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Serum Secretagogin and Focal Adhesion Kinase as Markers for Type 2 Diabetes Mellitus and Beta Cells Function

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Abstract

Diabetes mellitus (DM) is one of the world's major public health problems. The increasing incidence of DM worldwide makes it a leading cause of morbidity and mortality for the anticipated future. Secretagogin (SCGN) is a protein enriched and secreted from pancreatic islets, it demonstrates protective effects on β-cell function. Focal adhesion kinase (FAK) plays a critical role in β-cell survival and is a vital regulator of insulin secretion. In this research, serum SCGN, FAK, fasting blood glucose (FBG), HbA1c, C-peptide, lipid profile, blood urea nitrogen (BUN) and creatinine were measured in patients diagnosed with type 2 diabetes mellitus (T2DM) and in healthy volunteers. The results showed that there was a significant increase in the levels of serum SCGN in patients with T2DM compared to the control group. There was a significant decrease in levels of FAK and C-peptide in T2DM patients compared to the control group. In addition, a significant increase was seen in FBG and HbA1c levels in T2DM patients compared to the control group. The lipid profile of T2DM patients was altered compared to the control group. There was no significant difference in the levels of BUN and creatinine among the study subjects. The results of this study suggest that both SCGN and FAK may serve as potential biomarkers reflecting the state of islet cells dysfunction in T2DM patients which may present additional markers for the diagnosis of T2DM.

Keywords: *Serum secretagogin, focal adhesion kinase, C-peptide, glucose, HbA1c, type 2 diabetes mellitus.*

Introduction

Diabetes mellitus (DM) is a heterogeneous group of metabolic disorders commonly associated with hyperglycemia resulting from insulin secretion disorders, insulin action, or both[(1)].DM is a common public health issue worldwide and continues to increase its prevalence[(2, 3)]. DM is of the top five major death causes in developed countries. The International Diabetes Federation (IDF) estimated that in 2015, the global population of people with diabetes was 415 million and is expected to increase to 642 million by 2040[(4)].

Secretagogin (SCGN) is a Ca^{2+} sensor protein that has six EF-hand helix-loop-helix motifs for binding calcium. Originally, SCGN was cloned with pancreatic β cells[(5)]. It has been revealed recently that SCGN also plays key roles in insulin secretion in pancreatic β-cells by interacting with vesicle fusion, protein trafficking[(6)] and actin cytoskeleton[(7)]. The human protein atlas shows by systematic comparison of expression in various human tissues that SCGN has its largest amount of protein expression in Langerhans islets relative to other tissues[(8)].

Focal adhesion kinase (FAK) is a largely conserved 125 kDa non-receptor tyrosine kinase that plays a critical role in motility, survival and proliferation of adhesion-dependent cells in responding to signals of receptors of integrin and growth factor[(9)]. FAK protein is overexpressed in cancers such as ovarian, cervical, kidney, pulmonary, pancreatic, brain, colon, breast, and skin cancer[(10)]. In addition, FAK plays a critical role in regulating F-actin remodeling and insulin secretion[(11)]. In pancreatic β-cells, integrin β1-mediated intracellular signaling activates and phosphorylates FAK and paxillin upon glucose stimulation. The present study aims to investigate the role of SCGN and FAK in the pathogenesis of T2DM and the possibility of using these proteins as markers that may reflect β-cells dysfunction and thus aid as additional and more accurate markers in the diagnosis of T2DM.

Materials & Method

Study subjects:

The study subjects were divided into two groups. The first group included 40 patients diagnosed with T2DM (21 males and 19 females) with an age range of (70 – 36 and 65 – 44 respectively) and the second group included 40 healthy individuals (23 males and 17 females) with an age range of (70 – 25 and 70 – 26 respectively) serving as the control group. The patients enrolled in the present study were attending the Al-Imameen Al-Kademen Medical City and Al-Kindi Hospital. This study was approved by the Department of Chemistry, College of medicine, Al-Nahrain University, Baghdad, Iraq.

Exclusion criteria:

Patients were excluded from the study if they had one or more of the followings; treatment with statins or any hyperlipidemia drugs, type 1 diabetes, liver or pancreatic inflammation and any type of cancers or tumors.

