

Correlation of Human Protein Kinase A (PKA) and G-Glycoprotein in Patients with T2DM

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Abstract

Background: Type 2 diabetes usually starts with insulin resistance—a condition that happens when muscle, fat, and liver cells cannot use insulin to deliver glucose into the cells of the body for energy use. **Materials and Methods:** The group subjected to this study consists of 45 type 2 diabetic patients whose age ranges from 30 to 55 years, from both sexes (22 males and 23 females); the control group also includes 45 apparently healthy persons, and they were free from symptoms and signs of any diseases and ages of this group ranged between 30 and 55 years, from both sexes (23 females and 22 males). Protein kinase A (PKA) and G-glycoprotein concentrations were determined by Sandwich-ELISA kits by Sunlong (China) Company. **Results:** The results of this study revealed no significant differences in the concentration of PKA between patients and control groups, that is, PKA level of female patients and its control and of male patients and its control and between type 2 diabetes mellitus (T2DM) male and T2DM female ($P > 0.05$). Also, the results of the study revealed that there were no significant differences in the concentration of P-glycoprotein (PGP) between patient and control groups, T2DM females and T2DM males, and between T2DM male and its control group ($P > 0.05$), except for that between female T2DM and its control; the results revealed that there was a significant difference in the PGP concentration ($P < 0.05$). Finally, a moderate significant positive correlation was found between PKA and PGP concentrations ($P = 0.000$, $r = 0.51$). **Conclusion:** In our study, we measure the actual PKA concentration; it is important for future studies to measure PKA activity instead of concentration to find the role of active PKA in glucose hemostasis. Also, the study of G-glycoprotein gene expression, instead of concentration, is used to explore the accurate function of G-glycoprotein in the distribution and clearance of anti-diabetic drugs.

Keywords: Cyclic adenosine monophosphate, enzyme-linked immunosorbent assay, P-glycoprotein, protein kinase A, type 2 diabetes mellitus

INTRODUCTION

It is recently agreed that the essential characteristic common to all types of diabetes is the destruction of pancreatic β -cells.^[1] Many proposed mechanisms can lead to a drop in the function or complete destruction of β -cells.^[2] These mechanisms consist genetic susceptibility and anomalies, epigenetic processes, autoimmunity, insulin resistance, inflammation, concurrent illnesses, and environmental factors.^[3] Type 2 diabetes usually starts with insulin resistance—a condition that arises when fat, muscle, and liver cells cannot use insulin to deliver glucose into the body's cells for energy use. Consequently, the body requires more insulin to support glucose enter into the cells. First, the pancreas continues by making more insulin in order to fulfill all the demands.^[4] Protein kinase

A (PKA) is the main cAMP downstream effector. It is a serine/threonine kinase with wide specificity that controls numerous cellular processes, e.g., cell growth, metabolism, cardiac myocyte contraction, and cell division. It is a heterotetramer that contains two catalytic subunits ($C\alpha$, $C\beta$, or $C\gamma$) by two regulatory RI ($RI\alpha$ or $RI\beta$) or RII ($RII\alpha$ or $RII\beta$) subunits; it is kept in an inactive state which organized as homodimers in the holoenzyme.^[5] cAMP exerts its action through the activation of downstream

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effector proteins, i.e., cAMP-dependent PKA.^[6] C subunits are released when cAMP binds to the R subunits and thus activate and phosphorylate local substrates.^[7]

Current outcomes have supported the feasibility of cAMP/PKA pathway as a target for the management of various diseases such as polycystic kidney disease, cancers, and diabetes. Several approaches have been suggested for cAMP/PKA pathway targeting, containing the modulation of cAMP levels. As an example, metformin suppresses hepatic gluconeogenesis by inhibiting glucagon-induced cAMP production in hepatocytes.^[8] Extra class of drugs that have been clinically approved for type 2 diabetes mellitus (T2DM) treatment are GPCR agonists or antagonists.^[9] For example, the GLP1R agonists exenatide (Byetta) and liraglutide (Victoza) are FDA-approved anti-diabetic drugs and are capable of improving insulin secretion by stimulating cAMP production in β -cells. Few molecular antagonists for glucagon receptor are under preclinical or clinical trials for their inhibitory action on hepatic glucose production.^[10]

