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Glucagon-like peptide 1 and its association with dipeptidyl peptidase IV in subjects with various degrees of glucose tolerance

Ruqaya[1]*, Mohammad Saleh Soomro[1], Nazish Waris[2], Iftikhar Ahmed Siddiqui[3], Asher Fawwad[3] and Abdul Basit[2]

[1]Department of Physiology, Baqai Medical University, Karachi, Pakistan
[2]Baqai Institute of Diabetology and Endocrinology, Karachi, Pakistan
[3]Department of Biochemistry, Baqai Medical University, Karachi, Pakistan

Abstract: Dipeptidyl peptidase IV (DPP-4) and Glucagon like peptide 1 (GLP-1) has profound effect on insulin and glucagon secretion; ultimately decreasing glucose levels. We find out the association of GLP-1 levels and DPP-4 in normal, impaired and newly diagnose type 2 diabetic glucose tolerance. Prospective case control study was conducted at Department of Physiology, Baqai Medical University by the collaboration of Baqai Institute of Diabetology and Endocrinology; Karachi-Pakistan. Study groups were categorized into three groups Control, Impaired glucose tolerant (IGT) and newly diagnose type 2 diabetes mellitus (NDD). Biochemical parameters were estimated by international standard protocols. Logistic regression analysis and Chi square test with statistical significance at p value <0.05 were applied. DPP-4 concentrations were significantly lower in NDD participants compared to control and IGT participants (p=0.01), whereas GLP-1 levels were significantly higher in Control than Impaired glucose tolerant and NDD (p = 0.013). GLP1 levels and SBP were also found to be positively correlated with serum DPP4 levels in NDD group (p<0.05). GLP1 and DPP4 levels in NDD group (p<0.05) and in controls (p<0.001) respectively showed strong significant positive correlation. Effective correlation between GLP1 and DPP4 was found as both contribute to control hyperglycemia in NDD and impaired glucose tolerant people.

Keywords: Dipeptidyl peptidase IV (DPP-4), glucagon-like peptide 1(GLP1), newly diagnose Type 2 diabetes (NDD)

INTRODUCTION

A multi-functional protein, Dipeptidyl peptidase-4 (DPP-4), also known as CD26 T cell antigen, is catalytic, also functions as a binding integral membrane protein as well as ligand for a variety of extra cellular molecules; dispensed from the cell membrane and circulates as a plasma-soluble protein throughout the body (Zilleßen *et al*., 2016).

DPP-4 can split various bioactive molecules in vitro, but only a few have been observed as its physiological substrates. An incretin hormone glucagon-like peptide-1 (GLP-1) significantly maintains the homeostasis of glucose, and DPP-4; a key enzyme regulating biological activities and its pathway was pharmacologically targeted by the development of DPP-4 inhibitors, successfully used anti-hyperglycemic agents for the treatment of type 2 diabetes mellitus (Nargis *et al*., 2017).

DPP-4 researches noticeably plays a role in the release of incretin hormones as well as in regulating the glucose homeostasis (Ahmed *et al*., 2017). Besides, GLP-1 also delays food absorption, regulates satiety and appetite by modulating hypothalamus (Anandhakrishnan, 2016; Sanchez *et al.*, 2017). Subsequently, many studies estimated the use of DPP4 inhibitors raised GLP-1 or GIP levels. This effect is the primary goal of developing therapeutic targets for the treatment of T2DM (Janzen, Steuber & Nisly, 2016; Bastin & Andreelli, 2019; Holst *et al*., 2016).

The activity of DPP-4 in type 2 diabetes is not absolute however research has suggested that high plasma DPP-4 activity may be a risk factor related to worsening glycemic control (McShane *et al*., 2016). In this case, the degradation of GLP-1 (and other incretins, GIP) is greater, and their effect will change accordingly, which will lead to deterioration of blood sugar control. This may be the early onset of T2DM, which is one of the many risk factors that causes the initial hyperglycemia in susceptible individuals, or later due to the existing metabolic changes that increase the activity of DPP-4 and consequent uncontrolled high blood glucose. As a result, recent studies reported a positive correlation between DPP-4 plasma activity and fasting blood glucose or HbA1c (Sarkar *et al*., 2019).

The present study aimed to discover the association of GLP-1 levels and DPP-4 in normal glucose tolerance (NGT), impaired glucose tolerance (IGT) and newly diagnosed type 2 diabetic (NDD) subjects.

