Impact of Glimepiride and Metformin on the Biomarker miR-150-5p in Patients with Type 2 Diabetes and Left Ventricular Diastolic Dysfunction

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ABSTRACT

Background: Type-2 diabetes mellitus (T2DM) is a significant contributor to cardiovascular risk, with diabetic individuals having double the mortality rate compared to those without diabetes. Among the various early subclinical cardiac problems in type 2 diabetes, left ventricular diastolic dysfunction (LVDD) is the most easily identifiable impairment. Nevertheless, only a limited number of biomarkers can accurately forecast this group's cardiovascular events. Levels of miR-150-5p are reduced in patients with type 2 diabetes mellitus (T2DM), regardless of whether they have cardiovascular disease. Our findings also demonstrate a decrease in miR-150-5p levels in these patients. Lower levels of miR-150-5p in the blood have been observed in nearly all forms of cardiovascular disease, and its potential to predict various clinical outcomes has been extensively studied in patients.

Objective: To assess the effect of glimepride (Glm) and Metformin on cardiac parameter miR-150-5p in patients with type 2 diabetes and diastolic dysfunction.

Patients and methods: In a cross-sectional study that included 60 patients with type 2 diabetes and diastolic dysfunction in an age range of 45 to 65, the patients were divided into two groups: group G1 (received standard treatment for T2DM with Metformin 1g×2 max daily dose) and group G2 (received standard treatment for T2DM with Metformin + Glimepiride 4mg daily).

Results: Treatment with glimepride significantly increased miR-150-5p expression in patients with type 2 diabetes mellitus (T2DM) and left ventricular diastolic dysfunction (LVDD), according to this study. The rise was more pronounced in patients who were given Glimepiride and standard medical treatment.

Conclusions: Glimepiride plays an important role in improving cardiac biomarkers like miR-150-5p. Glimepiride use in diabetic patients with the addition of Metformin to usual diabetes type 2 treatment may benefit patients with cardiac complications.

Keywords: Glimepride, Metformin, left ventricular diastolic dysfunction, type2 diabetes mellitus, miR-150-5p.

تأثير جليميبيرايد والميتفورمين على العلامة الحيوية miR-150-5p في المرضى الذين يعانون من مرض السكري النوع ٢ والخلل الانبساطي للبطين الايسر

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الخلاصة

الخلفية : يعد داء السكري من النوع الثاني مساهمًا كبيرًا في مخاطر الإصابة بأمراض القلب والأوعية الدموية، حيث يكون الأفراد المصابون بالسكري معرضون للخطر مقارنة بأولئك الذين لا يعانون من مرض السكري. من بين مشاكل القلب المختلفة المبكرة في مرض السكري من النوع ٢ هو الخلل الوظيفي الانبساطي للبطين الايسر وهو الخلل الأكثر سهولة في التعرف عليه ,ومع ذلك لا يوجد سوى عدد محدود من المؤشرات الحيوية التي يمكنها التنبؤ بدقة بأحداث القلب والأوعية الدموية في هذه المجموعة بالذات. تقل مستويات miR-150-5p لدى الاشخاص الذين يعانون من داء السكري من النوع الثاني بغض النظر عما اذا كانوا يعانون من امراض القلب والاوعية الدموية. تظهر النتائج التي توصلنا اليها ايضا انخفاض في مستويات miR-150-5p لدى هؤلاء المرضى. وقد لوحظت مستويات منخفضة من miR-150-5p في الدم في جميع اشكال امراض القلب والاوعية الدموية تقريبا , وقد تمت دراسة قدرته على التنبؤ بالنتائج السريرية المختلفة على نطاق واسع لدى المرضى.