P-glycoproteins (PGPs) are transmembrane proteins, which are coded by the gene called multidrug resistance phenotype (MDR1). PGPs are present in various tissues such as peripheral blood lymphocytes.^[11] They are efflux pumps to reduce the intracellular accumulation of numerous immunosuppressants and lipophilic drugs, including cyclosporine A, anthracyclines, and vinca alkaloids. Therefore, the functions of PGP are to protect cells from their exposure to lipophilic drugs and help in developing resistance to them.^[12]

Furthermore, a large number of anti-diabetic drugs such as glibenclamide, rosiglitazone, metformin, and repaglinide in addition to numerous dipeptidyl peptidase-4 inhibitors consisting of sitagliptin/vildagliptin, saxagliptin, and linagliptin have been found to be substrates for PGP. Because of this development, it has been suggested that PGP expressed in numerous tissues may affect distribution, metabolism, and excretion routes of the anti-diabetic drugs mentioned earlier under diabetic conditions. In contrast, because anti-diabetic drugs are often prescribed orally in the clinic, it might be necessary to consider variations in the expression of intestinal PGP, which has an important role in the absorption process of its substrate drugs administered via the oral route. Glucose changes in intestinal PGP under diabetic circumstances may include a direct mechanism mediated by hyperglycemia. Some *in-vitro* studies had shown that high glucose level reduces PGP expression, leading to the accumulation of higher intracellular drugs in MCF-7 cells derived from human breast cancer cells.^[13] Totally, these outcomes focus on the influence of glucose concentration on PGP function and direct that changes in the form of protein expression, and drug efflux activity of intestinal PGP may show a discrepancy according to

hyperglycemia duration, the pathogenetic mechanism, and related diseases other than diabetes in each patient. It is important to monitor the changes in the expression, and functional activity of intestinal PGP under pathological conditions may become important means for patients to achieve effective pharmacotherapy.^[14]

MATERIALS AND METHODS

T2DM patients

This study was conducted at the Laboratory of College of Medicine, Babylon University, Babylon, Iraq. Blood samples were collected from November 1, 2020 to March 30, 2021. Diagnoses of all patients were done by physicians in the Merjan Teaching Hospital in Hilla city.

The diabetic group consists of 45 type 2 diabetic patients aged between 30 and 55 years, from both sexes (23 females and 22 males).

Control

The control group includes 45 apparently healthy persons; they were free from symptoms and signs of any diseases and age of this group ranged between 30 and 55 years, from both sexes (23 females and 22 males).

Exclusion criteria

The exclusion criteria are as follows:

- 1. Any subject with chronic liver disease;
- 2. Any subject with thyroid problem;
- 3. Pregnant;
- 4. Patient on corticosteroids or thyroxine treatment;
- 5. Any subject with retinopathy;
- 6. Any subject with neuropathy;
- 7. Any subject with hypertension;
- 8. Any subject with nephropathy.

Ethical consideration

The study was performed according to the ethical principles of the Declaration of Helsinki. It was accomplished with patients' verbal and analytical approval before the blood sample was taken. The informed consent was done by considering the study protocol. The subject information was reviewed and approved by a local Ethics Committee (College of Medicine, University of Babylon).

Blood samples collection

An aliquot of 5 mL of venous blood was withdrawn from patients and controls by venipuncture, pushed slowly into gel tubes. Blood was allowed to clot at room temperature for 30 min at 2000g for 5 min. Then the serum was divided into small Eppendorf tubes and kept at -20°C to be used later for biochemical estimation of PKA and PGP concentrations by the ELISA technique.

Determination of PKA concentration

Using Sandwich-ELISA kits from Sunlong (China) Company, the known concentrations of human PKA standard (x -axis) and its corresponding reading OD (y -axis) were plotted on a log scale [Figure 1]. The concentration of human PKA in the sample was estimated by plotting the sample's OD on the y -axis. By multiplying with the dilution factor, the original concentration of PKA was calculated.

Determination of PGP concentration

By using Sandwich-ELISA kits from Sunlong (China) Company, the concentration of PGP was determined. The known concentration on the log scale (x -axis) and its corresponding reading OD (y -axis) were plotted in Figure 2. The concentration of human PGP in the sample was calculated by plotting the sample's OD on the y -axis. By multiplying with the dilution factor, the original concentration was calculated.

