Study of Adiponectin and E-selectin in Type 2 Diabetic patients with Coronary Heart Disease(s)"

Thesis
Submitted for the partial fulfillment of Master Degree in
Pharmaceutical Sciences (Biochemistry)

By

Sherine Mahmoud Ibrahim

Demonstrator of Biochemistry, Faculty of Pharmacy,
Modern Sciences and Arts (MSA) University

Under Supervision of

Prof.Dr. Hala Osman ElMesallamy

Professor of Biochemistry
Head of Biochemistry Department,

Faculty of Pharmacy, Ain Shams University

Prof.Dr. Tarek Mahros Salman

Professor of Biochemistry
Head of Biochemistry Department,

Faculty of Pharmacy, Al-Azhar University

Dr. Nadia M. Hamdy El-Hefny

Lecturer of Biochemistry,Biochemistry Department,

Faculty of Pharmacy, Ain Shams University

Biochemistry Department,Faculty of Pharmacy,

Ain Shams University

2011

Acknowledgement

First and foremost, thanks to ***"Allah"*** who *provides me, with his unlimited generosity, the medical knowledge and for granting me the power to accomplish this work. Which I hope to be a benefit to people.*

I would like to express my endless gratitude and appreciation to ***Prof. Dr. Hazem Amin EL Zeniny*** *Professor of Obstetrics and Gynecology, Ain-Shams University for giving me the honor of working under his supervision and providing me with a lot encouragement and support.*

My deep thanks are to ***Dr. Mohammad Abdel-Hameed Abdel-Hafeez*** *lecturer of Obstetrics and Gynecology, Ain-Shams University. For his generous assistance and valuable guidance and unfailing efforts during the whole period of study.*

Finally, I can't fully express by words my deep gratitude to my family, to whom I dedicate this work.

Noha Mahmoud

Aim of the work

To compare the safety, tolerability, efficacy and hematological responses to intramuscular iron as (iron dextran) and oral iron (Biotron amino acid chelate) in the treatment of iron deficiency anemia in pregnacy.

INTRODUCTION AND AIM OF THE WORK

Type 2 diabetes mellitus (D.M) is an epidemic disease, increasing exponentially around the world particularly in developing countries ***(Seshasai et al., 2011)***.Diabetes mellitus increases the incidence of coronary heart disease (CHD), being the most common and clinically important complication in D.M***(Stirban and Tschoepe, 2008)***.

It's now widely accepted that obesity is associated with many metabolic disorders including type 2 D.M ***(Alhusseini et al., 2010)***particularly in children and adults ***(Jung et al., 2009)***, asbeing overweight in early life significantly increasesthe risk for and severity of obesity in adulthood. Moreover, the current obesity epidemic implies that obesity is becoming an increasingly important risk factor for CHD and type 2 D.M ***(Qasim et al., 2008)***.

Cardiovascular risk factors implying dyslipidaemia, obesity, insulin resistance (I.R), and hyperinsulinaemia create a state of constant and progressive damage to the vascular wall, manifested by a low-grade inflammatory process ***(El-Mesallamy et al., 2007)***and endothelial dysfunction***(Rochaand Libby, 2009)***. The realization that the adipose tissue acts as an endocrine gland affecting whole-body energy homeostasis was a major breakthrough toward a better molecular understanding of type 2 D.M ***(Wang and Nakayama, 2010).***Growing evidence implicates adipocyte-derived factors (adipokines) as major regulators of I.R ***(El-Mesallamy et al., 2010)***.Several inflammatory markers have been identified in the atherosclerotic lesions; among them are cytokines, including interleukin-1β (IL-1β) andmonocyte chemotactic protein-1 (MCP-1) ***(Reilly et al, 2007)***.Pro-inflammatory cytokines and acute phase reactants are involved in multiple metabolic pathways relevant to I.R, including reactive oxygen species (ROS) and adipocyte function***(Houstiset al,***

