"Study of Vitamin D and Thioredoxin Interacting Protein Levels in Diabetes Mellitus"

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

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بسم الله الرحمن الرحيم

" وقل رب زدني علما "

صدق الله العظيم

إهداء

إلى من مهدا لي طريق العلم بعد الله .....

إلى من ذللا لي الصعاب بدعواتهما الصالحة .....

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Contents

List of Abbreviations

$1,25(OH)_2D_3$	1α-25-dihydroxyvitamin D_3
$25(OH)D_3$	25-hydroxyvitamin D_3
AGEs	Advanced glycation end-product
ANOVA	Analysis of variance
ASK-1	Apoptosis signal-regulating kinase-1
ATP	Adenosine triphosphate
ChREBP	Carbohydrate-responsive element-binding protein
CRP	C-reactive protein
CYP	Cytochrome-P
DBP	Vitamin D binding protein
DM	Diabetes Mellitus
ELISA	Enzyme-linked immunosorbent assay
eNOS	Endothelial nitric oxide synthase
ER	Endoplasmic reticulum
FFA	Free fatty acid
FPG	Fasting plasma glucose
GCL	Glutamate-cysteine ligase
GLUT	Glucose transporter
GPx	Glutathione peroxidase
GR	Glutathione reductase
GSH	Reduced glutathione
GSIS	Glucose-stimulated insulin secretion
HbA_{1c}	Glycated hemoglobin
HDL-C	High density lipoprotein-cholesterol
HOMA2-IR	Homeostasis model assessment for insulin resistance
HOMA2-%β	Homeostasis model assessment for β-cell function
IAPP	Islet associated polypeptide
IDF	International diabetes federation
IKK	Inhibitor of kappa B kinase
IL	Interleukin
IFN-γ	Interferon gamma
iNOS	Inducible nitric oxide synthase
IR	Insulin receptor
IRS-1	Insulin receptor substrate-1
JAK	Janus kinase
JNK	Jun N-terminal kinase
LDL-C	Low density lipoprotein-cholesterol
MHC-1	Major histocompatibility complex-1
mRNA	Messenger Ribonucleic acid
mTOR	Mammalian target of rapamycin

NF-κB	Nuclear factor kappa B
nNOS	Neuronal nitric oxide synthase
NO	Nitric oxide
NO_2	Nitrogen dioxide
NOS	Nitric oxide synthase
NRF-1	Nuclear respiratory factor-1
$^{\bullet}O_2$	Superoxide radical
$^{\bullet}OH$	Hydroxyl radical
OHA	Oral hypoglycemic agent
$ONOO^-$	Peroxynitrite ion
PDK-1	Phosphoinositide-dependent protein kinase-1
PI	Proinsulin
PI/C	Proinsulin/C-peptide
PI/I	Proinsulin/insulin
PI3-K	Phosphatidylinositol-3-kinase
PKB/Akt	Protein kinase B
PKC	Protein kinase C
POD	Peroxidase
PPAR	Peroxisome proliferator-activated receptor
PGC-1α	Peroxisome proliferator-activated receptor gamma coactivator -1-alpha
RNS	Reactive nitrogen species
$^{\bullet}RO_2$	Peroxyl radical
RONOO	Alkyl peroxynitrite
ROS	Reactive oxygen species
SEM	Standard error of mean
SOD	Superoxide dismutase
T1DM	Type 1 diabetes mellitus
T2DM	Type 2 diabetes mellitus
TC	Total cholesterol
TG	Triglyceride
TGF-β	Transforming growth factor beta
TNF-α	Tumor necrosis factor-alpha
T_{reg}	Regulatory T-cell
Trx	Thioredoxin
TXNIP	Thioredoxin interacting protein
UCP-2	Uncoupling protein-2
VDR	Vitamin D receptor
VDREs	Vitamin D receptor elements
vit-D	Vitamin D
VLDL-C	Very low density lipoprotein-cholesterol

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Introduction and Aim of the Work

Diabetes mellitus (DM) is a growing public health issue characterized by either complete deficiency of insulin secretion by β-cells as in type 1 DM (T1DM), or peripheral insulin resistance and decompensation of the pancreatic β-cells that can no longer keep up with the increased insulin requirements as in type 2 DM (T2DM), resulting in hyperglycemia ***(Chiu et al., 2004; Wild et al., 2004).*** These elevated glucose levels have detrimental effects on various tissues including the pancreatic β-cell. β-cell glucotoxicity leads to progressive β-cell dysfunction, impaired insulin gene transcription, and irreversible β-cell loss by apoptosis, resulting in a vicious cycle with worsening hyperglycemia ***(Chen et al., 2008).***

Interestingly, several studies have shown an inverse correlation between basal serum vitamin D (vit-D) levels and glucose metabolism indices. One of these was in Finland, a low sunlight country, where it was shown that low serum vit-D level is associated with impaired glucose and insulin metabolism ***(Hurskainen et al., 2012)***. Another study showed that serum active vit-D is inversely correlated with glycated hemoglobin (HbA_{1C} %) ***(Hutchinson et al., 2011)***. Moreover, vit-D was proven to be closely related and one of the influential factors of islet β-cell function ***(Guo et al., 2013)***.

