Glucagon as a Predictor of Heart Failure with Preserved Ejection Fraction Evolution in Type 2 Diabetes Patients

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Abstract

Background: Background: Diabetes mellitus (DM) is a major risk factor for heart failure (HF), specifically for heart failure with preserved ejection fraction (HFpEF). The diagnosis of HFpEF is a complex matter, due to presence of many confounders that limit the diagnosis. Glucagon is a key player in DM pathogenesis, also, it has a positive inotropic and chronotropic effects on the failing hearts. **Objective:** It is of interest to investigate the role of fasting glucagon as marker for HFpEF evolution among patients with type 2 DM. **Methods:** This case-control study was conducted in Internal Medicine Department in collaboration with Cardiology Department and Clinical pathology Department, Faculty of Medicine, Zagazig University Hospitals. This study was conducted on 32 subject with type 2 DM and were allocated into two equal groups: Type 2 DM without HF group (control group), and Type 2 DM with HFpEF group (case group). All patients underwent trans-thoracic echocardiography, routine laboratory tests and measurement of fasting levels of serum glucagon by (ELISA) kits. **Results:** Diabetic patients without HF (group I) and those with HFpEF (group II) weren't statically different as regard the basic study parameters with exception of hypertension which was significantly higher in group II. Fasting glucagon level was significantly higher in group II when compared with group I. HFpEF correlated with fasting serum glucagon in a positive manner. Also, serum glucagon showed a positive correlation with AF. Fasting glucagon (pg/ml) at cut off value of > 63.56 pg/ml had a sensitivity of 93.7%, and specificity of 56.2% in predicting the presence HFpEF. **Conclusion:** Serum glucagon represents a clue for early detection of HFpEF among type 2 DM patients especially if measured in a serial manner.

Keywords: HFpEF, Type 2 Diabetes, Glucagon

Introduction

European Society of Cardiology defined heart failure with preserved ejection fraction (HFpEF) patients as those with preserved left ventricular EF (LVEF ≥ 50%), with evidence of diastolic dysfunction or structural heart disease, in the context of distinctive signs and symptoms of heart failure (HF) and elevated natriuretic peptides **(1).** Studies estimate that the prevalence of HFpEF is about 50% (range 40% to 71%) among patients with HF **(2).**In general; patients with HFpEF are usually old females with a history of hypertension. Diabetes mellitus (DM), coronary artery disease, obesity, atrial fibrillation (AF), and hyperlipidemia are also extremely common in the population with HFpEF **(3).**

Diabetes mellitus is a pivotal risk factor for HF ***(4).*** DM raises the danger for new-onset HF irrespective of other conventional risk factors. Each 1% rise in glycated hemoglobin (HbA1c) is associated with an 8 % increment in the risk of HF in type 2 DM **(5).**

Classically, the pathogenesis of type 2 DM is centralized on insulin resistance and *β* cell dysfunction; however the inappropriately raised *α* -cell function and resultant hyperglucagonemia have long been identified as a supporter of hyperglycemia in diabetic patients by promoting glucose production by the liver **(6).**

Farah and Tuttle were the first to document that glucagon operates on the heart to augment cardiac output by enhancing the potency and length of cardiac contractions. Glucagon's positive inotropic and chronotropic impacts were first shown in the isolated heart of cats, guinea pigs, rats, and dogs **(7)** and were thereafter proved in vivo in humans **(8).**

The diagnosis of HFpEF is more difficult than the diagnosis of HFrEF because it is generally one of excluding other potential non-cardiac etiologies of symptoms indicative of HF **(9).**Thus, in this study we aimed to assess fasting levels of serum glucagon as a predictor for development of HFpEF among patients with diabetes, for earlier detection and subsequently management.