2006).More recently, the adipose tissue is being regarded as a source of pro-inflammatory mediators which contribute to vascular injury, I.R, and atherogenesis ***(Attie and Scherer, 2009).***However, some of them have protective role against vascular inflammation and I.R, namely: adiponectin and the nitric oxide (NO) ***(Hopkins et al, 2007).***

Moreover, low-grade inflammatory state characterizing type 2 D.M may be the primary trigger of macrophages and endothelial cells (ECs)activation ***(Dandona et al., 2005)*** mediated through enhanced lipid peroxidation ***(Thornalley et al., 2007)***.Where the adhesion molecule; soluble endothelial-selectin (E-selectin)levels, as an index of endothelial dysfunction, have been shownto be increased during acute coronary syndromes and systemicinflammation***(Albert et al., 2008)***.Since it is well known that type 2 D.M subjects have multiple risk factors that potentiate each other, we limited our study to non-smoker adult males'and extended our study to explore the influence of childhood obesity in relation to markers ofglucose metabolism (glycated hemoglobin; (HbA_{1c}), insulin, and adiponectin) and/or endothelial damage (NO and sE-selectin).

Abbreviation	Definition
ABC	Avidin-biotin-peroxidase complex
ADIPOQ	Adiponectin
AGEs	Advanced glycation end products
AMs	Adhesion molecules
AMI	Acute myocardial infarction
ANOVA	Analysis of variance
ATP	Adenosine triphosphate
BMI	Body mass index
CAD	Coronary artery disease
CAMs	Cellular adhesion molecules
CC chemokine	Cytosine-cytosine- chemokine
CCL2	chemokine (C-C motif) ligand 2
CHD	Coronary heart disease
CRP	C-reactive protein
CV	Cardiovascular
CVD	Cardiovascular disease
D.M	Diabetes mellitus
ECs	Endothelial cells
EDTA	Ethelene diamine tetra acetic acid
ELISA	Enzyme linked immunosorbant assay
eNOS	Endothelial nitric oxide synthetase
E-selectin	Endothelial selectin
ET-1	Endothelin-1
FBG	Fasting blood glucose
GOD	Glucose oxidase
Hb	Hemoglobin
HbA_0	Non-glycosylated hemoglobin
HbA_{1c}	Glycated hemoglobin
HDL	High density lipoprotein

HDL-C	High density lipoprotein-cholesterol
HOMA- IR	Homeostasis assessment model assay for assessment of insulin resistance.
HRP	Horseradish peroxidase
hsCRP	High sensitivity C-reactive protein
HTN	Hypertension
ICAM-1	Intercellular adhesion molecule-1
IL	Interleukin
IL-1α	Interleukin-1 alpha
IL-1β	Interleukin-1 beta
I.R	Insulin resistance
LDL	Low density lipoprotein
LDL-C	Low density lipoprotein-cholesterol
LPL	Lipoprotien lipase
MAbs	Monoclonal antibodies
MCP-1	Monocyte chemoattractant protein-1
MDA	Malondialdehyde
$MgCl_2$	Magnesium chloride
MI	Myocardial infarction
NADPH	Nicotinamide adenine dinucleotidase phosphate.
NED	N-1-napthylethylenediamine
NF-κB	Nuclear factor kappaB
NIDDM	Non insulin dependent diabetes mellitus
NO	Nitric oxide
OS	Oxidative stress
Ox-LDL	Oxidized LDL
POD	Peroxidase
sE-selectin	Soluble endothelial selectin
ROS	Reactive oxygen species
S.D	Standard deviation

SMC	Smooth muscle cell
SPSS	Statistical package for the social sciences
TAG	Triacylglycerol
TBA	Thiobarbituric acid
TBS	Tris buffer solution
TC	Total cholesterol
TMB	3, 3\ - 5, 5\ tetra methyl benzidine
TNF-α	Tumor necrosis factor-alpha
VAT	Visceral adipose tissue
VCAM-1	Vascular cellular adhesion molecule-1
VLDL	Very low density lipoproteins

Contents

REVIEW OF LITERATURE

Type 2 Diabetes Mellitus (Type 2 D.M):

The more common form of diabetes, type 2 (non-insulin dependent D.M; NIDDM), sometimes called age-onset or adult-onset diabetes, recently type 2 D.M was regarded as a disease that typicallyaffected the middle-aged and elderly, even youth and adolescents are now part ofthe diabetes epidemic, this form of diabetes occurs most often in people who are overweight and who do not exercise ***(Patel et al., 2008)***. The prevalence of D.M in Egyptians less than 79 years of age is about 11.4% ***(International Diabetes Federation, 2011)***.

