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Insulin and Glucagon Levels in Diabetic, Diabetic Neuropathy Patient`s

Tamara Ala`a[1] Zeinab M. Al-Rubaei[2]
1.Department of Basic Sciences / Faculty of Dentistry / University of Kufa
2.Department of Chemistry / College of Education, (Ibn-Al-Haitham) for pure Science / University of Baghdad

Abstract:
Background: 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.**Objective:** The study aimed to determination of insulin, glucagon levels in diabetic and diabetic neuropathy patient`s and compare the results with control group. Also, Insulin/Glu ratio calculated that may be used in early diagnosis of diabetic neuropathy.**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), FBS, insulin, Glucagon were determined. Also, HOMA-IR and insulin/Glu ratio were calculated.**Results:** The results of this study revealed highly significant differences in ESC (Feet Mean), ESC (Hand Mean) and ESC (Risk of neuropathies) between G2 and G3 comparing with G1 and in G3 comparing to G2. Results revealed a highly significant increased in FBS, insulin and HOMA-IR levels in G2 comparing to G1 and in G3 comparing to G2 and G1. Results, also, showed a high significant increased in glucagon levels in G2 and G3 comparing to G1 while no significant increased was found in G3 comparing to G2. Results, also, showed no significant reduction was found in In/Glu ratio in G2 comparing to G1, while a high significant elevation was observed in G3 comparing to G2 and G1.**Conclusion:** The conclusion could be drawn from this study that insulin and glucagon increased in patients with neurodiabetic when comparing to diabetic patients. Also, In/Glu ratio increased significantly in neurodiabetic patients when comparing to diabetic and control group that may be considered important marker in diagnosis of development of neuropathy risk.
Keywords: Diabetic Neuropathy, Insulin, Glucagon, Insulin/ Glu Ratio.

1. Introduction:

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[(1)]. A loss of nerve fibers is the morphological identification of diabetic neuropathy, which assessing cutaneous innervation is considered to be a reliable means of diagnosing and staging of diabetic neuropathy[(2)]. Diabetic neuropathy also, can be diagnosed by bilateral symmetric polyneuropathy especially on the distal side, which develops from the lower limbs, feet and crura, than from the upper limbs [(3)].

Insulin is a peptide hormone produced by beta cells in the pancreas which regulates the metabolism of carbohydrates and fats by promoting the absorption of glucose from the blood to skeletal muscles and fat tissue and by stored fat as well as using for energy [(4)]. Release of insulin is inhibited by the norepinephrine which leads to increased blood glucose levels during stress. Release of catecholamines by the sympathetic nervous system has conflicting influences on insulin release by beta cells, because insulin release is inhibited by α_2-adrenergic receptors [(5)] and stimulated by β_2-adrenergic receptors [(6)]. The net effect of norepinephrine from sympathetic nerves and epinephrine from adrenal glands on insulin release is inhibition due to dominance of the α-adrenergic receptors [(7)]. Insulin resistance (IR) is another physiological condition associated with type 2 diabetes in which cells fail to respond to the normal actions of the insulin which contribute to a diagnosis of Type 2 diabetes or latent autoimmune diabetes of adults [(8)].

Glucagon which consists of 29 amino acids and molecular weight of 3485Dalton arranged in a single polypeptide chain [(9)] and its production bases on the central nervous system [(10)].

The ins/Glu ratio was a relatively frequently cited index assessed in various conditions, including nonglycemic states like burns and starvation, and dysglycemic syndromes including type 2 diabetes and gestational diabetes mellitus[(11)].

The study aimed to determination of insulin, Glucagon levels in diabetic and diabetic neuropathy patient`s and compare the results with control group. Also, Insulin/Glu Ratio calculated that may be used as index for neuropathy patient`s.

