SGLT2 inhibitors effects on cardiac function and plasma biomarkers of diabetic patients with preserved ejection fraction

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Research Article

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Abstract

BACKGROUND

Sodium-glucose cotransporter inhibitors (SGLT2i) have proven a reduction in cardiovascular events in diabetic patients with and without heart failure (HF), as well as in non-diabetic patients with HF. The mechanisms underlying this benefit are not well known, with contradicting data on the changes that SGLT2i produce on cardiac function and structure.

METHODS

Between October 2020 and October 2021, 31 diabetic patients without prior history of SGLT2i use with normal ejection fraction (> 50%), glycated hemoglobin < 6.5%, renal clearance rate of > 60 ml/min/1.73 m² and sinus rhythm were prospectively included. In all of them, SGLT2i were started. At inclusion and 6-months follow-up, different clinical, ECG, analytical and echocardiographic (standard, 3D and speckle tracking) variables were recorded.

RESULTS

The average age of our population was 66.4 years (± 8.4). 90% were male, 71% were hypertensive, and 77.4% were dyslipidemic. 77.4% of patients had a history of ischemic heart disease, and 10% had vascular disease in other territories. At the time of inclusion, 80.6% of the population was treated with ACEI or ARB, 54.8% with beta-blockers, 29% with diuretics, and 93.5% with statins. After an average follow-up period of 6.6 months (± 0.8), an average reduction of 9.9 g/m² (± 4.5, p = 0.048) in 3D-estimated left ventricle mass was observed. An increase in absolute left ventricle global longitudinal strain (LVGLS) of 0.74 (± 0.35, p = 0.05) was observed, as well as an increase in isovolumetric relaxation time (IVRT) of 9.8 ms (± 4.8, p = 0.05). Moreover, we observed a significant reduction in ANP (p = 0.008) and CK-MB levels (p = 0.006), after SGLT2i treatment. No relevant differences in LV dimensions and volumes were observed, as well as in the evaluated RV parameters and other biomarkers.

CONCLUSIONS

It is necessary to understand the mechanism underlying the clinical benefit of these drugs. This will allow us to better understand its effect in patients with HF. Our data shows that the use of SGLT2i is associated with cardiac improvements, both structural (myocardial mass) and functional (IVRT, LVGLS), in a population of patients with normal ejection fraction.

BACKGROUND
Heart failure (HF) continues to be a prevalent and relevant problem. The current prevalence in adult population is estimated to be 1–2%\(^1\). Despite great advances in recent years in treatment and management of these patients, the morbidity and mortality associated with HF remains high\(^2\). Diabetes mellitus (DM) is a poor prognosis factor related with HF, with a higher rate of events and mortality in this group of patients\(^3\).

On the other hand, sodium-glucose cotransporter type 2 (SGLT2) inhibitors (SGLT2i) are a new family of drugs developed for treatment of type 2 DM\(^4\). Initial studies in diabetic patients with some of these drugs showed a striking clinical benefit in cardiovascular diseases, particularly in regards to HF\(^5\)–\(^7\). Following these results, several relevant trials have been developed and published that have analysed the clinical effect of SGLT2i directly in populations of HF patients with reduced ejection fraction, with and without DM. Following the previously-mentioned trend, dapagliflozin showed a significant reduction in HF hospitalizations and all-cause mortality\(^8\), while empagliflozin showed a significant reduction in the combined primary end point of HF hospitalizations and cardiovascular death\(^9\). Promising data have been subsequently published on the treatment of HF with preserved ejection fraction with these drugs\(^10\)–\(^12\), resulting in the inclusion of both empagliflozin and dapagliflozin as first-line treatment for HF with reduced ejection fraction in the latest ESC HF Guidelines\(^13\).

The mechanisms underlying this clinical benefit are not well known. Said benefit cannot be explained solely by the hypoglycaemic effect for which they were developed, nor does it seem to stem from a reduction in atherosclerotic events, which is relatively scarce. This benefit also is not completely explained by its beneficial effect on renal function\(^14\) or the reduction in body weight and blood pressure associated with the use of SGLT2i. There are data that suggest that the reduction of HF hospitalization could be explained by a structural and functional improvement of the left ventricle (LV)\(^15\). In this regard, some authors have published results on the changes in LV structure and function derived from the use of SGLT2i in different populations, usually with reduced ejection fraction and DM, with not entirely congruent results \(^16\)–\(^22\). The use of speckle tracking imaging techniques is especially useful for detecting subtle and early changes in cardiac function, which could provide useful information. Additionally, antioxidant, anti-inflammatory, anti-fibrotic and anti-hypertrophic actions, as well as bioenergetic changes, have been associated to the use of SGLT2i\(^23\). In this sense, the use of circulating biomarkers representative of cardiac cellular events could help to better understand the mechanisms involved.

In short, although the efficacy and safety of SGLT2i has been demonstrated in different clinical trials, the mechanisms underlying their clinical benefits have not yet been fully elucidated, with this knowledge being necessary to achieve a better understanding of the effect of these drugs. In our work, we analysed the effect of SGLT2i in a population of diabetic patients with preserved ejection fraction by means of a deep structural and functional study with imaging techniques and specific biochemical markers.