In the early stage of type 2 D.M, the predominant abnormality is reduced insulin sensitivity. At this stage, hyperglycemia can be reversed by a variety of measures and medications that improve insulin sensitivity or reduce glucose production by the liver ***(Risérus et al., 2009)***. Diabetes is a growing concern. Its incidence is increasing rapidly and ispredicted to increase further, inparallel with the trends observed for obesity ***(Centers for disease control and prevention, 2008).***

Although type 2 D.M is widely diagnosed in adults, its frequency has markedly increased in the pediatric age group over the past two decades, coinciding with the increasing prevalence of obesity among children, the incidence of type 2 D.M in children and adolescents has markedly increased to the point that it accounts for as many as one third of all the new cases of diabetes diagnosed in adolescents ***(Dabelea et al., 2007)***.

Obesity as predisposing factor for Type 2 D.M:

Obesity is becoming a global epidemic. Around 1.1 billion adults and 10% of the world's youth are currently overweight or considered obese***(Susanet al., 2011)***.

Being overweight in early life significantly increases the risk for and severity of obesity in adulthood ***(Dietz and Robinson, 2005)***. Overweight and the related metabolic syndrome are dramatically increasing problems, particularly in children and adolescents***(Ogden et al., 2006).***Because childhood obesity often persists into adulthood and is associated with numerous chronic illnesses, children who are obese are often tested for hypertension (HTN), D.M, hyperlipidemia, and fatty liver ***(Flynn et al., 2006).***

In the past, the adipose tissue was regarded as a mere fat deposition. Now it is seen from a totally different standpoint, as an active endocrine and paracrine organ that produces several inflammatory cytokines, such as the adipokines ***(Lau et al., 2005)***. In obesity, adipocyte hypertrophy leads to many changes in adipocyte function and production of anti- and pro-inflammatory cytokines ***(Woestijne et al., 2011)***.

The overweight associated to fat accumulation in mesenteric region refers to a central, visceral or androgenic obesity. The so-called visceral obesity is well-known as associated to a higher mortality than the peripheral obesity ***(Zimmet and Albert, 2008)***. The cause of this difference lies in the fact that the visceral adipose tissue (VAT) is more metabolically active than the subcutaneous adipose tissue, causing for instance, a higher glucose production consequently type 2 D.M and hyperinsulinism ***(Sugerman, 2005)***. These conditions

characterize metabolic syndrome, considering currently a challenge for public health.Moreover, it represents a substantial increase in the risk of D.M as well as of cardiovascular disease (CVD) as shown in Figure (1) ***(Zimmet and Albert, 2008)***.