الهدف : تقييم تأثير الجليميبيرايد والميتفورمين على المؤشر القلبي miR-150-5p في المرضى الذين يعانون من مرض السكري من النوع الثاني والخلل الانبساطي.
طريقة أجراء الدراسة : دراسة مقطعية شملت ٦٠ مريضًا يعانون من مرض السكري من النوع ٢ والخلل الانبساطي في الفئة العمرية من ٤٥ إلى ٦٥ عامًا , تم تقسيم المرضى الى مجموعتين : المجموعة الاولى (تلقت علاجاً قياسياً للسكري النوع الثاني باستخدام الميتفورمين ١جرام × ٢) الحد الاقصى للجرعة اليومية
اما المجموعة الثانية (تلقت علاجاً قياسياً للسكري النوع الثاني باستخدام الميتفورمين + جليميبيرايد ٤ ملغ يومياً)
النتائج : ادى العلاج باستخدام جليميبيرايد الى زيادة كبيرة في التعبير miR-150-5p في المرضى الذين يعانون من داء السكري النوع الثاني والخلل الوظيفي الانبساطي للبطين الايسر وفقاً لهذه الدراسة, وكان الارتفاع اكثر وضوحاً في المرضى الذين تلقوا جليميبيرايد جنباً الى جنب مع العلاج الطبي القياسي.
الاستنتاجات : يلعب جليميبيرايد دوراً مهماً في تحسين المؤشرات الحيوية للقلب مثل miR-150-5p , وأن استخدام جليميبيرايد في مرضى السكري مع اضافة الميتفورمين الى العلاج المعتاد لمرض السكري النوع الثاني قد يفيد المرضى الذين يعانون من مضاعفات القلب.

الكلمات المفتاحية : جليميبيرايد , ميتفورمين , الخلل الوظيفي الانبساطي للبطين الايسر , داء السكري نوع ٢ , miR-150-5p.

INTRODUCTION

Diabetes Mellitus (T2DM) is a metabolic disorder that affects many people throughout the world. It is mainly caused by two main factors: a decrease in insulin production by pancreatic β-cells and a decrease in the responsiveness of insulin-sensitive tissues to insulin[1]. Approximately 6.28 percent of the world's population, or 462 million people, have type 2 diabetes. Over a million people died from this disease in 2017, making it the eighth leading killer[2].

The high mortality and morbidity rates, as well as the financial burden it places on healthcare systems around the world, make type 2 diabetes (T2D) an important concern in public health. The growing worldwide burden is accentuated by the rising incidence of chronic diseases such as obesity, Hypertension, and cardiovascular disease in both industrialized and developing countries, with type 2 diabetes (T2D) operating as a prevalent comorbidity[3].

Diastolic dysfunction refers to an anomaly in the left ventricle's relaxation and flexibility (LV) that affects the timing, speed, and degree of pressure decrease and filling of the LV during the diastolic phase. These alterations result in an atypical correlation between left ventricular pressure and volume, necessitating higher filling pressures to sustain normal left ventricular end-diastolic volume and cardiac output.

LVDD serves as a significant precursor to several cardiovascular illnesses. The dominant mechanism (accounting for 2/3 of patients) in the development of heart failure (HF) with preserved ejection fraction (HFpEF) is represented by this. HFpEF is increasingly prevalent in the older population, with an estimated 8% of people over 65 projected to have HFpEF by 2020. This condition is linked to a poor prognosis[4].

It is critical to identify the risk factors for left ventricular diastolic dysfunction and early-stage diabetic cardiomyopathy to delay or prevent the onset of heart failure in individuals with type 2 diabetes. This is of paramount importance, given the prevalence and severity of this illness. Whether or not a person has diabetes, age, Hypertension, or ischemic heart disease (IHD) is known to raise the risk of diastolic dysfunction[5-7].

Short, naturally occurring RNA molecules with a length of about 22 nucleotides, microRNAs (miRNAs) do not code for any genes.

These molecules can suppress gene expression during the post-transcriptional phase by attaching to certain messenger RNA (mRNA) molecules in their 3'-untranslated region (3'-UTR).

The mRNA is either degraded or its protein synthesis is inhibited due to this connection[8,9]. The given information is unclear and disjointed. It is important to remember that many miRNAs can target the same mRNA sequence and that one miRNA can target many mRNA sequences[10]. MicroRNAs specifically target oncogenes and tumor suppressor genes to regulate a wide range of biological processes, such as cell differentiation, proliferation, apoptosis, signal transduction, carcinogenesis, and cancer progression. There is no cohesion, and the user's writing is unclear[11,12]. Also, in some cancers, miRNAs can serve as diagnostic and prognostic biomarkers and therapeutic intervention targets[13,14]. Through its effects on oncogenes and tumor suppressor genes, miR-150 has been linked in multiple studies to tumor growth, cancer progression, aggressive behavior, and therapeutic benefits[15-17].

The role of miR-150 extends beyond hematological cancers to include a number of solid tumors, such as those of the breast, lungs, and stomach.