**METHODS**
We carried out a single-centre, observational prospective study. From October 2020 to October 2021, we prospectively enlisted 31 patients from cardiology or endocrinology outpatient clinics at our centre in whom the start of SGLT2i was considered based on clinical criteria. Inclusion criteria were the following: 1) Type 2 DM diagnosed according to the 2019 American Diabetes Association recommendations (24); 2) glycosylated haemoglobin > 6.5%; 3) LV ejection fraction (LVEF) higher than or equal to 50% as measured by echocardiography; 4) sinus rhythm; 5) one or more of the following echocardiogram findings: any degree of diastolic dysfunction, LV hypertrophy (defined as indexed LV mass ≥ 115 g/m² in men, ≥ 95 g/m² in women), and/or left atrial dilatation (defined as indexed volume > 34 mL/m²); 6) age > 18 years.

The major exclusion criteria were: 1) previous treatment with SGLT2i; 2) previous intolerance or allergy to SGLT2i; 3) glomerular filtration rate (GFR) lower than 60 mL/min/1.73 m²; 4) acute coronary syndrome within the last 3 months; 5) relevant valve heart disease, hypertrophic cardiomyopathy, diagnosed cardiac amyloidosis, complex congenital heart disease, or other specific heart diseases; 6) any other medical condition considered inappropriate by the study physician.

Our study included two visits. At the baseline visit (pre-treatment, first visit), all patients underwent a clinical assessment, anthropometric measurements (height and weight) and an echocardiographic study. Data about comorbidities, pharmacological treatment and diagnostic techniques (electrocardiography and echocardiography) were collected. We also obtained and preserved a blood and urine sample. After the first visit, all patients received 10 mg of dapagliozin daily for a minimum period of 6 months, according to usual clinical practice (3). After 6 to 9 months, a second visit was performed, in which blood and urine collection and an echocardiogram were repeated.

The study design and protocol have been revised and approved by the Clinical Research Ethics Committee of our institution (Ref. PIC192-19_FJD), and informed written consent was obtained from all participants. This investigation was conducted in accordance with the principles outlined in the Declaration of Helsinki.

Echocardiography

Comprehensive transthoracic echocardiography was performed using a commercially available system (EPIQ CVx 7.0.3, Philips Healthcare, Best, the Netherlands) equipped with a ×5–1 xMATRIX array transducer, according to a standardised protocol. Chamber size, and quantifications were measured according to American Society of Echocardiography (ASE) and the European Association of Cardiovascular Imaging (EACVI) guidelines (25, 26). Moreover, a specific 3D echocardiogram study of LV and left atrium (LA) was performed. We obtained standard measurements such as LV end-diastolic diameter, LA diameters, interventricular septum and posterior wall thickness, E, A, e' and a' waves, isovolumetric relaxation time (IVRT), TAPSE, S' wave and VD dimensions, among others. LVEF, indexed LV mass and indexed left atrium (LA) volume was measured using 3D-echocardiography. Strain imaging was performed with speckle tracking software, which measured LV, LA and right ventricle (RV) strain in...
accordance to the specific consensus documents and position papers published by the EACVI and ASE(27, 28). Longitudinal strain of the 17 segments of the LV was measured using two-, three- and four-chamber apical views. LV peak longitudinal strain and global longitudinal strain (LV-GLS) values for each patient were calculated automatically by the software. Tracking points were placed automatically by the software on an end-systolic and end-diastolic frame in each view, with mild manual changes if deemed necessary. RV longitudinal strain and LA strain analysis were performed. Strain analysis of the LA was performed in the four-chamber view, using the QRS complex as the baseline reference point. We also measured the following parameters: peak atrial longitudinal strain (PALS), peak atrial contraction strain (PACS), and LA strain during the conduit phase (LACS). Strain analysis of the RV was performed in the RV-focused apical four-chamber view. RV endocardial border was traced at both the end-diastolic and end-systolic frames automatically by the software, with mild manual changes if needed. We measured the following parameters: right ventricular four-chamber strain (RV4CSL) and right ventricular free-wall longitudinal strain (RVFWSL). RV4CSL included both the RV free wall and interventricular septum segments. RV free wall strain (RVFWSL) was defined as the strain value at the RV free wall.

Analysis of all cases was performed by only one observer, an expert cardiologist (M.C.) experienced in strain measuring and 3D echocardiography, using specific speckle tracking software (AutoStrain and Dynamic HeartModel, Philips Healthcare, Best, the Netherlands).

