GLYCATED LIPOPROTEINS AS A DIAGNOSTIC INDICATOR OF DIABETIC ATHEROGENESIS

Thesis

submitted for the partial fulfillment of
The Master Degree in Clinical and Chemical Pathology

By

MANAL MOHAMED ABD AL-AZIZ
(M.B., B.Ch.)

Under supervision of

PROF. DR. LAILA MOHAMED ABO EL-MAGD
Professor of Clinical Pathology

PROF. DR. IIANY SOBHI RUFAIL
Professor of Clinical Pathology

DR. NASHWA AHMED ADEL EL-BADAWY
Lecturer of Clinical Pathology

FACULTY OF MEDICINE
AIN SHAMS UNIVERSITY
1992

ACKNOWLEDGEMENT

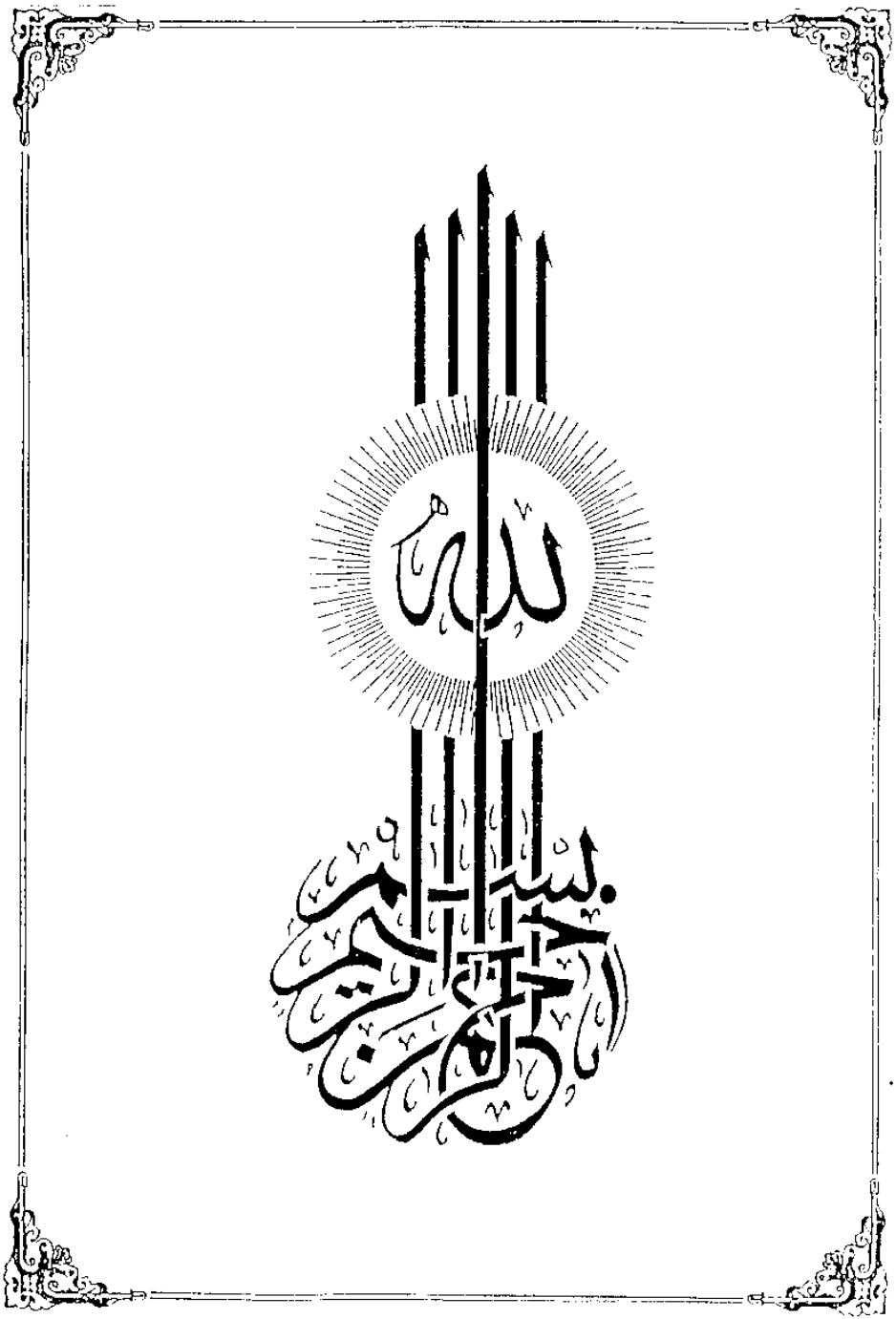
*I wish to express my deep appreciations and sincere gratitude to Prof. Dr. **LAILA MOHAMED ABO EL-MAGD**, Professor of Clinical Pathology, Ain Shams University, who offered much of her experience and support for providing me with adviceand suggestion.*

