**Secretion of glucagon- like peptide-1 (GLP-1) in type 2 diabetic patients: Study of its relation to glucose lowering effect of dipeptidyl peptidase IV (DPP-IV) inhibitors.**

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**Abstract: Background:** (1) Th aims of this study are to investigate the glycemic efficy and predictive parameters of DPP IV inhibitors therapy in Egyptian subjects with type 2 diabetes. (2) Investigate the level and the determinants that affect the secretion of glucagone like peptide-1(GLP-1) in Type2 diabetic patients. **Subjects and Methods:** This study was conducted on 70 type 2 diabetes patients【35 males & 35 females】under known antidiabetic drugs, Their ages range was (30-63y) and average mean (46.31±10.75 y), as well as 20 apparently healthy subject's volunteers served as control 【12 males & 8 females】with age range (28-50y) and SD (37.40±5.04 y). DPP-4 inhibitors were added to every patient after the start of the study. The patients were followed at monthly interval for 3 months after the beginning of DPP-4 inhibitors therapy. **Results:** GLP-1 levels were significantly decreased in DM subjects compared to controls (261.33± 9.37 vs.75.48 ± 20.81pg/mL, *p*<0.001) and it was negatively correlated with age, body mass index (BMI), DM duration, glycated hemoglobin (HBA1C %), fasting and 2 hour postprandial plasma glucose (FPPG, 2HPPG) and positively with plasma C-peptide level in all studied groups. Dpp-IV inhibitors significantly improved hemoglobin A1C (HbA1c) levels over 3 months. Th changes in HbA1c levels (ΔHbA1c) at month 3 were -3.97% (*P*<0.000).We investigated characteristics associated with the efficacy of dipeptidyl peptidase-4 inhibitors (DPP4i) in Egyptian patients with type 2 diabetes. We reviewed medical records of 70 patients who had taken DPP4i for 3 months. Response to Dpp-IV inhibitors was evaluated with HbA1c change after therapy.The Student's t-test between Responders (R: HbA1 c ≤7 .0%) and Non-Responders (NR: HbA1 c > 7%), a correlation analysis among clinical parameters, and a linear multivariate regressionanalysis were performed. The mean age was 46.31±10.75 yr, duration of diabetes 11 y rand HbA1c was 10.77%. Baseline C-peptide and GLP-1 were significantly higher in the R compared to the NR while age, BMI and DM duration were lower. DM duration, FPG, HbA1c, C-peptide and GLP-1were significantly correlated with HbA1c.In the multivariate analysis, only DM duration (P<0.02) was found to be an independent variable that could predict therapeutic efficacy of DPP-IV inhibitors as an addontherapy**. Coclusion:** In EgyptianT2DM subjects, DPP4i responders had lower BMI, shorter DM duration and were younger compared to non-resp. DPP4i was effctive when it was used in subjects with poor glycemic control.

**[**Atef Ahmed Ebraheem, Mohammed Shawky El-Sayed, Roshdy Khalf Allah, Khaled Mostafa Belal, Rizk Sayad Rizk Sarhan. **Secretion of glucagon- like peptide-1 (GLP-1) in type 2diabetic patients: Study of its relation to glucose lowering effect of dipeptidyl peptidase IV (DPP-IV) inhibitors.** *Nat Sci* 2015;13(6):75-87]. (ISSN: 1545-0740). <http://www.sciencepub.net/nature>. 12

**Key words:** glucagon- like peptide-1, Type2 DM, dipeptidyl peptidase IV (DPP-IV) inhibitors, GLP-1 level, incretin hormone, incretin based therapy.

**1. Introduction**

Glucagon-like peptide-1 (GLP-1) is an incretin hormone secreted from enteroendocreine L cells in response to ingested nutrients. GLP-1 exerts trophic action on pancreatic B-cell mass and survival. GLP-1 stimulates B-cell replication and DNA synthesis in experimental studies in vitro. GLP-1 induces the differentiation of progenitor cells in pancreatic duct epithelium towards that of B-cells. GLP- 1 also exerts antiapoptotic effects in rodent models of B-cells (1, 2, 3). GLP-1 induces satiety and reduces food intake in humans and animals. The two possibilities are that either peripheral GLP-1 acts on afferent neural fibers projecting to the appetite regulating areas of central nervous system (CNS) or that GLP-1 directly reaches CNS through areas, such as the area postrerna and subfornical organ that are devoid of blood-brain barrier and GLP-1Rs present in these locations project nerve fibers to appetite regulating areas of CNS (4, 5). Improved glycemic control by DPP-4 inhibitors may be attributed to enhanced pancreatic function and attenuated insulin resistance possibly secondary to reduction in glucose toxicity but an acute, insulin sensitizing effect of these agents, independent of improvement in glycemic control,has also been described (6). Extra pancreatic actions of GLP-1 include; delay of gastric emptying, reduction of appetite, increase satiety, increase glucose uptake by liver, muscle and adipocytes, decreased hepatic production by liver and increase of muscle glycogen synthase activity (7).