Samples collection:

Five milliliters of venous blood were taken from each patient and healthy control. (3 mL) of the blood was transferred into a gel tube to separate the serum. The remaining (2 mL) of the blood was transferred into an EDTA tube to prevent the blood from clotting. The obtained samples were stored at (-20 ºC) until assayed.

Biochemical Analyses:

Human serum secretagogin and FAK were measured by enzyme linked immunosorbent assay (ELISA) using Human secretagogin and Human FAK ELISA kits purchased from (Mybiosource/ USA) following the manufacturer's instructions. C-peptide was measured by cobas e411 using C-peptide kit purchased from (Roche Diagnostics/ Switzerland) following the manufacturer's directions. HbA1c, FBG, lipid profile, creatinine and blood urea nitrogen were measured by cobas c111 using HbA1c, FBG, lipid profile, creatinine and blood urea nitrogen kits purchased from (Roche Diagnostics/ Switzerland) following the manufacturer's directions.

Statistical Analyses:

Biochemical data were analyzed using statistical package for social sciences (SPSS) version 25. T-Test was used to calculate mean ± standard deviation (SD) and the p value.

Results & Discussion

Tables (1) show that there was a significant increase in the levels of SCGN in the sera of patients diagnosed with T2DM)104.52 ± 1.8 pg/mL) compared to the control group (59.1 ± 2.73 pg/mL) (p> 0.000). A significant decrease of FAK serum levels was also observed T2DM patients)402.82 ± 76.1 pg/mL) compared to the control group (1312.35 ± 163.35 pg/mL) (p> 0.000). there was a significant decrease in the levels of C-peptide in the sera of patients diagnosed with T2DM)0.7 ± 0.12 ng/ml) compared to the control group (3.54 ± 0.95 ng/ml) (p> 0.000). There was a significant increase in the levels of FBG in the sera of patients diagnosed with T2DM)203.15 ± 82.88 mg/dl) compared to the control group (97.70 ± 4.82 mg/dl) (p> 0.000). There was also a significant increase in the levels of HbA1c in the sera of patients diagnosed with T2DM)8.82 ± 2.11 %) compared to the control group (5.37 ± 0.30%) (p> 0.000). There was a significant increase in the level of Triglyceride in the sera of patients diagnosed with T2DM (189.26 ± 80.39 mg/dl) compared to the control group (101.07 ± 31.58 mg/dl) (p> 0.000). The result showed a significant increase in the level of cholesterol in the sera of patients diagnosed with T2DM (185.74 ± 53.56 mg/dl) compared to the control group (156.42 ± 24.44 mg/dl) (p> 0.003). There was a significant decrease in the levels of HDL in the sera of patients diagnosed with T2DM (40.64 ± 14.26 mg/dl) compared to the control group (49.21 ± 13.53 mg/dl) (p> 0.007). There was a significant increase in the levels of LDL in the sera of patients diagnosed with T2DM (106.21 ± 45.56mg/dl) compared to the control group (86.84 ± 22.37 mg/dl) (p> 0.018). There was also a significant increase in the levels of VLDL in the sera of patients diagnosed with T2DM (37.79 ± 16.09 mg/dl) compared to the control group (20.20 ± 6.32 mg/dl) (p> 0.000). In this study, there was no significant difference in the levels of creatinine and BUN among the study subjects.

Table (1): Levels of SCGN**, FAK and other clinical parameters in the study subjects.**

Clinical parameters	**T2DM patients Mean ± SD**	**Controls Mean ± SD**	**p-value**
SCGN (pg/mL)	104.52 ± 1.8	59.1 ± 2.73	0.000*
FAK (pg/mL)	402.82 ± 76.1	1312.35 ± 163.35	0.000*
C-peptide (ng/ml)	0.7 ± 0.12	3.54 ± 0.95	0.000*
FBG (mg/dl)	203.15 ± 82.88	97.70 ± 4.82	0.000*
HbA1c (%)	8.82 ± 2.11	5.37 ± 0.30	0.000*
S. Cholesterol (mg/dl)	185.74 ± 53.56	156.42 ± 24.44	0.003*
S. Triglyceride (mg/dl)	189.26 ± 80.39	101.07 ± 31.58	0.000*
S. HDL (mg/dl)	40.64 ± 14.26	49.21 ± 13.53	0.007*
S. LDL (mg/dl)	106.21 ± 45.56	86.84 ± 22.37	0.018*
S. VLDL (mg/dl)	37.79 ± 16.09	20.20 ± 6.32	0.000*
Blood urea (mg/dl)	28.04 ± 8.17	29.93 ± 8.03	0.301
Creatinine (mg/dl)	0.78 ± 0.18	0.77 ± 0.17	0.889

*Significant at the levels of ($p \leq 0.05$).

