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Study of GPCR levels in Iraqi diabetic and diabetic nephropathy

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Abstract

Aim of the study is to determined G-protein coupled receptor levels in diabetic and diabetic nephropathy patients and compare the results with control group. Study also, aimed to find relationship of GPCR with HbA1c , cholesterol and Triglyceride in these patients ,in order to that GPCR could be used as a marker combat diabetes and its complication . One hundred fifty subjects were involved in this study that divided into three groups as follows:- Control group (G1) consists of 50 healthy individuals. Diabetic group (G2) consists of 50 patients and diabetic Nephropathy group (G3) consists of 50 patients . levels of HbA1c % , urea , createnine , albumin , TC, TG, HDL-c, LDL-c , VLDL-c and GPCR were determined in all subjects. Conclusion could be drawn from study that the difference between GPCR levels among groups indicate GPCR may be used as a marker in development of diabetic nephropathy as well as their are a significant relation for GPCR with TC and TG .

Keywords: GPCR , diabetic and diabetic nephropathy

1. Introduction

Diabetic nephropathy (DN) is the leading cause of end-stage renal disease and the care of patients with diabetes and DN contributes significantly to health care costs[(1)]. In the past couple of decades, there have been notable advances in our knowledge regarding the DN, including the advent of interventions that can significantly slow or even reverse the course of progressive disease [(2)].More recently, there has been increasing interest in alternative mechanisms of glucose toxicity resulting in nephropathy, including lipotoxicity, activation of inflammatory pathways and disruption of mitochondrial DNA bioenergetics among others. Among the cytokines, most prominent in the development of diabetic nephropathy are the pro-fibrotic cytokines TGF (transforming growth factor) β1 and CTGF (connective tissue growth factor) [(3)].

GPCRs are the most abundant receptor family encoded by the human genome, and, accordingly, are the targets of a large percentage (~30%) of pharmaceuticals currently prescribed in the United States [(4)]. All GPCRs share basic structural features including seven trans membrane domains, three extracellular and three intracellular loops, and conserved cysteine residues in the second extracellular loop which may play a role in the formation of the ligand binding pocket [(5)]. Recently, several GPCRs have been identified as potential therapeutic targets for the treatment of diabetes and diabetes-associated complications, including retinopathy, nephropathy, and neuropathy [(6)] .With a few notable exceptions, the N- terminal region of GPCRs are extracellular and may function in ligand specificity, while the C-terminal cytoplasmic tail may play a role in GPCR signalling, including association with G protein Receptor Kinases (GRKs)[(7)].reported that principal cells of the cortical collecting duct in the mammalian kidney, when stimulated, showed an increase in ouabain binding sites and an increase in PKAc that was prevented by PKA inhibitors [(8)]. No increase in cAMP was observed, and PKAc activation was prevented by inhibiting the proteasome.Study revealed that GPCRs are abundantly expressed in the pancreatic islets and may play an important role in normal glucose homeostasis and microvascular function. Although many of the current therapies for diabetes and its complications target tyrosine kinase receptors (insulin) or ion channels (sulfonylureas), several GPCRs, including the GLP-1 receptor, D2 receptor, and the C-peptide receptor (GPR146), may provide novel targets for the treatment of this disease. At least 293 different GPCRs are expressed by pancreatic islets, and thus future studies may reveal even more targets for the development of GPCR-based therapeutics [(9)].

Aim of the present study is to determination of GPCR levels in diabetic and diabetic nephropathy patients and compare the results with control group Also, study aimed to find relationship of GPCR with HbA1c, TC and TG in these patients ,in order to that GPCR could be used as a marker combat diabetes and its complication .

2. Subjects &Methods:

One hundred fifty individuals with age ranged between (40-65) years were enrolled in this study. They were divided into three groups as follows:- Control group (G1) consists of 50 healthy individuals. Diabetic group (G2) consists of 50 patients and diabetic Nephropathy group (G3) consists of 50 patients.The patients attended the

diabetic & endocrinology center in Al- Yarmouk Teaching Hospital during June 2017 to September 2017. Patients with smoking and kidney disease were excluded .