In type 2 DM, the incretin effect is reduced or absent, suggesting a role for incretion hormones or their actions in the pathogenesis of type 2 diabetes (8). Toft-Nielsen *et al.,* 2012 (9) compared GLP-1 secretion (total and intact) in response to a standardized meal in subjects with type 2 diabetes, impaired glucose tolerance and normal glucose tolerance. In this study, meal stimulated GLP-1 secretion was significantly decreased in subjects with type 2 DM compared to glucose tolerant subjects. The subjects with impaired glucose Tolerance had a GLP-1 response that was intermediate between normal and diabetic subjects.

Multiple regression analysis revealed that GLP-1 response was determined positively by the presence of diabetes, male sex, greater BMI, glucagon and negatively by insulin and GIP (10). GLP-1 elimination rates are similar in subjects with type 2DM and normal controls, appearing to role out abnormal metabolism of GLP-1 as a reason for the decreased circulation GLP-1concentrations observed (11).

Whether impaired GLP-1 secretion is a primary phenomenon in the pathogenesis of diabetes or a consequence of diabetes is not known. First-degree relatives of patients with type 2 DM have normal GLP-1 secretion in response to oral glucose and meals, suggesting that the GLP-1 secretion abnormality seen in diabetes may be acquired (12). The actions of GLP-1 are very rapidly terminated by in vivo Nterminal peptide cleavage at the His-Ala by DDP-IV, giving rise to a biologically inactive from GLP-1 (9-36) amide which may act as GLP-1 receptor antagonist (9).The deficient secretion of GLP-1 in type 2 diabetic patients is the rational for replacing endogenous incretins with GLP-1 receptor agonists or renormalizing active GLP-1 concentrations with DPP-IV inhibitors (13).

**2. Material and methods:**

**Subjects:**

This study was conducted on 70 type 2 diabetes patients【35 males & 35 females】under known antidiabetic drugs, Their ages range was (30-63y) and average mean (46.31±10.75 y), as well as 20 apparently healthy subject's volunteres served as control 【12 males & 8 females】with age range (28-50y) and SD (37.40±5.04 y).Patients were recruited from those attending the outpatients diabetic Clinics at Benha University Hospitals. DPP-4 inhibitors were added to every patient after the start of the study. The patients were followed at monthly interval for 3 months after the beginning of DPP-4 inhibitors therapy.

**Study design:**

All individuals included in this study were subjected to:

1-Through history and clinical examination, with stress on the following: duration of DM, past and current antidiabetic drugs, complication of DM and any other associated and present medication.

2-Anthropometric data (height, weight and body mass index (BMI): BMI was calculated using the formula: weight (kg)/height (m2). Underweight <18.5, Normal BMI ranges from 18.5 to 24.9. Overweight: pre obese 25: 29.9, obese class I 30: 34.9, obese class II 35: 39.9, obese class III ≥ 40 (14).

3- The following Laboratory investigations were performed to all subjects; FPG levels were determined using Synchron CX9٭ system auto-analyzer applying enzymatic colorimetric method.and a standard glucose oxidase method. HbA1c levels were measured using high performance liquid chromatography.Total cholesterol, high density lipoprotein cholesterol (HDL-C), and low density lipoprotein cholesterol (LDL-C) levels were measured by an enzymatic method using a 7,150 autoanalyzer (Hitachi), and GLP-1 and c-peptide were determined by radioimmunoassay.

Diabetic were followed at mothly interval for three months regarding:

1-Treatment with DDP-IV inhibitors as add on therapy to current anti-diabetic drugs.

2- FPG and 2HPPG every month after starting treatment with DPP-4 inhibitors up to three months.

3- HBA1C% every month after starting treatment with DDP-IV inhibitors up to three months.

**Statistical analysis:**

The data were recorded on an “Investigation report form”. These data were tabulated, coded then analyzed using the computer program SPSS (Statistical package for social science) version 16 to obtain:

**Descriptive data:** Descriptive statistics were calculated for the data in the form of: Mean, standard deviation (±SD) and number and percent.

**Analytical statistics:** In the statistical comparison between the different groups, the significance of difference was tested using one of the following tests:-

Student's t-test:-Used to compare between mean of two groups of numerical (parametric) data.

ANOVA (analysis of variance):- Used to compare between more than two groups of numerical (parametric) data. Pearson correlation coefficient (r) test was used correlating different parameters.

Inter-group comparison of categorical data was performed by using chi square test (X2-value).

Some investigated parameters were entered into a logistic regression model to determine which of these factors is considered as a significant risk factor and identify its odds ratio.

*P* value <0.05 was considered statistically significant (S). And a P value <0.0001 was considered highly significant (HS) in all analyses.