Methods

Study Design A case-control study was conducted in Internal Medicine Department, Cardiology Department and Clinical pathology Department, Faculty of Medicine, Zagazig University Hospitals, during the period from December 2020 to December 2022. ***Patient Selection*** Sixteen subjects with type 2 DM (control group) and 16 subjects with type 2 DM and with HFpEF (case group), aged 43–80 years, were enrolled in the study. Enrolled Subjects in the two groups were patients of both sexes, diagnosed with type 2 DM of five years duration or more (based on the diagnostic criteria of American Diabetes Association, 2014) **(10).** ***Echocardiography and Biochemical measurement*** All patients underwent trans-thoracic echocardiography to exclude HFrEF, or HFmrEF, HF due to valvular heart diseases, cardiomyopathy (e.g. infectious or toxic), cor-pulmonale, and to assess ejection fraction, pulmonary artery systolic pressure and E/e$^{\square}$. HFpEF diagnosed according to H_2FPEF Score **Table (1) (11).** All patients subjected to routine laboratory investigations as well as HbA1c. Fasting levels of serum glucagon were assessed, after 8 hours fasting, using human double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) kits supplied by SunRed® Company. ***Ethical clearance*** This study protocol was approved by the Institutional Review Board (IRB) , Faculty of Medicine, Zagazig University, Egypt , before the study was conducted (registration no. IRB #5801/15-12-2019). Written Informed consent was taken from the patients involved in this study. This work followed the regulations of Declaration of Helsinki. ***Statistical analysis*** All data were analyzed using MedCalc Statistical Software version 15.8 (MedCalc Software bvba, Ostend, Belgium; http://www.medcalc.org; 2015). All normally-distributed data were analyzed using Independent Student t (t) test. Data found to be non-normally distributed were analyzed using the Mann-Whitney U (MW) test. Percent of categorical variables were compared using the Chi-square (χ2) test. Spearman's rank correlation coefficient (Spearman's rho) was calculated to assess correlation between glucagon and our study parameters. Receiver operating characteristic (ROC) curve analysis was used to

identify optimal cut-off value of fasting glucagon level (pg/ml) to predict HFpEF. $p < 0.05$ was considered statistically significant (S) and $p \geq 0.05$ was considered non statistically significant (NS).

Table (1): H_2FPEF score and probability of having HFpEF (10)

	Clinical Variable	Values	Points
H_2	***Heavy***	Body mass index > 30 kg/m^2	**2**
	Hypertensive	2 or more antihypertensive drugs	**1**
F	***Atrial Fibrillation***	Paroxysmal or persistent	**3**
P	***Pulmonary Hypertension***	Doppler echocardiographic estimated pulmonary systolic artery pressure >35 mm Hg	**1**
E	***Elder***	Age > 60 years	**1**
F	***Filling pressure***	E/e´ > 9	**1**
H_2FPEF score	H_2FPEF score of 0–1 : low probability (<20%), H_2FPEF score of 2–5 : intermediate probability, H_2FPEF score of 6–9 : High probability (>90%), HFpEF is likely		**Sum (0–9)**

.**Results**

In this study, Thirty two individuals' were involved [15 male (46.9%) and 17 female (53.1%)]. The mean age of the study subjects was 63.46 ± 9.1 years. Twenty-four subjects (75%) were non smoker; however 8 subjects (25%) were smoker. The mean BMI was 34.9 ± 7.47, where the mean diabetes duration was 11.5 ± 4 years. Patients with hypertension were 27(84.4%), in opposite to 5 (15.6%) non-hypertensive.

We compared diabetic patients without HF (group I) and those with HFpEF (H_2FPEF ≥ 6) (group II) as regard the basic demographic parameters as well as the laboratory data which were summarized in **Table (2)** and **(3),** respectively.

Regarding glycemic parameters, A Mann-whitney test indicated that there isn't any difference of FBG for group I (Mdn=173) and group II (Mdn=163); U= 116.5, p = 0.66. There wasn't a significant difference in 2hppG level for group I (M=235.43, SD= 72.6) and group II (M=204.8, SD= 68.4); t = -1.228, p =0.229. There wasn't a significant difference in HBA1c for group I (M=9.31, SD= 2.28) and group II (M=8.16, SD= 1.31); t = -1.74, p = 0.09. As regard fasting serum glucagon, a Mann-whitney test indicated that there is a significant difference of fasting serum glucagon for group I (Mdn=93.1) and group II (Mdn=173.5); U= 52, p = 0.0042.

The comparison between the two study groups regarding the echocardiography parameters highlighted in **Table (4).**

The correlation between H_2FPEF Score and other study parameters were tested, after exclusion of clinical signs and symptoms of heart failure and the six items of H_2FPEF score, using appropriate correlation

analysis, as shown in **Table (5).** Positive correlation between H_2FPEF Score and fasting serum glucagon was found in total population (n= 32, r = 0.445, *P* < 0.00108), **Figure (1)**.

Utilizing ROC curve, fasting glucagon (pg/ml) at cut off value of > 63.56, had AUC = 0.797, with sensitivity of 93.7%, and specificity of 56.2% in predicting the presence HFpEF, **Figure (2)**.

The correlation between fasting glucagon and other study parameters showed a positive correlation between fasting glucagon and AF in total population (n= 32, r = 0.515, *P* < 0.0026).