MATERIALS AND METHODS

Study population and data collection

The prospective case-control study was carried out at Department of Physiology, Baqai Medical University

**Corresponding author:* e-mail: ruqnanrs2009@yahoo.com

(BMU) with the collaboration of Baqai Institute of Diabetology and Endocrinology (BIDE-BMU), Karachi-Pakistan. The duration of the study was from February 2018 to November 2019. Subjects visiting the out-patient department (OPD) of BIDE invited in fasting state of at least 8:00 hours. Subjects recruited based on oral glucose tolerance test (OGTT); each subject was given 75-gram glucose and advised to be seated for 2 hours. Pre and post glucose blood samples were acquired as biochemical parameters. Details for demographic and anthropometric measurements were noted on the pre-designed questionnaire. Subjects were categorized into three groups based on OGTT. Group A: Controls (C) n=100, Group B: IGT n=100 and Group C: NDD n=100.

Sample size by using Fleiss with CC method

Two-sided significance level=0.05
Power (% of chance of detecting) =80
Proportion of control with exposure=0.1
Ratio of sample size=1
Odd ratio=3.2
The required sample size n= 300 (n=100 per group).

Measurement of anthropometrics and clinical parameters

The anthropometric clinical variables includes weight (Kilograms), height (inches) and blood pressure (mmHg) measured by using standard protocols. Body mass index (BMI) of each study participant was calculated by dividing their weight (kilograms) to the height (meter squared) (kg/m^2). For the measurement of blood pressure a stethoscope and a sphygmomanometer was used and the participants were in sitting position (Bakris, 2016).

Measurement of biochemical parameters

Measurement of Plasma GLP-1 level was performed by ELISA using kit (Ahmed *et al*., 2017), Cat.No. RSCYK 160R, Bio Vendor Research and Diagnostic Products. The dipeptidyl peptidase IV inhibition assay was used for the estimation of DPP-4 (Liu *et al*., 2016). Nongonierma and FitzGerald described the assay as triplicate; helps in determination of freeze-dried samples (hydrolysate and control) which were isolated in HPLC-grade water at a concentration of 2.8×10^{-2} to 2.8 mg mL^{-1} (the final concentration is expressed in mg protein equivalent /mL). The DPP-IV half maximal inhibitory concentration (IC50) value is depicted the percentage of inhibition as a function of the tested compound concentration. Glucose oxidase peroxidase method was used for the measurement of fasting and random blood sugar concentration. After 8 to 12 hours fast fasting blood sugar was measured (Sharma, Ajankar and Kale, 2017). High-performance liquid chromatography method was used for estimation of HbA1c (Fawwad *et al*., 2016).

Ethical Approval and informed consent

The ethics committee of Baqai Medical University has approved the present study with reference number BMU-EC/2018-03. Each participant before sample collection signed the consent form.

STATISTICAL ANALYSIS

All the parameters were statistically analyzed by Statistical Package for Social Sciences (SPSS) Version 20. The significant difference of variables between the groups and within the group was assessed by on way Anova. To examine the relationship between two variables *X* and *Y*, giving a value between +1 and -1 inclusive, where 1 (total positive correlation), 0 (no correlation) and -1 (total negative correlation) was done by Pearson's correlation analysis.

RESULTS

The Clinical and baseline parameter were compared among the individuals of three study groups mentioned in table 1. It was observed that RBS, HbA1c, DPP4, GLP1 and SBP were found to depict high significant difference among the groups of NDD, IGT and controls. Whereas, Age, BMI, FBS & DBP indicated no significance on comparison between three groups.

The statistics of correlation was applied to examine the effect of DPP4 levels on various baseline and clinical parameters in all three groups. The incidence of positive correlation was obtained for serum DPP4 level with fasting ($p<0.01$) and random blood glucose ($p<0.001$) in IGT group. The serum GLP1 levels and systolic blood pressure was also found to be positively correlated with serum DPP4 levels in NDD group ($p<0.05$). However, no correlation was indicated in controls (table 2).

The correlation was also tested for serum GLP1 levels with targeted variables of this study among three study groups. A strong positive correlation witnessed for GLP1 levels with DPP4 levels in NDD group ($p<0.05$) and in controls ($p<0.001$) respectively. Whereas, systolic blood pressure exhibited a negative correlation with serum GLP1 levels in controls ($p<0.05$). In contrast, no clear depiction of correlation was revealed with any parameter of the IGT group (table 3).

