A STUDY ON THE ASSOCIATION OF SERUM LIPID PROFILE WITH RETINAL HARD EXUDATES AMONG PATIENTS WITH TYPE 2 DIABETES MELLITUS IN HOSPITAL USM

by

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ABSTRAK

Pengenalan: Eksudat keras retina adalah komponen retinopati diabetik yang disebabkan oleh kegagalan dalam fungsi halangan-retina-darah (blood-retinal-barrier) menyebabkan kebocoran salur darah halus pada retina.

Objektif: Untuk mengakaji assosiasi di antara profil lipid dengan eksudat keras retina di kalangan pesakit diabetes jenis 2 yang menghidapi retinopati diabetik.

Metodologi: Satu kajian keratan rentas telah dilakukan terhadap 40 orang pesakit diabetes jenis 2 yang menghadapi retinopati diabetik dan 40 orang pesakit diabetes jenis 2 yang tiada sebarang retinopati. Data demografi dan pemeriksaan mata komprehensif telah dijalankan. Sembilan cc darah vena diambil untuk memeriksa kandungan kolesterol, triglyceride, HDL, LDL, oxLDL dan HbA_{1C}. Data deskriptif bagi min dan 'standard devation' digunakan bagi data demografi. pelbagai.

Keputusan: Min tahap kolesterol bagi pesakit dengan retinopati diabetik ialah 5.9 (1.86) mmol/L berbanding 5.0 (1.03) mmol/L bagi pesakit tanpa retinopati diabetik, (P= 0.001). Min tahap LDL juga lebih tinggi di kalangan pesakit dengan retinopati diabetik iaitu 3.6 (1.69) mmol/L berbanding 3.0 (1.02) mmol/L, (P=0.005). Tahap kolesterol, triglyceride dan LDL di kalangan pesakit dengan eksudat retina yang teruk adalah lebih tinggi berbanding kumpulan sederhana (moderate) atau kurang teruk (mild), tetapi perbezaan ini tidak signifikan (P= 0.082, 0.116, 0.218). Min oxidized-LDL adalah lebih tinggi di kalangan mereka yang mempunyai eksudat retina teruk berbanding kumpulan sederhanan dan kurang teruk. Begitu juga terdapat perbezaan

yang tidak signifikan di antara min oxidized-LDL dengan penyakit 'ischemic heart disease', darah tinggi dan penyakit dyslipidaemia.

Kesimpulan: Terdapat assosiasi di antara tahap kolesterol dan LDL dengan retinopati diabetik. Walau bagaimanapun tiada hubungan didapati di antara kandungan profil lipid dengan kadar keterukan eksudat retina. Kandungan paras oxLDL juga tidak mempunyai hubungan yang signifikan di antara retinopati atau penyakit sistemik yang lain di dalam kajian ini.

ABSTRACT

Introduction: Retinal hard exudate is a component of diabetic retinopathy which is formed due to breakdown of the inner blood retinal barrier, as a process of microangiopathy.

Objectives: To study the association between lipid profile and retinal hard exudates in diabetic retinopathy and the association between oxidized-LDL with systemic diseases among type 2 diabetic patients.

Methodology: A cross sectional study was conducted in 40 patients with diabetic retinopathy and another 40 patients without diabetic retinopathy. Demographic data was collected and comprehensive ocular examination was performed. Nine ml venous blood was taken for fasting serum cholesterol, triglycerides, HDL, LDL, ox-LDL, and for HbA_{1C}.

Results: The mean serum cholesterol level was 5.9 (1.86) mmol/L in diabetic retinopathy group compared to patients without retinopathy 5.0 (1.03) mmol/L (P= 0.001). The mean serum LDL was 3.6 (1.69) mmol/L in retinopathy group compared to 3.0 (1.02) mmol/L in the contol group (P=0.005). There was a higher concentration of serum cholesterol, triglyceride and LDL in patients with severe retinal hard exudates compared to those with mild and moderate,however it was not statistically significant (P= 0.082, 0.116, 0.218) respectively. The mean serum oxidized-LDL concentration was higher in diabetic retinopathy with severe retinal hard exudates compared to mild and

moderate. There was no statistically significant difference in the mean oxidized LDL with other systemic diseases or duration of diabetes.