*I would like to thank Prof. Dr. **HANY SOBHI RUFAIL**, Professor of Clinical Pathology, Ain Shams University, for his supportive advice, generous help and revision of this work.*

*I am deeply indebted to Dr. **NASHWA AHMED ADEL EL-BADAWY**, Lecturer of Clinical Pathology, Ain Shams University, for her kind advice and guidance, her meticulous supervision and revision of this thesis and for her continuous and enthusiastic stimulation throughout the whole work.*

***My sincere** appreciation is to all the staff members of the **Department** of Clinical Pathology, Ain Shams University, for their interest and help.*

*Last but not least, no words could ever thank my evergiving **parents** and my **husband**, they are unforgettable.*



LIST OF ABBREVIATIONS

- ACAT : Acyl Co A cholesterol transferase.
- CHE : Cholesteryl esters.
- DMF : 1-deoxy-1-morpholino fructose.
- 2,3 DPG : 2,3 diphospho-glycerate.
- GBM : Glomerular basement membrane.
- Glc-beta : Glycated beta lipoproteins.
- Glc-LPs : Glycated lipoproteins.
- HDL : High density lipoproteins.
- HLA : Human leukocyte antigens.
- HMF : 5-hydroxy methyl furfuraldehyde.
- HMG Co A reductase : 5-hydroxy methyl glutaryl Co A reductase.
- ICA : Islet cell antibodies.
- IDDM : Insulin dependent diabetes mellitus.
- IDL : Intermediate density lipoproteins.
- LCAT : Lecithin-cholesterol acyl transferase.
- LDL : Low density lipoproteins.
- LPL : Lipoprotein lipase.
- NBT : Nitroblue tetrazolium.
- NIDDM : Non-insulin dependent diabetes mellitus.
- TBA : Thiobarbituric acid.
- TCA : Trichloroacetic acid.
- TG : Triglyceride.
- VLDL : Very low density lipoproteins.

CONTENTS

INTRODUCTION AND AIM OF THE WORK

INTRODUCTION AND AIM OF THE WORK

INTRODUCTION:

Non enzymatic glycation, i.e., the formation of a stable ketoamine adduct from glucose and free amino groups of proteins, was found to occur in the plasma of diabetic patients (Kennedy and Bayens, 1984).

Glycated hemoglobin and glycated albumin (fructosamine) are widely accepted as reliable indicators of metabolic control in diabetes mellitus (Johnson et al., 1982).

Glycation of lysine or arginine residues of apolipoprotein B, the major protein of low density lipoprotein (LDL) was found to inhibit the physiological ability of LDL to be metabolized by the LDL receptor pathway (Witztum et al., 1982).

The LDL receptor pathway (the receptor mediated endocytosis of LDL leads to an orderly regulated handling of internalized cholesterol and cellular cholesterol homeostasis) has been proposed to be antiatherogenic and interferance with it may lead to accelerated atherogenesis (Goldstein and Brown, 1977).

As glycation of LDL causes the various severe complications, for example, generalized vascular diseases (microangiopathy and atherosclerosis) in critical organs as the kidneys, the eyes and the nerves system. Thus measurement of glycated lipoproteins (glc LPs) in the serum may be diagnostically important in diabetes mellitus with hyperglycemia.

AIM OF THE WORK:

The aim ofthis work is to describe a simple method for determining glycated lipoproteins (Glc LPs) and to assess the importance of glycated lipoproteins in the serum as a diagnostic indicator of atherogenesis in diabetes mellitus.

REVIEW OF LITERATURE

DIABETES MELLITUS

Diabetes mellitus is one of the most common diseases that is seen in the physicians' offices. As a chronic disease, diabetes and its complications are the cause of major morbidity and martality.

Diabetes mellitus is a heterogeneous primary disorder of carbohydrate metabolism with multiple etiologic factors that generally involve absolute or relative insulin deficiency; or insulin resistance or both. All causes of diabetes ultimately lead to hyperglycemia, which is the hallmark of the disease syndrome (Olefsky, 1988).

