NOTE TO USERS

Anterior Lens Capsule Thickness in Diabetic And Non-diabetic Patients

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Abstract

Diabetes has been shown to affect the thickness of the basement membrane in various human tissues and organs. The purpose of this thesis is to determine whether diabetes can cause thickening of the anterior capsule basement membrane (ACBM), and correlate the findings with the duration and the severity of diabetes. For the purpose of this study, anterior lens capsule specimens of diabetic patients (DP), and non-diabetic patients (NDP) are collected from phaco-emulsification cataract surgeries. All cases are formalin-fixed, paraffin-embedded and stained with H&E and periodic acid-Schiff. The ACBM thickness is measured and reviewed under a light microscope. The results of this thesis showed that the mean thickness of ACBM is significantly thicker in DP as compared to NDP. The ACBM thickness is increased with patient age in both DP and NDP. Moreover, in DP the ACBM thickness increased with the duration and the severity of diabetes.

Resumé

Il est démontré que le diabète a un effet sur l'épaisseur de la membrane basale de divers tissus et organes humains. L'objectif de cette thèse consiste à déterminer si le diabète peut causer l'élargissement de la capsule antérieure de la membrane basale et à fournir, suite aux résultats expérimentaux, une corrélation entre l'épaisseur de cette dernière et la durée ainsi que la sévérité du diabète. Pour atteindre cet objectif, des spécimens de capsule antérieure de la membrane basale de lentille prélevés sur des patients diabétiques et non diabétiques ont été recueillis des chirurgies de cataracte utilisant la phacoémulsification. Après avoir fixé tous les échantillons à l'aide du formol, noyé dans le paraffine et taché de H&E et periodic acide-Sciff (PAS), l'épaisseur de la capsule antérieure de la membrane basale a été mesurée et examinée sous un microscope lumineux. Les résultats de la thèse montrent que l'épaisseur moyenne de la capsule antérieure de la membrane basale des patients diabétiques est plus large comparée à celle des patients non diabétiques. L'épaisseur de la capsule antérieure de la membrane basale est augmentée en fonction de l'âge des patients diabétiques et non diabétiques. De plus, dans le cas des patients diabétiques, les résultats montrent une augmentation de l'épaisseur en fonction de la durée et de la sévérité du diabète.

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List of Abbreviations

ACBM	Anterior capsule basement membrane
D1	Duration of diabetes less than five years
D2	Duration of diabetes between six to ten years
D3	Duration of diabetes more than ten years
DM	Diabetes mellitus
DP	Diabetic patients
G1	Age of diabetic and non-diabetic patients less and equal to fifty five
G2	Age of diabetic and non-diabetic patients between fifty six to sixty five
G3	Age of diabetic and non-diabetic patients between sixty six to seventy five
G4	Age of diabetic and non-diabetic patients more and equal to seventy six
HbA1c	Glycosylated hemoglobin
H&E	Hematoxylin and Eosin
M	Mean
NDP	Non-diabetic patients
NPDR	Non proliferative diabetic retinopathy
PAS	Periodic acid-Schiff
PDR	Proliferative diabetic retinopathy
SD	Standard Deviation
u	Units

CHAPTER 1

Introduction and Literature Review

1.1 Justification

The prevalence of diabetes mellitus (DM) is 8% of the general population over 20 years of age. Diabetes represents a heterogeneous group of disorders that have hyperglycemia as a common feature. Diabetes has been classified into: type 1 diabetes, type 2 diabetes, gestational diabetes, and other specific types of diabetes (Alberti and Zimmet, 1998). Type 2 diabetes is the most common form of diabetes (accounting 90%-95% of diabetic population), and is becoming more prevalent due to obesity and a sedentary life style (Kapellen et al., 2004). Cataract formation is one of the most common causes of visual impairment in diabetic patients (Chenault et al., 2002).

Furthermore, diabetes has been shown to increase the thickness of the basement membrane in various human tissues and organs such as the retinal capillary basement membrane and glomerular basement membrane (Kim et al., 1998; Obineche et al., 2001).

The purpose of the present study is to determine for the first time in human eyes whether diabetes can cause thickening of the anterior lens capsule basement membrane. The results in diabetic patients will be correlated with the duration of diabetes as well as the severity of retinopathy.

1.2 Diabetes

Diabetes is a common chronic metabolic disease that can affect many organs and tissues, in which impaired glucose utilization, caused by a defect in insulin production or action, induces hyperglycemia (Crawford and Cotran, 1994). The prevalence of diabetes

increases with the age of the patient. Three percent of people between 35 and 64 years of age have the disease, while these numbers increase to 10% of people older than 65 years of age. The most frequent types of diabetes are type 1 diabetes, and type 2 diabetes. Type 1 diabetes may account for 5-10% of all diagnosed cases. While type 2 diabetes is the most frequent form of the disease, accounting for 90-95% of all diagnosed cases (Health, 1995; Prevention, 1997).

1.2.1 Type 1 diabetes

This form of the disease usually develops in childhood and manifests at puberty. It had previously been known as insulin-dependent diabetes or juvenile onset diabetes. Type 1 diabetes results from a severe and absolute lack of insulin due to reduced islet beta cells (Crawford and Cotran, 1994).