Conclusion: There was significant association between serum cholesterol and LDL with diabetic retinopathy. However there was no association between serum lipid profile with the severity of retinal hard exudates. Serum ox-LDL was also not associated with diabetic retinopathy and other systemic co-morbidities in our study.

1

Introduction

CHAPTER I

1. INTRODUCTION

Diabetes mellitus is a syndrome of altered metabolism characterized by chronic hyperglycemia due to an absolute deficiency of insulin secretion and/or a reduction in the biological effectiveness of insulin. Diabetes mellitus is increasing at epidemic proportions throughout the world and is currently considered one of the main threats to human health in the 21st century. It is estimated that about 100 million individuals currently suffer from diabetes, with more than 16 million diabetics in the United States alone (Farhad R, et al 2004).

Diabetes mellitus is one of the leading causes of blindness in developed as well as in developing countries (Thomas A et al, 2003). The World Health Organization (WHO) estimates that there are currently 150 million people with diabetes, and that this number will double by the year 2025 (Charlis P et al, 2003). Diabetic retinopathy is the most common complication of diabetes present in around 25%-33% of persons with diabetes at any point in time (Bhavsar AR, 2002).

Diabetic retinopathy is the leading cause of blindness among persons aged 20-64 years in the United State (Bhavsar AR, 2002). The prevelance of diabetic retinopathy in Asian India population varies from 4-16% (Kaushik Sen et al, 2000). In Malaysia about 30% of the diagnosed diabetic population has diabetic retinopathy (Ministry of Health Malaysia, 1997).

The largest population-based survey of diabetic retinopathy, the Wisconsin Epidemiologic Study of Diabetic Retinopathy (WESDR), showed diabetic maculopathy was a common complication of diabetes mellitus and frequently accompanied by lipid exudation.

A direct toxic effect of low density lipoprotein (LDL) on retinal capillary pericytes has been demonstrated, and this toxic effect can be enhanced by LDL glycation or oxidation (Guerci B et al, 1999 , Kim CH et al 1998).

High concentration of glucose promote oxidative modification of LDL and this results in increased accumulation of LDL-derived lipids in macrophages. Therefore diabetes and hyperglycaemia are associated with increased oxidative stress and oxidation of lipoproteins. These abnormalities are hypothesized to contribute to the accelerated development of exudative maculopathy in diabetic type 2 patients (Rottenidge et al, 1999).

1.1 Type 2 Diabetes Mellitus

Type 2 diabetes mellitus (DM) is a heterogeneous disorder characterized by reduced tissue sensitivity to insulin and impaired insulin secretion. The disease usually develops in adults, with increased prevalence in obese persons and in the elderly. Recently, Type 2 DM has been appearing in increasing numbers in younger adults and adolescents, owing to worsening status of nutrition, obesity and lack of exercise in this age group.

1.2 Pathogenesis of Type 2 Diabetes Mellitus

Diabetes mellitus results from a complex interplay between underlying resistance to the action of insulin in its target metabolic tissues (liver, skeletal muscle and adipose tissue). A reduction in glucose level stimulates insulin secretion, which fails to compensate for the increased demand for insulin. Progression of overt diabetes in susceptible populations occurs most commonly in patients exhibiting both of these defects.

In a normal person, the extracellular concentration of glucose in fed and fasting state is maintained in a tightly limited range. This limited control is mediated by the opposing actions of insulin and glucagon. Following a carbohydrate rich meal, absorption of glucose from the gut leads to an increase in blood glucose, which stimulates insulin secretion by the pancreatic β cells and the subsequent insulin-mediated increase in glucose uptake by skeletal muscle and adipose tissue. At the same time, insulin suppresses hepatic glucose production by inhibiting gluconeogenesis, enhancing glycogen synthesis, blocking the effects of glucagon on the liver and antagonizing the release of glucagon from the pancreas (Raphael Rubin et al, 2005).

