ORIGINAL ARTICLE

Effect of Linagliptin on Incretin-axis and Glycaemic Variability in T1DM

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Abstract

Backgrounds & Objectives: Short-term studies have demonstrated potential therapeutic efficacy of dipeptidyl peptidase 4 inhibitors (DPP4 inhibitors) in patients with poorly controlled T1DM. In this study we evaluated the effect of DPP4 inhibitor, linagliptin, on glycaemic control and variability, and incretin-axis in well controlled T1DM patients to mitigate the effect of glucotoxicity on incretin secreting cells.

Methods: Twenty T1DM patients were randomized to receive either linagliptin (10 patients, dose-5 mg/day) or placebo (10 patients), in addition to insulin for 3 months. HbA1C, continuous glucose monitoring (CGM) and mixed meal test (MMT) were performed before and at the end of the study period.

Results: HbA1C reduction and change in glycaemic variability and insulin requirement in the linagliptin group did not attain the level of statistical significance. The increase in AUC_{GLP1} (Area under curve for GLP1) and decrease in $AUC_{glucagon}$ (Area under curve for glucagon) during the MMT in linagliptin group were also statistically insignificant.

Interpretations & Conclusions: Linagliptin is not effective in reducing HbA1C and glycaemic variability in relatively well controlled T1DM patients.

Introduction

Type 1 diabetes mellitus (T1DM) is the commonest endocrine disorder of young adult population. Although, reduction in HbA1c is important in improving diabetes-related microvascular complications; glycaemic variability is increasingly recognised as an important contributing factor.[1,2] T1DM is characterised by absolute insulin deficiency and hence insulin is the mainstay of treatment. Insulin therapy is challenging and often complicated with frequent hypo- and hyperglycaemic episodes. To overcome these difficulties, investigators have used oral anti-diabetic drugs as an adjunct to insulin therapy in T1DM with limited benefit.[3,4]

Dipeptidyl peptidase 4 (DPP4) inhibitor increases endogenous glucagon-like peptide 1(GLP1) levels by inhibiting its rapid metabolism through the DPP4 enzyme. The raised GLP1 level causes increase in insulin release from the β-cells and decrease glucagon secretion from the α-cells; thereby, resulting in better glycaemic control.[5] These drugs are currently Food and Drug Administration (FDA) approved for the treatment of T2DM as they have been shown to be GLP1 deficient.[6] Hyperglucagonemia has been reported in patients with T1DM in many studies;[7] hence, incretin-based therapy has been tried to target this pathophysiological defect in T1DM.

In a pilot study sitagliptin, a DPP4 inhibitor, when used along with insulin was found to be effective in poorly controlled T1DM patients.[9] However, the mechanism of action remains elusive as the incretin response was not assessed in this study. Further, Lugari et al. has demonstrated blunting of GLP1 response during mixed meal test (MMT) in T1DM subjects and proposed chronic hyperglycaemia could have resulted in intestinal 'L cell failure' due to glucotoxicity. Linagliptin, another DPP4 inhibitor, which unlike sitagliptin does not require dose modification in renal failure patient, has not been studied yet in T1DM patients.

In the present study we investigated the effect of linagliptin on HbA1c and glycaemic variability in patients with T1DM who are relatively well controlled to obviate the effect of glucotoxicity on intestinal L cells.

Subjects and Methods

The research proposal was approved by the Institute Ethics Committee and registered in ClinicalTrial.gov (id.NCT02725502). Written informed consent was obtained from each of the patient participating in the study. The study was conducted according to the Declaration of Helsinki and ICH-GCP (International Conference on Harmonization-Good Clinical Practice) guidelines. During the study ICMR's Ethical guidelines for biomedical research on human participants (2006) were strictly followed.

It was a 12 week randomized double-blind placebo control prospective study conducted in PGIMER, Chandigarh from 2013-2016. Euthyroid individuals with T1DM of either gender with age between 15-30 years, duration of DM between 6 months to7 years, having BMI of < 25 kg/m^2 and HbA1c < 8% were enrolled in this study. The diagnosis of T1DM was based either on diabetic ketoacidosis (DKA) as the 1st presenting manifestation of the disease or on insulin requirement since the diagnosis along with anti-GAD65 Ab positivity.

