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GLP-1, GPCR Levels and their Relationshipwith Some Parameters in Non-diabetic Dyslipidaemia in Iraqi Patients

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ABSTRACT

Abnormalities in the Lipid and lipoprotein levels are common in the general population and are considered as very important risk- factors for cardiovascular disease .In this context the effect of cholesterol, which is one of the most clinically relevant lipids is very important. Aim of the present study was to determine the levels of GLP-1 and GPCR in non- diabetic dyslipidaemic patients and compare the results with the control group, which may be used as a novel biomarker to predict heart disease in these patients. The study was also aimed to find the relationship between GLP-1 and GPCR with lipid profile and glucagon in the patient group. The study involved 90 non-diabetic dyslipidaemia patients, with 90 healthy controls. The subjects were matched by age (35-50 years) and body mass index (BMI) (28 kg/m2). Blood samples were collected from healthy controls and dyslipidaemic patients after 12-14 hours of fasting. The study was conducted between January 2015– September 2015 in the Ibn- Al Naphes hospital in Baghdad province / Iraq .Diabetic patients were exclusion from this study. BMI were determined for all student groups.FBG, Lipid Profile ,glucagon, GLP-1, GPCR was determined in the control and patient groups. The results are expressed as mean ± SEM. Student's t-test was used to compare the significance of the variation between dyslipidaemia and control groups. Results showed non-significant elevations in BMI, FBS, and HbA1c levels in the patient group compared with the control group. There was a significant elevation in TC, TG, LDL-c, and VLDL-c levels in the patient group compared with the control group, while a significant decrease was noticed in HDL-c level in the patient group compared with the control group .There was also a significant elevation in glucagon, while a highly significant elevation in GLP-1 and GPCR levels in the patient group when compared with the control group. A significant correlation was observed between GLP-1 with TC, TG, HDL-c, and GPCR in the patient group. There was also a significant correlation between GPCR with TC, TG and HDL-c in the patient group. From this study, it is concluded that a significant increase in GLP-1and GPCR levels, in addition to their correlation with TC, TG, HDL-c and glucagon in the patient group, compared to the control group indicate that these parameters could be used as a novel biomarker to predict heart disease in these patients in future.

Keywords: GLP-1, GPCR, non-diabetic dyslipidaemia patients.

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INTRODUCTION

Hyperlipidaemia is the presence of raised levels of lipids and/or lipoproteins in the blood [1]. It can be classified into two types, the first one caused by specific genetic defects (familial) ,also known as primary, and the second type (acquired), also known as secondary and is caused by other tacit disorders due to changing in plasma lipid and lipoprotein metabolism [2,3].

Glucagon-like peptide-1(GLP-1) is an incretin hormone, the active forms of which are GLP-1- (7-36) and GLP-1- (7-37) amides. GLP-1 is produced by intestinal L-cells in response to meal intake. The subsequent increase in the levels of nutrients derived from carbohydrate, lipid, and protein leads to insulin secretion. The half-life of this hormone is ~2 minutes and is degraded by dipeptidyl peptidase-4 (DPP-4) [4,5] .

Glucagon is a 29- amino acid peptide hormone which plays an important role in regulating blood glucose levels. Glucagon is released into bloodstream by the α-cells of the Islets Langerhans in the pancreas. The arrangement of a core of insulin-secreting β-cells surrounded by glucagon-secreting α- cells, indicates the close relevance between the insulin and glucagon [6,7] .

The G-protein-coupled receptors (GPCRs) are integral membrane proteins having owns seven membrane-spanning domains. Some types of this family involves rhodopsin, adrenergic receptors, neurotransmitter receptors, olfactory receptors, muscarinic cholinergic receptors, and chemokine receptors [8].

GPCR have been found in eukaryotes, including yeast, choano-flagellates, animals, and plants. These receptors are activated by linking the ligands such as neurotransmitters, hormones, pheromones, odours, and light-sensitive compounds, which rang and these differ in size from small molecules to peptides to large proteins. GPCR are the targets of most modern medicinal drugs, and are also involved in many diseases [9] .