A variety of biochemical mechanisms have been proposed to account for the development of the pathogenesis of diabetes and chronic hyperglycaemia is now accepted as the common pathway leading to diabetic retinopathy (Klein R et al, 1988, Nathan DM et al, 1996).

Many different pathways appear to link glucose metabolism to the development of diabetic retinopathy, including the sorbitol or aldose reductase pathway, increased protein kinase C activity with increased vasodilatory prostaglandins, increased non-enzymatic glycation or glycosylation of proteins, production of vascular endothelial and other growth factors in the retina, glucose induced auto-oxidative damage, as well as retinal capillary blood flow changes and increased capillary permeability.

1.2.1 Protein Glycosylation

Protein glycosylation is one of the biochemical mechanisms which have been proposed to account for the development of the pathological changes in diabetes. The glucose binds non enzymatically, by attaching to a wide variety of proteins.This glycosylation process occurs in proportion to the severity of hyperglycemia.

Numerous cellular proteins are modefied in this manner, including heamoglobin, and proteins in cellular basement membranes. A specific fraction of the glycosylated haemoglobin in circulating red blood cells (haemoglobin A_{1C}) is measured routinely to monitor the overall degree of hyperglycemia that occusr during the preceding 6-8 weeks (Barry J, 2005).

The non enzymatic glycosylation of haemoglobin is irreversible, and the level of haemoglobin A_{1C} , therefore serves as a marker for glycemic control, as well as ongoing protein damage in the body due to excessive blood glucose. The initial glycolysation products (known chemically as Schiff bases) are labile and can dissociate rapidly (Barry J, 2005).

With time this labile products undergo complex chemical rearrangements to form stable advanced glycosylation products, consisting of a glucose derivative covalently bound to a protein amino group. As a result, the structure of protein is permanently altered, and its function may be affected. For example, albumin and IgG do not normally bind to collagen, but they adhere to glycosylated collagen (Barry J, 2005).

Unstable chemical bonds in the proteins containing advanced glycosylation products can lead to physical cross of nearby proteins, which may contribute to the characteristic thickening of the of the vascular basement membranes in diabetes. Importantly, unlike the initial labile glycosylation products, advanced glycosylation products can continue to cross-link protein despite a return of blood glucose to a normal level (Barry J, 2005).

Patients with diabetic retinopathy have higher levels of these products than those without this complication. Moreover, compounds that inhibit the formation of advanced glycosylation products provide some protection against diabetic complications in experimental animals (Raphael Rubin et al, 2005).

1.2.2 Aldose Reductase (Sorbitol) Pathway

The aldose reductase (sorbitol) pathway is active during hyperglycaemia (Gabbay KH, 1973, Larkins RG et al, 1996) with accumulation of sorbitol. In critical retinal vascular tissues, it damages retinal pericytes via apoptosis, as well as basement membrane thickening, with closure of retinal capillaries (Klein R, 1988). These changes have long been identified as the key initial lesions of diabetic retinopathy (Bloodworth JMB, 1962, Kern TS, 1995).

Aldose reductase is an integral enzyme in the polyol pathway and catalyses the reduction of glucose to sorbitol. Its inhibition has been shown to arrest the impairment of pericytes (Horie S et al 1998, Naruse K, 2000).

The loss of pericytes is thought to play a crucial role in the development of diabetic retinopathy. Studies have shown that pericytes synthesise transforming growth factor ß and inhibit proliferation and migration of vascular endothelial cells so that loss of pericytes would contribute not only to vasodynamic changes in the early stage of diabetic retinopathy, but also to neovascularisation in proliferative diabetic retinopathy (Naruse K, 2000).

A hyperglycaemia-induced increase in the activity of aldose reductase also results in a build-up of sorbitol concentration which is thought to cause osmotic damage to vascular cells (Crabbe MJ, 1998). Aldose reductase inhibitors (ARIs) also have the potential to influence the sorbitol pathway and have been the subject of many trials (Henry DN et al, 1993, Ko BC et al, 1995).