Table (2): Demographic and clinical data in studied groups:

	Diabetic without HF (n=16)		**Diabetic with HFpEF (n=16)**		**Test**	**P**
	No	**%**	**No**	**%**		
Age (Years) *Mean± SD* *Median (Range)*	62.06 ± 8.33 61 (43 – 74)		64.8 ± 9.92 64 (47 – 80)		**T** 0.868	0.39 (NS)
Sex *Male* *Female*	9 7	56.2% 43.7%	6 10	37.5% 62.5%	χ^2 1.094	0.29 (NS)
Past History						
Smoking Status *Non Smoker* *Smoker*	11 5	68.7% 31.2%	13 3	81.2% 18.8%	χ^2 0.646	0.421 (NS)
HTN *No* *Yes*	5 11	31.2% 68.7%	0 16	0% 100%	χ^2 5.74	**0.016** **(S)**
Diabetes duration (Years) *Mean± SD* *Median (Range)*	12.18 ± 4.18 61 (43 – 74)		10.81 ± 3.88 64 (47 – 80)		**T** - 0.96	0.34 (NS)
BMI *Mean± SD* *Median (Range)*	32.85 ± 7.66 30.98 (22.86 – 46.6)		36.98 ± 6.9 34.8 (30.12 – 55.25)		**T** 1.60	0.11 (NS)
SBP *Mean± SD* *Median (Range)*	134 .37 ± 19 130 (110 – 170)		135.62 ± 22.5 140 (100 – 170)		**T** 0.17	0.866 (NS)
DBP *Mean± SD* *Median (Range)*	134 .37 ± 19 75 (60 – 100)		135.62 ± 22.5 85 (60 – 100)		**MW** 88.5	0.127 (NS)

Table (3): Basic laboratory data of the studied population (n=32):

	Group I	***Group II***	**Test**	***P***
	Diabetic without HF (n=16)	**Diabetic with HFpEF (n=16)**		
WBC (x10^3/mm^3)			**T test**	0.56
Mean± SD	10.75 ± 3.64	11.44 ± 3.07	0.585	(NS)
Median (Range)	10.85 (4.2 – 18.4)	10.7 (7.4 – 17)		
Hemoglobin (g/dl)			**T test**	0.8
Mean± SD	11.88 ± 1.71	11.7 ± 2.18	- 0.253	(NS)
Median (Range)	12.15 (9 – 14.6)	11.8 (9.2 – 15.4)		
Platelet count (x10^3/mm^3)			**T test**	0.95
Mean± SD	281.87 ± 139.5	284.43 ± 136.97	0.05	(NS)
Median (Range)	245 (145 – 562)	241 (134 – 673)		
Creatinine (mg/dl)			**MW**	0.82
Mean± SD	1.2 ± 0.50	1.18 ± 0.53	122	(NS)
Median (Range)	1.05 (0.57 – 2.15)	1.05 (0.71 – 2.88)		
ALT (U/L)			**MW**	0.57
Mean± SD	25.78 ± 21.8	101.5		(NS)
Median (Range)	20 (7 – 95)	24 (4 – 427)	113	
AST (U/L)			**MW**	0.62
Mean± SD	28.2 ± 20.29	24.96 ± 14.9		(NS)
Median (Range)	20.8 (4.8 – 75)	22.0 (8 – 60)	115	

Table (4): Comparison of echogardiographic data of the studied population (n=32):

	Group I **Diabetic without HF (n=16)**	***Group II*** **Diabetic with HFpEF (n=16)**	**Test**	**P**
EF *Mean± SD* *Median (Range)*	64.5 ± 8.44 61.7 (50 – 84)	61.46 ± 4.68 61 (54 – 69)	**MW** 101.5	0.31 (NS)
E/e *Mean± SD* *Median (Range)*	8.75 ± 2.5 7.95 (4.87 – 13)	9.1 ± 0.88 9.1 (7.6 – 11.1)	**T** 0.55	0.58 (NS)
PASP *Mean± SD* *Median (Range)*	31.93 ± 7.28 30.45 (20 – 52)	35.72 ± 7.11 37.9 (22 – 46)	**T** 1.49	0.146 (NS)
HFpEF score *Median (Range)*	3 (1 – 5)	8 (7 – 9)	**MW** 0	**0.0001 (S)**

Table (5): Correlation between HFpEF and different study parameters:

	Total population (n=32)		
	R	***P***	
Sex	0.157	0.3918	(NS)
Smoking	0.181	0.3227	(NS)
DM_Duration	0.0170	0.9262	(NS)
A1C	-0.187	0.30	(NS)
Glucagon	0.445	**0.00108**	**(S)**
HDL	0.429	0.0746	(NS)

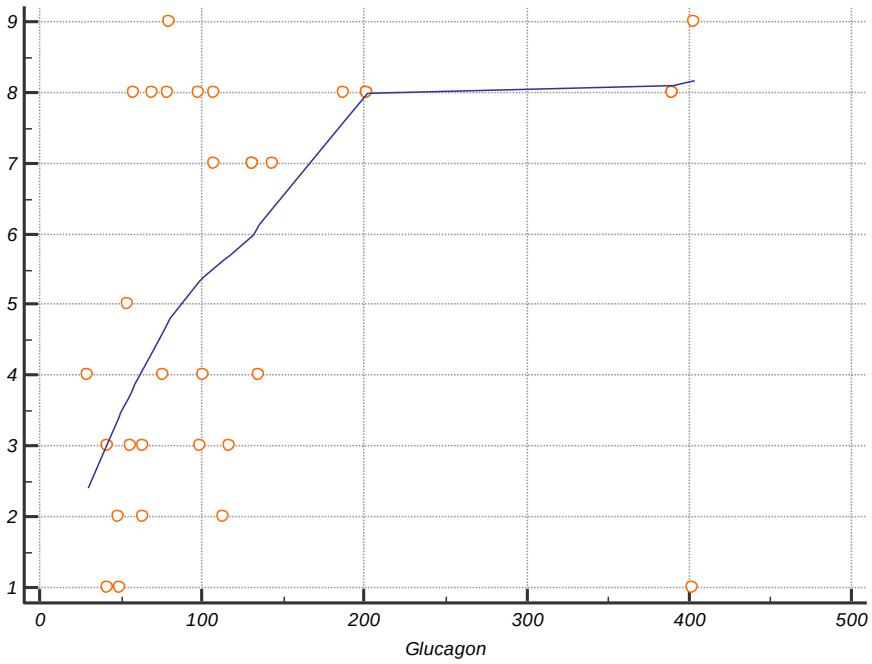

Figure (1): Correlation between HFpEF score and fasting glucagon.