Some studies regarding the expression of GPCR in α-cells have indicated that these were expressed from a few up to 20% [8] . De Marinis et al. , reported that the expression of GPCRs such as GLP-1R in α-cells is <0.2% of that in β-cells and that GLP-1 stimulates repression of glucagon secretion ,which is dependent of protein kinase A (PKA) and independent of glucose or porcine effects mediated by insulin or somatostatin [10]. De Heer et al. have previously demonstrated that GLP-1 inhibitory effect on glucagon secretion is mediated by somatostatin acting on somatostatin receptor subtype-2 (SSTR-2) [11] .

The aim of the present study was to determine the levels of GLP-1 and GPCR in dyslipidaemia patients and to compare the results with the control group, in order to establish whether these could be used as novel biomarkers to predict heart disease in these patients. Another aim was to find the relationship between GLP-1 and GPCR with triglycerides (TG) , total cholesterol (TC) and high density lipoprotein-cholesterol (HDL-c).

MATERIALS AND METHODS

Collection of Blood Samples:

The study involved 90 non-diabetic dyslipidaemia patients, with 90 healthy controls. Type of study is case control study that subjects were matched by age (35-50 years) and body mass index (BMI) (28 kg/m^2).

Blood samples were collected from healthy controls and dyslipidaemic patients after 12-14 hours of fasting. The study was conducted between January 2015–September 2015 in the Ibn- Al Naphes hospital in Baghdad province / Iraq. Diabetic patients were exclusion from this study

Determination of Body Mass Index (BMI) :

BMI wascalculated using below formula [12] .

$$\mathbf{BMI} = \frac{\mathbf{weight(kg)}}{(\mathbf{height\ (m)}\ )\mathbf{2}}$$

Determination of Fasting Blood Glucose :

Serum glucose was measured by using kit (Randox Company, France), which were based on the PAP (phenol+ aminophenazone) enzymatic determination of glucose [13] , as per the manufacture's instruction.

Determination of Glycated Haemoglobin (HbA1c) :

The kit was obtained from Stanbio (USA) [14], to determine the glycohaemoglobin that formed progressively and irreversibly in the erythrocyte during its 120-day life cycle .

Determination of Lipid Profile :

The TC and TG were determined by enzymatic method (Randox Company, France) [15,16] , while the HDL-c was determined using the deposition method (Randox Company, France) [17] . The levels of low density lipoprotein-cholesterol (LDL-c) and very low density lipoprotein-cholesterol (VLDL-c) were calculated by using the Friedewald equation [18] .

$$\mathbf{VLDL-c\ (mg/dl)} = \frac{\mathbf{TG}}{\mathbf{5}}$$

$$\mathbf{VLDL-c\ (mg/dl)} = \frac{\mathbf{TG}}{\mathbf{5}}$$

Determination of Glucagon GLP-1 and GPCR Levels in Blood Serum :

The enzyme-linked immunosorbent assay (ELISA) kit was purchased from (Elabscience, China) and used for determination of the levels of glucagon, GLP-1.and GPCR. Sandwich ELISA format was employed and performed as per the manufacture's instructions.

Statistical Analysis:

The results are expressed as mean ± SEM. Student's t-test was used to compare the significance of the variation between dyslipidaemia and control groups. The *p*-values (p>0.05), (p_<0.05), and (p_<0.001) were considered statistically non-significant, significant, and highly significant respectively. The correlation coefficient (r) test was used for describing the association between the different studied parameters.

RESULTS

Table 1 represents the BMI, fasting blood sugar (FBS) , HbA1c, TC, TG, HDL-c , LDL-c, VLDL-c, glucagon, GLP-1 and GPCR levels in the patient and control group. Results showed a non- significant elevation in BMI, FBS, and HbA1c in the patient group when compared to the control group. Results showed a highly significant elevation in TC, TG, LDL-c and VLDL-c levels in the group compared to the control group, while a highly significant decrease in HDL-c levels was found in the patient group compared to the control group.