Biochemical analysis

Serum, plasma and urine samples were extracted and stored at two times in the study. First, at the inclusion of the patient in the study, before starting treatment with SGLT2i, and second, after 6–9 months of follow-up. We measured the usual parameters in urine and blood (complete blood count, kidney function, liver function, lipid profile, urine sediment, proteins...). Additionally, we analysed the levels of the following specific biomarkers related to possible cardiac remodelling responses: monocyte chemoattractant protein-1 (MCP-1) and interleukin-6 (IL-6) as heart pro-inflammatory biomarkers(29); cardiotrophin-1 as a marker of myocardial hypertrophy(30); and heart-fatty acid binding protein (H-FABP) as a marker of myocardial steatosis(31). We also measured known biomarkers such as N-terminal prohormone of brain natriuretic peptide (NT-proBNP) as markers for HF, myocardial hypertrophy and vasodilation; high-sensitivity C-reactive protein (hsCRP), matrix metallopeptidase-2 and − 9 (MMP-2 and MMP-9) and T-cell immunoglobulin and mucin domain-1 (TIM-1) as markers of inflammation; atrial natriuretic peptide (ANP) as a marker of myocardial hypertrophy and vasodilation; galectine-3 (GAL-3) as a marker of inflammation and fibrosis; and creatine kinase MB (CK-MB) as a marker of myocardial injury. Enzyme-linked immunosorbent assays (ELISA) were performed to quantify ANP (DANP00, Bio-Techne, Minnesota, USA), MMP-2 (MMP200, Bio-Techne, Minnesota, USA), and FABP3 (DY1678, Bio-Techne, Minnesota, USA). The technique was carried out following the manufacturer's instructions. The absorbance reading was taken at 450nm with a 570nm wavelength correction in the plate reader (EnSpire® Multimode Reader Perkin Elmer, Massachusetts, USA). MCP-1 (SPCKA-PS-008756), IL-6 (SPCKA-PS-008755), TIM-1 (SPCKA-PS-008754), HS-CRP (SPCKB-PS-000200), MMP-9 (SPCKB-PS-000661), and Galectin-3 (SPCKB-PS-000490) were quantified by the ELLA™ automated immunoassay
Follow-up, adverse events and outcomes

After and during follow-up, clinical outcomes and adverse events were monitored. The outcomes analysed in our study were admission due to HF and deaths from any cause. HF admission was defined as admission to a healthcare facility lasting more than 24 h due to the onset or worsening of HF symptoms and followed by specific treatment for HF. Adverse events recorded were hypoglycaemia, urinary or genital infections and pharmacological changes, among others. All these clinical events during follow-up were collected from patient visits or electronic health records.

Statistical analysis

Data were subjected to descriptive statistical analysis with frequency measurements (absolute frequencies and percentages) for qualitative variables and with mean and standard deviation or median and interquartile range, for quantitative variables. We performed a comparative analysis of the different variables at baseline and after 6 to 9 months of treatment with SGLT2i. The analysis of the different variables was performed using the paired Student t-test when distribution of the variable was assumed to be normal, and the Mann-Whitney U-test (Wilcoxon) when distribution was not assumed to be normal were not. A p-value of less than 0.05 was considered statistically significant. We randomly selected 8 patients from the study cohort and analysed the intraobserver reproducibility of the IVRT, E and e' wave measurements, among others. A variability analysis of variables such as myocardial mass, LV strain, or LA volume was not performed, since these variables were obtained automatically by the echocardiography software. The intraclass correlation coefficients (absolute agreement) of IVRT, E and e’ waves were 0.9025, 0.961 and 0.877, respectively. To ensure reproducibility, these variables were measured again in the same sample of 8 studies by a second experienced operator (M.T.) in a blinded fashion to determine interobserver variability. The interobserver intraclass correlation coefficients of IVRT, E and e’ were 0.802, 0.993 and 0.965, respectively.

Statistical analyses were performed with SPSS version 22.0 (SPSS, Inc., Chicago, USA).

RESULTS

Characteristics and follow-up of the study population

A total of 31 type 2 diabetic patients were enrolled in the study. The average age of our population was 66.4 years (± 8.4). 90% of our patients were male, 71% were hypertensive, 77.4% were dyslipidaemic, and 87.1% were current or former smokers. 77.4% of patients had a history of ischemic heart disease, and 10% had vascular disease in other territories. At the time of inclusion, 80.6% of the population was treated with angiotensin-converting enzyme inhibitors (ACEI) or angiotensin receptor blockers (ARB), 54.8% with
beta-blockers, 29% with diuretics, and 93.5% with statins. Baseline characteristics of our study population are shown in Table 1 and in Fig. 1.