1.3 Pathophysiology of Diabetic Retinopathy

Retinopathy is the most devastating ophthalmic complications in diabetes. Chronic hyperglycemia, as well as hyperlipidemia and hypertension, contribute to the pathogenesis of diabetic retinopathy (DR) (Klein R et al 1988, Klein R et al 1998). The exact mechanisms by which elevated glucose initiates the vascular disruption in retinopathy remain poorly defined, and not surprisingly, several pathways have been implicated. The vascular disruptions of DR and diabetic macular edema are

characterized by abnormal vascular flow, disruptions in permeability, and/or closure or non perfusion of capillaries.

A hallmark of early DR is the change in the structure and cellular composition of the microvasculature (Kubawara T et al, 1962, Antonelli Orlidge A, et al 1989). Endothelial cells are responsible for maintaining the blood-retinal barrier, and damage to them results in increased vascular permeability. In early stages of DME, breakdown of the inner blood-retinal barrier may occur, resulting in accumulation of extracellular fluid in the macula (Ferris FL et al, 1984, Antscliff RJ, et al 1999).

Pericytes are essential cellular components in the regulation of retinal capillary perfusion, and damage to these cells in diabetes leads to altered retinal hemodynamics, including abnormal autoregulation of retinal blood flow (Ciulla TA et al, 2002). Loss of retinal pericytes represents another early feature of DR (Speiser P, et al 1968, Paget C et al 1998) and correlates with microaneurysm formation (Cogan DJ et al, 1961).

Another common feature of DR is the thickening of the capillary basement membrane and increased deposition of extracellular matrix components. This feature may contribute to the development of abnormal retinal haemodynamics (Mogensen CE et al 1979, Koya D et al 1998) including abnormal autoregulation of retinal blood flow.

There is evidence that retinal leukostasis may also play an important role in the pathogenesis of DR. Leukocytes possess large cell volume, high cytoplasmic rigidity, a natural tendency to adhere to the vascular endothelium, and a capacity to generate toxic superoxide radicals and proteolytic enzymes (Miyomoto K et al 1999).

In diabetes, there is increased retinal leukostasis, which affects retinal endothelial function, retinal perfusion, angiogenesis, and vascular permeability. In particular, leukocytes in diabetes are less deformable, a higher proportion are activated, and they may be involved in capillary nonperfusion, endothelial cell damage, and vascular leakage in the retinal microcirculation (Miyomoto K et al 1999).

There are many capillary occlusions by leukocytes and capillary dropout or degeneration associated with leukocytes in the diabetic retina. Serial acridine orange leukocyte fluorography and fluorescein angiography (FA) show trapped leukocytes directly associated with areas of downstream nonperfusion in the diabetic retinal microcirculation (Miyomoto K et al 1999). Whereas leukostasis probably plays a key role in the pathogenesis of DR, platelets and erythrocytes are also involved in this process.

As a result of occluded capillaries, retinal ischemia stimulates a pathologic neovascularization mediated by angiogenic factors, such as vascular endothelial growth factor (VEGF), which results in proliferative diabetic retinopathy (PDR) (Aiello LP et al 1994, Miller JW et al 1997). This neovascularization is the predominant feature of PDR. Hemorrhaging of new vessels into the vitreous may also lead to tractional retinal detachment (Riordan Eva, 2003).

1.4 Pathogenesis of Retinal Hard Exudates

Retinal hard exudates are the component of diabetic retinopathy most likely to be related to plasma lipoproteins, because the exudates are lipid rich. Hard exudates are small

yellowish white deposits with sharp margins, they appear waxy, shiny, or glistening. They are located in the outer plexiform layer of the retina, deep to the retinal vessels. They can be arranged as individual dots, confluent patches, or in rings or crescents surrounding zones of retinal edema or groups of microaneurysms. Retinal hard exudates are composed of lipid and proteinaceous material, such as fibrinogen and albumin (Yanoff M et al, 1969).