**3. Results:**

Out of a total of 70, type 2 diabetic patients, 35 were men, and 35 were women. The mean age was 46.31±10.75 years, Out of a total of control subjects, 12 were men and 8 were women. The mean age was 37.40±5.04 years.For diabetic patients baseline the mean value of HbA1c was 10.77±1.00%, FPG was 269.49±74.98mg/dL, post prandial 2 hour blood glucose (PP2) was 370.13±86.94mg/d. GLP1 level was 26.33±9.37pg/ml and C-peptide was 1.02±0.51ng/mL, the average BMI was 28.72±2.84kg/m2, the mean T.CHOL was 181.33±31.8 mg/dl, LDL 97.96±12.66 mg/dl, HDL 47.51±13.98 md/dl and TG 148.00±27.51mg/dl for patients.For control the mean value of HbA1c was 4.98±0.49%, FPG was 93.20±23.17 mg/d, post prandial 2 hour blood glucose (PP2) was 122.90±86.94 mg/dL.GLP1 level was 75.48±20.81pg/ml and C-peptide was 2.97±0.62ng/ml. The average BMI was 25.16±1.39kg/m2, the mean T.CHOL was 139.10±9.23 mg/dl, LDL 89.75±3.23 mg/dl, HDL 59.95±2.82 md/dl and TG 84.80±4.96 mg/dl.

This table shows that FPG, PPG, HBA1C, serum total cholesterol, LDL, and TG are significantly high in diabetic patients compared to control subjects, whereas C-peptide, HDL, GLP-1 are significantly low in diabetic patients compared to control subjects.



**Figure (1): Comparison between cases and control regarding C-peptide and GLP1 level**

Table (1): comparison of the baseline clinical and laboratory data between diabetic patients and control subjects.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | | Cases (n=70) | | Control (n=20) | | t | *p*-value |
| Mean | ±S. D | Mean | ±S. D |
| Age (year) | | 46.31 | 10.75 | 37.40 | 5.43 | 5.04 | <0.001 |
| BMI 【BW(kg)/Height(m)²】 | | 28.72 | 2.84 | 25.16 | 1.39 | 5.4 | <0.001 |
|  | | No. | % | No. | % | X2 | *p*-value |
| Sex | Male | 35 | 50.0% | 12 | 60.0% | 0.3 | >0.05 |
| Female | 35 | 50.0% | 8 | 40.0% |
|  | | Cases (n=70) | | Control (n=20) | | t | *p*-value |
| Mean | S. D | Mean | S. D |
| FPG (mg/dl) | | 269.49 | 74.98 | 93.20 | 23.17 | 17.03 | <0.001 |
| 2HPPG (mg/dl) | | 370.13 | 122.90 | 86.94 | 7.87 | 23.5 | <0.001 |
| HBA1C % | | 10.77 | 1.00 | 4.98 | 0.49 | 35.6 | <0.001 |
| C-PEPTIDE (ng/ml) | | 1.02 | 0.51 | 2.97 | 0.62 | 12.9 | <0.001 |
| GLP1 (pg/ml) | | 26.33 | 9.37 | 75.48 | 20.81 | 10.3 | <0.001 |
| T.CHOL(mg/dl) | | 181.33 | 31.80 | 139.10 | 9.23 | 9.8 | <0.001 |
| LDL (mg/dl) | | 97.96 | 12.66 | 89.75 | 3.23 | 4.9 | <0.001 |
| HDL (mg/dl) | | 47.51 | 13.98 | 59.95 | 2.82 | 6.9 | <0.001 |
| TG (mg/dl) | | 148.00 | 27.51 | 84.80 | 4.96 | 18.2 | <0.001 |

BMI: body mass index, FPG: fasting plasma glucose, 2HPPG: 2hour postprandial plasma glucose, HBA1C: glycated hemoglobin A1c, C-peptide: plasma c-peptide level, GLP-1: glucagon like peptide-1,T.CHOL:total cholesterol, LDL:Lowdensity lipoprotein, HDL: high density lipoprotein, TG: triglycerides. Significant *p* <0.001 and *p* <0.05, no significant: *p* >0.05.

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**Figure (2): Comparison between cases and control regarding lipid profile**

Table (2): Effect of DPP-IV inhibitors as an add on therapy on FPG, PPG, HBA1C % during the follow up period. HbA1c % was 6.8±0.47%, FPG was 116.74±12.81mgl/dl, and PP2 was 133.19±16.31mmol/L.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | | N | Mean | ±S.D | f | *p*-value |
|
| FPG (mg/dl) | Basal | 70 | 269.49 | 74.98 | 205.6 | <0.001 |
| follow up one month | 70 | 157.59 | 19.76 |
| follow up two months | 70 | 132.61 | 17.51 |
| follow up three months | 70 | 116.74 | 12.81 |
| 2HPPG (mg/dl) | Basal | 70 | 370.13 | 86.94 | 333.4 | <0.001 |
| follow up one month | 70 | 200.09 | 32.82 |
| follow up two months | 70 | 158.97 | 25.49 |
| follow up three months | 70 | 133.19 | 16.31 |
| HBA1C % | Basal | 70 | 10.77 | 1.00 | 340.2 | <0.001 |
| follow up one month | 70 | 8.50 | 0.91 |
| follow up two months | 70 | 7.53 | 0.64 |
| follow up three months | 70 | 6.80 | 0.47 |