<table>
<thead>
<tr>
<th>Variables</th>
<th>N: 31</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years, ± SD)</td>
<td>66.4 (± 8.4)</td>
</tr>
<tr>
<td>Male (n (%))</td>
<td>28 (90.3)</td>
</tr>
<tr>
<td>Hypertension (n (%))</td>
<td>22 (71.0)</td>
</tr>
<tr>
<td>Dyslipidaemia (n (%))</td>
<td>24 (77.4)</td>
</tr>
<tr>
<td>Tobacco (n (%))</td>
<td>27 (87.1)</td>
</tr>
<tr>
<td>Obesity (n (%))</td>
<td>8 (25.8)</td>
</tr>
<tr>
<td>Cerebrovasc disease (n (%))</td>
<td>1 (3.2)</td>
</tr>
<tr>
<td>Peripheral vasc. disease (n (%))</td>
<td>3 (9.7)</td>
</tr>
<tr>
<td>Chronic pulmonary disease (n (%))</td>
<td>3 (9.7)</td>
</tr>
<tr>
<td>Glomerular filtration (media, ±SD)</td>
<td>83.2 (± 13.2)</td>
</tr>
<tr>
<td>Ischemic heart disease (n (%))</td>
<td>24 (77.4)</td>
</tr>
<tr>
<td>Heart failure (n (%))</td>
<td>0 (0)</td>
</tr>
<tr>
<td>QRS &gt; 120 (n (%))</td>
<td>5 (16.1)</td>
</tr>
<tr>
<td>Sinus rhythm (n (%))</td>
<td>31 (100)</td>
</tr>
</tbody>
</table>

After an average follow-up period of 6.6 months (± 0.8), dapagliflozin had to be withdrawn in 5 patients. SGLT2i were withdrawn in 3 patients due to repeated genital or urinary infections. In the other two patients, withdrawal was due to patient choice. No other adverse effects such as hypoglycaemia, ketoacidosis or hypotension were observed. In addition, no adverse events (admission due to HF or death of any cause) were observed during the follow-up. One patient refused to undergo the echocardiogram and laboratory tests at the end of follow-up. Another patient refused follow-up analysis. Both patients were in the group of 5 patients in whom SGLT2i were discontinued. The remaining 29 patients completed the study.

Finally, we performed a comparative analysis before and after treatment with dapagliflozin in those patients who maintained the drug throughout the follow-up (26 patients). This analysis shows a significant weight reduction after 6 months of treatment, as expected. We observed a mean reduction in glycosylated haemoglobin of 0.5% (± 1.4, p = 0.07), with no significant reduction in renal function. Treatment with SGLT2i significantly reduces blood ferritin levels (p = 0.02), and increases HDL cholesterol.
(p = 0.03) and haemoglobin (p = 0.02). Table 2 shows the comparative analysis of the different variables included in our study.
Table 2
Comparative analysis before and after 6 months of follow-up.

<table>
<thead>
<tr>
<th>N:26</th>
<th>Baseline</th>
<th>SGLT2i (6 months)</th>
<th>Δ (mean (SD))</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biochemical variables (*)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemoglobin (g/dl)</td>
<td>14.4 ± 1.4</td>
<td>14.9 ± 1.8</td>
<td>0.5 (1.08)</td>
<td>0.023</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>42.9 ± 3.8</td>
<td>45.1 ± 4.5</td>
<td>2.2 (2.9)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Creatinin (mg/dl)</td>
<td>0.9 (0.2)</td>
<td>1 (0.4)</td>
<td>0.03 (0.11)</td>
<td>NS</td>
</tr>
<tr>
<td>GF (ml/min/1.73m2)</td>
<td>86.2 (22.2)</td>
<td>84.4 (26.0)</td>
<td>-2.1 (8.3)</td>
<td>NS</td>
</tr>
<tr>
<td>Potassium (mmol/l)</td>
<td>4.5 ± 0.4</td>
<td>4.5 ± 0.4</td>
<td>-0.04 (0.4)</td>
<td>NS</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>136.5 (58)</td>
<td>135 (37)</td>
<td>3.5 (28)</td>
<td>NS</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dl)</td>
<td>61 (31)</td>
<td>64.5 (18)</td>
<td>4.5 (24)</td>
<td>NS</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>47.5 (14)</td>
<td>50 (19)</td>
<td>3 (6)</td>
<td>0.03</td>
</tr>
<tr>
<td>Glyc. hemoglobin (%)</td>
<td>6.9 (0.8)</td>
<td>6.8 (0.9)</td>
<td>-0.2 (0.9)</td>
<td>NS</td>
</tr>
<tr>
<td>Ferritin (ng/ml)</td>
<td>55.5 (183)</td>
<td>41.5 (123)</td>
<td>-10 (57)</td>
<td>0.018</td>
</tr>
<tr>
<td>NT-ProBNP (pg/ml)</td>
<td>52.4 (76.5)</td>
<td>51.5 (116)</td>
<td>-0.5 (35.6)</td>
<td>NS</td>
</tr>
<tr>
<td>CK-MB (ng/ml)</td>
<td>1.34 (1.1)</td>
<td>1.32 (1.5)</td>
<td>0.3 (0.6)</td>
<td>0.006</td>
</tr>
<tr>
<td>MCP-1 (pg/ml)</td>
<td>212 (95)</td>
<td>228 (88)</td>
<td>11.84 (61.08)</td>
<td>NS</td>
</tr>
<tr>
<td>IL-6(pg/ml)</td>
<td>3.26 (2.78)</td>
<td>2.68 (2.47)</td>
<td>-0.37 (2.28)</td>
<td>NS</td>
</tr>
<tr>
<td>MMP-9(ng/ml)</td>
<td>489.9 (470.5)</td>
<td>554.9 (473.7)</td>
<td>52.8 (34.4)</td>
<td>NS</td>
</tr>
<tr>
<td>CRP-HS (mg/ml)</td>
<td>1.05 (2.07)</td>
<td>1.26 (1.86)</td>
<td>-0.03 (1.42)</td>
<td>NS</td>
</tr>
<tr>
<td>GAL-3 (ng/ml)</td>
<td>6.9 ± 1.7</td>
<td>7.5 ± 2.4</td>
<td>5.7 (2.9)</td>
<td>NS</td>
</tr>
<tr>
<td>TIM-1(pg/ml)</td>
<td>91.3 (67.7)</td>
<td>103 (72.5)</td>
<td>5 (35.8)</td>
<td>NS</td>
</tr>
</tbody>
</table>