Natural microRNAs are a small subset of RNA molecules that do not encode proteins; they are about 22 nucleotides long. These molecules can act as post-transcriptional negative regulators of gene expression by binding to specific messenger RNA (mRNA) molecules in their 3'-untranslated region (3'-UTR). Because of this connection, the messenger RNA is degraded, or its translation is impeded[8,9]. Clarity and significance are lacking in the provided input. Remember that a single microRNA can have several mRNA targets[10], and numerous miRNAs can target the same mRNA sequence. MicroRNAs (miRNAs) regulate various biological processes targeting particular genes like tumor suppressors and oncogenes, including cell proliferation, differentiation, apoptosis, signal transduction, carcinogenesis, and cancer progression. Cohesion and clarity are absent in the user's writing[11,12].

On top of that, microRNAs can potentially be diagnostic and prognostic biomarkers in addition to therapeutic targets for a range of malignant disorders[13,14]. Many studies have shown that abnormal miR-150 expression is associated with tumor formation, cancer advancement, aggressive behavior, and possible therapeutic effects by influencing oncogenes and tumor suppressor genes[15-17]. There are a variety of solid tumors that involve miR-150, including hematological malignancies, stomach cancer, lung cancer, and breast cancer.

AIM OF THE STUDY

To assess the effect of glimepride (Glm) and Metformin on cardiac parameter miR-150-5p in patients with type 2 diabetes and diastolic dysfunction.

PATIENTS AND METHODS

Study Design

A cross-sectional study involved 60 patients aged between 45 and 65 years. These patients' information was collected from the file sheet in the inpatient ward; these patients were previously treated for type 2 diabetes mellitus. Each patient fulfilled the inclusion criteria, and the patients were contacted, and written informed consent was obtained from them. Patients were divided into group G1 (received standard treatment for T2DM with Metformin 1g×2 max daily dose) and group G2 (received standard treatment for T2DM with Metformin+Glimepride 4mg daily). All patients' variables collected include. These variables include age, gender, body mass index (BMI), glycated hemoglobin (HbA1c), creatinine, urea, cholesterol, CRP, and Triglycerides.

Study Settings

The patient's data was gathered from the inpatient ward of Al-Diwaniyah Teaching Hospital in Al-Diwaniyah Province, Iraq. The data files collected information from September 1, 2023, to March 1, 2024.

Inclusion Criteria

T2DM, HbA1C (6.5-9), Age 45-65 years, Onset of Diseases >3 years, Onset of current Therapy > 6 months.

Exclusion Criteria

HbA1C > (9.5), Renal (eGFR < 30 ml/min/1.73m2 of body surface area) or end-stage renal failure or dialysis, hepatic impairment, Pregnancy, Heart failure, Arrhythmia AF.

Laboratory Analysis

The patients' Names, ages, genders, weights, heights, BMIs, comorbidities, smoking, and LLVD were taken. Biochemical parameters were measured for all patients with Glimepride: HbA1C, FBS urea, creatinine, C-reactive protein, Cholesterol, Triglyceride, and Magnesium.

Blood samples of 4 ml were collected from the patients that were aspirated from an antecubital vein and divided into two portions:

The patient's complete blood was drawn into a tube containing EDTA for DNA extraction, and one milliliter (ml) of the blood was kept at -20 C until the moment of DNA extraction.

❖Three milliliters (ml) of the patient's whole blood were collected in a gel tube, spun at 5,000 revolutions per minute for five minutes, and the serum was collected for biochemical tests.

Demographics Assessment

Assessment of Body Mass Index BMI

The study's subjects' weight is expressed in kilograms using an electronic scale. The body mass index (BMI) was computed as weight/height2 (kg/m2). Height is determined using the height scale in m2.

RNA Extraction

Quantitative Reverse Transcription Real-Time PCR (RT-qPCR)

RNA extraction: After adding 600 µl of Triazol to a 1.5 ml micro-centrifuge tube, each 200 µl whole blood sample was lysed and allowed to sit at room temperature for five minutes. The mixture was centrifuged for two minutes at a rate of 13,000 revolutions per minute. At the same time, 600 µl of ethanol was added, and the liquid portion above the sediment was carefully transferred into a new

tube. Next, a vortex was used to mix the resultant slurry thoroughly. The lysate was carefully added to the spin column's upper reservoir in preparation for RNA binding. After that, it was centrifuged at 13,000 revolutions per minute for one minute, and the flow-through was disposed of. After the spin column was rinsed with washing buffer, 45 µl of DNase buffer and five µl of DNase were added to treat it with DNase. After that, the combination was given a 15-minute incubation period. Following that, 500 µl of washing buffer was added to the spin column, and it was centrifuged for one minute at 13000 rpm to rinse it three times. The silica gel was dried out by spinning the column empty to elute the RNA. Subsequently, 50 microliters of elution buffer were added, and the mixture was centrifuged for two minutes at 13,000 rpm.