Ten milliliters of venous blood was drawn from the cases and control, that placed in a plane tube, left for (15 min) at room temperature, then centrifuged at 35,000 ×g for 10 min. Serum that obtained stored at (-20^{0}C) unless used immediately. whole blood was used in determination of HbA1c . the HbA1c was determined by HPLC method .A hemolyzed whole blood was mixed with a weakly binding cation-exchange resin. The non-glycosylated hemoglobin (HbA_0) linked to the resin, leaving (HbA_1) free to removed by a resin separator. The percent of HbA_1 was measured by evaluation of the absorbance values at 415nm of HbA_1 fraction and of the total Hb fraction,by calculating the ratio of absorbances (R).This ratio comparing was compared to the ratio of a glycohemoglobin standard that obtained from the same procedure[10].The urea level was estimated after enzymatic hydrolysis by urease enzyme . Indophenol compound was generated from Salicylate and Hypochlorite as shown in below .The intensity of the green complex is proportional to the urea found in the sample[11] .Creatinine in the sample was reacted with picrate in alkaline medium forming a coloured complex (Jaffe method)[12] . The method for determination of albumin was based on the specific binding of bromocresol green (BCG), an anionic dye , and the protein at acidic pH with the resulting shift in the absorption wavelength of the complex . the intensity of the color formed was proportional to the concentration of albumin in the sample[13] .The cholesterol was evaluated by spectrophotometeric method after enzymatic hydrolysis of cholesterol ester and oxidation of free cholesterol. The marker quinoneimine was generated from 4-aminophenazone and phenol in the presence of hydrogen peroxide and peroxidase as shown in below .The intensity of the pink complex was proportional to the total cholesterol found in the sample[14].The triacylglycerol (TG) level was estimated after enzymatic hydrolysis by lipoprotein lipase. Quinoneimine which was formed from hydrogen peroxide, 4-aminophenazone and 4-chlorophenol under the catalytic influence of peroxide was an indicator [15]. . Phosphotungstic acid was added in the presence of magnesium ions led to LDL, VLDL and chylomicron fractions precipitation quantitatively. The cholesterol concentration in the HDL fraction, which remains in the supernatant, after centrifugation was determined [16].The LDL-c and VLDL-c were calculated according to Friedewald formula [17] equation as displayed below:

$$\mathbf{LDL-c\ (mg/dL)} = \mathrm{TC} - \left[\mathbf{HDL-c} + \frac{\mathbf{TG}}{\mathbf{5}}\right]$$

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

Sandwich ELISA technique was used in determination of GPCR by ready kit . The results expressed as mean ± SEM. Students t-test was applied to compare the significance of the difference between DN, Diabetic patient`s and control groups .p- value ($P \geq 0.05$),($P \leq 0.05$) ,($P \leq 0.001$) considered statistically nonsignificant , significant and highly significant respectively. The correlation coefficient (r) test is used for describing the association between the different studied parameters.

3. Analytical & discussion :

Results in table (1) illustrated levels of (HbA_{1C} ,urea ,creatinine and albumin) in G1, G2 and G3. Results in table (1) reveled a significant elevation in HbA1c , urea and creatinine levels in G2 comparing to G1. results ,also, showed a highly significant elevation in these parameters in G3 comparing to G1 and G2 . While results display a significant decrease in albumin levels in G2 and G3 comparing to G1 and in G3 comparing to G2 .

Table 1. Descriptive Parameters for G1,G2,G3

Parameters	(G1)	(G2)	(G3)	T-Test G1 vsG2	T-Test G1 vsG3	T-Test G2 vsG3
HbA_{1C} (%)	5.32±0.475	8.42±1.068	9.77±1.47	S	S	S
Urea (mg/dL)	20.31±3.40	33.67±4.62	107.37±57.21	S	HS	HS
Creatinine (mg/dL)	0.47±0.119	0.92±0.164	3.94±2.42	S	HS	HS
Albumin (mg/dL)	4.029±0.30	3.97±0.215	2.4±0.836	S	S	S

Table (2) display levels of lipid profile (TC, TG, HDL-c, LDL-c, VLDL-c) in all studied groups . Results revealed a significant elevation in levels of (TC, TG, LDL-c and VLDL-c) in G2 and G3 comparing to G1 while a significant decrease was found in HDL levels in G2 and G3 comparing to G1 . Results , also showed a significant increase in (TC, TG, LDL-c and VLDL-c) in G3 comparing to G2 , while there are a significant decrease was found in G3 comparing to G2 in HDL levels .