Glucagon was a significantly elevated in the patient group (63.30 ± 6.33) , compared to the control group (41.82 ± 2.62).

Results showed a highly significant increase in GLP-1 and GPCR levels in the patient group (3.87 ± 0.19: 2.32 ± 0.17) respectively, compared to the control group (2.45 ± 0.1: 1.45 ± 0.08).

Table 1: Parameters studied in the patient and control groups.

Parameters	Control group	Patient group	t-test
BMI (kg/m^2)	28.06 ± 0.36	28.61 ± 0.61	NS

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FBS (mg/dl)	93.65 ± 1.69	96.25 ± 2.16	NS
HbA1c	5.26 ± 0.07	6.03 ± 0.15	NS
TC (mg/dl)	160.35 ± 6.25	370.35 ± 9.50	HS
TG (mg/dl)	140.55 ± 4.37	248.60 ± 7.03	HS
HDL-c (mg/dl)	42.3 ± 1.31	25.44 ± 0.77	HS
LDL-c (mg/dl)	89.91 ± 6.44	295.20 ± 9.12	HS
VLDL-c (mg/dl)	28.15 ± 0.88	49.72 ± 1.41	HS
GLP-1 (ng/ml)	2.45 ± 0.1	3.87 ± 0.19	HS
Glucagon (ng/ml)	41.82 ± 2.62	63.30 ± 6.33	S
GPCR (ng/ml)	1.45 ± 0.08	2.32±0.17	HS

˚(S) significant (p<0.05), (HS) highly significant (p<0.001), (NS) non-significant (p>0.05)

Correlation of GLP-1 with GPCR, TC, TG, and HDL-c

Table 2 shows the results for the correlation of GLP-1 with TC, TG, HDL-c , GPCR. There was a significant positive correlation of GLP-1 with TC in the control group (r= 0.277) and a significant negative correlation in the patient group (r= -0.421) (Figure 1).

There was a significant positive correlation of GLP-1 with TG in the control group (r= 0.673) and a significant negative correlation in the patient group (r= -0.011) (Figure 2).

There was a significant negative correlation of GLP-1 with HDL-c in the control group(r=-0.600) and a significant positive correlation in the patient group (r= 0.031) (Figure 3).

There was a significant negative correlation of GLP-1 with GPCR in the control group(r= -0.098) and a significant positive correlation in the patient group (r= 0.239) (Figure4).

Table 2: Correlation of GLP-1 levels with some studied parameters in the patient and control group.

Groups / Parameters	Control group		Patient group	
	r-value	*p*-value (S)	r-value	*p*-value (S)
GLP-1 vs TC	0.277	S	-0.421	S
GLP-1 vs TG	0.673	S	-0.011	S
GLP-1 vs HDL-c	-0.600	S	0.031	S
GLP-1 vs GPCR	-0.098	S	0.239	S

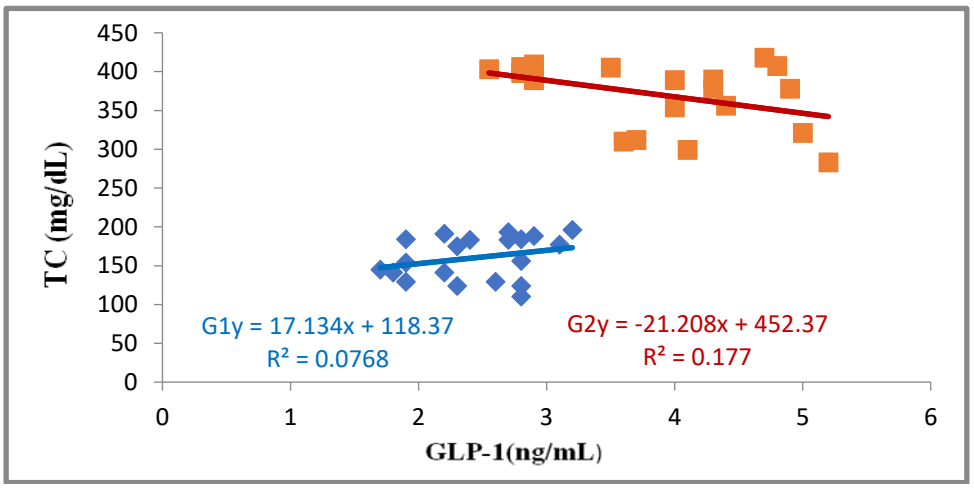

Figure 1: Correlation between GLP-1 and TC for the control and patient groups .