All the participants were on stable doses of insulin for the last one month. Patients with creatinine >1.5mg/dl or calculated creatinine clearance of < 50 ml/min or having overt proteinuria, celiac disease, pregnancy, serious illness and gastroparesis were excluded from the study. Those, who were on metformin, GLP-1 agonist, DPP 4

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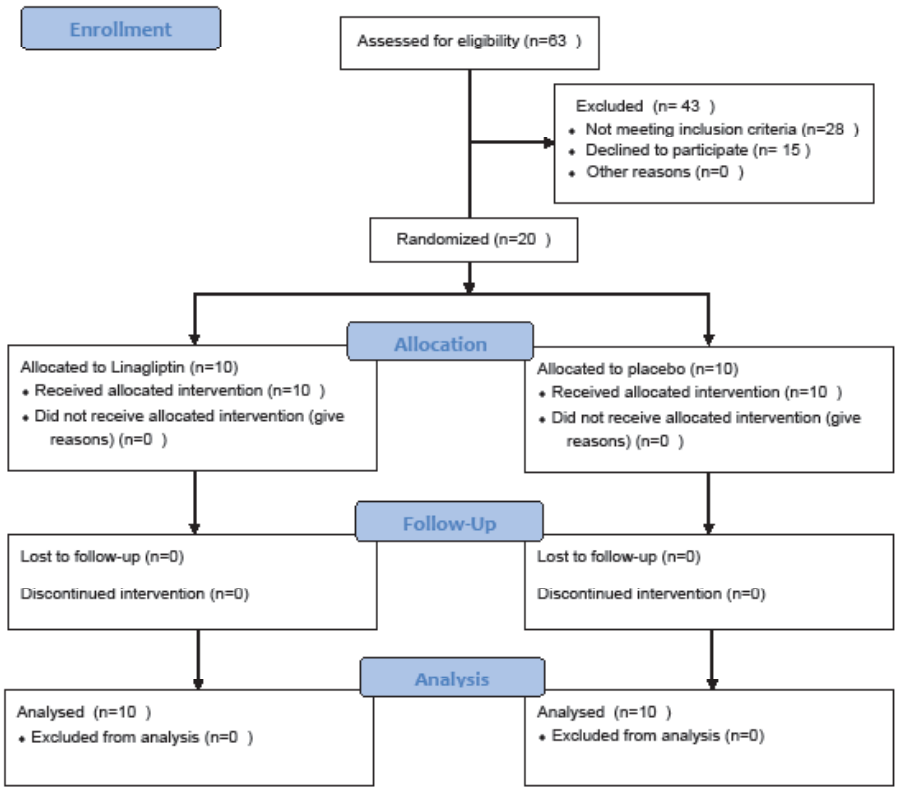

Fig. 1: Flow diagram for participant recruitment

inhibitor, prokinetics and proton pump inhibitors, were also excluded from the study.

A flow diagram for participant recruitment has been shown in the Figure 1. Twenty T1DM patients were included for analysis. The primary end point of the study was reduction in HbA1c. From the previous pilot study (Estimated difference of HbA1C between intervention and placebo group was -0.27±0.11%) it was estimated that 7 patients were required in each group to get a significant difference with 80% power and statistical significance of 5%[6]. The secondary end points include, change in glycemic variability, insulin requirement, $AUC_{Glucagon}$ (Area under curve for glucagon) and AUC_{GLP-1} (Area under curve for GLP1) At the onset of the study, patients' HbA1c and daily insulin requirement were calculated.

Seventy two hour blood glucose profile with continuous glucose monitoring system (CGMS) was monitored and mixed meal test (MMT) was performed. During CGMS monitoring indices of glucose control [mean continuous glucose monitor glucose, M120, J-index, Glycemic Risk Assessment Diabetes Equation (GRADE), High and Low Blood Glucose Index and time spent in hyper, hypo and euglycemic ranges] and glucose variability [mean amplitude of glycaemic excursion (MAGE), and glucose standard deviation] were calculated.[7] On the next day, after the continuous glucose monitoring had been completed, the patients were subjected to MMT with the standard protocol.[8,9] After overnight fast, mixed meal (Ensure, Abbott) was administered at a dose of 10 Kcal/kg mixed in water. Morning dose of insulin was omitted on the day of MMT. On the day of MMT, if blood glucose was <4 or >10 mmol/L, then the procedure was deferred till the blood glucose was in the desirable range. After the mixed meal was administered, blood samples for glucose, C-peptide, GLP1 and glucagon was collected at 0, 30, 60, 120 and 180 minutes. Blood samples for GLP1 and glucagon were collected in pre-chilled EDTA tube containing protease inhibitors and plasma was separated immediately and stored at -80 ^{0}C, till analysed.