Moreover, It was shown that vit-D administration to type 2 diabetic patients showed improvements in serum fasting blood glucose and insulin levels, indicating that vit-D supplement may reduce insulin resistance ***(Talaei et al., 2013)***. In addition, a previous study suggested that vit-D might affect basic functions of pancreatic β-cells, with the potential to render them more resistant to the detrimental conditions encountered during T1DM and T2DM ***(Wolden-Kirk et al., 2013)***. Interestingly, an oxidant protein, thioredoxin-interacting protein (TXNIP), also called vit-D-upregulated protein was found to be highly stimulated by vit-D administration ***(Masutani et al., 2012)***.

Recently, TXNIP was identified as a highly glucose regulated pro-apoptotic factor in β-cells, suggesting that it may represent a potential mediator of β-cell glucotoxicity ***(Chen et al., 2008)***. The TXNIP binds to and inhibits thioredoxin, an antioxidant, and thereby can modulate the cellular redox state and promote oxidative stress ***(Hwang et al., 2014)***. In

addition, TXNIP has been shown to exert anti-proliferative effects by inducing cell-cycle arrest at the G0/G1 phase ***(Shalev, 2014)***.

Moreover, it was demonstrated that glucose stimulates TXNIP transcription through a carbohydrate response element in the TXNIP promoter, resulting in elevated TXNIP messenger ribonucleic acid (mRNA) expression. Interestingly, this TXNIP over expression induced apoptosis in pancreatic β-cells ***(Minn et al., 2005)***. Together, these findings raised the possibility that TXNIP might play a role in the glucotoxic β-cell death associated with DM.

As shown previously, that in contrast to the detrimental effects of TXNIP in DM, there is an inverse association between vit-D status and risk of DM due to its role in insulin resistance and β-cell function ***(Sung et al., 2012)***. Therefore, we assumed that determination of serum level of vit-D and its association with a well-established DM closely related player as TXNIP would be very useful in illuminating the role played by vit-D in pathogenesis of DM. This attracts more interest with the observed discrepancy between vit-D and TXNIP actions on DM parameters, although both of them follows same axis, TXNIP being a direct target of vit-D.

Moreover, there is a recent public ever-going interest and awareness of vit-D administration to diabetic patient. This inspired us to study the exact association between vit-D and DM.

Accordingly, this study aimed to:

1- Investigate the serum levels of 25-hydroxy vit-D and TXNIP in both type1 and type 2 DM patients.

2- Study the association between both these parameters and parameters of β-cell dysfunction and insulin resistance in a trial to undertand how these markers might interact together in pathogenesis of DM.

Literature Review

1. Diabetes Mellitus

Diabetes mellitus is one of the most common chronic diseases of the 21st century. Globally, more than 415 million adults recognized to have DM in 2015 compared to 108 million in 1980 ***(IDF, 2015)***. The global prevalence of DM was raised from 4.7% in 1980 to 8.5% in 2014 and that is meaning nearly duplication during 35 years. This raise was recognized to be faster in low- and middle-income countries than in high-income countries ***(Roglic and World Health Organization, 2016)***.

Unfortunately, Egypt occupies eighth position in top ten countries for number of adults with DM. Prevalence of diabetic patients in Egypt reached 14.9% (7.8 million patients) in the year 2015 and suspected to be increased to 15.1 million in the year 2040 ***(IDF, 2015)***.

It is well known that DM is caused either due to decreased insulin production due to autoimmune destruction of pancreatic β-cell as in T1DM ***(Foulis, 1987)***, or due to insulin resistance and subsequent declining β-cell function in T2DM ***(Nesher et al., 1987)***.

1.1. Type1 and Type2 diabetes mellitus:

Type 1 diabetes mellitus is characterized by autoimmune destruction of insulin-producing pancreatic β-cell ***(Atkinson and Maclaren, 1994)***. It occurs in genetically susceptible persons after triggering by one or more environmental factors, such as viral infection. This destruction progresses for many months or years. The patient remains asymptomatic until enough functional β-cells are lost, then hyperglycemia occurs, **table (1)**, ***(Pietropaolo and Le Roith, 2001; Atkinson, 2012)***.