*Values of variables (baseline and follow-up) are expressed as mean ± SD, or median (IR), depending on whether their distribution is normal or not.

ANP: atrial natriuretic peptide; BMI: Body mass index; CK-MB: creatininphosphokinase-MB; CRP-HS: high sensitivity C-reactive protein; GAL-3: Galectin-3; GF: glomerular filtration; H-FABP: heart-fatty acid binding protein; IL-6: interleukin-6; IR: interquartil range; IVRT: isovolumic relaxation time; LA: left atrium; LV: left ventricle; LVEF: LV ejection fraction; LVEDV: LV end-diastolic volume; LVESV: LV end-systolic volume; LV-GLS: LV global longitudinal strain; MCP-1: monocyte chemoattractant protein-1; MMP-2: Matrix metallopeptidase 2; MMP-9: Matrix metallopeptidase 9; PALS: Peak atrial longitudinal strain; PACS: peak atrial contraction strain; LACS: LA strain during the conduit phase; RVFWSL: right ventricular free-wall longitudinal strain; RV4CSL: right ventricular four-chamber strain; SD: standard deviation; TIM-1: T cell immunoglobulin and mucin domain 1.
<table>
<thead>
<tr>
<th>N:26</th>
<th>Baseline</th>
<th>SGLT2i (6 months)</th>
<th>Δ (mean (SD))</th>
<th>p</th>
</tr>
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<tr>
<td><strong>Biochemical variables (*)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ANP (ng/ml)</td>
<td>19.4 (17.99)</td>
<td>14.3 (12.29)</td>
<td>-4.75 (11.7)</td>
<td>0.007</td>
</tr>
<tr>
<td>MMP-2 (ng/ml)</td>
<td>277.1 ± 100.2</td>
<td>297.2 ± 88.7</td>
<td>20.1 (77.5)</td>
<td>NS</td>
</tr>
<tr>
<td>H-FABP (ng/ml)</td>
<td>1.01 (0.60)</td>
<td>0.85 (0.8)</td>
<td>0.11 (0.51)</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Clinical variables (*)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>83 (19.5)</td>
<td>74.0 (22.0)</td>
<td>-2.0 (2.0)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.68 (5.02)</td>
<td>26.5 (5.2)</td>
<td>-0.69 (0.73)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td><strong>Ecocardiographic variables (*)</strong></td>
<td></td>
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</tr>
<tr>
<td>LVEF (%)</td>
<td>59.8 ± 3.5</td>
<td>60.5 ± 3.0</td>
<td>1.4 (4.4)</td>
<td>NS</td>
</tr>
<tr>
<td>3D LVEDV (ml)</td>
<td>121.9 ± 31.6</td>
<td>132.1 ± 37.4</td>
<td>11.8 (23.9)</td>
<td>0.05</td>
</tr>
<tr>
<td>3D LVESV (ml)</td>
<td>49.4 ± 14.3</td>
<td>52.7 ± 15.2</td>
<td>3.8 (10.5)</td>
<td>NS</td>
</tr>
<tr>
<td>3D LV mass (gr/m²)</td>
<td>156.1 ± 35.3</td>
<td>146.3 ± 31.2</td>
<td>-9.9 (18.4)</td>
<td>0.05</td>
</tr>
<tr>
<td>LV-GLS (%)</td>
<td>-18.7 ± 1.6</td>
<td>-19.3 ± 1.9</td>
<td>-0.7 (1.7)</td>
<td>0.05</td>
</tr>
<tr>
<td>LA 3D-volume (ml/m²)</td>
<td>37.7 ± 10.1</td>
<td>37.8 ± 13.4</td>
<td>-1.7 (8.3)</td>
<td>NS</td>
</tr>
<tr>
<td>PALS (%)</td>
<td>34.1 ± 12.8</td>
<td>32.5 ± 9.7</td>
<td>0.5 (8.7)</td>
<td>NS</td>
</tr>
<tr>
<td>PACS (%)</td>
<td>-16.7 ± 8.2</td>
<td>-16.1 ± 7.2</td>
<td>-0.3 (4.7)</td>
<td>NS</td>
</tr>
<tr>
<td>LACS (%)</td>
<td>-17.0 (11.3)</td>
<td>-13.9 (8.1)</td>
<td>-0.8 (7.7)</td>
<td>NS</td>
</tr>
<tr>
<td>TAPSE (%)</td>
<td>20.6 ± 3.2</td>
<td>21.7 ± 3.2</td>
<td>1.8 (3.3)</td>
<td>0.01</td>
</tr>
<tr>
<td>RVFWSL (%)</td>
<td>-23.1 ± 5.8</td>
<td>-23.4 ± 3.5</td>
<td>-1.3 (6.4)</td>
<td>NS</td>
</tr>
<tr>
<td>RV4CSL (%)</td>
<td>-19.1 ± 4.1</td>
<td>-18.4 ± 2.4</td>
<td>-0.1 (4.6)</td>
<td>NS</td>
</tr>
</tbody>
</table>

