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GLP-1, DPP-4 and GPCR Levels in Diabetic and Diabetic neuropathy Patient`s

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Abstract:
Background: Neuropathy is a common disorder in DM patients which results from poor glycaemic control and long duration of diabetes. Higher body mass index, smoking, hypertension, hypercholesterolemia and hypertriglyceridemia are associated with the incidence of neuropathy. **Objective:** The aim of this study is to estimate glucagon like Peptide-1, DPP-4 and G-Protein coupled Receptor levels in diabetic and diabetic neuropathy and to compares the results with control group. Also, to find the correlation of GPCR with GLP-1 and DPP-4 in all studied groups.**Subjects and Methods:** ninety subjects were enrolled in this study with aged ranged (40-65) years and BMI with (30-35) Kg/m^2 that divided into three groups as follows: group one (G1) consists of 30 healthy individuals as a control group, group two (G2) consists of 30 patients with diabetic and group three (G3) consists of 30 patients with diabetic and neuropathy as complication. ESC(Feet Mean), ESC(Hand Mean), ESC(Risk of neuropathies), HbA_{1C}, GLP-1, DPP-4 and GPCR were determined.**Results:** The result showed highly significant elevation in HbA_{1C} levels in patients groups comparing to control group. Results, also a highly significant elevation in GLP-1 and DPP-4 levels in G2 comparing to G1, and in G3 comparing to G2 and G1 were noticed. A highly significant elevation in GPCR levels in G2 comparing to G1and G3, while no significant increased noticed in G3 comparing to G2. A high significant positive correlation was found between GLP-1 and DPP-4 in G1, while highly significant negative correlation was found in G2 and G3. A high significant positive correlation was observed between GLP-1 and GPCR in G1. Moreover, No significant positive correlation was observed in G2, but a significant positive correlation was observed in G3. High significant negative correlation was found between GPCR and DPP-4 in G1 and G2, while a high significant positive correlation was found in G3. **Conclusion:** the conclusion is obtained from this study that GLP-1and GPCR have important role in development of neuro injury in neurodiabetic patients. Also, correlation was found between GPCR with GLP-1 and DPP-4.
Keywords: Diabetic neuropathy, GLP-1, DPP-4 and GPCR.

1. Introduction:

Diabetic neuropathy affects 30–50% of patients with diabetes mellitus. It encompasses several neuropathic syndromes, the commonest being distal symmetrical polyneuropathy or 'diabetic peripheral neuropathy' (DPN). Risk factors for DPN include poor glycaemic control and drivers of macrovascular disease including hypertension[(1)].

Glucagon-like peptide-1 (GLP-1) is an incretin with 42 amino acids [(2)] derived from the transcription product of the proglucagon gene which the intestinal L cell that secretes GLP-1 as a gut hormone is a major source of GLP-1. The GLP-1-(7-37) and GLP-1-(7-36) NH_2 are the active form of GLP-1 that due to selective cleavage of the proglucagon molecule [(3)]. Study investigated that GLP-1 protects myenteric neurons in two various culture systems. Separate receptors, suggested to be neuronally expressed, were activated. The results refereed towards a powerful therapeutic promise for these three peptides in the prevention of enteric neuropathy in diseases like diabetes, in addition to inflammatory and neurodegenerative diseases [(4)]. In the gastro intestinal tract, GLP-1 delays gastric emptying and acts as a postprandial satiety signal to the brain to suppress appetite and food intake. The GLP-1 plays an important role in the enteric and central nervous system. The release of GLP-1 is controlled and includes the gut-to-brain and the brain-to-periphery axis [(5)]. Pannacciulli et.al, 2007, found that postprandial GLP-1 response is positively associated with changes in neuronal activity of brain areas implicated in safiety and food intake regulation in humans [(6)].

DPP-4 (DP-IV, CD26, EC 3.4.14.5) is an enzyme with a 240 kDa. It is widely distributed in kidney, liver, intestine, spleen, adrenal glands, lymphocytes, endothelial cells and placenta which specifically removes N-terminal dipeptides from substrates containing proline, and to some extent alanine, at the penultimate position as in GLP-1 and GIP. *In vivo*, GLP-1 has a very short half-life of 1.5 minutes because the rapid proteolytic degradation by dipeptidyl peptidase-IV (DPP-IV), serine protease, which cleaves the active GLP-1 (7-36) to its inactive GLP-1 (9-36) form by removing two amino acids at the N-terminus of the peptide. Studies indicate that DPP-4 is also involved in the regulation of other homeostatic mechanisms, such as neurogenic inflammation, blood pressure and the immune system[(7-9)].