Randomization and intervention: These patients were randomly divided into two groups - group A & group B, 10 patients in each group with the help of a computer-generated randomization list. In addition to insulin regimen, patients of group A received linagliptin (5 mg/day), while patients of group B received placebo for the next 3 months. During this period the patients were followed-up by weekly telephonic conversation and monthly visits. Insulin dose adjustment was made to achieve target blood glucose level (Fasting blood glucose 80-130 mg/dl and 2h post meal blood glucose 140-180 mg/dl).

HbA1c, 72 hour blood glucose profile (with CGMS), and MMT were repeated at 3 months to document any change. Daily dose of insulin per kg body weight before and after the study were also compared.

Biochemistry: Blood glucose was estimated by glucose oxidase peroxidase (GOD-POD) enzymatic method and HbA1c was measured by ion exchange chromatography (D 10 Biorad USA). C- Peptide was measured by electrochemiluminescence immunoassay (Roche diagnostic Germany). Plasma glucagon (Sigma Aldrich, USA) and total GLP-1(Cusabio, China) were analyzed by ELISA.

Statistical Analysis

Data was analyzed using the SPSS 22.0 software package. Values were expressed as median along with interquartile range. The area under curve for glucose ($AUC_{glucose}$), GLP1 (AUC_{GLP1}), glucagon ($AUC_{glucagon}$) and C-peptide ($AUC_{C\text{-}peptide}$) were calculated according to the trapezoidal rule. Pre- and post-treatment values within each group were compared by Wilcoxon signed Ranks test. Mann-Whitney test was used to compare the parameters between the two groups. Data were considered significant if $p < 0.05$.

Results

A total of 20 patients (n=10 in each group) completed 3 months follow-up period without any dropout. The baseline parameters were similar between the two groups (Table 1).

Change in Weight and BMI

There was no significant change with respect to body weight and BMI after 3 months of treatment with linagliptin [51kg (IQR,45-55) to 50.5kg (45-61), p=0.43 and 18.9 kg/m^2 (17.5-20) to 18.4 kg/m^2 (17.5-20.9), p=0.72, respectively] and placebo [49kg (45-54) to 49.5kg (44-54), p= 0.26 and 18.9 kg/m^2 (17.3-

Table 1: Baseline study subjects

Parameters	Linagliptin (n=10)	Placebo (n=10)	P value
Age (year)	20 (17-24)	20 (18-22)	0.84
Duration (year)	4.3 (1.5-7)	2 (1-4)	0.09
Weight (kg)	51 (45-55)	49 (45-54)	0.63
BMI (Kg/ m^2)	18.9 (17.5-20)	18.9 (17.3-19.8)	0.75
FBG (mmol/L)	6.1 (5-6.9)	6.7 (5.8-8.2)	0.11
PPBG (mmol/L)	9.7 (8.0-9.9)	9.3 (8.3-10.1)	0.68
HbA1C (mmol/mol)	54 (50-55)	52 (48-56)	0.42
MMT parameters at baseline			
AUC $_{Glucose}$ (mmol/L X minute)	3583 (2906-3717)	3689 (3340-3796)	0.36
AUC $_{Glucagon}$ (pg/ m X minute l)	7164 (2811-9257)	2231 (1424-5477)	0.15
AUC $_{GLP\ 1}$ (ng / ml X minute)	1549 (345-5339)	2087 (974-2822)	0.67
AUC $_{C\text{- peptide}}$ (ng / ml X minute)	776 (419-940)	287 (186-377)	0.82
Baseline CGMS parameters			
CGMS Mean Glucose (mmol/L)	7.1 (5.8-7.9)	7.5 (5.7-9)	0.37
Glucose standard deviation (mmol/L)	2.6 (2.3-3)	2.9 (2.2-4.4)	0.49
J Index	33.7 (24.1-36.1)	34.8 (22.8-75.4)	0.28
LBGI	6.6 (2.5-8.3)	2.3 (1.1-13.9)	0.96
HBGI	6.9 (4.3-7.7)	6.9 (4.4-22.1)	0.28
GRADE	4.3 (2.9-7.1)	5.7 (2.5-11.4)	0.36
MAGE	5 (4.3-9.1)	5.9 (4.5-8.1)	0.60
M 120	8.9 (5.3-15.4)	21.4 (5.6-39.7)	0.14
Time spent >10 mmol/L (%)	17.5 (7-29)	23.5 (9-29)	0.35
Time spent 4-10 mmol/L (%)	65.5 (59-77)	60.5 (38-76)	0.30
Time spent < 4 mmol/L (%)	8.5 (3-31)	5.5 (0-35)	1.000
Baseline Insulin requirement:			
Daily total insulin requirement /kg body weight (unit/Kg)	0.83 (0.75-1.12)	0.85 (0.53-1.05)	0.62