*Values of variables (baseline and follow-up) are expressed as mean ± SD, or median (IR), depending on whether their distribution is normal or not.

ANP: atrial natriuretic peptide; BMI: Body mass index; CK-MB: creatininphosphokinase-MB; CRP-HS: high sensitivity C-reactive protein; GAL-3: Galectin-3; GF: glomerular filtration; H-FABP: heart-fatty acid binding protein; IL-6: interleukin-6; IR: interquartil range; IVRT: isovolumic relaxation time; LA: left atrium; LV: left ventricle; LVEF: LV ejection fraction; LVEDV: LV end-diastolic volume; LVESV: LV end-systolic volume; LV-GLS: LV global longitudinal strain; MCP-1: monocyte chemoattractant protein-1; MMP-2: Matrix metallopeptidase 2; MMP-9: Matrix metallopeptidase 9; PALS: Peak atrial longitudinal strain; PACS: peak atrial contraction strain; LACS: LA strain during the conduit phase; RVFWSL: right ventricular free-wall longitudinal strain; RV4CSL: right ventricular four-chamber strain; SD: standard deviation; TIM-1: T cell immunoglobulin and mucin domain 1.
Echocardiographic data: functional and structural changes.

We performed a comparative analysis of echocardiographic variables before and after treatment with dapagliozin in patients who maintained the drug throughout follow-up. We observed an average reduction in 3D-estimated LV mass of 9.9 g/m² (± 18.4, p = 0.048) after 6 months of treatment. Also, an increase in absolute LV-GLS of 0.74 (± 0.7, p = 0.05) was observed, as well as an increase in IVRT of 9.8 ms (± 23.9, p = 0.05) and a significant decrease of E/e′ ratio of 1.4 (± 2.6, p = 0.02). A mild increase in e′, LA and VD strain, as well as a decrease in LA indexed volume were noted, although differences did not achieve statistical significance. Table 2 and Fig. 2 show the echocardiographic variables analysed in our study before and after starting dapagliozin.

Biochemical analysis: changes in hypertrophy biomarkers

Following the described methodology, we analysed plasma and urine samples from our study population at baseline and after follow-up in patients who maintained dapagliozin during follow-up. We observed a significant reduction in ANP (biomarker with vasodilatation and anti-hypertrophic functions, p = 0.008) and CK-MB levels (biomarker related with myocardial injury, p = 0.006), after SGLT2i treatment. The comparative analysis showed a trend towards increase of pro-inflammatory biomarkers, without reaching statistical significance. Other biomarkers, such as GAL-3, H-FABP or NT-proBNP, also showed slight, non-statistically significant increases at the end of follow-up. Table 2 and Fig. 2 show the comparative analysis of all biochemical variables included in our study.

Association between biomarkers and echocardiographic variables.
We performed a specific analysis to evaluate the possible relationships that could be established between those variables in which a statistically significant change was observed after the period of treatment with dapaglifozin. Specifically, we studied the possible association between all those clinical and analytical variables with statistically significant changes with respect to the echocardiographic variables that in turn showed significant differences. This analysis did not show any association between the different clinical and analytical variables assessed, and the echocardiographic variables. Thus, the reduction in weight or glycemia, as well as the reduction in ANP and circulating CK-MB after 6 months of dapaglifozin treatment, did not explain the improvement in diastolic function or cardiac hypertrophy in our population. Figure 3 shows the data matrix with the results of this analysis.

**DISCUSSION**

Our study shows that the use of dapaglifozin in diabetic patients with preserved ejection fraction and without HF, but data suggesting incipient functional and/or structural damage, is associated with an improvement in functional heart parameters (both systolic and diastolic function) as well as a reduction in myocardial mass, in just 6.6 months of treatment. Our data could help explain the clinical benefits of SGLT2i in HF.