2. Materials 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. 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. Serum obtained that used in determination of FBS, insulin and glucagon. 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 [(12)]. Serum glucose was measured by using kits from (Randox Company, United Kingdom) which based on the PAP enzymatic determination of glucose[(13)]. Insulin and Glucagon measured by using ELISA kit that produced from (Elabscience, China) the levels of these hormones can be measured according to the procedure along with kit. HOMA-IR was calculated according to Matthews *et al.* in 1985 equation as follows[(14)]:

$$\text{HOMA} - \text{IR} = \frac{\text{Glucose} \times \text{Insulin}}{405}$$

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:

The ESC (Feet Mean), ESC (Hand Mean) and ESC (Risk of neuropathies) were display in table (1). Results revealed highly significant differences in ESC (Feet Mean), ESC (Hand Mean) and ESC (Risk of neuropathies) between G2 and G3 comparing with G1 and in G3 comparing to G2.

Table (1):- ESC (Feet Mean), ESC (Hand Mean) and ESC (Risk of neuropathies) 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
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

Electrochemical skin conductance (ESC) of hands and feet is decreased in G3 (diabetic neuropathy) diagnosed using the current Toronto classification of DPN (diabetic peripheral neuropathy), compared with G1 and G2. Feet ESC was significantly decreased in patients with painful DN compared with the value in patients with nonpainful neuropathy. These results suggest that sudomotor function, evaluated through reverse iontophoresis (Sudoscan), is a reliable option when evaluating diabetes patients for the detection of small fiber neuropathy and peripheral autonomic neuropathy. Combined with a simple bedside test as the NIS-LL (Neurologic Impairment Score—Lower Legs), Sudoscan may increase the effectiveness in detecting neuropathy. Dyck et al. [(15)] have shown significant inconsistencies on clinical neurological evaluations performed by different blinded physicians; the advantage of incorporating Sudoscan for the detection of neuropathy is that it eliminates the subjective component of the clinician error. Study evaluated different neuropathy assessments on 265 diabetes patients and found that ESC measurements between the left and right side varied by 9.5% for hands and 6.0% for feet, compared with 14.2% for the vibration perception threshold test[(16)].

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) [(12, 17)].

More recent study showed that Diabetic autonomic neuropathy is associated with an impaired vasodilator response of coronary resistance vessels to increased sympathetic stimulation, which is related to the degree of SND [(18)].

Table (2) represented the levels FBS, insulin, Glucagon, HOMA-IR and insulin/Glu ratio for all studied groups. Results revealed a highly significant increased in FBS, insulin and HOMA-IR levels in G2 comparing to G1 and in G3 comparing to G2 and G1. Results, also, showed a high significant increased in glucagon levels in

G2 and G3 comparing to G1 while no significant increased was found in G3 comparing to G2.

Table (2):- FBS, insulin, Glucagon, HOMA-IR and insulin/Glu ratio 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
FBS (mg/dL)	92.57±1.7	191.13±9.7	284.43±10.3	H.S	H.S	H.S
Insulin (μIU/mL)	7.13±0.2	21.65±0.8	29.98±1.1	H.S	H.S	H.S
Glucagon (pg/mL)	59.1±4.9	233.7±17.1	234.1±67.7	H.S	N.S	H.S
HOMA-IR	1.62±0.06	10.09±0.58	20.95±0.95	H.S	H.S	H.S
In/Glu ratio	6.2±0.6	4.9±0.6	13.4±2.1	N.S	H.S	H.S

Recent study demonstrated that the neural mechanisms involved in neuroendocrine system insulin mechanism in the body[19], also, the results show that the influence of insulin on the molecular and cellular mechanisms of neurodegeneration is important in contemporary neuroendocrinology the body. Many diseases with dysfunction of insulin are progressively growing day by day such as senile dementia, diabetes [20]. Neuronal insulin receptors are intensive at synapses. In addition, peripheral sensory and autonomic ganglia have insulin receptors[21]. Insulin resistance and deficiency are a serious metabolic and functional problem on central nervous system. These problems can lead to cerebral atrophy, subcortical, brain stem damages, and cognitive dysfunctions [19, 21]. Study showed that insulin affects the liver apolipoprotein production and regulates the enzymatic activity of lipoprotein lipase and cholesterol ester transport protein, which causes dyslipidemia in diabetes mellitus[22]. This results is in agreement with the present study. Data was accumulating to suggest that neurons could also develop insulin resistance, resulting in neuronal injury [23].