RNA Concentration Measurement by Quantus™ Fluorometer (Promega, USA):

1. 1X TE buffer was created by diluting 20X TE Buffer (pH 7.5) 1.1 times with nuclease-free water.
2. A 1:400 dilution was obtained by combining 10µl of QuantiFluor® Dye with 3,990µl of 1X TE buffer to form the QuantiFluor® Dye working solution. A 1X TE buffer was used for the mixing procedure.
3. 200 µl of QuantiFluor® RNA Dye was added to a 0.5 ml PCR tube to create the empty sample for the QuantiFluor® ONE RNA System.
4. As directed by the manufacturer, two µl of the RNA nucleic acid standard was added to 200 µl of working solution in a 0.5 ml PCR tube.
5. Establish a reference measurement using the Quantus Fluorometer reading, then measure the RNA samples following the suggested protocols of the Quantus machine.

cDNA Synthesis

Table (1): The entire amount of RNA was subjected stayed inverted and copied to cDNA using the kit as of ADDBio (Korean) as below**:**

Material	Quantity
H2O	Six µl
Reverse transcriptase (RT) 2X enhance script Cdna	20 µl
dNTPs	Four µl
accidental oligos hexamer	2 µl
RNA	Eight µl
Whole size	40 µl

Table 2: The thermal conditions were as follows:

Heat	Period	Reason
25 C	10 min	Get ready
50 C	60 min	(RT)
80 C	5 min	inactivation of RT
4 C	On embrace	Stock cDNA

Gene expression of miR-150-5p

This study utilized the comparative Ct technique (ΔΔCt) to compare the transcript levels of the experimental group to those of the control group, with normalization to the level of GAPDH mRNA. This was accomplished based on the suggestion of[18].

3. To achieve this objective, the miR-150-5p gene was amplified using the primers provided by[19].

Table (3): Gene of Interest (miR-150-5p):

Sequence (5'->3')	Template strand (5'……3')
Forward of miR-150-5p-	TCAATGCCCTGTCTCCCAAC
Reverse of miR-150-5p-	TTCCCAAGTCCCTATCCCCC

Table (4) Housekeeping gene (HKG) or interior orientation gene; hominid dehydrogenase of Glyceraldehyde 3-phosphate :

Sequence(5'->3')	Template strand (5'……3')
GAPDH-F	CAGAACATCATCCCTGCCTCTA
GAPDH-R	CCAGTGAGCTTCCCGTTCA

Quantitative Reverse Transcriptase PCR (RT-qPCR) Preparation

RT-qPCR Amplification

Initially, The amplification was carried out using AddBio's (Korea) AddScript RT-qPCR Syber master.

Table (5): A/ the response stayed comprising :

Material	Quantity
H2O	Four µl
Add Script RT-qPCR	Ten µl
primer Forward	Two µl
primer Reverse	2 µl
cDNA	Two µl
Complete	20 µl

Table (6): B/ shows the thermal conditions that were measured using BioRAD (USA):

Temperature		Time	Repeat
Early denaturation	95 C	5 min	1
Denaturation	95 C	20 sec	40x
Annealing	55 C	30 sec	40x by mechanism read
Extension	72 C	30 sec	40x
Melting investigation	95 C	15 sec	1
Melting examination	60 C	60 sec	1
Melting examination	+0.3 C of 95C	15 sec	Through mechanism read

RT-qPCR Data Normalisation

The transcript levels were normalized using the delta-delta Ct approach, as described by Schmittgen and Livak (2008). This method involved employing the following calculation and comparing the results to the levels of GAPDH mRNA.

To obtain 2-ΔΔCT, we can use the following formula: 2-ΔΔCT can be expressed as the difference between (CT gene of interest - CT internal control) sample A and (CT gene of interest - CT internal control) sample B.

Note: Sample A refers to a specific group.

Sample B refers to a distinct and specific group.

Glycated Hemoglobin (HbA1c), (Linear, Sapin)

An enzymatic approach is used to selectively quantify the N-terminal fructosyl dipeptides of the HbA1c -chain in the hemoglobin A1c assay.