Today, HF continues to be a prevalent and relevant issue. It is estimated that 1 to 2% of the adult population suffers from HF, reaching a prevalence of over 10% in elderly patients(1, 32). Despite the great advances in treatment and management of these patients in the last years, mortality and morbidity associated with HF remains high(2). This problem is accentuated in diabetic patients, being one of the main causes of hospital admission in this population(33, 34). Moreover, DM is a poor prognosis factor in HF, elevating the rate of events and mortality even higher in the aforementioned population(3). In this regard, it has been shown in test animals and patients that type 2 DM produces inflammatory phenomena and cardiac hypertrophy and steatosis, which can derive in cellular apoptosis and necrosis and cardiac remodelling, profoundly affecting cardiac function(33) and potentially leading to a direct myocardial damage in relation to DM, producing the so-called diabetic cardiomyopathy (DCM)(35). DCM, together with other factors such as arterial hypertension and coronary artery disease causes a significant percentage of diabetic patients to present both systolic and diastolic functional alterations, even with preserved ejection fraction(36, 37).

SGLT2i are a family of drugs recently developed for the treatment of type 2 DM(4), which inhibit SGLT2 receptors in the proximal tubule of the nephron, increasing the urinary excretion of glucose(38). Safety studies of these drugs in diabetic patients showed a striking beneficial clinical effect in cardiovascular disease, particularly in HF. Empagliflozin showed a significant reduction in mortality (5.7% vs 8.3%) and HF hospitalizations (2.7% vs 4.1%)(5). Canagliflozin significantly reduced the combined end point of cardiovascular mortality, stroke or myocardial infarction, as well as a marked reduction in HF hospitalization (HR 0.68; IC 95%, 0.51–0.90)(6). Lastly, dapagliflozin showed a significant reduction in HF hospitalizations (HR 0.73; IC 95% 0.61–0.88)(7). Following these studies, the effect of SGLT2i was studied directly in populations of patients with HF with reduced ejection fraction, both with and without
DM. Important trials such as DAPA-HF were published, showing a significant reduction in both HF hospitalizations (HR 0.7; IC 95%, 0.59–0.83) as well as all-cause mortality (HR 0.83; IC 95%, 0.71–0.97) with the use of dapagliozin. In the EMPEROR-Reduced trial, empagliozin also significantly reduced the combined end point of cardiovascular death and HF hospitalization in a similar population. More recently, promising data have been published on the treatment of HF with preserved ejection fraction with these drugs, as well as several meta-analyses that confirm the clinical benefit of SGLT2i in HF. All this evidence has led to SGLT2i being included in clinical practice guidelines as one of the four pillars of HF with reduced ejection fraction.

The mechanisms that explain the aforementioned clinical benefits are not well known. The alterations in myocardial function and structure that are commonly found in patients with DM, together with the relative scarcity of reduction in acute arteriosclerotic events that SGLT2i use causes, has suggested that the reduction in HF hospitalizations might be caused by an improvement in LV function and not a reduction in atherosclerotic burden. This hypothetical improvement in LV structure and function might occur in response to indirect mechanisms on the myocardium (hemodynamic, metabolic...) and not due to a direct mechanism, considering the absence of SGLT2 receptors in the heart. Following this line of research, authors have studied the changes in LV structure and function derived from the use of SGLT2, using imaging techniques such as echocardiography and magnetic resonance imaging (MRI). These studies included few patients, but were able to show that few months (3 to 6) of treatment produced an increase in diastolic function and a reduction of LV mass, without a clear improvement in ejection fraction or LV volumes. Cohen et al, however, showed a reduction in LV volume as measured with MRI after 6 months of treatment with empagliozin. Few data exist with respect to myocardial strain in these patients. Speckle tracking echocardiography has shown its usefulness in being able to detect initial alterations of cardiac function, which could advance diagnosis and treatment of patients. In particular, speckle tracking echocardiography can identify early functional improvement or deterioration, allowing the detection of cardiac alterations in type 2 diabetic patients and the understanding of SGLT2i in the prevention of structural deterioration of the LV.

In our study, we performed an in-depth imaging study with speckle tracking echocardiography in a population of diabetic patients without prior SGLT2i treatment, completing the obtained results with a wide array of cardiac biomarkers of fibrosis, hypertrophy, necrosis, pressure... Our data show that the use of SGLT2i is associated with an early improvement (at 6.6 months) in both cardiac structure and function, in a population of diabetic patients with normal ejection fraction. The use of SGLT2i in our population showed a significant reduction in indexed LV mass, as well as a significant improvement in LV systolic function as evidenced by an increase in LV-GLS absolute value, and an improvement in LV diastolic function as illustrated by a significant increase in IVRT and decrease in the E/e’ ratio. This last finding is particularly relevant considering that diastolic function deterioration is the main cardiac alteration associated with DM.

In addition, a significant reduction in ANP levels was observed in our study population. ANP regulates salt-water balance and blood pressure by promoting renal sodium and water excretion and stimulating
vasodilation. Furthermore, ANP has an anti-hypertrophic effect on the heart, independent of its systemic anti-hypertensive effect(49). The SGLT2i-mediated reduction of ANP levels could be related to a reduction in biological hypertrophic responses in the heart, in line with our result of a reduction in myocardial mass, as well as the reduction in LA pressure after improving systolic and diastolic function parameters. We have also observed a significant decrease in CK-MB levels in our study population, which indicates a reduction in myocardial injury, mediated by SGLT2i use. As with ANP, the improvement in diastolic function leads to less myocardial overload, which could explain the reduction in myocardial injury. The aforementioned comments must be valued as just possible etiological hypothesis of the findings, and specific studies are needed to determine the exact relation between SGLT2i and the production of ANP and release of CK-MB.