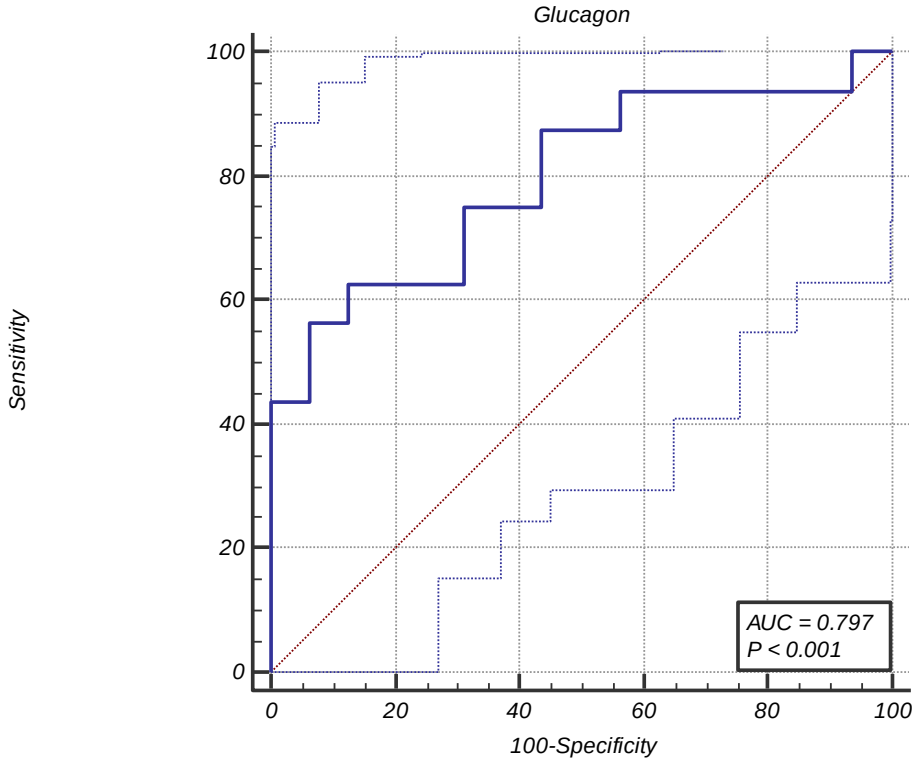

Figure (2) ROC curve of serum glucagon as a predictor of HFpEF

Discussion

The prevalence of HFpEF is more common than HFrEF with a prevalence of 4.9% in comparison to 3.3% **(12)**. Patients with type 2 DM exhibit increased risk of HF. Of note, 25% of type 2 DM patients exhibit various types of HF, including HF with preserved, reduced, and midrange ejection fraction **(13).** Moreover, type 2 diabetes is more strongly associated with the development of HFpEF than with HFrEF **(14)**. The diagnosis of HFpEF is a complex matter (**9)**; thus, it must be supported by elevated levels of natriuretic peptides (NPs). However, NPs can be lower in obese patients with HF, which complicates the diagnosis of HFpEF **(15).** In this setting, our goal in this study was to assess fasting glucagon as a marker to detect HFpEF as early as possible among patients with type 2 diabetes.

Regarding demographic and clinical data in our study, there wasn't statistically significant difference between diabetic patients without HF and those with HFpEF as regard age and sex. Among diabetic patients with HFpEF 37.5% of the patients were males, however 62.5% were females, this in agreement with the Framingham Heart Study, that stated that female sex was associated with a two-fold increased risk for HFpEF **(16).** The age was slightly higher in group II, 64.8 ± 9.92 versus 62.06 ± 8.33 in group I , this result in agreement with *Bhatia et al., 2006*, who compared HFpEF with HFrEF, and found that patients with HFpEF tend to be older than those with HFrEF (75 vs. 72 years) **(17).** BMI did not differ statically between diabetic patients without HF and those with HFpEF , however it was higher in HFpEF patients ,this in the context with *Obokata et al., 2017,* that stated that both obesity and DM are important risk factors for the development of HFpEF **(18).** Hypertension prevalence was significantly higher in group II than group I, 100% versus 68.7%. This consistent with the epidemiology of HFpEF , as shown in by the meta-analysis implemented by *Lam, et al. 2011* **(19).**

In our study, there wasn't a statically significant difference between the studied groups regarding FBG, 2hppG, or HbA1c. In both groups the glycemic control parameters showed a poor glycemic control, however slightly better among HFpEF group. *Blecker et al., 2016,* in a retrospective study conducted on 4723 patients with type 2 DM and HF, found that HbA1c ≥9.0% carried a 13% higher risk of all cause mortality and a 33% higher risk of hospitalization compared to HbA1c of 8.0–8.9% **(20).** In our results the slight improvement in HbA1c in HFpEF group, HbA1c was 9.31±2.28 in Group I in comparison to 8.16±1.31in Group II; t = -1.74, p = 0.09, could be explained by intensification of therapy among HFpEF patients. In this context, The ADA recommendations targeted HbA1c of less than 7 % for most patients with DM but suggest that "less stringent A1c goals (such as < 8 %) may be appropriate" for subpopulations such as those with limited life expectancy or significant co-morbidity **(10).** This could be explained in light of targeting a tight glycemic control had generally failed to improve cardiovascular outcomes in patients with type 2 DM **(21).**

Our results revealed a statically significant difference in fasting glucagon level between group I and group II, where serum glucagon markedly increased among group II, notably both groups were nearly matched where there weren't statically difference in demographic, basic laboratory data and glycemic control parameters. Moreover, a significant positive correlation between H_2FPEF Score and serum glucagon was found. Our results in agreement with *Ceriello A et al., 2016* who stated that the ''plasma levels of glucagon

may contribute to maintain the heart function when the HF is not severe'' **(22)** due to its positive inotropic and chronotropic effect on failing hearts**(8).**

Packer, 2018, extrapolated that **''**in clinical practice, the diagnosis of HFpEF is missed in many patients who are obese or have diabetes **''(23).** *In 2020, Packer, 2020,* found that AF may be the earliest indicator of HFpEF in patients with obesity or type 2 DM **(24).** Our results showed a statically positive correlation between fasting serum glucagon and AF. This could explain the significant difference between diabetic patients without HF and those with HFpEF, moreover, confirm the importance of fasting glucagon as a predictor for HFpEF especially if measured serially.

Conclusion Fasting levels of serum glucagon rose in diabetic patient with HFpEF in comparison with those without HF. Glucagon showed a positive correlation with AF. Serum glucagon represents a clue for early detection of HFpEF among type 2 DM patients especially if measured in a serial manner. Further studies on a large scale should be conducted to confirm the beneficial role of glucagon as a predictor of HFpEF and document the best cutoff ratio for HFpEF detection.

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