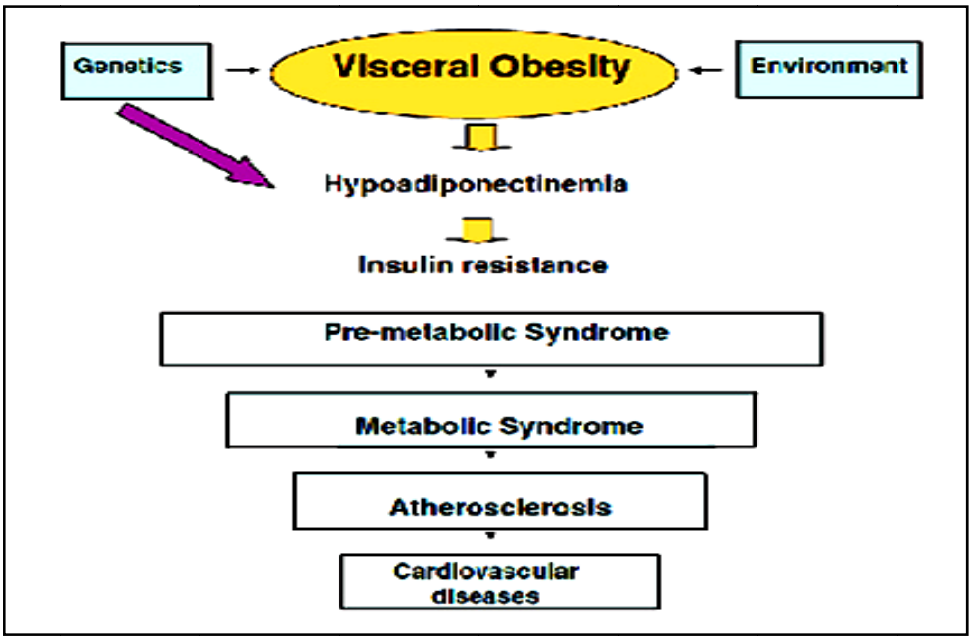

Figure (1): Role of visceral obesity in the pathogenesis of MS *(Zimmet and Albert, 2008).*

Currently, an estimated onethirdof the world's population is at risk for CVD, with an estimated one billion of those due to being overweight or obese ***(WHO, 2007).***

Generally associated with classic risk factors for CVD such as arterial high blood pressure, D.M, dyslipidemias, and MS which is known for very long time now. However, more recent findings reveal that, even after the control of these associated diseases, the risk ofcardiovascular (CV) events remains high, which makes obesity to be considered an independent CV risk factor ***(Haslam and James, 2005)***.

Early onset type 2 D.M appears to be a more aggressive disease. Recently reported youngersubjects with type 2 D.M are obese and severely insulin resistant with markedly abnormal CV risk markers, similar to the findings in patients 30 years older ***(Mcquaid et al., 2005).***

Diabetic Complications:

Premature coronary artery disease (CAD) and cardiac failure are vastly overrepresented in the diabetic population, with significant morbidity and mortality. In fact, the rate of cardiac events in patients with diabetes is equivalent to non-diabetic patients with a previous myocardial infarction (MI) ***(Connelly et al., 2011).***

Factors Promoting Diabetic Complication:

The Cardiovascular risk factors HTN, dyslipidaemia, obesity, insulin resistance (I.R), hyperinsulinaemia, and impaired fibrinolysis cluster in the metabolic syndrome. Allof the above-mentioned factors create a state of constantand progressive damage to the vascular wall, manifestedby a low-grade inflammatory process and endothelial dysfunction ***(Capewell, et al., 2010)***.

The so-called visceral obesity is well-known as associated to a higher mortality than the peripheral obesity. The cause of this difference lies in the fact that the VAT is more metabolically active than the subcutaneous adipose tissue causing for instance, a higher glucose production and consequently type 2 D.M and hyperinsulinism. This higher insulin secretion causes sodium retention, resulting in systemic arterial high blood pressure ***(Sugerman, 2005).***

Diabetic Vascular Dysfunction:

The pathogenesis of endothelial dysfunction in type 2 D.M is multifactorial, with principal contributors being oxidative stress (OS), dyslipidaemia, and hyperglycaemia. Endothelial dysfunction and increased arterial stiffness occur early in the pathogenesis of diabetic vasculopathy. They are both powerful independent predictors of CV risk.Endothelial dysfunction primarily reflects decreased availability of NO; a critical endothelium-derived vasoactive factor with vasodilatory and anti-atherosclerotic properties (***Woodman et al., 2005)***.

Acute Myocardial Infarction (AMI):

Myocardial infarction or acute myocardial infarction (AMI), commonly due to occlusion (blockage) of a coronary artery following the rupture of a vulnerable atherosclerotic plaque, which is an unstable collection of lipids (fatty acids) and white blood cells (especially macrophages) in the wall of an artery ***(Mallinson, 2010)***.