Chronic insulin stimulation was shown to induce insulin resistance in mouse DRGs, as evidenced by decreased activation of Akt and its downstream effectors, and could attenuate the neurotrophic effects of insulin, resulting in mitochondrial dysfunction, subsequent development of PN [24]. These findings indicated that in the MetS insulin resistance might play an important role in the development of PN [25]. In 2007, Ferrannini et al.[26] measured insulin resistance by the euglycaemic–hyperinsulinaemic clamp in 1,296 non-diabetic individuals and demonstrated that whole-body insulin resistance is independently associated with elevated fasting glucagon concentrations, possibly as a result of alpha-cell insulin resistance.

The progressive impairment of β cell function and increased insulin demand as tissue becomes insulin resistance are core pathophysiologic defects in the development of hyperglycemia in type 2 diabetes[27]. Stimulation of insulin release by glucagon was observed in experiments on human subjects nearly 50 years ago - glucagon's insulinogenic effect [28]. There may be no direct information on this effect when both are at "high" levels and when the concentration of the proposed complex is also high and inhibitory. At higher doses, perfused insulin has been shown to stimulate glucagon secretion in whole rats [29]. Also, the insulin receptor is required in alpha-cells for glucagon secretion induced by low glucose because siRNA-mediated "knockdown" of the insulin receptor in a pancreatic alpha-cell line (alpha-TC6), abolishes this glucagon secretion [28]. Increased alpha-cell function and consequent hyperglucagonemia has long been recognised as a contributor to hyperglycemia in diabetic patients, by stimulating hepatic glucose production[30]. Indeed, elevated fasting concentrations of glucagon, as well as impaired glucose-induced glucagon suppression and a disrupted insulin–glucagon interaction in the postprandial period, were described in T2D patients, differently from healthy subjects who present plasmatic glucagon and insulin concentrations inversely related in the postprandial state. The loss of the inverse relationship between these two hormones in T2D patients might be secondary to the observed diminished mass of insulin pulses, and suggests that alterations in the cross-talk between beta- and alpha-cells may underlie hyperglucagonemia [31].

Results, also, showed no significant reduction was found in In/Glu ratio in G2 comparing to G1, while a high significant elevation was observed in G3 comparing to G2 and G1.

Insulin glucagon ratio can be used as an index of anabolism; insulin is the most potent anabolic hormone in the body, and a high In/Glu ratio reflects its action, as opposed to that of glucagon, which has glycogenolytic or catabolic activity in the liver [32]. The authors suggest that SGLT‐2 expression in alpha cells, when down‐regulated, is associated with an increase in the expression of SGLT 1 and glucagon genes, as well as genes related to hepatic gluconeogenesis. The same research team reports that SGLT 1 and 2 gene expression is lower in islets of type 2 diabetes as compared to normal subjects. Lower gene expression is associated with increased glucagon gene expression. Similar results are obtained when SLCSA‐2 gene is knocked out, or when it is inhibited with dapaglifozin. This may explain the paradoxical increase in plasma glucagon and hepatic glucose production noted with SGLT‐2 inhibition. Some studies have demonstrated that hyperglucagonemia, or an elevated glucagon-to-insulin ratio, plays an important role in the development of hyperglycemia in diabetic subjects.

Hyperglucagonemia destabilizes normal blood glucose control mostly because of glucagon-mediated increases in blood glucose levels. These studies are in agreement with present study[33-35].

4. Conclusion:

The conclusion could be drawn from this study that insulin and glucagon increased in patients with neurodiabetic when comparing to diabetic patients. Also, In/Glu ratio increased significantly in neurodiabetic patients when comparing to diabetic and control group that may be considered important marker in diagnosis of development of neuropathy risk.

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