G-protein coupled receptors (GPCRs) which pass through the cell membrane seven times are consisting of a single polypeptide chain of up to 1100 amino acid residues. This membrane structure due to in an

extracellular N-terminal domain, seven transmembrane α-helices joined by three extracellular loops and three intracellular loops followed by an intracellular C-terminal domain that interacts with G proteins[10,11]. GPCRs are abundantly expressed in the pancreatic islets and may have an important role in normal glucose homeostasis and microvascular function[12]. Many therapies for the treatment diabetes and its complications target tyrosine kinase receptors (insulin) or ion channels (sulfonylureas), several GPCRs, including the GLP-1 receptor, dopamine D2 receptor, and the C-peptide receptor (GPR146). At least 293 different GPCRs are expressed by pancreatic islets, so future studies may investigate more targets for the development of GPCR-based therapeutic [13]. The five GPCR families are associated with various physiological functions, but the roles of the glutamate and the rhodopsin families are particularly well documented for neurotransmission and regulation of the nervous system [14].

The aim of this study is to estimation glucagon like Peptide-1, DPP-4 and G-Protein coupled Receptor levels in diabetic and diabetic neuropathy and to compare the results with control group. Also, to find the correlation of GPCR with GLP-1 with all studied groups, that may be GLP-1 and GPCR considered as a marker to diagnosis neuropathy in diabetic patients.

2. Materials and Methods:

Ninety individuals with age ranged between (40-65) years and BMI with (30-35) Kg/m^2 were enrolled in this study. They were divided into three groups as follows:-control group (G1) consists of 30 healthy individuals, diabetic group (G2) consists of 30 patients and diabetic Neuropathy group (G3) consists of 30 patients. Blood samples were collected from healthy control, diabetic and diabetic neuropathy patients after a period of fasting 12-14 hours. The study was conducted between March 2015– October 2015 in the diabetic & endocrinology center in Al-sader medical city / Iraq. Whole blood used in determination of HbA_{1C}. Serum was obtained from other part of blood that used in determination of GLP-1, DPP-4 and GPCR. Electrochemical Skin Conductance (ESC) were determined by using Sudoscan™ instruments which based on different electrochemical principles (reverse iontophoresis and chronoamperometry) to measure sudomotor function than prior technologies, affording it a much more practical and precise performance profile for routine clinical use with potential as a research tool. The device consists of a simple desktop computer connected to two sets of large surface stainless steel electrodes: two for application of the palms, and two for the soles [15]. HbA_{1C} was determined in whole blood by kit was produced from (Stanbio, USA). Glycohemoglobin is formed progressively and irreversibly in the erythrocyte during its 120-day life .and by using this measurement kit the membranes of erythrocytes are break down and edit hemoglobin associated with glucose and measure its quantity[16]. The GLP-1 determined by using ELISA kit produced from (Elabscience, China), the measurement is based on Sandwich principle, and the levels of this hormone can be measured according to the procedure along with kit, The Dipeptidyl Peptidase -4 estimated using ELISA kit produced from (Cloud-clone corp.Wuhan USCN Business Co. Ltd, USA) and GPCR determined also by ELISA technique using kit from (BlueGene Biotechnology Co. Ltd, China).

2.1 Statistical analysis:

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 with ($P \geq 0.05$), ($P \leq 0.0001$), ($P \leq 0.00001$) considered statistically no significant, significant and highly significant respectively.

3. Results & Discussion:

Table (1) display the results of HbA1c, ESC (Feet Mean), ESC (Hand Mean) and ESC (Risk of neuropathies) levels for all studied groups, G1 represent control group, G2 diabetic group and G3 diabetic neuropathy group. The result showed highly significant elevation in HbA_{1C} levels in patients groups comparing to control group.

Table (1):- HbA1c, ESC (Feet Mean), ESC (Hand Mean) and ESC (Risk of neuropathies) levels for all studied groups.