On the other hand, T2DM is a progressive disease caused due to interactions between genetic and environmental factors which are responsible for onset of the disease. These interactions adversely affect tissue responses to insulin (insulin sensitivity) and insulin secretion (pancreatic β-cell function). The most important environmental factor is obesity (especially visceral obesity) ***(Kohei, 2010)***.

Table (1): *Difference between T1DM and T2DM* ***(Pietropaolo and Le Roith, 2001; Atkinson, 2012)***.

Type 1 Diabetes	Type 2 Diabetes
Occurs at any age but specifically in childhood.	Usually affects those over 30 years old.
Immunity origin leads to β-cell destruction and no insulin or very low insulin production	Body cell resist insulin → Insulin overproduction with no effect on blood glucose → Pancreas exhaustion → Less insulin level
Associated with weight loss	Mainly in obese individuals
Ketonuria and acidosis are common in these patients	Ketonuria and acidosis are rare in these patients
Managed with external insulin injection	Managed by diet, hypoglycemic drugs and sometimes insulin.

Nowadays, it is obvious that both types of diabetes are associated with decreased β-cell mass, although through different etiologies ***(Saisho, 2015; Inaishi et al., 2016)***.

2. β-cell mass and function in diabetes

2.1. β-cell dysfunction in diabetes

In both types of DM, marked reduction in β-cell mass occurs. Autoimmunity is the cause in T1DM while inadequate mass and function are the main causes in T2DM that lead to inability to compensate insulin resistance ***(Saisho, 2015)***. This mass reduction is due to inadequate replication or neogenesis and/or increased cell death ***(Rahier et al., 2008)***.

β-cell mass is affected directly by glucose level as chronic hypoglycemia leads to its atrophy while β-cell hypertrophy and hyperplasia were shown in chronic hyperglycemia ***(Cerf, 2013; Weir and Bonner-Weir, 2013)***. Actually, partial pancreactomy in rats leads to increased β-cell mass by 85% ***(Jonas et al., 1999)***.

Although, T2DM is due to insulin resistance, however, not all patients with insulin resistance will show frank DM. Only those with genetic susceptibility will develop hyperglycemia and DM as shown in **figure (1)**. All theses genetic susceptibilities are related to progressive β-cell dysfunction ***(Mota, 2013)***.

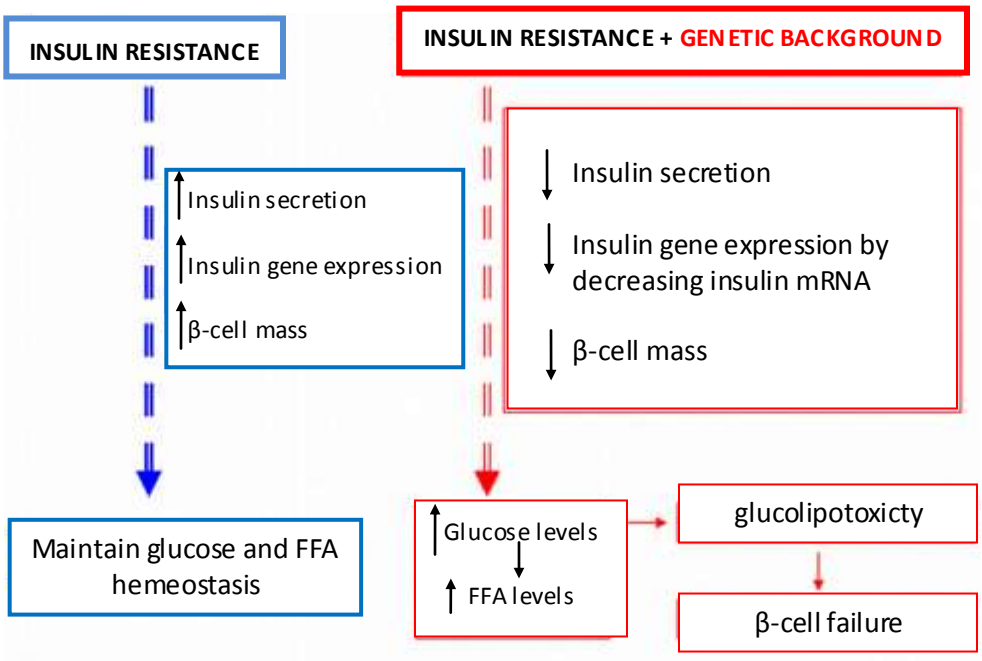

Figure (1): *Progression of T2DM from insulin resistance into β-cell failure.*

Genetic predisposition firstly initiate β-cell defect, whereas the subsequent β-cell failure may be a consequence of concomitant environmental conditions ***(Mota, 2013)***.

