Association of some cytokines with pancreatic β-cell dysfunction in diabetes mellitus

A Thesis Submitted for Partial Fulfillment of Master Degree in Pharmaceutical Sciences (Biochemistry)

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List of Abbreviations

AIR	Acute insulin response
BMI	Body mass index
cAMP	Cyclic AMP
CE	Cholesterol esterase
CO	Cholesterol oxidase
CXCL	Chemokine (C-X-C motif) ligand
DM	Diabetes mellitus
ELISA	Enzyme-linked immunosorbent assay
ER	Endoplasmic reticulum
FBG	Fasting blood glucose
FFA	Free fatty acids
GLP-1	Glucagon-like peptide-1
GSIS	Glucose-stimulated insulin secretion
HbA_{1c}	Glycated hemoglobin A_{1c}
HDL-C	High density lipoprotein- cholesterol
HepG2 cells	Human hepatocellular carcinoma cell line
HGP	Hepatic glucose production
HOMA	Homeostasis model assessment
HOMA2	Updated computer solved homeostasis model assessment
HPLC	High-performance liquid chromatography
HRP	Horseradish peroxidase
IAPP	Islet amyloid polypeptide
IFN-γ	Interferon-gamma
IGT	Impaired glucose tolerance
IL	Interleukin
IR	Insulin resistance
JNK	c-Jun N-terminal kinase
LDL-C	Low density lipoproteins- cholesterol
LPL	Lipoprotein lipase
MAPK	Mitogen activated protein kinase
MCP-1	Monocyte chemoattractant protein-1
NIDDM	Non-insulin-dependent diabetes mellitus
NIDE	National Institute of Diabetes and Endocrinology

NOD	Non-obese diabetic
OGTT	Oral glucose tolerance test
OHA	Oral hypoglycemic agent(s)
PANDER	Pancreatic-derived factor
PDX-1	Pancreatic and duodenal homeobox-1
PI	Proinsulin
PI/C-pep	Proinsulin/C-peptide
PI/I	Proinsulin/Insulin
ROS	Reactive oxygen species
T1DM	Type 1 diabetes mellitus
T2DM	Type 2 diabetes mellitus
TC	Total cholesterol
TG	Triglycerides
TNF-α	Tumor necrosis factor alpha
VLDL	Very low-density lipoprotein
WC	Waist circumference
4-PL	Four parameter logistic

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1. Introduction and Aim of the Work

Type 2 diabetes mellitus (T2DM) is a major worldwide health concern. Its prevalence is increasing dramatically worldwide with expectations to reach around 592 million by 2035 *(Guariguata et al., 2014)*. It is well known that T2DM is sustained by both insulin resistance (IR) and impaired insulin secretion.

Nowadays, impaired insulin secretion due to beta (β)-cell dysfunction is greatly recognized in the pathogenesis and progression of T2DM *(Russo et al., 2014)*. At any rate, whenever it appears, impaired β-cell function leads to the progressive failure of islet-cells to secrete sufficient amounts of insulin to overcome peripheral IR, ultimately resulting in failure to maintain normal glucose homeostasis over time. Therefore, it became strongly believed that β-cell dysfunction surpasses IR in inducing the onset and progression of T2DM *(Cerf, 2013)*, hence being regarded as the critical determinant for T2DM.

Several pathological processes were suggested to play a role in β-cell dysfunction including; glucotoxicity, lipotoxicity, amyloid deposition and inflammation ***(Goldberg, 2009; Van Raalte and Diamant, 2011; Westermark et al., 2011)***. Nowadays, there is an increasing evidence that pancreatic islet cells-secreted and produced cytokines are involved in the development and progression of β-cell dysfunction, apoptosis and eventually diabetes ***(Lee and Pervaiz, 2007)***.

Interestingly, a novel islet-secreted cytokine-like peptide, PANcreatic-DERived factor (PANDER), has been suggested to be implicated in β-cell dysfunction. Interestingly, it is assumed that PANDER is co-secreted with insulin in the β-cells in response to glucose via the same regulatory mechanisms ***(Xu et al., 2005; Yang et al., 2005)***.