Statistical analysis

Descriptive statistics and graphs were carried out by using SPSS 19 (SPSS Inc., Chicago, IL, USA). By using Student's t -test, the parameters (mean $\pm$ SD) were compared. All data were expressed as mean $\pm$ SD.

RESULTS

The results of this study revealed that there were no significant differences in the concentration of PKA between patient and control groups and between T2DM males and T2DM females ($P > 0.05$). Also the results revealed that there was no significant difference in the

PKA level between female patients and its control and male patients and its control ($P > 0.05$). The means, standard deviation, and statistical parameters are listed in Table 1.

The results of the study revealed that there were no significant differences in the concentration of PGP between patients and control groups and T2DM female and T2DM male ($P > 0.05$). Also, the results revealed that there was a significant difference in PGP concentration between female T2DM and its control ($P < 0.05$), whereas the results showed that there was no significant difference between T2DM male and its control group ($P > 0.05$). The means, standard deviation, and statistical parameters are listed in Table 2.

DISCUSSION

Our data showing normal fasting blood glucose and insulin levels with the continuation of the effect reveal the benefits to diabetes therapy by changing the insulin secretion profile, rather than producing a huge increase in the release of insulin. Pharmacological and genetic studies suggested that both selective activation in pancreatic β -cells of cAMP/PKA and selective inhibition of the cAMP/PKA pathway in the liver can improve glycemic control in T2DM. Further, the cAMP/PKA pathway can be clinically challenging to yield tissue-specific manipulation, the findings that metformin can exert its anti-diabetic effect by suppressing the hepatic cAMP/PKA/CREB pathway. He *et al.* and Pernicova and Korbonits established these signaling events as a target for the progress of new T2DM treatment.^[15] Kelly *et al.* demonstrated in their study that signaling of cAMP via PKA in β -cells provides valuable