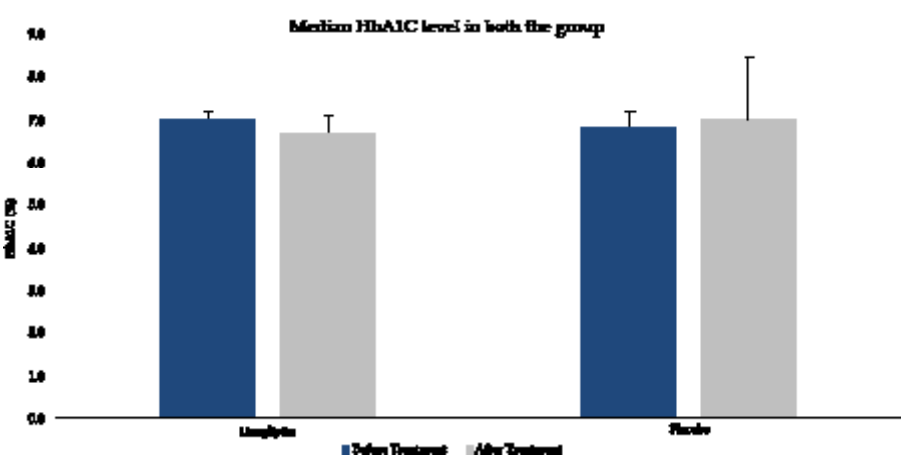

Fig. 2: Median HbA_1C level in both the group

19.8) to 18.9 kg/m^2 (17.4-19.8), p=0.56, respectively]

Change in FBG, PPBG and HbA1c

There was a modest decrease in HbA1C from 54mmol/mol (50-55) [7.1% (6.7-7.2)] to 50mmol/mol (45-54) [6.75% (6.3-7.1)] in linagliptin group; whereas there was a mild increase in HbA1C from 52 mmol/mol (48-56) [6.9% (6.5-7.3)] to 54 mmol/mol (46-69) [7.1% (6.4-8.5)] in placebo group; however, these changes were statistically insignificant (p=0.39, 0.2; respectively, Figure 2). Similarly, in linagliptin group FBG remained unchanged [6.3 mmol/L (4-7.5) to 6.1 mmol/L (5.1-6.9), p=0.8]; whereas, PPBG showed a decreasing trend [9.7 mmol/L (8.0-9.9) to 7.6 mmol/L (6.7-10.7), p=0.45] after 3 months of therapy and in placebo group both FBG and PPBG showed a rising trends [6.7 mmol/L (5.8-8.1) to 7.1 mmol/L (4.8-8.8), p=0.77 and 9.3 mmol/L (8.3-10.1) to 11.4 mmol/L (9.2-12.0), p=0.10, respectively]; however these alterations were statistically insignificant.

Changes in CGMS Parameters

CGMS data which represents glycaemic control and variability remained similar even after 3 months of treatment (Table 2). Neither the time spent in euglycemic range (4-10 mmol/L) nor the time spent in hypo- or hyperglycaemic range changed significantly in both the groups from baseline to completion of the study (Table 2).