Parameters	Mean ±SEM(G1)	Mean ±SEM(G2)	Mean ± SEM(G3)	T-Test G1 vs G2	T-Test G2 vs G3	T-Test G1 vs G3
HbA1c (%)	5.44±0.07	7.18±0.08	8.08±0.19	H.S	H.S	H.S
ESC (µS) Feet Mean	89.08±0.77	84.88±1.01	62.67±3.08	H.S	H.S	H.S
ESC Hand (µS) Mean	81.17±1.88	74.08±1.76	49.08±2.35	H.S	H.S	H.S
ESC (%) Risk of neuropathies	10.13±2.31	37.33±2.55	58.96±3.75	H.S	H.S	H.S

During the past three decades, elevated HbA_{1C} has been associated with long-term risk of microvascular complications and currently, HbA_{1C} assessment is being used ubiquitously for monitoring effective glycaemic

control as a keystone of diabetes care [17].

Diabetic patients with DPN had significantly worse ESCs of both feet and hands than patients without DPN and HC [62.67±3.08 vs. 84.88±1.01 and 89.08±0.77 ($p<0.0000$) for feet and 49.08±2.35 vs. 74.08±1.76 and 81.17±1.88 ($p<0.0000$) for hand]. ESCs correlated significantly with clinical neuropathy scores of sensory, motor, and reflex function; somatic (quantitative sensory testing and nerve conduction studies) and autonomic (quantitative autonomic function testing) measures of DPN suggesting that the measure captures both somatic and autonomic nerve function.

As in the study by Casellini and collaborators, a number of projects have shown that ESC measurement may be a simple tool for early identification of autonomic neuropathy, and may be useful in screening for sub-clinical cardiac autonomic neuropathy(CAN) [15, 18].

The presence of correlation of some central neurological abnormalities with the glycated hemoglobin suggests the importance of identifying these risk factors in the assessment of central neuropathic affection. This might lead to risk-reduction strategies. In this respect, there is evidence from in *vitro* and animal studies that lipid lowering therapy has multiple potentially neuroprotective effects through improvement in Schwann cell and polyol pathway function and improved neuronal blood supply[19].

Table (2) represented the levels of GLP-1, DPP-4 and GPCR in all studied groups. A highly significant elevation in GLP-1 and DPP-4 levels in G2 comparing to G1, G3 comparing to G2 and G1 were noticed.

Table (2):- GLP-1, DPP-4 and GPCR levels for all studied groups.

Parameters	Mean ±SEM(G1)	Mean ±SEM(G2)	Mean ± SEM(G3)	T-Test G1 vs G2	T-Test G2 vs G3	T-Test G1 vs G3
GLP-1 (ng/mL)	0.62±0.09	1.65±0.08	2.31±0.14	H.S	H.S	H.S
DPP-4 (ng/mL)	23.55±1.50	73.16±2.59	154.92±4.66	H.S	H.S	H.S
GPCR (ng/mL)	0.71±0.09	1.67±0.14	1.83±0.15	S	N.S	S

Study from separate groups have determined that BMI is negatively associated with nutrient-induced GLP-1 secretion[7]. Carroll et.al, 2007, shown that elevated circulating non-esterified fatty acids, as a consequence of obesity, impair GLP-1 release in humans[20].

Rask et al. recently determined that insulin resistance is associated with impaired GLP-1 release in human's subjects, which suggested that hyperinsulinemia has deleterious effects on L cell function[21]. Recently, Ellingsgaard et al. demonstrated that the intestinal L cell is a target of insulin due to its stimulatory effects on proglucagon expression and GLP-1 synthesis[22].

The recent study suggested that both hybVIP(hybrid neurotensin 6-11 and VIP 7-28; hybVIP) and exendin(9-39)amide to antagonize the GLP-1-induced neuroprotective effects, that GLP-1 binds to GLP-1 receptors localized on VIP-expressing myenteric neurons, thereby causing release of VIP (Vasoactive intestinal peptide). Thus released VIP is responsible for GLP-1-induced neuroprotection of cultured myenteric neurons. Neuroprotection induced by GLP-1 was reversed by the addition of the GLP-1 receptor antagonist[23].

Fisman et.al, 2009, illustrated that circulating GLP-1 may be a novel biomarker for improving insulin sensitivity in high -risk patients for cardiovascular disease. Concerning the glucose metabolism, excess glucagon secretion, abnormally accelerated gastric emptying during hyperglycemia, obesity, and increased food intake all contribute to hyperglycemia. The GLP-1 receptor enhancement is used clinically to ensure glucose homeostasis in patients with type 2 diabetes in addition to its ability to mediate neuroprotection and neurotrophic effects. GLP-1 illustrated to protect central neurons against excitotoxicity as well as to neuroprotective properties effectors in neurodegenerative diseases like Alzheimer's and Parkinson's diseases [24,25].