Significant *p* <0.001 and *p* <0.05, no significant: *p* >0.05

This table shows that DPP-IV inhibitors as an add on therapy had a significant impact on reduction of FBG, 2HPPG and HBA1C after one month, two months and three months.

Table (3): Comparison between responders and non-responders cases regarding different variables at the end of the study (three months) after addition of DPP-IV inhibitors regarding HBA1C ≤7%.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | responders (n=53) | | non-responders (n=17) | | T | *p*-value |
| Mean | ±S.D | Mean | ±S.D |
| C-PEPTIDE (ng/ml) baseline | 1.5 | 0.51 | 0.91 | 0.50 | 4.2 | <0.05 |
| GLP1 (pg/ml) baseline | 27.20 | 10.41 | 21.62 | 3.99 | 2.2 | <0.05 |
| Age (year) | 44.91 | 11.35 | 50.71 | 7.20 | 2.5 | <0.05 |
| Baseline BMI【BW(kg)/Height(m)²】 | 27.4 | 2.6 | 28.9 | 2.4 | 2.1 | <0.05 |
| DURATION(DM) | 5.15 | 4.33 | 9.82 | 4.63 | 3.8 | <0.001 |
| T.CHOL (mg/dl) | 181.92 | 33.99 | 179.47 | 24.52 | 0.9 | >0.05 |
| LDL (mg/dl) | 98.15 | 13.78 | 97.35 | 8.54 | 0.2 | >0.05 |
| HDL (mg/dl) | 46.42 | 11.16 | 50.94 | 20.55 | 0.9 | >0.05 |
| TG (mg/dl) | 148.45 | 24.98 | 146.59 | 35.14 | 0.2 | >0.05 |

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**Figure (3): Comparison between responders and non-responders cases regarding different variables.**

Table (4): correlation between serum GLP-1 level and different variables (diabetic patients and control subjects).

|  |  |  |
| --- | --- | --- |
|  | r | *p*-value |
| Age | -.389\*\* | <0.001 |
| BMI | -.409\*\* | <0.001 |
| DURATION(DM) in years | -.247\* | <0.05 |
| Basal FPG (mg/dl) | -.630\*\* | <0.001 |
| Basal 2HPPG (mg/dl) | -.705\*\* | <0.001 |
| Basal HBA1C % | -.840\*\* | <0.001 |
| C-PEPTIDE | .666\*\* | <0.001 |
| T.CHOL (mg/dl) | -.477\*\* | <0.001 |
| LDL (mg/dl) | .347\*\* | <0.01 |
| HDL (mg/dl) | -.203 | >0.05 |
| TG (mg/dl) | -.637\*\* | <0.001 |

Significant *p* <0.001 and *p* <0.05, no significant: *p* >0.05

This table shows a significant negative correlation between serum GLP-1 level and age, BMI, duration of DM, FPG, 2HPPG, HBA1C, serum total cholesterol, TG, LDL and C-eptide and a positive correlation between serum GLP-1 level and HDL.

Table (5): Multivariate logistic regression analysis for all factors associated with prediction of control.

|  |  |  |
| --- | --- | --- |
|  | *p*-value | OR |
| C-PEPTIDE (ng/ml) | 0.3 | 1.8 |
| GLP1 (pg/ml) | 0.06 | 1.2 |
| Age | 0.056 | 0.9 |
| BMI | 0.5 | 1.1 |
| DURATION(DM) in years | 0.02 | 0.8 |
| FPG (mg/dl) | 0.07 | 1.5 |

Significant *p* <0.001 and *p* <0.05, no significant: *p* >0.05, OR, odds ratio.

Showed that only DM duration (*P*<0.02) was found to be an independent variable that could predict therapeutic efficacy of DPP-IV inhibitors as an add on therapy.

**4. Discussion**

In humans, two hormones-gastric inhibitory polypeptide (GIP) and glucagon like peptide-1 (GLP-1) account for the incretin effect. GIP and GLP-1 are polypeptides that are released from gut in response to food ingestion and potentiate insulin secretion in a glucose dependent manner. GIP is secreted from K-cells in the small intestines, which are found primarily in the duodenum, while GLP-1 is secreted from L- cells, primarily located in the distal ileum but also found throughout the small intestine, the large intestine, and pancreatic a-cells (4, 16). GLP-1 has additional effects including reducing glucagon secretion, slowing gastric emptying, inducing satiety, causing weight loss, as well as trophic effects on pancreas. GIP increases fatty acid uptake and lipogenesis by adipocytes, leading to suggestions that GIP may contribute to obesity (17, 18, 19).