Of interest, PANDER was discovered to play important role in β-cells. Under physiological conditions, PANDER is involved in the regulation of β-cell function by regulating the normal insulin secretory process and controlling glucose homeostasis ***(Wilson et al., 2011)***. On the other hand, excessively produced PANDER in islet-cells, under inflammatory and hyperglycemic conditions, can exert a negative impact on β-cell function ***(Wang et al., 2012)***, as it was found to promote apoptosis of both human and rodents β-cells in dose- and time-dependent manner ***(Cao et al., 2003)***. In addition, *in vivo* studies showed that PANDER expression is markedly increased in diabetic mice islets when compared with those of control mice ***(Wang et al., 2012)***.

Therefore, it could be suggested that PANDER may play a vital role in inducing the onset and/or the progression of T2DM under pathological conditions, through negatively influencing pancreatic β-cell function ***(Wang et al., 2012)***. However, to date, the circulating levels of PANDER and how these levels can be used to reflect the β-cell status in T2DM haven't been widely elucidated yet.

Another interesting cytokine suggested to be implicated in the process of β-cell dysfunction is the well-known Monocyte Chemoattractant Protein-1 (MCP-1), which is established to be increased in states of low-grade inflammation as obesity, atherosclerosis, IR and T2DM ***(Harsimran et al., 2009; El Mesallamy et al., 2012)***.

Similar to PANDER, MCP-1 was suggested to play important role in β-cells. Interestingly, it was discovered that pancreatic β-cells normally express and secrete MCP-1 ***(Piemonti et al., 2002)***. On the other hand, under inflammatory conditions, it was discovered that the failure of transplanted islets to function properly is attributed to increased MCP-1 expression and secretion in β-cells ***(Sell and Eckel, 2007)***, thus predicting the outcome of islet transplantation ***(Cardozo et al., 2003)***. Therefore, it is expected that MCP-1 may be associated with β-cell dysfunction in T2DM, although this potential association hasn't been validated in T2DM patients yet.

Interestingly, a previous study proposed that MCP-1 may indirectly contribute to β-cell dysfunction by upregulating PANDER gene expression in β-cell line MIN6 and pancreatic islets, under inflammatory conditions ***(Hou et al., 2011)***. However, to date, the clinical association between PANDER and MCP-1 together and individually in relation to β-cell dysfunction in T2DM patients hasn't been investigated yet.

Thus, the aim of this study was to:

1- Determine the serum levels of PANDER and MCP-1 in T2DM patients with different degrees of β-cell dysfunction, as two interesting cytokines being suggested to play a role in β-cell deficit.

2- Study the correlation between each of PANDER and MCP-1 with other biochemical/metabolic parameters and β-cell assessment markers in an attempt to evaluate and understand how these cytokines might clinically reflect the β-cell status in diabetic patients.

3- Demonstrate the clinical association between both cytokines, as a potential novel pathogenic mechanism for β-cell dysfunction in T2DM.

2. Review of Literature

2.1 ***Diabetes Mellitus Prevalence and Statistics***

Diabetes Mellitus (DM) is the most common endocrine disorder representing one of the main threats to human health in the 21st century and is a leading cause of morbidity and mortality worldwide ***(Vetere et al., 2014)***. The World Health Organization (WHO) projects that DM will be the 7th leading cause of death in 2030 ***(WHO, 2015)***.

Nearly 350 million people worldwide currently suffer from DM. **This number is projected to increase to 592 million worldwide in 2035 and much of the anticipated increase will occur in the economically developing countries *(Guariguata et al., 2014)*. Concerning Egypt, the number of diabetic patients was found to be** 2,623,000 patients in the year 2000 and is predicted to reach 6,726,000 patients by the year 2030 as expected by the WHO ***(WHO, 2015)***.

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia, resulting from defects in insulin secretion, insulin action, or both. This chronic hyperglycemia is associated with long-term damage and failure of different organs including diabetic microangiopathy such as retinopathy, nephropathy and neuropathy, as well as an increased in risk of cardiovascular diseases ***(Rossi, 2010; Seshasai et al., 2011)***. Consequently, a substantial reduction in life expectancy, decreased quality of life as well as increased costs of care are usually expected in diabetic patients.