Diabetic complications include: retinopathy which is the leading cause of blindness in young persons. Nephropathy which contributes to end-stage renal disease. In fact, approximately 25% of all renal transplants are performed in patients who have diabetic nephropathy. Two other complications, neuropathy and peripheral vascular disease, are the leading causes of non traumatic lower extremity amputation. The presence of diabetes also doubtes the risk for coronary disease. Finally, in pregnancy it increases the risk for congenital anomalies and fetal wastage as a result of spontaneous abortions and perinatal morbidity and mortality (Byron, 1991).

The currently accepted classification and criteria for the diagnosis of diabetes are based on the report of National Diabetes Data Group (1979) and are compared to the standard set forth by the World Health Organization (Olefsky, 1988).

Classification of diabetes according to the National Diabetes Data Group:

1) Primary Diabetes:

a) Insulin dependent or type I diabetes (IDDM):

It was formerly called juvenile onset, ketosis-prone or brittle diabetes. It is present in patients with little or no insulin secretory capacity. The treatment of associated symptomatology is entirely dependant on exogenous insulin therapy (Olefsky, 1988).

The common characteristics of this form are a sudden clinical onset, severe hyperglycemia and easy appearance of ketoacidosis. This clinical onset is usually abrupt but there is evidence of long subclinical prediabetic period in some individuals (Gorsuch et al., 1981). It is still not clear which precipitating factors aggravate and reveal insulin deficiency state.

b) Non insulin dependant, or type II diabetes (NIDDM):

It was formerly called adult onset, maturity onset, or non ketosis diabetes.

About 80% of type II diabetics are obese. In obesity, hyperinsulinemia, insulin resistance and reduced concentration of insulin receptor sites are well recognized features (Freychet, 1976). Olefsky (1981) suggested that hyperinsulinemia may be responsible for the reduced receptor site concentration in mild obesity due to insulin-induced receptor loss. In more severely insulin resistance obese subjects, the post receptor defect take prominence (Kolterman et al., 1980).

Not all diabetic patients exhibit receptor defects. However, when such defects exist they appear to be associated with insulin resistance which is manifested by the coexistence of hyperinsulinemia and hyperglycemia (Ginsberg et al., 1975).

2) Secondary Diabetes:

Secondary diabetes is associated with the followings:

a) Pancreatic disease (e.g., pancreatectomy, pancreatic insufficiency, hemochromatosis).

b) Excess counter insulin hormones (e.g Cushing's syndrome, acromegaly, pheochromocytoma).

c) Drug induced diabetes (e.g., thiazide diuretics, steroids, phenytoin).

d) diabetes associated with specific genetic syndromes (e.g., lipodystrophy, myotonic dystrophy and ataxia telangiectasia).

3) Impaired Glucose Tolerance:

Impaired glucose tolerance is a clinical state characterized by partial compensation of insulin resistance and associated with normal fasting plasma glucose values but postprandial hyperglycemia (Henning et al., 1990).

4) Gestational Diabetes:

Gestational diabetes means glucose intolerance that develops during pregnancy.

This classification divided primary diabetes according to insulin dependence. Nevertheless, the sole criterion of insulin dependence may be misleading as non insulin dependant subjects may be treated with insulin for various causes or may develop clear insulin dependence within few months oryears of diagnosis. To overcome these limits, other criteria are put into consideration as the detection of islet cell antibodies (ICA) and HLA typing. However, only a few diabetic clinics are able to perform genetic and immunological studies and the results may differ among different populations (National Diabetes Data Group, 1979).

Pathogenesis of type I diabetes mellitus:

[1] The genetic factor:

The main data of genetic factors involved in type I diabetes are derived from studies of human leucocyte antigens (HLA) of the major histocompatibility system and from epidemiological researches conducted with different approaches: population, family and twin studies.

A) HLA system and population studies:

The genes that encode the antigens, present on all nucleated cell surfaces (HLA-A, B, C) or an some cells as B lymphocytes and macrophages only (HLA-D, DR), are located on the short arm of chromosome 6. However, between HLA-A, B, C, DR there is marked linkage disequilibrium.

At present there is clear evidence of an association between certain HLA antigens and type I diabetes with an increased relative risk when possessing certain HLA-A and HLA-B antigens (Cudworth, 1978). In 1980, it become clear that the strongest associations with the disease lie with the DR antigens, HLA-DR3 (Deschamps et al., 1980), whereas association of HLA-DR2 with the disease is significantly low.