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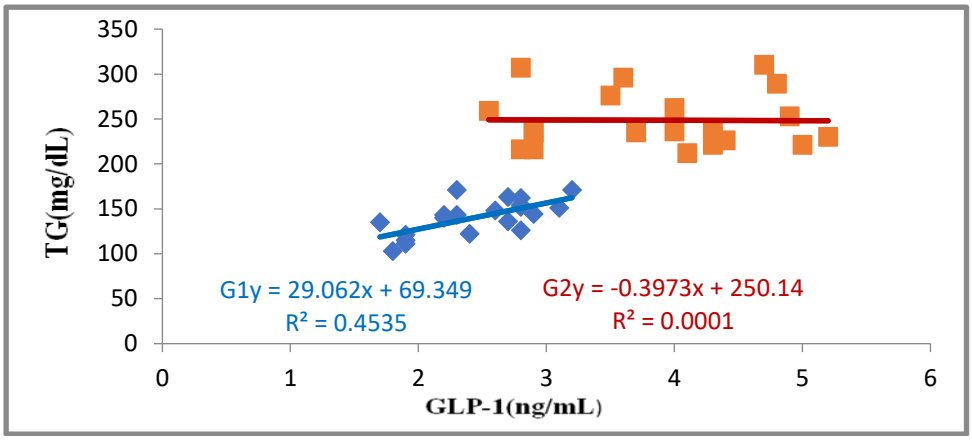

Figure 2:Correlation between GLP-1 and TG for the control and patient groups.

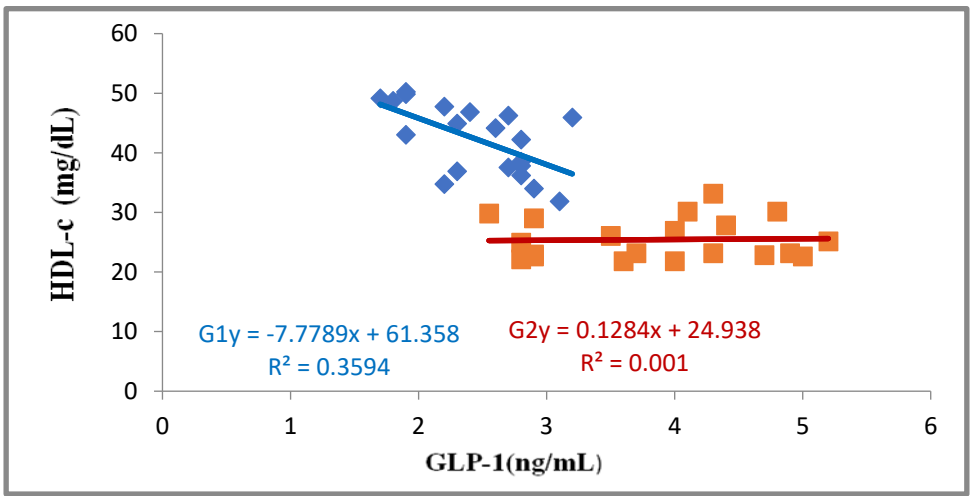

Figure 3: Correlation between GLP-1 and HDL-c for the control and patient groups.