We have not found significant changes in the rest of analysed biomarkers associated, especially in regards to fibrosis or inflammation markers, but we have observed a significant increase in haemoglobin and haematocrit, in line with previous observations that SGLT2i use leads to an increase in erythropoietin levels(50). A higher haemoglobin levels could enhance oxygen delivery to the myocardium, theoretically improving its function, but the low increase in haemoglobin does not appear to be sufficient to fully explain the improvement in function.

All the results point to an improvement in intracardiac pressures as a basic mechanism in the clinical improvement associated with SGLT2i, in favour of all other mechanisms. These effects could be explained by the renal effect of SGLT2i, although the improvement in contractile function, as evidenced by a significant increase in LV-GLS, also suggests a more direct effect on the cardiomyocyte, supporting theories that postulate a direct effect of SGLT2i on cellular metabolism of these cells(51), improving their contractility and reducing myocardial damage, a possibility that is further supported by our finding of a significant reduction in CK-MB in our test population.

In conclusion, our data show that the use of SGLT2i is associated with cardiac improvement, both structural (myocardial mass) and functional (IVRT, LV-GLS) in a population of diabetic patients with normal EF.

LIMITATIONS

This study has three limitations. Firstly, although this was a prospective study, the study was an uncontrolled observational study with a small number of patients included and, therefore, larger randomized controlled trials are needed. Secondly, we could not verify whether the improvement in diastolic function was solely attributable to SGLT2i. Finally, the relationship between the administration of SGLT2i inhibitors and cardiovascular events could not be verified due to the short research period.

List Of Abbreviations

ANP
atrial natriuretic peptide
BMI
Body mass index
CK-MB
creatining phosphokinase-MB
CRP-HS
high sensitivity C-reactive protein
DM
diabetes mellitus
EF
ejection fraction
GAL-3
Galectin-3
GFR
glomerular filtration rate
HF
heart failure
H-FABP
heart-fatty acid binding protein
IL-6
interleukin-6
IR
interquartil range
IVRT
isovolumic relaxation time
LA
left atrium
LV
left ventricle
LVEF
LV ejection fraction
LVEDV
LV end-diastolic volume
LVESV
LV end-systolic volume
LV-GLS
LV global longitudinal strain
MCP-1
monocyte chemoattractant protein-1
MMP-2
Matrix metallopeptidase 2
MMP-9
Matrix metallopeptidase 9
MRI
magnetic resonance imaging
PALS
Peak atrial longitudinal strain
PACS
peak atrial contraction strain
LACS
LA strain during the conduit phase
RVFWSL
right ventricular free-wall longitudinal strain
RV4CSL
right ventricular four-chamber strain
SD
standard deviation
SGLT2
sodium-glucose cotransporter type 2
SGLT2i
sodium-glucose cotransporter type 2 inhibitors
TIM-1
T cell immunoglobulin and mucin domain 1.

Declarations

Ethics approval and consent to participate
The study design and protocol have been revised and approved by the Clinical Research Ethics Committee of our institution (Ref. PIC192-19_FJD), and informed written consent was obtained from all participants. This investigation was conducted in accordance with the principles outlined in the Declaration of Helsinki.

Consent for publication
Not applicable.

Availability of data and materials
The datasets used and analysed during the current study are available from the corresponding author on reasonable request.
Competing interests

The authors declare that they have no competing interests.

Funding

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Authors' contributions

MC and OL designed the study, researched and analyzed data, and wrote and edited the manuscript. MC performed the echocardiograms. OL, JL and SM handled the biological samples and analyzed the results of the plasma and urine tests. MT, AMP and JAB researched data and reviewed the manuscript. JT participated in the design of the study and reviewed the manuscript. All authors have read and approved the final manuscript.

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Not applicable.

References


**Figures**

**Figure 1**

Pharmacological treatment of the study population at inclusion.
Figure 2

Changes in left ventricle mass, global longitudinal strain, E'/e ratio and ANP after dapagliflozin treatment.

Dapagliflozin is associated with greater reduction in 3D-left ventricular mass (A), left ventricular global longitudinal strain (B), E'/e ratio (C), and ANP (D). Graphs represent mean and 95% confidence interval.
Figure 3

Association between biomarkers and echocardiographic variables: data matrix.

\(\Delta\): absolute difference from baseline; \(p\): Spearman’s rho (correlation coefficient); \(r\): Pearson’s r (correlation coefficient). ANP: atrial natriuretic peptide; BMI: Body mass index; CK-MB: creatininphosphokinase-MB; IVRT: isovolumic relaxation time; LV: left ventricle; LVEDV: LV end-diastolic volume; LV GLS: LV global longitudinal strain;