2.2 ***Types of Diabetes Mellitus***

The vast majority of cases of diabetes fall into two broad etiopathogenetic categories; Type 1 DM (T1DM); previously known as juvenile or childhood-onset or insulin-dependent diabetes mellitus (IDDM), and the other more prevalent category is T2DM; formerly

called adult-onset or non-insulin-dependent diabetes mellitus (NIDDM) ***(TA, 2014)***.

Of all diabetic patients, about ~5-10% only have T1DM, which results from absolute loss of insulin production, whereas ~90-95% of patients have T2DM which suffer from impaired insulin sensitivity as well as problems with insulin secretion ***(Vetere et al., 2014)***.

Several differences exist between T1DM and T2DM, as shown in **Table 2.1** ***(Harvey and Ferrier, 2010)***, however, both types of disease share a common pathogenic center; *the pancreatic β-cell*, which is considered the focal point in both T1DM and T2DM, because of its capacity to produce and secrete insulin ***(Cernea and Dobreanu, 2013)***.

Table 2.1: Differences between T1DM and T2DM (Harvey and Ferrier, 2010)

	Type 1 Diabetes	Type 2 Diabetes
AGE OF ONSET	Usually during childhood or puberty; symptoms develop rapidly	Frequently after age 35; symptoms develop gradually
NUTRITIONAL STATUS AT TIME OF DISEASE ONSET	Frequently undernourished	Obesity usually present
PREVALENCE	900,000 = 10% of diagnosed diabetics	10 Million = 90% of diagnosed diabetics
GENETIC PREDISPOSITION	Moderate	Very strong
DEFECT OR DEFICIENCY	β Cells are destroyed, eliminating production of insulin	Insulin resistance combined with inability of β cells to produce appropriate quantities of insulin
FREQUENCY OF KETOSIS	Common	Rare
PLASMA INSULIN	Low to absent	High early in disease; low in disease of long duration
ACUTE COMPLICATIONS	Ketoacidosis	Hyperosmolar coma
TREATMENT WITH ORAL HYPOGLYCEMIC DRUGS	Unresponsive	Responsive
TREATMENT	Insulin is always necessary	Diet, exercise, oral hypoglycemic drugs, +/– insulin

2.3 ***Beta-cells and Diabetes***

Beta-cells constitute about 70-80% of pancreatic islet-cells. Normally, upon β-cell exposure to various stimuli including: [glucose, amino-acids, free-fatty acids (FFAs), gastrointestinal hormones and neural stimuli], stored proinsulin (PI) in granules is rapidly cleaved into insulin and C-peptide, which are released into the circulation ***(In't Veld and Marichal, 2010)***.

Interestingly, it was found that the pancreatic β-cell function and mass are decreased from the clinical onset of both types of DM accompanied by a correspondent deterioration of glycemic control ***(Weir et al., 2001)***. In both types of diabetes, β-cells are lost, though with different degrees and due to different causes ***(Wajchenberg, 2007)***.

In T1DM, the phenomenon is more severe and is mainly due to the autoimmune attack of auto-reactive T-cells against islet β-cells, where the immune system recognizes the β-cell as foreign body, probably owing to a combination of genetic and environmental factors ***(Bluestone et al., 2010)***. This ensuing autoimmune attack results in the destruction of the β-cell population, and patients are therefore completely dependent on exogenously administered insulin for survival ***(Vetere et al., 2014)***. It is assumed that about 70-90% of the β-cell mass in T1DM is lost at the time of clinical presentation, which is usually abrupt, with acute metabolic decompensation ***(Cernea and Dobreanu, 2013)***.

In T2DM, although the autoimmune destruction of β-cells does not occur, unlike T1DM ***(Bluestone et al., 2010)***, yet the pathogenesis remains to be highly complex. In fact, although it is well established that T2DM is characterized by IR and β-cell dysfunction, yet the significance of the latter in patients with T2DM has been often ignored. However, nowadays, the β-cell dysfunction is taking a higher priority in T2DM research since it is being regarded as the critical determinant for