

Serum Visfatin as a Novel Marker of Type 2 Diabetes in Obese Patients

Protocol of Thesis

Submitted for the Partial Fulfillment of Master Degree
in Clinical and Chemical Pathology

By

Marwa Ahmed Ahmed Saleh
M.B., B.Ch., Mansoura University
Supervised by

Professor / Sawsan Said Hafez

*Professor of Clinical and Chemical Pathology
Faculty of Medicine, Ain Shams University*

Doctor / Eman Saleh El Hadidi

*Assistant Professor of Clinical and Chemical Pathology
Faculty of Medicine, Ain Shams University*

Doctor/ Rania Salah El Din Kamle Shahin

*Lecturer of Clinical and Chemical Pathology
Faculty of Medicine, Ain Shams University*

Faculty of Medicine
Ain Shams University
2012



Acknowledgement

*Above all and first of all; all thanks to **ALLAH**, the source of all knowledge, by whose abundant aid this work has come to fruition.*

*It has been a great honor to proceed into this work under the supervision of **Prof.Dr. Sawsan Said Hafez**, Professor of Clinical and Chemical Pathology, Faculty of Medicine, Ain shams University. I am greatly indebted to her for suggesting and planning the subject, supervising the whole work, reading and criticizing the manuscript. I will never forget her unlimited help, continuous support, kind encouragement, constructive criticism and wise guidance.*

*I would like also to express my sincere gratitude and appreciation to **Dr.Eman Saleh El Hadidi**, Assistant Professor of Clinical and Chemical Pathology, Faculty of Medicine, Ain shams University, who offered much of her time and advice for suggesting, reading and supervising throughout this work. To her words of praise are not sufficient.*

*I'm particularly very grateful to **Dr.Rania Salah El Din Kamle Shahin**, Lecturer of Clinical and Chemical Pathology, Faculty of Medicine, Ain Shams University, for her helpful guidance, valuable advice, meticulous care, great effort and generous help and support. She offered me much of her time and advice to accomplish this work.*

List of Contents

<u>Title</u>	<u>Page</u>
• LIST OF CONTENTS	i
• LIST OF ABBREVIATIONS	iii
• LIST OF TABLES	ix
• LIST OF FIGURES	x
• INTRODUCTION AND AIM OF THE WORK	1
• REVIEW OF LITERATURE	
I- OBESITY	
A- Definition	4
B- Epidemiology	4
C- Adipose tissue	5
D-Measurement of obesity	16
E-Causes of obesity	18
F-Complications of obesity	23
II-TYPE 2 DIABETES AND INSULIN RESISTANCE	
A-Type 2 DM	29
B-Insulin resistance	40
III-VISFATIN	
A-Discovery of visfatin	50

B-Structure of visfatin	50
C-Biochemical activities	52
D-Mode of action	52
E-Tissue and cellular distribution	52
F-Recombinant visfatin	54
G-Physiological effect of visfatin	54
H-Pathological effect of visfatin	55
I-Methods of assay	62
• SUBJECTS AND METHODS	69
• RESULTS	85
• DISCUSSION	95
• SUMMARY AND COCLUSION	100
• RECOMMENDATIONS	103
• REFERENCES	104
• ARABIC SUMMARY	

List of Abbreviations

ADA	American Diabetes Association
BMI	Body mass index
CE	Cholesterol esterase
DM	Diabetes mellitus
DNO	Diabetic non obese
DO	Diabetic obese
EIA	Enzyme immunoassay
ELISA	Enzyme linked immunosorbant assay
FBG	Fasting blood glucose
FBI	Fasting blood insulin
FPG	Fasting plasma glucose
GDM	Gestational diabetes mellitus
H²	Height Square
HbA1C	Hemoglobin A1C (Glycated hemoglobin)
HDL-C	High density lipoprotein cholesterol
HOMA-IR	Homeostatic model assessment of insulin resistance
HS	Highly significant
IDDM	Insulin dependent diabetes mellitus
IFG	Impaired fasting glucose
IGT	Impaired glucose tolerance
IR	Insulin resistance
LDL-C	Low density lipoprotein cholesterol
NAMPT	Nicotinamide 5- Phosphorybosil-1 pyrophosphate Transferase

NAPRTase	Nicotinic acid phosphoribosyl transferase
NDNO	Non diabetic non obese
NDO	Non diabetic obese
NIDDM	Non insulin dependent diabetes mellitus
NS	Non significant
PBEF	Pre-B Cell Enhancing Factor
PCR	Polymerase chain reaction
RIA	Radio immunoassay
RT-PCR	Reverse Transcriptase Polymerase Chain Reaction
S	Significant
SD	Standard deviation
TC	Total cholesterol
TG	Triglycerides
TNF-α	Tumour Necrosis Factor- α
Type 1 DM	Type 1 diabetes mellitus
Type 2 DM	Type 2 diabetes mellitus
VLDL	Very low density lipoprotein
W	Weight
WAT	White adipose tissue
WC	Waist circumference
WHO	World Health Organization
WHR	Waist Hip Ratio