Glucose and C-Peptide level during MMT

Five patients in linagliptin and 3 patients in placebo group had undetectable C-peptide level (0.01ng/ml). Changes in AUC $_{Glucose}$ during MMT in linagliptin group [3583 mmol/L X minute (2906-3717) to 3458 mmol/L

Table 2: Comparison with in each group with respect to CGMS parameters after 3 months

CGMS parameters	Linagliptin			Placebo			Inter- group comparison at 3 months
	Baseline	After 3 months	P value	Baseline	After 3 months	P value	P value
Mean Glucose (mmol/L)	7.1 (5.8-7.9)	7.1 (6.7-7.9)	0.39	7.5 (5.7-9)	8.5 (7.6-10.7)	0.85	0.31
Glucose standard deviation (mmol/L)	2.6 (2.3-3)	3.4 (2.4-4.8)	0.11	2.9 (2.2-4.4)	3.5 (2.5-3.9)	0.78	0.46
J Index	33.7 (24.1-36.1)	32.4 (28.1-47.4)	0.16	34.8 (22.8-75.4)	46.1 (35.3-67.6)	0.85	0.72
LBGI	6.6 (2.5-8.3)	5.7 (3.4-11.4)	0.41	2.3 (1.1-13.9)	4.2 (1.3-5.8)	0.28	0.07
HBGI	6.9 (4.3-7.7)	7.9 (4.8-19.1)	0.15	6.9 (4.4-22.1)	10.3 (5.3-16.9)	0.65	0.81
GRADE	4.3 (2.9-7.1)	4.6 (2.6-12.7)	0.46	5.7 (2.5-11.4)	5.6 (4.6-13.3)	0.82	0.71
MAGE	5 (4.3-9.1)	5.5 (4.9-7.4)	0.82	5.9 (4.5-8.1)	6.2 (5.1-8.9)	0.71	0.98
M value	8.9 (5.3-15.4)	14.9 (5.4-32.2)	0.21	21.4 (5.6-39.7)	13.4 (8.5-21.6)	0.18	0.40
Time spent >10 mmol/L (%)	17.5 (7-29)	18.5 (12-34)	0.55	23.5 (9-29)	31.5 (19-56)	0.64	0.25
Time spent 4-10 mmol/L (%)	65.5 (59-77)	69 (33-81)	0.64	60.5 (38-76)	58.5 (35-65)	0.92	0.44
Time spent <4 mmol/L (%)	8.5 (3-31)	10 (5-17)	0.94	5.5 (0-35)	4.5 (0-12)	0.73	0.67