Ethical Consideration

The Ethics Committee of the College of Medicine, University of Al-Qadisiyah, authorized the study. Before any procedures, all patients were provided with a comprehensive description of the therapies, and their consent was obtained. All patients will be informed verbally and in writing about the procedure and the purpose of the study; before enrolling, all participants will provide their written informed consent.

Statistical Analysis

The data analysis was conducted using GraphPad Prism software, specifically version 8.4.3. The data were presented as the mean ± standard deviation (SD), and a difference with a P value <0.05 was deemed statistically significant.

RESULTS

The assay amplification efficiency in tested groups for (miR-150-5p).

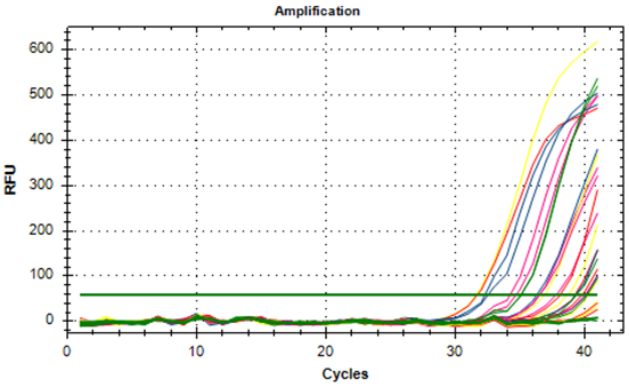

Figure (1): amplification curve of Metformine group

Figure (1): amplification curve of the tested samples for expression of the gene of interest (miR-150-5p) in the Metformin group. The successful amplification curves with the corresponding crossing threshold (CT) are the number of cycles with the round forming unit (RFU).

Glimepiride Group

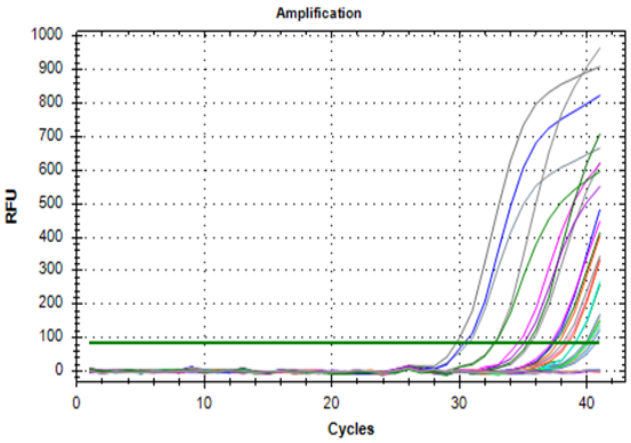

Figure (2): amplification curve of Glimepiride group

Figure (2): amplification curve of the tested samples for gene expression of interest (miR-150-5p) in the Glimepiride group. The successful amplification curves with the corresponding crossing threshold (CT) are the number of cycles with the round forming unit (RFU).

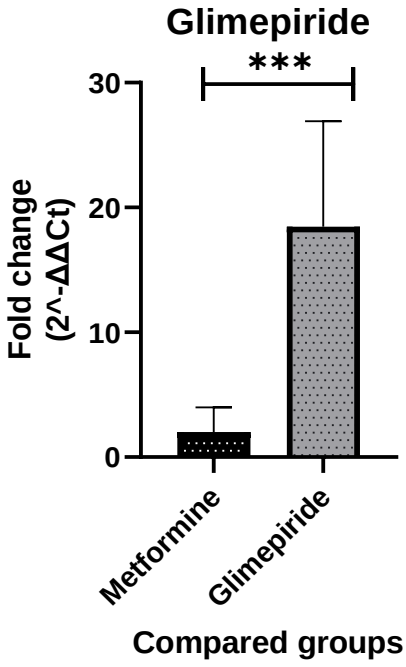

Figure (3): Gene expression of miR-150-5p

Figure (3): Gene expression of miR-150-5p in the glimepiride treatment group. This shows a significant upregulation of miR-150-5p in the Glimepiride treated group (P<0.0001) compared to metformin treatment. This was analyzed using GraphPad Prism software (version 8.4.3).

Descriptive statistics in the miR-150-5p expression in Glimepiride and metformin-treated groups. This was analyzed by t-test, as shown in Table (7)

Table (7): Descriptive statistics in the miR-150-5p expression as follows:

Characteristic	Metformin group	Glimepiride plus Metformin group	P value
Micro RNA 150-5P	2.025	18.47	<0.0001
Mean			
Std. Error of Mean	0.3600	1.540	

DISCUSSION

The present study found that glimepride medication considerably elevated miR-150-5p expression in individuals with type 2 diabetes mellitus (T2DM) and left ventricular diastolic dysfunction (LVDD). Diastolic function markers also showed improvement compared to the group that got standard care with Metformin.