Table 2. Lipid Profile Levels for G1,G2,G3

Parameters	(G1)	(G2)	(G3)	T-Test G1 vs G2	T-Test G2 vsG3	T-Test G1 vs G3
TC (mg/dL)	90.79±4.01	145.91±29.1	249.62±48.38	S	S	S
TG (mg/dL)	105.04±20.111	177.01±52.8	268.62±80.97	S	S	S
HDL-c (mg/dL)	48.166±4.444	35.167±4.32	35.2±4.211	S	S	S
LDL-c (mg/dL)	82.2±14.37	110.1±32.47	138.04±18.51	S	S	S
VLDL-c (mg/dL)	22.475±6.34	34.42±9.21	53.62±12.41	S	S	S

Diabetic dyslipidemia comprises a triad of raised triglycerides, reduced HDL-c and excess of small, dense LDL particles, which is in agreement with the present study[18]. The dyslipidemia are found in diabetes patients due to insulin resistance or deficiency affect key enzymes and pathways in lipid metabolism[19].

Table (3) illustrated levels of GPCR in G1 , G2 and G3 . Results display a significant elevation in GPCR levels in G2 comparing to G1 and a highly significant increase was found in G3comparing to G1 .There are a significant elevation was absorbed in G3 comparing to G2 , that indicate GPCR may be used as a marker in development of diabetic nephropathy in these patient .

Table 3. GPCR Levels for G1,G2,G3

Parameters	(G1)	(G2)	(G3)	T-Test G1 vs G2	T-Test G2 vs G3	T-Test G1 vs G3
GPCR (ng/mL)	0.54±0.158	1.307±0.299	2.10±0.59	S	S	HS

GPCRs that have received recent attention in the field of diabetes therapeutics include the incretin receptors (GLP1R, GIPR), GPR119, FFAR1 (GPR40), FFAR4 (GPR120) and the bile acid receptor GPBAR1 (TGR5) . Activation of these receptors is normally associated with the postprandial state, as they are variously the targets for nutrients, bile acids and gut hormones [20]. As approximately 30% of current pharmaceutical agents work through GPCRs because the role of GPCRs in islets is of great interest to the scientific community[21] .

3.1 Correlation relation for GPCR with HbA1c , TC and TG were studied for all groups r- value and p- value were shown in table (4) .

Table 4. r- value and p- value for GPCR with HbA1c , TC and TG for G1,G2 and G3

Parameters	GPCR			P- values		
	r-value					
	r1	r2	r3	G1	G2	G3
HbA1c %	0.172	0.427	-0.042	NS	S	S
TC (mg/dl)	0.306	0.514	-0.288	NS	S	S
TG (mg/dl)	-0.0009	-0.292	0.276	NS	S	S

Results in table (4) revealed a non significant positive correlation between GPCR and HbA1c in G1 (r_1=0.172) while a significant positive correlation was found in G2 (r_2=0.427) and a significant negative correlation in G3 (r_3=-0.042) in these parameters . as shown in figure (1) .