Our study showed that total serum cholesterol, LDL, and TG were significantly high in diabetic patients compared to control subjects whereas HDL-C was significantly low in diabetic patients compared to control subjects.

Insulin resistance and the ensuing hyperinsulinemia are associated with hypertriglyceridemia and low serum high-density lipoprotein (HDL) cholesterol concentrations (20, 21). Jain Meenu, *et al.* (22) evaluated the correlation between levels of HbA1c and lipid profile in 150 non obese, non hypertensive type2 diabetic patients. They found that HbA1c showed direct and significant correlations with cholesterol, triglycerides, LDL &VLDL and inverse correlation with HDL. Daniel Nii Aryee Tagoe and Philip Amo-Kodieh (23) staded that (93%) of diabetic patients were dyslipidaemic compared with (38%) of control patients. Of the dyslipidaemic subjects, (83.5%) were in the age range 40-69 years. (50.4%) had abnormal HDL only whilst (17.3%) had abnormal TC, HDL and LDL. There was a significant association of abnormal lipid parameters in type 2 diabetic subjects which was positively and significantly correlated.

Our study showed that C-peptide was significantly low in diabetic patients compared to control subjects. This result was consistent with Bilal Bin Abdullah; *et al.* (24) estimated the C–peptide levels in elderly diabetics to assess the endogenous insulin secretory function, HbA1c and BMI. They found that the fasting C-peptide levels in the obese patients were increased compared to the non obese individuals, indicating insulin resistance. The fasting plasma glucose levels were elevated despite elevated C-peptide levels in the obese patients, proving the role of insulin resistance. The levels of HbA1c were increased more in obese patients indicating poor glycaemic control due to insulin resistance. The fasting c-peptide levels decreased as the duration of diabetes increased.

Our study showed that GLP-1 was significantly low in diabetic patients compared to control subjects, 26.33±9.37 pg/ml versus 75.48±20.81pg/ml respectively. Our result was consistent with Knop, 2007 who found that defective GLP-1 secretion does not appear to predate the development of glucose intolerance; rather diabetes itself seems to be associated with the acquired defect in GLP-1 secretion and GIP action. Multiple regression analysis revealed that GLP-1 response was determined negatively by the presence of diabetes.

Our result was in agreement with Lugari, et al, (25) who reported that a GLP-1 response, as observed in healthy subjects after ingestion of a small meal (230 kcal), was absent in type2 diabetic patients. They also stated that, even after ingestion of 700 kcal, a GLP-1 response could not be detected in type 2 diabetic patients. Toft-Nielsen *et al.* (26) found a pronounced impairment of the postprandial GLP-1 response, particularly during the later postprandial phase. Similar findings were made in subsequent studies which also indicated that the postprandial levels of intact GLP-1 were strongly reduced in subjects with Type 2 diabetes (27).

Our result was in consistent with Agus Lastya, *et al.* (28) who revealed that the levels of GLP-1in fasting and post-prandial states in subjects with T2DM were lower than in subjects with NGT. Muscelli and co-workers found that with OGTT, plasma GLP-1 levels were similar in IGT and NGT but were reduced markedly in diabetics (29).

GLP-1 is rapidly degraded by the enzyme dipeptidyl peptidase-4 [DPP-4] and the concentrations of the active intact hormone are much lower than that in the late postprandial phase.The decreased secretion was thought to be secondary to the disease, since it was noted only in the diabetic twin of identical twins discordant for diabetes (30).

In addition, there is evidence from preclinical and clinical studies that hyperglycemia further reduces the insulinotropic effect of GLP-1, possibly through down regulation of the GLP-1 receptor (31). Ultimately, the diminished incretin effect in patients with type2 diabetes may be a consequence of the inability of the B-cells to provide an appropriate secretory response to a stimulus.On the basis of such reasoning, the reduction of the incretin effect in patients with diabetes may simply be an epiphenomenon of chronic hyperglycemia, independent of any primary defect in GLP-1 action. Reducing hyperglycemia and enhancing B -cell function in general terms may therefore also improve the incretin effect, independent of specific interventions related to circulating levels of GLP-1 (32).

Our result was in disagreement with Ryskjaer, *et al.* (33), who studied incretin concentrations in subjects with normal, impaired glucose tolerance and diabetic and found that GLP-1levels were not different between the groups both after oral glucose and meal ingestion. Sou Shou, *et al.* (34) found that the levels of GLP-1 secretion stimulated by glucose or mixed meal loading were not lower in the T2DM patients than in the subjects with NGT or IGT. They proposed that impaired insulin secretion can develop in the absence of impaired GLP-1 secretion.