Research has shown that miR-150 expression is much lower in osteosarcoma (OS) cell lines (HOS, SAOS2, MG-63, and U2OS) compared to normal osteoblast cells. Additional studies for various therapies have validated this. When miR-150 was overexpressed, it greatly reduced osteosarcoma cell proliferation. Osteosarcoma (OS) cells can be more sensitive to the chemotherapeutic drug doxorubicin (DOX) by enhancing their miR-150 expression. It has been noted that miR-150 expression is down in osteosarcoma cells and tissues[20]. It has been discovered that miR-150 inhibits the PI3K-AKT pathway, which in turn suppresses tumors[21]. One of the parameters that can predict the overall and disease-free survival of osteosarcoma patients is the combination of miR-150 and insulin-like growth factor 2 mRNA binding protein 1 (IGF2BP1) or both factors alone[22]. We measured cell proliferation using CCK-8 to see how miR-150 affected OS cell growth. The results showed that miR-150 overexpression dramatically reduced HOS and U2OS cell growth. A DOX-based chemotherapy model was developed to evaluate the impact of miR-150 on OS cell resistance to chemotherapy.

The effect of DOX therapy on cell viability was determined by assessing the viability of OS cells. The dosage-response relationship of DOX treatment was investigated in an experiment. 0, 25, 50, 75, and 100 µM were used as concentrations. Depending on the dosage, the results showed that DOX considerably reduced the cell viability of both HOS and U2OS. As the DOX concentration reached 50 µM, there was no more decrease in cell viability. In addition, compared to the group treated with the NC mimic + DOX, the DOX group had a much lower survival rate for HOS and U2OS cells due to the overexpression of miR-150. Thus, these results show that miR-150 reduced OS cell growth and increased DOX sensitivity.

Following this, studies showed that compared to a control group, children with ASD had abnormally high quantities of several miRNAs in their blood. The microRNAs zeroed in on genes that play critical roles in brain function and processing. Our conclusion is based on the evidence that suggests SSRI usage may affect miRNA expression in developing brain cells. In this in vitro investigation, we use undifferentiated human neuroblastoma cells to look at how fluoxetine might affect the expression of miRNAs that were either upregulated or downregulated in ASD children[23].

The main discovery of this research was that fluoxetine, in two different neuroblastoma cell lines, SH-SY5Y and SK-N-SH, consistently increased the levels of miR-572 and miR-663a at concentrations varying from 1 to 10 µM. A greater dosage of 25-µM proved harmful to both cell lines, indicating that the activity-promoting effects of fluoxetine were dose-dependent. After 30 days of taking 40 mg of fluoxetine orally as prescribed, the blood concentration of the drug reaches approximately one µM[24,25]. However, the concentration is much lower than needed to cause miRNA overexpression in our research. Fluoxetine is more concentrated in the brain than in plasma because of its lipophilic nature, which allows it to build up in brain tissue[26,27]. Thus, it is probable that a dosage of 5-10 µM of fluoxetine falls within the range of dosages meaningful for therapeutic purposes. Over 100 mg of fluoxetine daily has been prescribed to patients by psychiatrists in therapeutic settings for long durations without major side effects[28].

We can conclude from the existing clinical data that SSRI fluoxetine has no negative impact on developing brain-specialized neurons and has favorable effects on them. A number of neurological illnesses, including autism spectrum disorder (ASD), have been linked to miR-572 [29,23].

One of miR-572's targets, neuronal cell adhesion molecule 1 (NCAM1), seems to be related to cognitive impairment in studies showing its presence[30]. NCAM1 has a significant role in neuronal plasticity, axonal development, and neuronal cell variety[31,32]. Yu et al. (2015) found that people with postoperative cognitive impairment lose neuronal protection due to decreased NCAM1 expression from elevated miR-572 levels[30].

Study Limitations

Several limitations were identified in this study. The trial was limited by its short duration, and no significant morbidity or death outcomes were observed due to this short timeframe.

CONCLUSIONS

Glimepride is essential in improving cardiac events and cardiac biomarkers like miR-150: adding glimepride to usual diabetes type 2 treatment may benefit patients with cardiac complications like LVDD.

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