SCGN appears to have an extra role in cytoskeleton reorganization during insulin release, it affects the dynamics of F-actin to promote the transportation of the vesicle to the periphery and the remodeling of the focal adhesion[7]. In the present study, there was a significant increase in the levels of the SCGN in the sera of patients diagnosed with T2DM compared to the control group, and the results were in accordance with the results of a recent study that found increased levels of SCGN in T2DM patients[12]. The study showed that SCGN release was not fluctuating in relation to insulin release and that it could enable SCGN to reflect a certain disease condition that cannot be captured by insulin, C-peptide or proinsulin levels. These results support that SCGN release from the islets was sufficient to create quantifiable levels in the plasma as part of islet failure (i.e. increased islet stress)[12]. Another study supported that intracellular SCGN protected β-cells from apoptosis, in which apoptosis was inhibited by overexpression of SCGN[13]. In addition, a recent study suggested that intracellular SCGN promoted the survival of pancreatic β-cells and reduced Endoplasmic reticulum (ER) stress by stabilizing deubiquitinating proteins[14].

FAK is an exercise-sensitive protein that plays a part in skeletal muscle morphology, metabolism and insulin sensitivity[15, 16]. FAK regulates insulin-mediated cytoskeletal rearrangement in skeletal muscle cells, which is essential to normal glucose transportation and glycogen synthesis[17]. FAK's role in regulating glycogen synthesis was also revealed in HepG2 cells and hepatic insulin signaling in vitro[17, 18]. In the current study, there was a significant decrease in the levels of the FAK in the sera of patients diagnosed with T2DM compared to the control group. This study represents the first attempt to measure the levels of FAK in the blood of T2DM patients however, there are a number of previous studies describing the role of FAK in tissues. A previous study showed that activated FAK-paxillin complexes were integrated into nascent focal adhesions when main rat β-cells were stimulated by glucose. Focal adhesion remodeling in response to glucose is Ca^{2+} -dependent, fast, reversible and connected to short-term glucose-induced signaling pathway activation of ERK1/2. Finally, these glucose-mediated occurrences are crucial for controlled β-cell insulin secretion[19]. Another study showed that FAK is needed to maintain both pancreatic β-cell mass and in vivo function so that glucose homeostasis is disturbed in its absence. The study also showed that deletion of FAK in β-cells has been shown to result in impaired cell proliferation, survival and function. In the absence of FAK, deficiencies in actin dynamics related to impaired focal protein dynamics and insufficient trafficking of insulin granules resulted

in decreased insulin exocytosis. The study concluded that FAK has a critical dual role in controlling both the viability of β-cells and cell functions and can be a prospective therapeutic target for T2DM[20].

C-peptide is a helpful and commonly used technique for evaluating the function of pancreatic β-cells[21]. The concentration of C-peptides has been shown to decrease over decades with diabetes length[22]. Lower concentrations of C-peptide and lower function of β-cells were associated with higher rates of glucose variability[23]. In the current study, FBG and HbA1c levels were higher in the T2DM patients compared to the controls. Our findings were supported by a number of previous studies that showed increased levels of FBG and HbA1c in T2DM patients compared to controls[24].

In the present study, levels of serum triglycerides were higher in the patients diagnosed with T2DM compared to the control group. Previously reported studies had suggested that the levels of TG in serum were positively associated with DM. Moreover, it has also been shown that increased serum TG levels over time enhanced the risk of developing DM in various populations[25]. In the current study, serum TC, VLDL and LDL in the patients diagnosed with T2DM were higher than the control.

Conclusion

The results of the present study suggest that both SCGN and FAK may serve as potential biomarkers reflecting islet cell dysfunction in T2DM patients and that they may aid as additional markers for the diagnosis of T2DM. SCGN and FAK may differentiate β-cells dysfunction T2DM from insulin resistant T2DM. The patients of the present study suffered from β-cells dysfunction. Alterations in the lipid profile of the patients was associated with T2DM. The kidney function reflected by BUN and creatinine levels was not affected by T2DM in patients of the present study.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

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