Our result was in disagreement with Orskov, *et al.* (35) who described increased GLP-1 responses during OGTT in patients with type2 diabetes with an average BMI of 28.2kg/m2.Velasquez-Mieyer *et al.* (36) found a significant difference in fasting and glucose-stimulated GLP- 1 levels among different races.They attributed this variance to racial differences. Nauck, *et al.* (16), suggested that inter-individual differences in GLP-1 secretion, age of patients, duration of diabetes, concomitant drug therapy, and other variables, preclude definitive conclusions that diabetes is invariably associated with reduced GLP-1 secretion.

It is now known that antidiabetic treatment may influence GLP-1 secretion, most clearly demonstrated for metformin, which increases proglucagon expression in the L-cells (36, 37), and enhances postprandial GLP-1responses. Therefore, concurrent therapy of patients with type 2 diabetes represents an important confounding factor. Impaired secretion of the incretin hormones may not be a constant finding, but a decreased secretion of GLP-1 after mixed meals is observed in most studies. Given that the sensitivity of the pancreatic islets to the actions of the incretin hormones is decreased in type2 diabetes, it is evident that impaired secretion, when present, will aggravate the loss of incretin effect in these patients (38).

Our study showed that there was a significant negative correlation between serum GLP-1 level and duration of DM, glycemic control (FPG, 2HPPG, HBA1C).

Our result was consistent with Jens *et al.* (38), and Muscelli, *et al.* (39) who stated that long duration and severity of type 2 diabetes (poor glycemic control, high HbA1c levels, and poor insulin secretory reserve) are associated with poor GLP-1 responses. Ioannis, *et al.* (40), carried out a study to establish any difference in the fasting state concentration of GLP-1in type 2 diabetes as compared with the normal state.using linear regression analysis; they found that GLP-1 was more negatively correlated with insulin in the type 2 diabetic patients than in the normal subjects. In addition, GLP-1 was also negatively correlated with HbA1c in type 2 diabetic subjects at a statistical level. Interestingly, the results were independent of diabetes duration.Our result was consistent with several studies which revealed that GLP-1 response was determined positively by insulin which declined with increasing duration of diabetes (41, 42).

Fang Zhang, *et al.* (43). found that there were observable correlations between impaired GLP-1 secretion and β cell function, IR and IS (insulin sensitivity).There is no substantial clarity yet, as to what happens to GLP‑1 level on varying degree of dysglycaemia.Therefore, any conclusion regarding GLP‑1 level on varying degree of worsening the glycaemia, remains elusive (44).

Our study showed that there was a significant negative correlation between serum GLP-1 level and dyslipidemia.

Our result was consistent with Marjan Alssema, *et al.* (45) who compared incretin responses to oral glucose and mixed meal of diabetic patients with the normoglycaemic population and whether incretin responses are associated with hypertriglyceridaemia and alanine aminotransferase (ALT) as liver fat marker. They found that GLP-1 was inversely related to fasting triglycerides and that this hormone may reflect risk for dyslipidemia and liver fat accumulation in an opposite way. Also that the inverse relationship between GLP­1 response after oral glucose load and fasting triglycerides is consistent with clinical study results among patients with type2 diabetes, showing that treatment with a GLP­1 analogue or GLP­1 agonist improves blood lipid profile (46, 47).

Our study showed that there was a significant negative correlation between serum GLP-1 level and BMI.

Our result was in agreement with several studies which revealed that GLP-1 response was determined negatively by greater BMI (40, 41). Verdich, *et al.* (48) and Muscelli, *et al.* (39) found that BMI is a powerful regulator of the GLP-1 response and comes out as a significant determinant in larger studies.

Our result was in concordance wih holst (49) and Vaag, *et al.* (29) who found that low GLP-1 has been implicated in obesity and type2 diabetes. several investigators have shown that GLP-1 responses following orally ingested nutrients are reduced among obese subjects (25), and a study by Muscelli, *et al.* (50) who described separate impacts of obesity and reduced glucose tolerance, on the secretion of GLP-1.In fact, in patients with morbid obesity, GLP-1 may not be measurable at all (51). Thus, obesity appears to be one of the factors responsible for impaired secretion of GLP-1 in type 2 diabetes (50).

Our result was in consistent with the results reported by Verdich *et al.* (48) who found that post-prandial GLP-1was reduced in obese subjects. Following weight reduction GLP-1 response in obese subjects apparently rose to a level between that of obese and lean subjects. Magda, *et al.* (52) evaluated the effect of obesity and pre-diabetes on GLP-1 levels. They found that GLP-1 levels were significantly decreased in obese subjects compared to controls and it was negatively correlated with body mass index (BMI) and waist circumference in all studied groups. Levels of GLP-1 were negatively correlated with HOMA-IR in all obese groups and morbidly obese cases had significantly higher fasting insulin, higher HOMA-IR, and lower GLP- 1 compared to non morbid obese cases.