List of Tables

<i>Table No.</i>	<i>Table Title</i>	<i>Page No.</i>
1	Risk factors for diabetes	33
2	The ADA recommendation for diabetes diagnosis and other high risk categories	36
3	Diagnosis of GDM	37
4	Classification of diabetic complications	39
5	Descriptive and comparative statistics of studied parameters in diabetic and non diabetic groups (Student Test for parametric data and Wilcoxon Rank Sum Test for non parametric data)	88
6	Descriptive and comparative statistics of studied parameters in diabetic obese and diabetic non obese groups (Student Test for parametric data and Wilcoxon's Rank Sum Test for non parametric data)	89
7	Descriptive and comparative statistics of studied parameters in diabetic obese and non diabetic obese groups (Student Test for parametric data and Wilcoxon's Rank Sum Test for non parametric data)	90
8	Descriptive and comparative statistics of studied parameters in non diabetic obese and non diabetic non obese groups (Student Test for parametric data and Wilcoxon's Rank Sum Test for non parametric data)	91
9	Descriptive and comparative statistics of studied parameters in diabetic non obese and non diabetic obese groups (Student Test for parametric data and Wilcoxon's Rank Sum Test for non parametric data)	92
10	Person correlation between visfatin and all measured parameters in diabetic obese group using Ranked Sperman Correlation Test	93

List of Figures

Figure No.	Figure Title	Page No.
1	Different adipokines secreted by adipose tissue	6
2	Factors influencing the development of obesity	23
3	Features of different disorders associated with obesity and insulin resistance	28
4	Pathophysiology of type 2 diabetes	34
5	Mechanism of insulin resistance	44
6	Structure of visfatin	51
7	Principle of sandwich Enzyme Linked Immunosorbant Assay	63
8	Polymerase chain reaction	65
9	Western blot method	68
10	Bar chart showing difference between all studied groups as regards median values of visfatin	94
11	Box-plot chart showing difference between study groups concerning serum visfatin level	94



INTRODUCTION



INTRODUCTION

The escalating international epidemic of obesity is now the most contributor to ill health (*Caballero, 2005*). It is a major risk factor for cardiovascular diseases, pulmonary diseases, metabolic diseases, osteoarticular diseases, different forms of cancer and serious psychiatric illness (*Samanic et al., 2006*).

Obesity is a rapidly growing disorder in industrialized and developing countries, that when weight is gained, hyperplasia and hypertrophy of adipose tissue are found (*Seeger et al., 2007*).

The metabolic complications of obesity consist of dyslipidemia, hypertension, premature heart disease, insulin resistance, that often ends in β cell failure, impaired glucose tolerance and type 2 diabetes (*Parati et al., 2007*).

Type 2 diabetes is a complex disorder that affects between 6% and 20% of the population in western industrialized societies (*Singh et al., 2004*).

The epidemic of type 2 diabetes continues to grow worldwide that 171 million individuals are currently affected and the number of cases may double by 2030 (*Wild et al., 2004*). The rising numbers of people with diabetes, caused by an increasing prevalence of obesity, may soon start to increase cardiovascular disease mortality (*Sicree et al., 2006*).

Adipose tissue represents an active endocrine organ that releases a large number of bioactive mediators (adipokines) that signal to organs of metabolic importance including brain, liver, skeletal muscles and the immune system thereby modulating hemostasis, blood pressure, lipid and glucose metabolism, inflammation and atherosclerosis. These adipokines include adiponectin, leptin, omentin, resistin, retinol binding protein, tumor necrosis factor- α , interleukin-6, vaspin, chemerin and visfatin (*Rabe et al., 2008*).

Visfatin was originally identified as a 52kd protein that is primarily expressed in liver, muscles and bone marrow as a growth factor for B lymphocytes precursor (thus its alternative name, Pre-B Colony Enhancing Factor) (*Fukuhara et al., 2005*).

Circulating visfatin levels are closely correlated with WAT (white adipose tissue) accumulation, and its mRNA levels increase in the course of adipocyte differentiation (*Jia et al., 2004*).

It has been reported that visfatin mimics actions of insulin by activating the insulin signal transduction pathway through binding to the same receptors. Therefore, it is implicated in the development of obesity-associated insulin resistance and diabetes mellitus (*Fukuhara et al., 2005*).

Recently visfatin concentrations are acutely regulated by glucose and insulin and elevated in patients with insulin resistance, obesity and diabetes (*Dominik et al., 2007*).



AIM OF THE WORK



AIM OF THE WORK

The aim of the present study was to evaluate serum visfatin as a marker for type 2 diabetes in obese patients.



Review Of Literature





Obesity