2.2. Stages of β-cell dysfunction

Actually, β-cell destructions usually occurs through different stages of β-cell characteristics including mass, phenotype or function. ***Weir and Bonner-Weir (2004)***, made a very nice review about these stages of β -cell dysfunction from insulin resistance until progression to severe β-cell

dysfunction. For use of this valuable information, we summarized these stages in **table (2)**.

Table (2): *Summarization of the five stages of β-cell dysfunction during progression of diabetes* ***(Weir and Bonner-Weir, 2004)***.

Stage 1	Compensation	• Insulin resistance • High fasting blood glucose level • Increased insulin secretion • Increased β-cell mass • β-cell phenotype is kept intact
Stage 2	Stable adaptation	• Fasting blood glucose level 89-130mg/dL • Loss of acute glucose-stimulated insulin secretion (GSIS). • β-cell change either in differentiation or function
Stage 3	Early unstable decompensation	• Fasting blood glucose level 285-350 mg/dL. • Less efficient insulin secretion • Critical decline of β-cell mass and/or insulin resistance. • Unstable due to it may be progressed to stage 4 or remission occurred and fall back to stage 2
Stage 4	Stable decompensation	• Less efficient insulin secretion • 50% reduction in β-cell mass • β-cell dedifferentiation is more severe at higher glucose levels
Stage 5	Severe decompensation	• Blood glucose > 350mg/dL • Last stage of diabetes • Severe marked loss of β-cells • Patients become ketotic and depend on external insulin for survival

In diabetic β-cells, progression from stage 2 (stable adaptation) to stage 4 (stable decompensation) through an unstable transient stage 3 of decompensation may take short time as in T1DM or many years as in T2DM. Autoimmunity and imbalance between regulatory T-cell (T_{regs}), in T1DM, or inflammatory responses associated with proinflamatory cytokines as C-reactive protein (CRP), tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) , in T2DM, might push β-cell through stage 3 to stage 4 and these marked reduction in β-cell mass and differentiation lead to less efficient insulin secretion as shown in **figure (2)** ***(Pietropaolo et al., 2007)***.

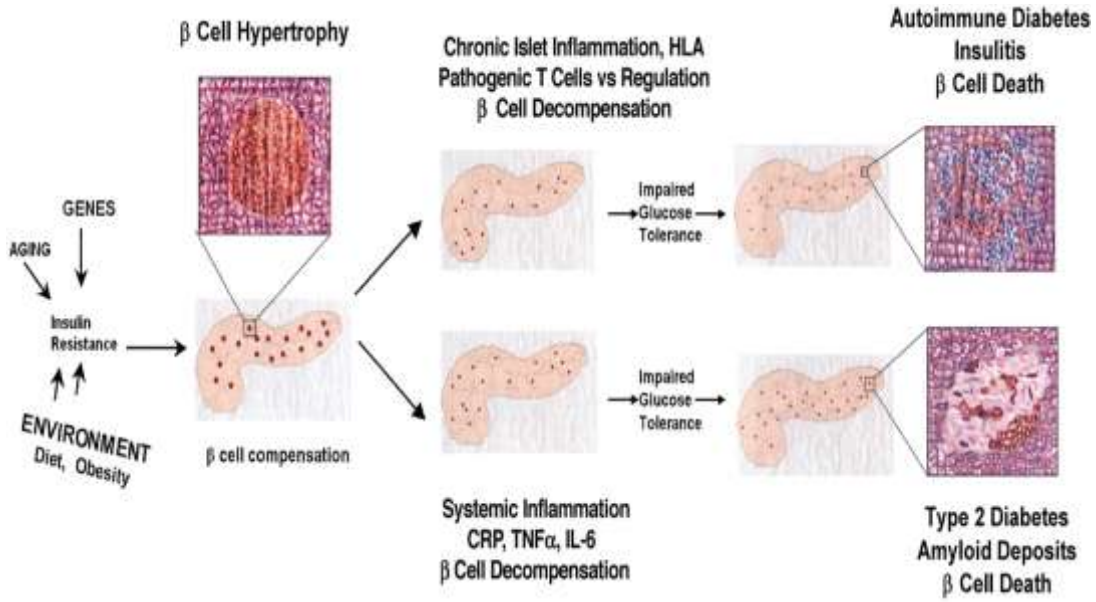

Figure (2): *Stages of β-cell failure in DM*

Firstly, multiple factors lead to insulin resistance that may lead to hyperglycemia. Secondly, β-cell tries to compensate by increased mass and insulin production. However, due to over stress, β-cell will be unable to compensate, apoptosis occurred and severe hyperglycemia persist ***(Pietropaolo et al., 2007)***.

2.3. β-cell dysfunction mechanisms in diabetes

Various mechanisms can lead to defects in the functional β-cell mass. This loss plays an important role in diabetes pathogenesis ***(Path and Seufert, 2003)***. These mechanisms are illustrated in **figure (3).**