However Lee *et al.* (52) found no correlation between postprandial GLP-1 levels and BMI. Marjan Alssema, *et al.* (45) found no association between postprandial GLP-1 levels and BMI, age, gender, or glucagon response. In addition, BMI ­stratified analyses clearly showed no effect of BMI on incretin responses in the present population.Laferre're *et al.* (54) found that in obese diabetic patients undergoing gastric by pass surgery, GLP-1 release and insulin secretion were enhanced early postoperatively at a time when body weight was unchanged but glycemia was improved.

Our study showed that there was a significant negative correlation between serum GLP-1 level and age.

Aging is a risk for development of insulin resistance and T2DM (55). Although some studies document a significant reduction in meal-stimulated GLP-1 levels, the available evidence, summarized by Nauck and colleagues (15) suggest that inter-individual differences in GLP-1 secretion, age of patients, duration of diabetes, concomitant drug therapy, and other variables, preclude definitive conclusions that diabetes is invariably associated with reduced GLP-1 secretion.

Our result was in disagreement with Marjan Alssema, *et al.* (45), Magda, *et al.* (52) and Muscelli et al (39) who found no significant correlation between postprandial GLP-1 levels and age. Conversely Vollmer and co-workers (42) found a significant positive correlation between increasing age and GLP-1 levels although the cause of this positive correlation not known by them.

Our study showed that DPP-IV inhibitors as an add on therapy had a significant impact on reduction of FBG, 2HPPG and HBA1C after one month, two months and three months. Our results were in agreement with Jin-Sun Chang, *et al.* (56) who found that vildagliptin signifiantly improved hemoglobinA1c (HbA1c) levels over 6 months in combination theraby with metformin and sulfonylurea. Zhang, *et al.* (57) reported that DPP-4 inhibitor as a third-line add-on therapy can achieve signifiant glycaemic improvement in patients with type 2 diabetes inadequately controlled on the combination of metformin and sulphonylurea.

Our result was in consistent with Soo Lim, *et al.* (58) who found that initial combination therapy with sitagliptin and metformin in drug-naïve, type 2 diabetic patients resulted in a significant decrease in mean HbA1c levels, fasting and post load 2h glucose from baseline. Koichi Hirao1, *et al.* (59), investigated the effect of addition of sitagliptin, to ongoing metformin and sulfonylurea therapy in three female Japanese patients with T2DM who refused insulin therapy. They found that combined treatment with all three drugs resulted in marked improvements in HbA1c with HbA1c levels decreased from11.1% to 6.1%, from 7.9% to 6.0% and from 8.6% to 7.1%.

Our result was in consistent with Arnolds, *et al.* (60) who found that addition of sitagliptin to insulin plus metformin resulted in a statistically significantly greater mean reduction in HbA1c from baseline (-1.49%; *p*<0.05) and a greater proportion of patients achieving HbA1c <7% (88%; *p*<0.05) versus metformin plus insulin. Fonseca, *et al.* (61) reported that vildagliptin in combination with insulin in a 24-week study, the mean HbAl C levels were reduced by 0.5% in the group given vildagliptin along with insulin as compared to 0.2% in the group given insulin alone.

DDP-1V enzymes block through DPP-IV inhibitors and cause reduction of hyperglycaemia and glucotoxicity, and also improve B-cell function with the recovery of the incretin system and effect (62). GLP-1 stimulates insulin gene transcription, biosynthesis, B-cell proliferation, and survival in experimental studies (4).

Our study showed that age, duration (DM), and BMI were significantly high in the non-responders diabetic patients compared to the reponders diabetic patients, whereas baseline serum C-peptide and baseline GLP-1 levels were significantly low in the non-responders diabetic patients compared to the reponders diabetic patients. However Multivariate logistic regression analysis for all factors associated with prediction of control showed that only DM duration (*P*<0.02) was found to be an independent variable that could predict therapeutic efficacy of DPP-IV inhibitors as an add on therapy. This could be explained by:

1- The Hyperglycemia-Induced “Metabolic Memory: Hyperglycaemia can leave an early imprint in cells of the vasculature and of target organs, favoring the future development of complications. This “memory” can appear even when agood control of glycaemia is achieved. Potential mechanisms for propagating this “memory” are the non-nzymatic glycation of cellular proteins and lipids, and an excess of cellular reactive oxygen and nitrogen species, originated at the level of glycated-mitochondrial proteins, perhaps acting in concert with one another to maintain stress signaling (63, 64).

2-The relation between DM duration and age: Aging is a risk for development of insulin resistance and T2DM (65). Suastika *et al.* (66) showed that the prevalences of T2DM were higher in the elderly than in the younger age group. Studies conducted by Kilpatrick *et al.* (67) in diabetic patients have shown a significant positive correlation between HbA1c and age aswell as duration of diabetes.