DISCUSSION

In this study, we established that DPP4 levels were significantly higher in control compared to NDD and IGT participants. This verdict is consistent with the reports of previous studies (Chia, Egan, & Ferrucci, 2018; Neidert *et al*., 2016; Senyigit *et al*., 2017). Moreover, it is seen that GLP1 levels were decreased in NDD, IGT as compared to control subjects. It is seen that the DPP4 activity was positively correlated with the FBG and RBG of IGT and GLP1 of the NDD.

The present study also observed that the DPP4 and GLP1 levels in controls was highly positively associated NDD

Table 1: Distribution of anthropometric and clinical parameters

		Sum of Squares	Df	Mean Square	F value	p value
Age	Between Groups	7429.407	2	3714.703	31.133	0.03
	Within Groups	35437.51	297	119.318		
BMI	Between Groups	49053.529	2	24526.764	.859	0.425
	Within Groups	8477875.697	297	28545.036		
FBS	Between Groups	219572	2	109786	47.312	0.029
	Within Groups	689178.6	297	2320.466		
RBS	Between Groups	83712.51	2	41856.25	5.05	0.007**
	Within Groups	2461443	297	8287.687		
HbA1c	Between Groups	975.722	2	487.861	33.343	0.0001**
	Within Groups	4345.562	297	14.632		
DPP4	Between Groups	14.152	2	7.076	4.803	0.009**
	Within Groups	437.564	297	1.473		
GLP1	Between Groups	13441.05	2	6720.523	4.383	0.013**
	Within Groups	455386	297	1533.286		
*S*BP	Between Groups	3347.167	2	1673.583	5.953	0.003**
	Within Groups	83497.75	297	281.137		
*D*BP	Between Groups	2737.5	2	1368.75	9.916	0.09
	Within Groups	40996.75	297	138.036		

Sum of Squares: Mean square of all three groups, df: degree of freedom, **P <0.01.
NDD = New ly diagnosed diabetics, IGT = Impaired glucose tolerant

Table 2: Correlation of DPP4 levels (ng/µl) with parameters of study groups

Parameters	IGT (n=100)	NDD (n=100)	Controls (n=100)
Correlation (r values)			
Age (Year)	0.043	0.058	0.024
BMI (Kg/m^2)	0.019	0.066	0.012
FBG (mg/dl)	0.256**	0.014	0.026
RBG (mg/dl)	0.315***	0.109	0.127
HbA1c (%)	0.117	0.078	0.024
GLP1 (ng/ml)	0.019	0.195*	0.124
SBP (mmHg)	0.107	0.191*	0.113
DBP (mmHg)	0.053	0.031	0.078

NDD = Newly diagnosed diabetics, IGT = Impaired glucose tolerant

Table 3: Correlation of GLP1 levels (ng/ml) with parameters of three groups

Parameters	IGT (n=100)	NDD (n=100)	Controls (n=100)
Correlation (r values)			
Age (Year)	0.060	0.085	0.013
BMI (Kg/m^2)	0.043	0.068	0.063
FBG (mg/dl)	0.052	0.081	0.028
RBG (mg/dl)	0.120	0.061	0.049
HbA1c (%)	0.051	0.083	0.016
DPP4 (ng/µl)	0.019	0.227*	0.493***
SBP (mmHg)	0.136	0.012	0.065
DBP (mmHg)	0.052	0.061	-0.130*

Potential association Pearson correlation coefficient (r<0.20)
NDD = New ly diagnosed diabetics, IGT = Impaired glucose tolerant

subjects. One of the previous researches described that the GLP1 is associated with insulin secretion; which shows circulating DPP4 in insulin-resistant subjects were raised (Valerio *et al*., 2017). Same was found in the present study with NDD and IGT groups. However, the mechanism by which the activity of DPP4 levels is increased remains unclear. Recent studies show that resistance to insulin due to enhanced fat accumulation might be involved in augmented levels of circulating DPP4 in diabetic subjects; which may be because of improved expression and release of DPP4 from the fatty tissues (Ramesh & Gupta, 2018). In addition, the inflammatory markers are also produced and disseminated in obesity and DM (Nauck, 2016; Gluvic *et al*., 2017). This plays a crucial role in the DPP4 concentration and hence its indirect role in inflammation (Altenhofen *et al*., 2017) and further deterioration of insulin sensitivity aggravating obesity and type 2 DM (Altenhofen *et al*., 2017; Ottobelli Chielle *et al*., 2016). These findings hence contribute towards the outcomes as increased FBS, RBS, HbA1c, and BMI, which were found to be affected in both NDD and IGT patients. However, the BMI, FBS and RBS were highly affected in the NDD than the IGT subjects, portraying that the initiation of the disease has severe effects as in NDD and tones down upon time as observed in the present study as presented in table 1. Recently, it has been also been suggested that the insulin resistance in type 1 diabetes (T1D) patients is associated with DPP4 activity that DPP4 activities may result in inflammation, insulin resistance and T2DM.