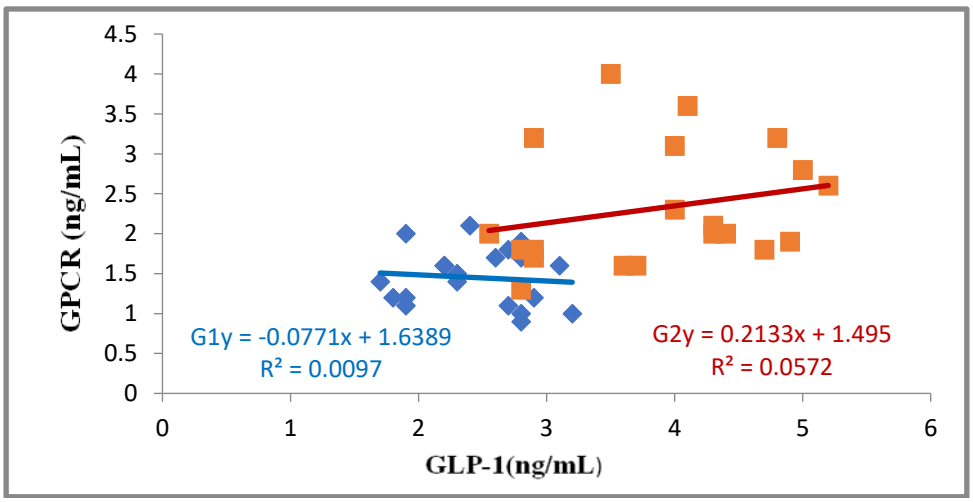

Figure 4: Correlation between GLP-1 and GPCR for the control and patient groups.

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Correlation of GPCR with TC, TG, HDL-c and Glucagon:

Correlation for GPCR with TC, TG , HDL-c, and glucagon was studied (Table 3). There was a significant positive correlation of GPCR with TC in the control group (r= 0.347) and a significant negative correlation of GPCR with TC in the patient group (r = - 0.147) (Figure 5). There was a significant negative correlation in the control and patient group of GPCR with TG (r=-0.133 : - 0.042) respectively (Figure 6).

There was a significant negative correlation in the control group for GPCR with HDL-c (r= -0.073) and a significant positive correlation in the patient group of GPCR with HDL-c (r = 0.463) (Figure 7) .There was a significant positive correlation of GPCR with glucagon in the control group (r = 0.251) , and a significant negative correlation in the patient group (r= - 0.244**)** (Figure 8).

Table 3: Correlation coefficient for GPCR levels with some studied parameters in the patient and control groups.

Groups / Parameters	Control group		Patient group	
	r-value	*p*-value (HS)	r-value	*p*-value (HS)
GPCR vs TC	0.347	< 0.001	-0.147	< 0.001
GPCR vs TG	-0.133	< 0.001	-0.042	< 0.001
GPCR vs HDL-c	-0.073	< 0.001	0.463	< 0.001
GPCR vs Glucagon	0.251	< 0.001	-0.244	< 0.001

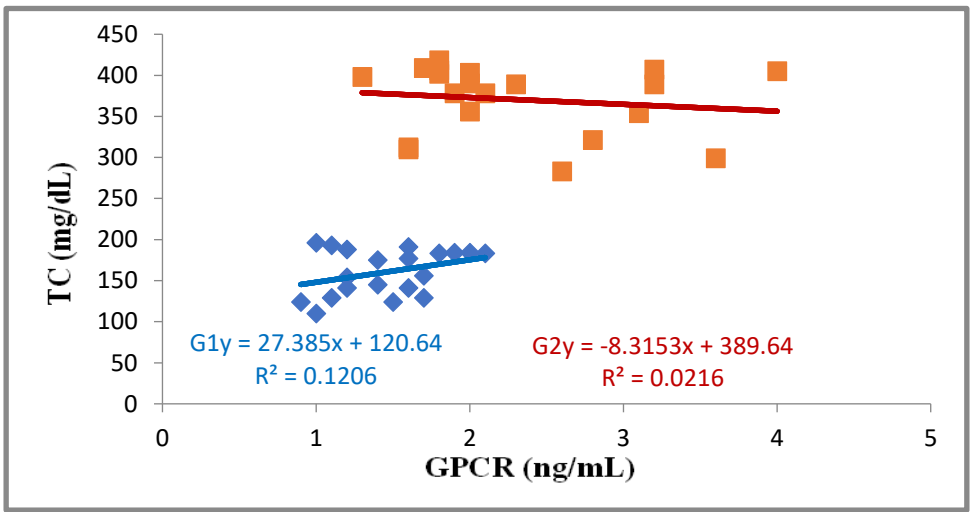

Figure 5: Correlation between GPCR and TC for the control and patient groups.