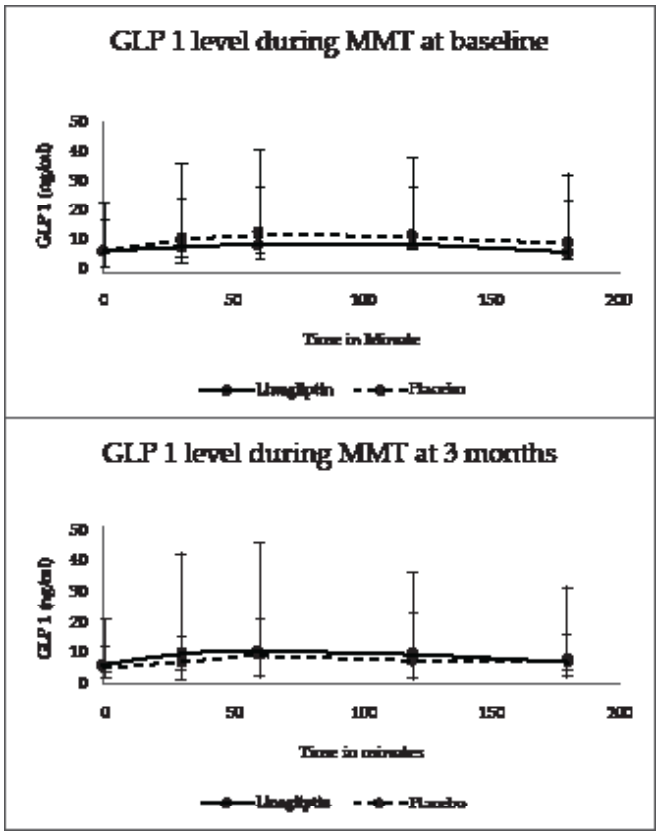

Fig. 3: GLP1 level during MMT

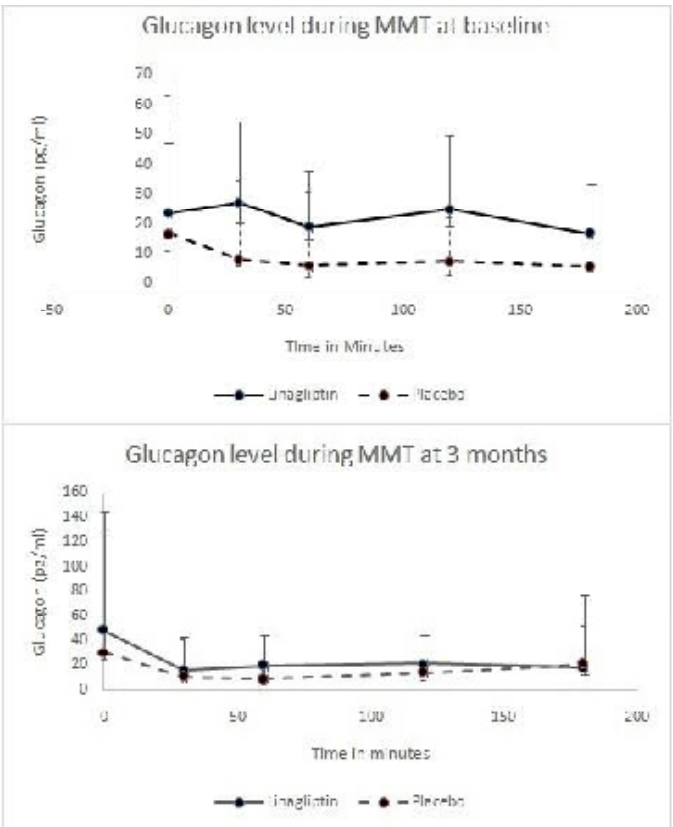

Fig. 4: Glucagon level during MMT

X minute (1979-3845), p=0.41] and in placebo group [3689 mmol/L X minute (3340-3796) to 3668 mmol/L X minute (3132-3867), P=0.75] were statistically insignificant. Similarly, AUC $_{C\text{-peptide}}$ did not alter significantly in linagliptin [351 ng/ml X minute (242-380) to 208 ng/ml X minute (182-243), p=0.11] and placebo group [113 ng/ml X minute (94-188) to 134 ng/ml X minute (101-184), p=0.59].

GLP-1 Level during MMT

The median GLP-1 levels during MMT between the two groups were similar at baseline and at the end of the study (Figure 3). The AUC $_{GLP\text{-}1}$ during the MMT showed a rising trend in linagliptin group [1549 ng/ml X minute (345-5339) to 1821 ng/ml X minute (524-5650), p=0.13]; while it showed a decreasing trend in placebo group [2087 ng/ml X minute (974-2822) to 1459 ng/ml X minute (1057-2165), p=0.57], however these changes were statistically insignificant.

Glucagon Level during MMT

One patient in linagliptin group and two patients in placebo group had undetectable glucagon level (0.1pg/ml). Although glucagon levels were not significantly different between the two groups during the MMT at baseline and after 3 months (Figure 4), the median AUC $_{glucagon}$ tend to decrease in linagliptin group [7164 pg/ml X minute (2811-9257) to 6103 pg/ml X minute (2946-10244), p=0.35], while it showed rising trend in placebo group [2231 pg/ml X minute (1424-5477) to 5688 pg/ml X minute (1790-9497), p=0.15], but these changes were statistically insignificant.

Insulin Requirement

Daily total insulin requirement decreased from 40 unit (34-48) to 32 unit (20-46) in linagliptin group after 3 months (P=0.58), while in the placebo group it decreased from 40.5 unit (28-48) to 35.3 unit (29-46) after 3 months (P=0.11). Insulin requirement per kg body weight did not change significantly in both linagliptin [0.84 unit/kg (0.75-1.12) to 0.66 unit/kg (0.44-0.81); p=0.84] and placebo group [0.86 unit/kg (0.53-1.05) to 0.73 unit/kg (0.48-0.91), p=0.09].

