impact on cardiovascular outcomes in patients with diabetes.

CONCLUSION

Gliclazide, through its effect on Galectin-3, may have no any beneficial impact on LVDD in patients with T2DM. The increase in galectin-3 levels indicates it has no potential as an adjunct therapy targeting LVDD. To confirm these results and investigate the further implications, more research with bigger sample sizes and longer follow-up times is required underlying mechanisms in more detail**.**

Author's Contribution:

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Final Approval of version:	By all above authors

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