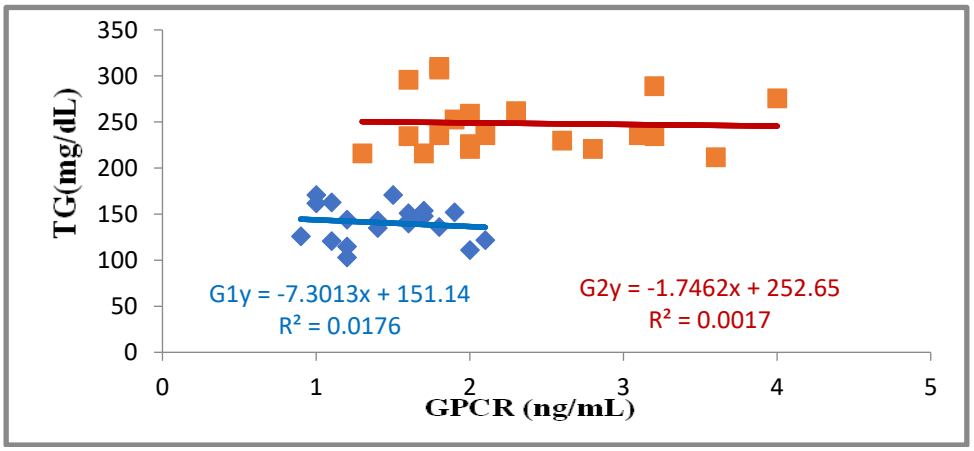

Figure 6: Correlation between GPCR and TG for the control and patient groups.

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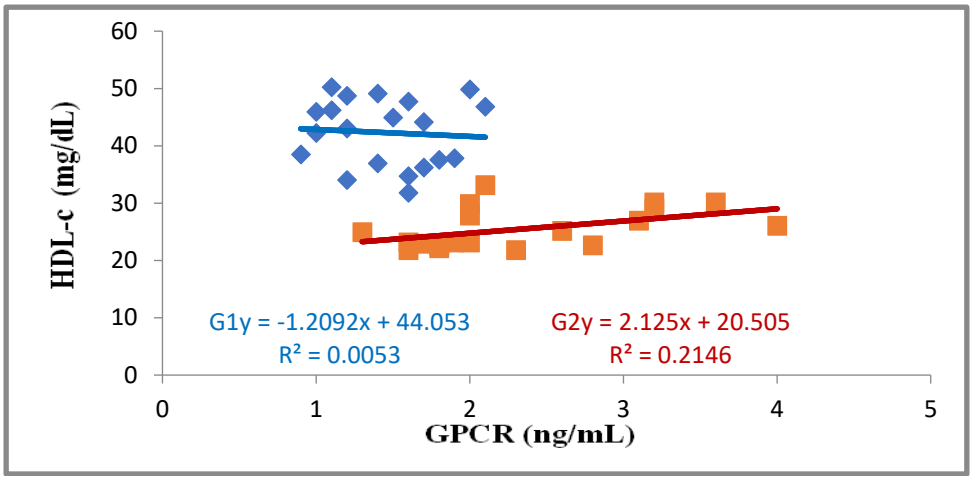

Figure 7: Correlation between GPCR and HDL-c for the control and patient groups.