Aging induces decrease insulin sensitivity and alteration or insufficient compensation of beta cell functional in the face of increasing insulin resistance (68). Also, decrease in beta cell proliferation capacity and enhanced sensitivity to apoptosis are the states related with aging (69). A study by Szoke *et al.* (70) showed that the first and second phase of insulin secretion normally decreases at the rate of approximately 0.7% per year with aging.

3-The relation between DM duration and C-peptide level: Bilal Bin Abdullah, *et al.* (23); found that the fasting C-peptide levels decreased as the duration of diabetes increased. Farhad Zangeneh, *et al.* (71); found that fasting C-peptide, 6-minute C-peptide, or postglucagon increment in C-peptide concentrations, declined with increasing duration of diabetes in approximately half of the patients.

Jin-Sun Chang, *et al.* (56) investigated the glycemic efficacy and predictive parameters of vildagliptin therapy in Korean subjects with type2 diabetes. They found that initial HbA1c and history of sulfonylurea use were independently associated with responsiveness to vildagliptin treatment. Our results were in consistent with Kim, *et al.* (72) and Lim *et al.* (73) who evaluated factors predicting therapeutic efficacy of combination treatment with sitagliptin and metformin. They found that high baseline HbA1c and short duration of diabetes were independent parameters for the reduction of HbA1c. However, fasting C-peptide was not different between the highest and the lowest quartiles of HbA1c reduction in the study. Also Bando, *et al.* (74) and Nomiyama, *et al.* (75) found that high BMI was an independent predictor for poor response to DPP4i in 2 Japanese studies.However Lim *et al.* (753) did not report any relationships between BMI and DPP4i efficacy.

Zhang, *et al.* (57), examined the efficacy of adding a dipeptidyl peptidase-4 (DPP-4) inhibitor in patients with type2 diabetes inadequately controlled by metformin and sulphonylurea combination treatment. They found that poor baseline glycaemic control, lower BMI, and younger age were associated with a better response, but duration of diabetes and gender did not affect outcome. Soon Ae Kim, *et al.* (76) obtained data from 251 Korean T2DM subjects who had recently started sitagliptin as add-on therapy. They found that age, duration of diabetes, BMI, and HOMA-β were an independent variables and regression analysis showed that there was no significant relationship between them, excluding BMI which was discovered to be a dependent variable. They concluded that in Korean T2DM subjects, sitagliptin responders had lower body mass index and were younger compared to non-responders. Soo Lim, *et al.* (58) assessed the predictive parameters for therapeutic efficacy of initial combination therapy with sitagliptin and metformin in drug-naïve, type 2 diabetic patients. They found that the reduction in HbA1c was significantly associated with high baseline HbA1c, low IGI, and short duration of diabetes. They concluded that these results suggest that drug-naïve type 2 diabetic patients with low β-cell function would benefit the most from early initial combination therapy of sitagliptin and metformin.

Our results were in consistent with Tae Jung, *et al.* (77) who investigated characteristics associated with the efficacy of dipeptidyl peptidase-4 inhibitors (DPP4i) in Korean patients with type2 diabetes. They found that in the multivariate analysis, duration of diabetes and HbA1 c were independent predictors for the response to DPP4i and those BMI and insulin resistances were not associated with the response to DPP4inhibitors. F-Wind and Chikushi-JRN Group, (78), Maeda, *et al.* (79) and Bando, *et al.* (74) reported that low BMI and high HbA1c were common predictive factors for HbA1c reduction contributing to the efficacy of sitagliptin in Japanese diabetic patients.

**Limitations Of Our Study:**

This study has several limitations. First, small numbers of cases, short follow up period and little information about life style and drug compliance. Therefore, individual treatment regimens need to be customized for each T2DM patients. Even though; further prospective study will be needed to confirm our results.

**Conclusions:**

DPP-4 inhibitors are new pharmacological approaches to correct incretin system impairment in patients with type2 diabetes which cause reduction in glucose levels towards normal values of HbA1c with lower risk of hypoglycemia and potential long -term benefits. The study showed that age, duration (DM), and BMI were significantly high in the non-responders diabetic patients compared to the reponders diabetic patients, whereas baseline serum C-peptide and baseline GLP-1 levels were significantly low in the non-responders diabetic patients compared to the reponders diabetic patients. However Multivariate logistic regression analysis for all factors associated with prediction of control showed that only DM duration (*P*<0.02) was found to be an independent variable that could predict therapeutic efficacy of DPP-IV inhibitors as an add on therapy.

# Recommendation

Diabetic patients most likely to get good benefit from DPP-IV inhibitors either as monotherapy or add on therapy are the drug naïve patients, the younger in age and the shorter the DM duration. The control of dyslipidemia and body weight would provide more benefits and better prediction of response of treatment with DPP-IV inhibitors. Further studies are needed which should entail a larger number of diabetic patients and a longer follow up period.

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5/25/2015