Adverse Events

No serious side effects related to linagliptin (nausea, vomiting or pancreatitis) therapy were observed during the study period.

Discussion

In this study, we examined the effect of linagliptin on incretin-axis and on glycaemic variability in relatively well controlled T1DM patients. Although we observed a decreasing trend in HbA1C and PPBG in linagliptin group; it did not attain statistical significance. Neither the glycaemic variability nor the insulin requirement changed significantly with linagliptin therapy. The increase in AUC $_{GLP1}$ and decrease in AUC $_{glucagon}$ during the MMT in linagliptin group were also statistically insignificant.

In a study liraglutide,[13] a GLP 1 agonistic analogue, when used in T1 DM patients for 4 weeks period showed a trend towards lowered HbA1c with significantly lower total daily insulin requirement. In a pilot study[9] sitagliptin, another DPP4 inhibitor was shown to be effective in reducing HbA1c and glycaemic variability in T1DM patients. But it was a short duration (4 weeks) cross-over trial and the baseline mean HbA1c was around 9.5% (80 mmol/mol). Our study was of 12 weeks duration and showed a decreasing trend in PPBG and HbA1c with no alterations in insulin requirement and glycaemic variability with linagliptin therapy. The baseline median HbA1c in our study group was around 7% (53

mmol/mol). The non-significant change in glycaemic profile in our study may be explained by inclusion of well controlled DM patients, and hence the magnitude of HbA1c reduction was too meagre to attain the level of statistical significance.

Linagliptin is a DPP4 inhibitor. DPP4 is an enzyme which is responsible for degradation of GLP 1. So DPP4 inhibitor increases GLP1 level which in turn increases insulin release from pancreatic β cells and decreases glucagon release from α cells.[14] Kielgast[15] et al. showed that GLP-1 infusion reduces gastric emptying rate and glucagon levels in T1DM patients and increases fasting C-peptide in C peptide positive T1DM patients. Lugari R et al.[8] evaluated endogenous GLP 1 concentrations, both at fasting state and in response to nutrient ingestion, in type 1 and 2 diabetes patients and in healthy controls. They observed that there was no increase in postprandial GLP-1 in patients with T1DM and proposed that chronic hyperglycaemia could result in desensitization of the L-cells with consequent peptide failure response i.e. "L cell glucotoxicity". The AUC_{GLP-1} tended to increase in the linagliptin group, while in the placebo group it tended to decrease after 3 months of study period; however, these changes were statistically non significant. Chronic hyperglycemia and consequent L cell failure or relatively small sample size may be responsible for this. In our study $AUC_{glucagon}$ tend to decrease in linagliptin group; while it tend to increase in placebo group, however these changes were also statistically insignificant. Farngren J et al. have shown that during the meal, glucagon levels were lower with vildagliptin treatment, (another DPP4 inhibitor) than with placebo.[16] However, they took relatively less well controlled T1DM subjects than we did [Baseline mean HbA1C 7.5% (58 mmol/mol) and fasting blood glucose 10.5 mmol/L vs. baseline median HbA1C 7.1% (54 mmol/mol) and FBG 6.1 mmol/L in linagliptin group in our study]. Decrease in glucagon level could not attain level of statistical significance in linagliptin group. This is possibly due to use of supraphysiologic dose of insulin which can suppress α-cells.[17] This fact is further substantiated by frequent hypoglycemic episodes and undetectable glucagon level in some of the patients.

Limitations of our study are inclusion of relatively well controlled diabetes patients, small sample size and heterogeneity in β-cell reserve with respect to C peptide positivity. Further, we have used total GLP1 assay; however, intact GLP1 assay may be more useful to assess the DPP4 effect.[18, 19]

The status of incretin-axis in patients with T1DM remains elusive. DPP-4 inhibitor may not be effective in well controlled patients with T1DM possibly because of α-cell inhibition of supra-physiologic dose of exogenous insulin.

Acknowledgment

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