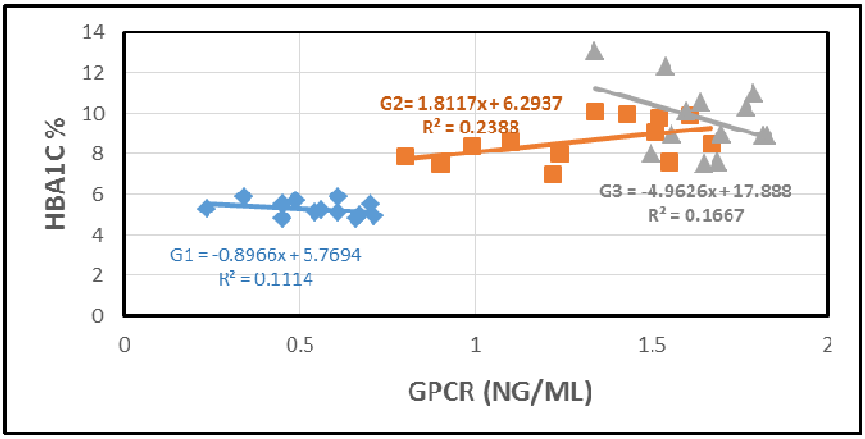

Figure 1. Correlation between GPCR and HbA1C% for G1,G2,G3

Results , also, showed a non significant positive correlation was observed in G1 between GPCR and cholesterol (r_1=0.306 , P≥0.05) . while there are a significant positive correlation in G2 (r_2=0.514, P≤0.05) . and a significant

negative correlation between GPCR and cholesterol in G3 (r_3= -0.288, P≤0.05) . as shown in table (4) and figure (2) .

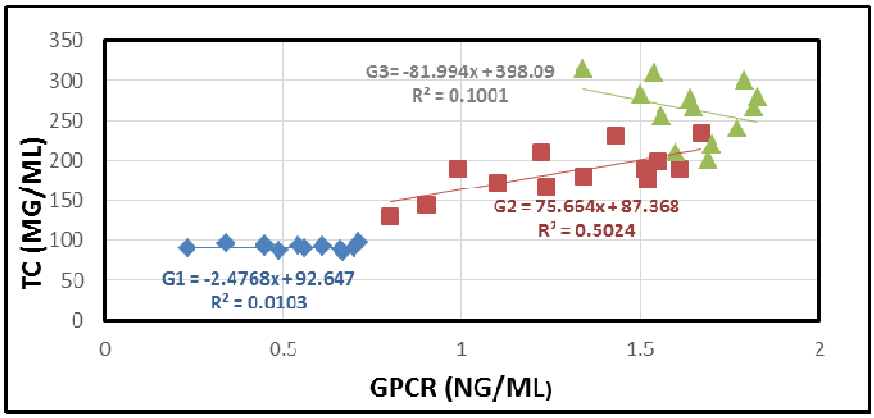

Figure 2. Correlation between GPCR and TC for G1,G2,G3

Table (4) showed a non significant negative correlation between GPCR and TG in G1 (r_1=-0.0009, P≥0.05). while there are a significant negative correlation in G2 was found (r_2=-0.292, P≤0.05) . Also, results revealed a significant positive correlation between GPCR and triglyceride in G3 (r_3= 0.276, P≤0.05) . as shown in figure (3) .

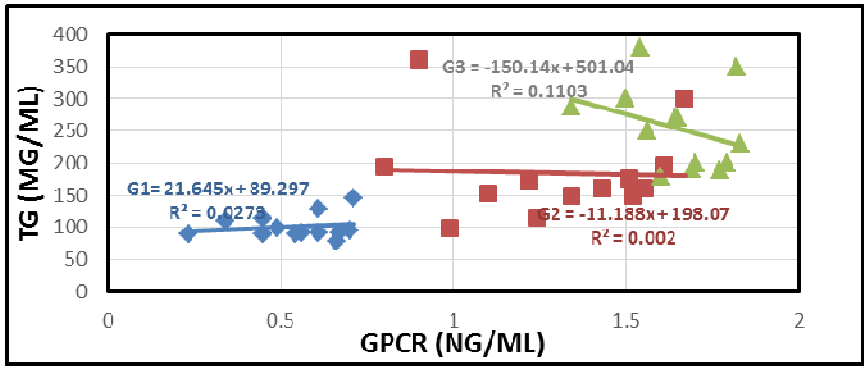

Figure 3. Correlation between GPCR and TG for G1,G2,G3

3.2 Conclusion could be drawn from study that the difference between GPCR levels among groups indicate GPCR may be used as a marker in development of diabetic nephropathy as well as their are a significant relation for GPCR with TC and TG .

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