Although the source of this extravascular lipid may be extravascular cells that contain such lipid, it is more likely that the large amounts of exudate observed in these cases originated from the plasma. The extravasation of lipid and protein most likely occurs as a result of the breakdown of the blood–retina barrier (Vinores S.A, 1999). Such a breakdown has also been suggested by the presence of perivascular fibrinogen (Murata T et al, 1992).

Damage to the blood–retina barrier may be associated with a localized inflammatory process and adherance of leukocytes in the retinal vessels (Miyamoto K et al, 1999). Monocytes and neutrophils make up most of this leukocyte population (Schröder S et al, 1991). These activated leukocytes are thought to promote microvascular damage through the release of cytotoxic products and growth factors such as vascular endothelial growth factor. Macrophages and vascular endothelial growth factor have also been shown to be present in the tissue of excised hard exudates and vascular canals in patients with hard exudate (Ferris F. L et al,1999, Takagi H et al,1999).

The Early Treatment Diabetic Retinopathy Study (ETDRS) showed that severity of retinal hard exudates was strongly associated with total triglycerides, total cholesterol,

and LDL cholesterol (Davis MD et al, 1998). Also, elevated levels of these lipids conferred increased risk for future hard exudates and subsequent visual deterioration.

Atherosclerosis Risk in Communities (ARIC) Study found that each 10 mg/dl increase in LDL, there is 18% increase in risk of hard exudates (Su DHW et al, 2000). Increasing amounts of hard exudate in the posterior pole seem to be associated independently with increased risk of visual impairment. Accumulation of retinal hard exudates can lead to vision loss either from a foveal lipid plaque or from the development of fibrosis (Michael C, 2004).

1.5 Classification of Diabetic Retinopathy

Previously proposed grading scales recognized and recorded the following abnormalities: hemorrhages, microaneurysms, hard exudates, soft exudates ("cotton-wool patches"), intraretinal microvascular abnormalities (IRMA), venous beading (VB), new vessels < 1 disc diameter from the optic nerve (NVD), new vessels elsewhere (NVE), vitreous hemorrhages (VH), preretinal hemorrhages (PRH), and fibrous proliferations in disc and else where (FPD, FPE) (Charles P et al, 2003).

In the proposed new classification, in addition to documenting the presence of some of the specific lesions mentioned above, the absence of diabetic retinopathy is documented as a distinct stage (Charles P et al, 2003).

Table 1.1: International Clinical Diabetic Retinopathy Disease Severity Scale (Charles P 2003).

Proposed Disease Severity Level	**Findings Observable With Dilated Ophthalmoscopy**
No apparent DR	No abnormalities
Mild nonproliferative DR	Microaneurysms only
Moderate nonproliferat DR	More than "mild" but less than "severe"
Severe nonproliferative DR	Any of the following: •20 or more intraretinal hemorrhages in 4 quadrants •Definite venous beading in 2 or more quadrants •Prominent IRMA in 1 or more quadrants and no neovascularization
Proliferative DR	1 or more of the following: •Definite neovascularization •Preretinal or vitreous hemorrhage

1.6 Classification of Diabetic Macular Edema

Diabetic macular edema (DME) is defined as retinal thickening, and this requires a 3-dimensional assessment that is best performed through a dilated pupil examination using slit-lamp biomicroscopy with an accessory lens and/or stereo fundus photography. Hard exudates are a sign of current or previous macular edema. The initial grading decision involves a determination of the presence of any retinal thickening in the posterior pole. Further defining retinal thickening in terms of its extent, 3 grades of edema severity are differentiated. These are related to the distance of the thickening from the center of the retina. The distinction between "distant" and "approaching" is admittedly vague, but it is especially important to differentiate eyes that have no thickening of the central macula from those that do.

The International Clinical Disease Severity Grading Scale For Diabetic Retinopathy and Diabetic Macular Edema classify DME into Mild DME which show retinal thickening or hard exudates in the posterior pole but distant from the center of the macula, Moderate DME shows retinal thickening or hard exudate approching the macula but not involving the center and Severe DME show retinal thickening or hard exudates involving the center of the macula (Table 1.1) (Charlis P et al, 2003).