The levels of DPP4 are also of main focus recently due to its metabolic effects on the degradation of GLP-1 (Zheng *et al*., 2017). Table 2 shows an increase in the level as well as activity of DPP4 may contribute to elevated SBP, FBS, RBS with significant correlation with GLP1. The GLP-1 levels were considerably lesser in NDD and IGT compared to normal subjects. The present study was in association with previously reported reduced levels of GLP1 stimulated by glucose and GLP-1 secretion in insulin resistance, (Ahmed *et al*., 2017; Hussein *et al*., 2014) but in contrast to the results obtained in previous studies (Mak *et al*.,2020 and Tanaka *et al*., 2016). In addition there is other study that shows a balanced concentrations of the DPP4 and GLP1 and this research show that DM with or without insulin resistance show equilibrium between DPP4 and GLP1 (Silver Junior *et al*., 2019). However, it is not clear due to certain conflicting findings that whether the connection and relative outcome of DPP4 and GLP1 on insulin resistance and hence diabetes is due to the reduction in insulinotropic efficiency or decrease secretion of resGLP-1 or both. Thus, the effect of GLP-1 in the development of diabetes and insulin resistance with respect to DPP4 has been suggested partially to the physiological factors which includes hormones, food intake, appetite as well as body weight of the individual (Nauck & Meier., 2017). A recent study of Carr and his colleagues has estimated the increased DPP4 activity affect the levels of active GLP-1 as well as GIP. Although DPP1 is known to metabolize GLP1 and GIP but scientists has found that augmented DPP4 activity can also lead to reduction of GLP-1 secretion and hence can alter normal hemostasis of the body (Nauck & Meier, 2018). Furthermore, Previous studies showed reduced secretive response of GLP-1 interconnected to insulin resistance which endorse the modifications in body hemostasis during disturbed DPP4 and GLP1 secretion towards development of insulin resistance and DM (Lund *et al*., 2016).In addition, all these allied factors are also responsible for the impediments in FBS, RBS, SBP, DBP and BMI as presented in table 3. This is in support of the reports by study of Rask that insulin resistance is associated with increased weight, reduced secretory response of GLP-1 and insulin resistance (Ormazabal *et al*., 2018). Hence diminished levels of GLP-1 in Type 2 Diabetes mellitus endorsed to either depressed bowel secretion or increased elimination with degradation of DPP4 enzyme (Kennedy & Freeman, 2009). Thus the findings of the present study and the supporting evidence from the past research establishes the fact that GLP-1 might play a vital role in obesity, insulin resistance as well as Diabetes Mellitus with evidences that DPP4 is valuable early marker for increased hazard of this metabolic syndrome. We realize and understand the limitations of our study as being one with relatively smaller sample size, however, future study on large size of sample, must be warranted to fully explain the relationship of DPP4 and GLP1 with obesity, insulin resistance and DM.

CONCLUSION

It is evident that GLP-1 receptor agonists and DPP-4 inhibitors has an important role in the second-line treatment of Type 2 Diabetes Mellitus. Their hypoglycemic effect is impressive, although it is not comparable to the hypoglycemic effects of metformin and sulfonylureas. Compared with DPP-4 inhibitors, GLP-1 receptor agonists seem to decrease blood sugar to a maximum level and help lowering weight. Besides improving blood lipids and systolic blood pressure, they also have enhanced cardiovascular protection. However long-term data is collected from clinical trials that assess morbidity and mortality, the definitive answer to the most effective treatment cannot be given. The incretin therapy is safe and tolerable even in most cases it is better than traditional therapy. However, DPP-4 inhibitors appear to have advantages over well-tolerated GLP-1 receptor agonists.

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