Heart disease, particularly CHD is a major cause of morbidity and mortality among patients with D.M compared to non-diabetics; diabetics are more likely to have CHD or multivessel disease and to have episodes of silent ischemia. As a result of these and other factors, diabetics with CHD have a worse outcome and poorer long-term survival compared to non-diabetics with CHD ***(Sarwar et al., 2010)***.

Risk factors for atherosclerosis, are generally the risk factors for MI which are D.M, hypercholesterolemia, inflammation, thrombosis, and obesity as shown in Figure (2) ***(Biondi-Zoccai et al., 2003)***.

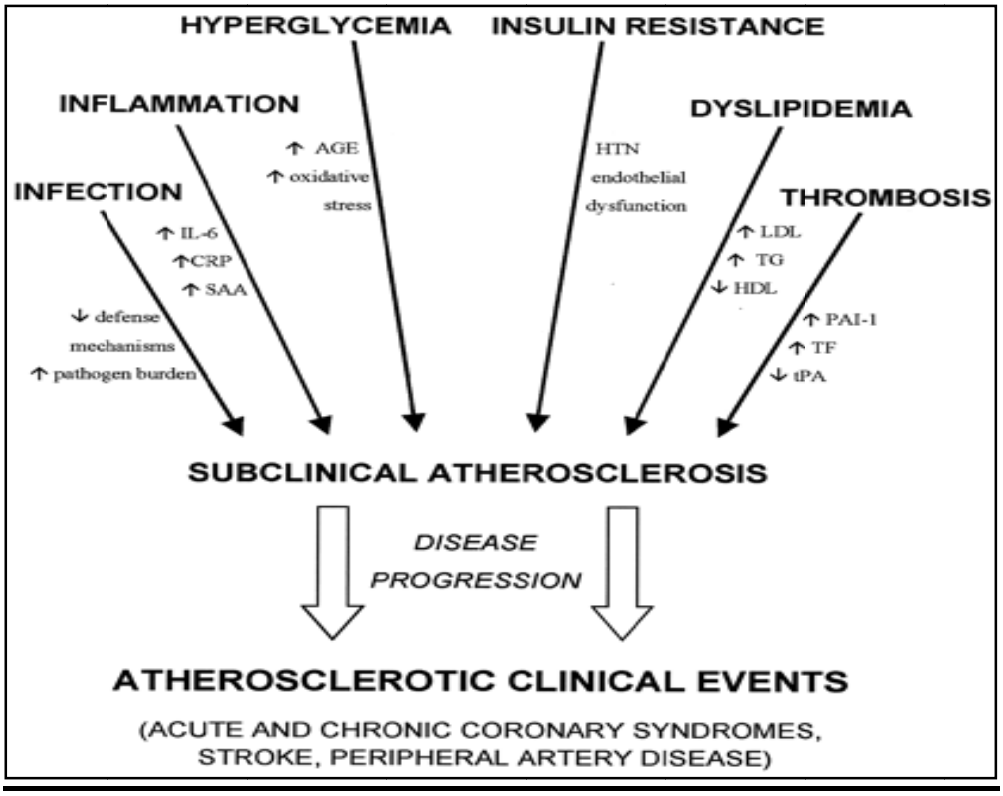

Figure (2): Risk factors involved in the initiation of subclinical atherosclerosis and progression to atherosclerotic clinical events in diabetic patients *(Biondi-Zoccai et al., 2003)*.

Reported prevalence of silent myocardial ischemia ranges from 10% to 60% in diabetic patients, possibly reflecting the differing numbers of atherogenic risk factors ***(Verhaert and Thomas, 2009)***.

Early onset type 2 D.M (diagnosed age 12-15 years) has been associated with more aggressive CVD than later-onset type 2 D.M, suggesting that CVD complications resulting from type 2 D.M diagnosed in youth may be even more unfavorable ***(Milicevic et al., 2008)***.

For many years, the physiopathology of arteriosclerosis was considered merely as an accumulation of lipids on the arterial wall. However, over the two