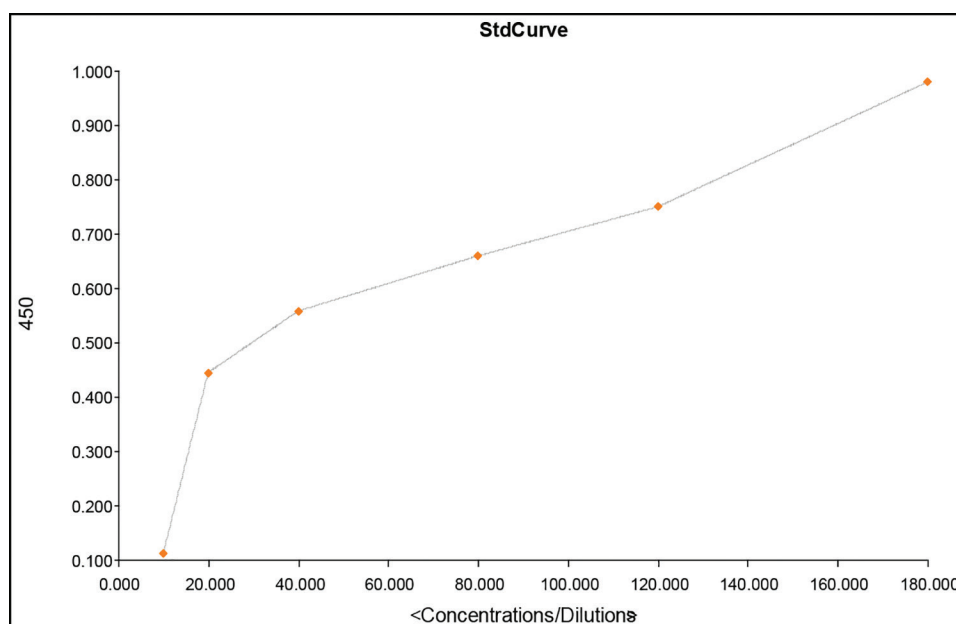


Figure 1: Standard curve for determination of PKA concentration

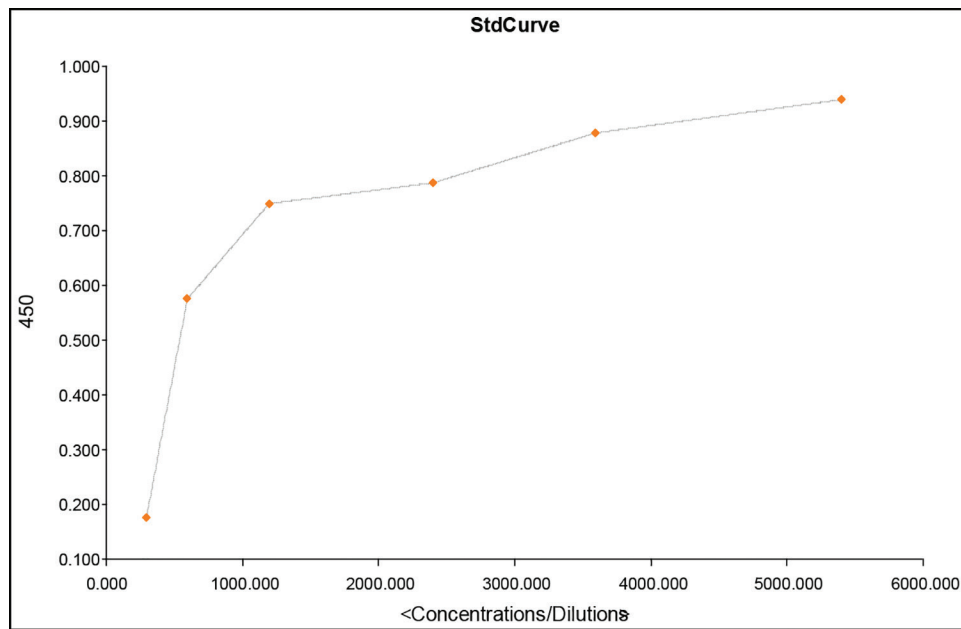


Figure 2: Standard curve for determination of permeability glycoprotein concentration

Table 1: PKA concentrations for all groups

Group		N	PKA, mean $\pm$ SD, ng/L	P-value*
Subjects	T2DM	45	73.9 $\pm$ 21.2	0.17
	Control	45	81.4 $\pm$ 28.5	
Patients only	Female	23	76.8 $\pm$ 17.0	0.25
	Male	22	121.1 $\pm$ 25.1	
Female	T2DM	23	76.8 $\pm$ 17.0	0.77
	Control	23	78.4 $\pm$ 19.9	
Male	T2DM	22	121.2 $\pm$ 181.5	0.98
	Control	22	119.9 $\pm$ 128.9	

*P-value of student test

effects to insulin secretion and the protection of β -cell tissues with reduced risk for opposing effects. Also, they do establish that the PKA activation is not enough to drive proliferation of β -cell numbers. It has been suggested that the increment in glucose associated with an inadequate β -cell mass may be a participating factor to β -cell expansion. The secretion of insulin activated by PKA does not occur in chronic hyperinsulinemia, but rather it delivers acute development of insulin secretion in order to lower glucose levels rapidly.^[16]

The expression of PGP protein was different in DM and non-DM patients, proposing that the PGP protein expression was affected by DM and resistance of the drug.^[17] Earlier studies suggest the changes in the expression of PGP under many pathological conditions at numerous tissue levels.^[18] Yeh *et al.*^[19] reported that in rats hyperglycemia suppresses renal expression of PGP. As peroral bioavailability of drugs will be affected by intestinal PGP and patients with DM are usually recommended multiple treatments, it is of great efforts

to estimate the intestinal PGP expression in these T2DM patients.^[20]