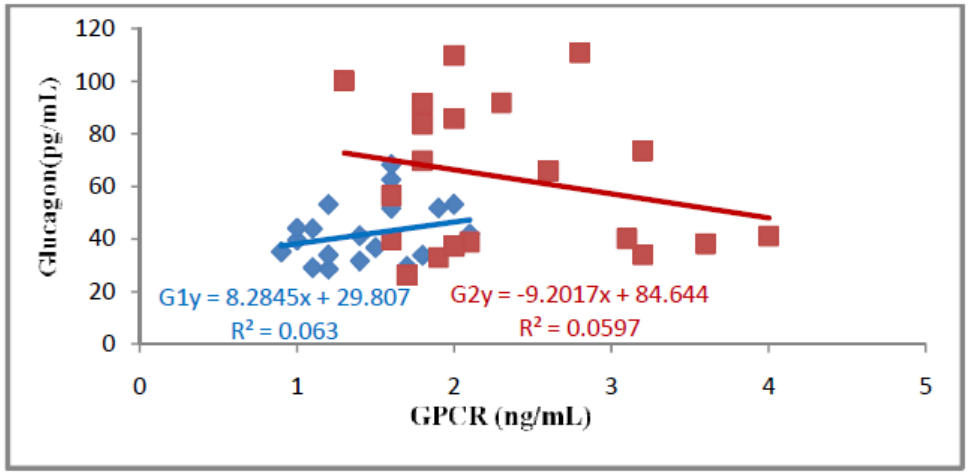

Figure 8: Correlation between GPCR and glucagon for the control and patient groups.

DISCUSSION

As per our knowledge this is the first study to determine the levels of GPCR in non-diabetic dysjipidaemia patients and compare the results with the control group, as well as to find the relationship for GPCR with GLP-1, TC, TG, HDL-c, and glucagon in these patients.

This study assumed that dyslipidaemia (high TG and/or low HDL-c levels) was significantly associated with circulating levels of GLP-1. It was also proposed that metastasis patients have high levels of GLP-1 and are at a high-risk of developing patients for cardiovascular disease, independent of the existence of diabetes. The results agree with a previous study [19] . Studies have illustrated that GPCRs (such as GLP-1R) have an impact on cardiovascular actions, like myocardial contractility, blood pressure vascular tone and modulation of heart rate [4,20] .

A previous study has demonstrated that GLP-1 exerts a cardioprotective effect in experimental dilated cardiomyopathy, myocardial infarction, and hypertensive heart failure. Infusions may improve cardiac contractility in myocardial infarction patients after successful angioplasty, as well as in chronic heart failure patients (with and without diabetes) [21] .

The relation of GLP-1 with levels of TG can be secondary to elevated hepatic synthesis of TG under the influence of obesity, glucagon, glucocorticoids, and/or hyperlipidaemia that stimulate the sympathetic nervous system [22] . Concerning glucose metabolism, glucagon excretion and gastric emptying are accelerated abnormally through hyperglycemia, obesity, and elevated food intake , which all contribute to hyperglycemia [23] . A recent study demonstrated that both short- term peripheral and central GLP-IR stimulation induce

weight loss, enhanced satiety and prevent fructose-induced dyslipidaemia. The inability to confirm expression of a hepatic GLP-IR suggests an indirect mechanism. While vagotomy alone caused a modest reduction in fasting plasma TG, the exendin-4 mediated lipid lowering effects were negated in absence of vagal signaling. This suggests the involvement of a parasympathetic signaling pathway; however, the mechanism also appears to involve changes in whole body energy utilization [24] . Studies showing both GLP-IR agonism and raising endogenous levels of GLP-1 decrease hepatic lipid levels by enhancing β-oxidation and decreasing de novo lipogenesis [25,26]. More recently, a study indicated that the lack of a hepatic GPCR led to the reduction in hepatic VLDL-c production and hepatic de novo lipogenesis that are likely due to indirect mechanisms [27] .

In conclusion this study indicated that significant increases in GLP-1and GPCR levels and their relationship with TC, TG and HDL-c in the patient group compared to control group may make these useful as a novel biomarkers for in predicting heart disease in these patients.

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