Study suggested that may be relation between autoimmune-based diabetes and the functioning of DPP-4 protein, which led to the exploration of additional information on the pathomechanism of the disease and might enhance knowledge of its diagnosis and treatment[26].

Results in table (2), also, revealed a highly significant elevation in GPCR levels in G2 comparing to G1and G3, while no significant increased noticed in G3 comparing to G2.

The GPCRs bind peptide hormones and have causal roles in many diseases, ranging from diabetes and osteoporosis to anxiety[27]. Most small peptide hormones that signal via a GPCR, such as glucagon and GLP-1, interact with either Class A (Rhodopsin-Like) or Class B (Secretin-Like) GPCRs[28]. Both the sympathetic and parasympathetic nervous systems are regulated by GPCR pathways, responsible for control of many automatic functions of the body such as blood pressure, heart rate, and digestive processes[29].

A high significant positive correlation was found between GLP-1 and DPP-4 in G1 ($p<0.000$, $r=+0.414$), while A highly significant negative correlation was found in G2 and G3 ($p<0.000$, $r=-0.096$, $r=-0.177$), also, A high significant positive correlation was observed between GLP-1 and GPCR in G1 ($p<0.000$, $r=+0.213$).

Moreover, No significant positive correlation was observed in G2 ($p<0.90$, $r=+0.286$), but a significant positive correlation was observed in G3 ($p<0.02$, $r=+0.430$) and high significant negative correlation was found between GPCR and DPP-4 in G1 and G2 ($p<0.000$, $r=-0.383$, $r=-0.391$), while a high significant positive correlation was found in G3 ($p<0.000$, $r=+0.169$).

In β-cells, the main action of GLP-1 through the GPCR is the formation of cAMP and its insulinotropic activity[30]. Upon agonist binding, the Gαs subunit dissociates from the receptor, couples to AC and generates cAMP [31]. When blood glucose levels rise, it enters the β-cell through GLUT1 and GLUT2 transporters. Glucose is phosphorylated by glucokinase to glucose-6-phosphate, and results in the ATP/ ADP ratio in the cytosol increasing and the plasma membrane depolarizing by closing KATP channels. The closure of KATP channels, in turn opens calcium channels, releasing intracellular stores of calcium. Te increase of cytosolic calcium causes secretory granules containing insulin to fuse to the plasma membrane and insulin is exocytosed [32]. It is also likely that human glucokinase activity is more important in glucose-induce insulin secretion than the rate at which glucose enters the β-cell [33].

Metabotropic receptors consist of GPCRs that bind to neurotransmitters and cause slow synaptic transmission through intracellular signaling pathways as well as induction of gene expression necessary for exerting antipsychotic actions. Notably, most neuropharmacological drugs are known to regulate GPCR activity in the central nervous system (CNS) [14]. Some functions include synaptic transmission, synapse formation, axon guidance and development of neuronal circuits. Of the five GPCR families, the rhodopsin family is the largest, serving as molecular targets for the neurotransmitters serotonin, dopamine, acetylcholine, histamine, adrenaline and norepinephrine. GPCRs in this family also react to several neuropeptides, such as somatostatin, melanocortins, neuropeptide Y and neuropeptide FF. Similarly, glutamate GPCRs are molecular targets for the principal excitatory neurotransmitter of the central nervous system – glutamate. Upon activation, glutamate GPCRs crucially modulates synaptic transmission and neuronal excitability throughout the central nervous system. Adhesion GPCRs are a growing research field, and several of these receptors have shown an important role in the CNS [34-37].

To the best of our knowledge, this is the first study determined the levels of GLP-1, DPP-4 and GPCR in neurodiabetic patients, also, this is the first study reported the relationship of GPCR with GLP-1, DPP-4 in diabetic and neurodiabetic patients.

4. Conclusion

The conclusion that obtained from this study is GLP-1and GPCR have important role in development of neuro injury in neurodiabetic patients. Also, correlation was found between GPCR and GLP-1, which may be GLP-1 and GPCR considered as a marker in diagnosis of neuropathy in early stage in diabetic patients**.**

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