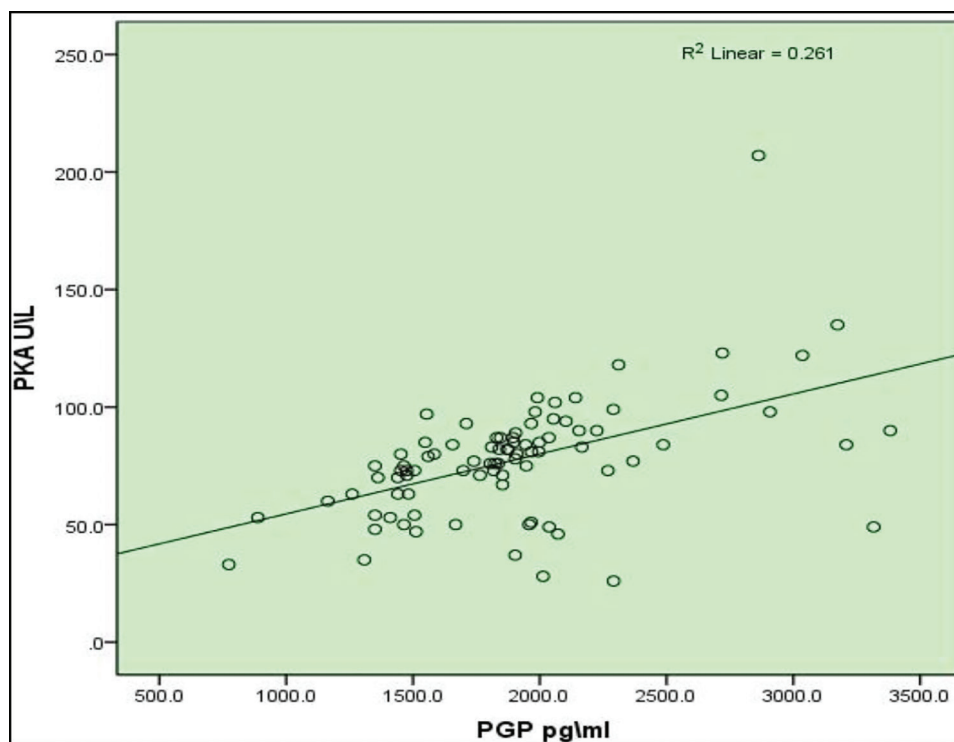
In this study, the result in female T2DM and its control revealed that the concentration of PGP in T2DM is higher than that in controls, and these results did not agree with Abbasi *et al.*,^[21] which reported in their study that anti-diabetic drugs gliclazide, metformin, and pioglitazone inhibited PGP efflux activity and also down-regulated PGP expression. The inhibitory effects of the drugs on PGP should be considered when drugs are co-administered with drugs that are PGP substrates. Further investigation is necessary to confirm these results and to define the underlying PGP inhibitory mechanism of the drugs.

In this study, a moderate significant positive correlation between PKA and PGP concentrations is obtained. Phosphorylation by Vanoye *et al.* reported in their study that activation of PGP by PKA and PKC will alter swelling-activated Cl⁻ fluxes. Some proteins of the ATP-binding cassette superfamily can affect the function of ion channel, which include the cystic fibrosis transmembrane conductance regulator, sulfonylurea receptor, and the

Table 2: PGP concentration for all groups

Group		N	PGP, mean $\pm$ SD, pg/mL	P-value*
Subjects	T2DM	45	2037.7 $\pm$ 681.9	0.42
	Control	45	1919.2 $\pm$ 702.4	
Patients only	Female	23	2080.913 $\pm$ 431.5	0.66
	Male	22	1990.571 $\pm$ 889.0	
Female	T2DM	23	2080.9 $\pm$ 431.5	0.013
	Control	23	1753.2 $\pm$ 424.2	
Male	T2DM	22	1990.5 $\pm$ 889.0	0.69
	Control	22	2101.1 $\pm$ 892.5	

Finally, a moderate significant positive correlation between PKA and PGP concentrations is shown in Figure 3 ($P = 0.000$, $r=0.51$).

**Figure 3:** Correlation between PKA and PGP concentrations

multidrug resistance protein of PGP (MDR1). Activation of PKA with cyclic monophosphate swells only in MDR1-expressing cells. The effects of PKA stimulation were absent in cells expressing the phosphorylation-defective mutant.

CONCLUSION

In this study, we measure the actual PKA concentration, and it is important for future studies to measure PKA activity instead of concentration to find the role of active PKA in glucose hemostasis. Also, the study of G-glycoprotein gene expression instead of concentration is to explore the accurate function of G-glycoprotein in the distribution and clearance of anti-diabetic drugs.

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Conflicts of interest

There are no conflicts of interest.

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