New studies indicate that there are two subgroups of this type of diabetes.

- Type 1A, caused by autoimmune destruction of islet beta cells.
- Type 1B, associated with severe insulin deficiency, but with no evidence of autoimmunity (Swenne, 1992).

Moreover genetic susceptibility such as having certain HLA-D genes, and some environmental factors such as viruses and toxins may also play a role in development of this type of diabetes (Powers, 2001).

1.2.2 Type 2 diabetes

Type 2 diabetes usually develops in adults over the age of 40, with some degree of obesity. It was previously known as non-insulin dependent diabetes. There is no

evidence that autoimmune mechanisms are involved in the pathogenesis of this type of diabetes. Genetic factors, sedentary lifestyle, and visceral obesity are the most important risk factors for the development of this type of the disease. In families with a history of this type of diabetes, the risk of developing the disease is 5 to 10 times higher when compared to people that do not have a positive family history (Crawford and Cotran, 1994).

Two metabolic defects are seen in type 2 diabetes.

1- Insulin secreting defect

2- Insulin resistance due to:

- Decrease in the number of insulin receptors
- Post-receptor defects such as post-binding abnormalities (Swenne, 1992).

Type 2 diabetes frequently goes undiagnosed for many years, since the hyperglycemia develops quite gradually and is generally asymptomatic initially. Despite this mild presentation, these patients are at increased risk of developing macrovascular and microvascular complications (Powers, 2001).

1.3 Complications of diabetes

Most patients with diabetes develop a number of pathological changes, which occur at variable intervals during the course of the disease. These changes involve the vascular system for the most part. However, they also occur in the nerves, skin, and in the lens. Diabetic vascular disease is divided into two main categories: microvascular and macrovascular disease. Microvascular disease affects the capillary, the precapillary arterioles and venules, while the macrovascular disease affects the arteries and veins.

Microvascular disease involving the retina leads to diabetic retinopathy, and microvascular disease involving the kidney causes diabetic nephropathy. Macrovascular disease in diabetes is essentially an accelerated form of atherosclerosis. It accounts for the increased incidence of myocardial infarction, stroke, and peripheral gangrene in diabetic patients (Crawford and Cotran, 1994; Powers, 2001).

1.3.1 Renal complications of diabetes

The pathogenesis of diabetic nephropathy is related to chronic hyperglycemia. The incidence of nephropathy differs between the two types of diabetes. Patients with type 1 diabetes who have not received intensive insulin therapy and have had poor glycemic control have a 30-40% chance of having nephropathy after 20 years. In contrast the frequency of nephropathy in type 2 diabetes patients is much lower and about 15-20%. Since so many more individuals are affected with type 2 diabetes, end-stage renal disease is much more prevalent in type 2 diabetes in North America and also throughout the world (Raptis and Viberti, 2001; Shumway and Gambert, 2002). Diabetic nephropathy is initially manifested by proteinuria; subsequently, as kidney function declines, urea and creatinine accumulate in the blood. Thickening of capillary basement membranes and of the mesangium of renal glomeruli produces varying degrees of glomerulosclerosis and renal insufficiency (Crawford and Cotran, 1994; Powers, 2001; Sheetz and King, 2002).

1.3.2 Neural complications of diabetes

Peripheral and autonomic neuropathies are the two most common complications of both type 1 and type 2 diabetes. They occur in approximately 50% of individuals with long standing diabetes. The development of neuropathy correlates with the duration of diabetes and glycemic control (Sheetz and King, 2002). The pathogenesis of diabetic neuropathy is poorly understood. Some lesions, such as the acute cranial nerve palsies and diabetic amyotrophy, have been attributed to ischemic infarction of the involved peripheral nerve. The much more common symmetric sensory and motor peripheral neuropathies and autonomic neuropathy are felt to be due to metabolic or osmotic toxicity somehow related to hyperglycemia (Perkins and Bril, 2003; Powers, 2001; Sheetz and King, 2002)

1.3.3 Cardiovascular complications of diabetes

The risk of cardiovascular disease increases in individuals with either type 1 or type 2 diabetes. Microvascular disease has recently been recognized to occur in the heart of diabetic patients, which may explain the existence of congestive cardiomyopathies found in diabetic patients without demonstrable coronary artery disease (Boffa et al., 2001). However, heart failure in diabetic patients as a consequence of coronary atherosclerosis is much more common (Ledru et al., 2001). Myocardial infarction is three to five times more common in diabetic patients than in age-matched controls and is the leading cause of death in patients with type 2 diabetes. A loss of the protection against myocardial infarction usually present in women during the childbearing years is particularly evident in diabetic women. The exact reason for the increased incidence of

myocardial infarction in diabetics is not clear. It may reflect the combination of hyperlipidemia, abnormalities of platelet adhesiveness or coagulation factors (or both), and/or hypertension (Ginsberg and Tuck, 2001; Howard, 1996).

Arteriosclerosis is markedly accelerated in the larger arteries. It is often diffuse, with localized enhancement in certain areas of turbulent blood flow, such as at the bifurcation of the aorta or other large vessels. Clinical manifestations of peripheral vascular disease include ischemia of the lower extremities, impotence, and intestinal angina.

The incidence of gangrene of the feet in diabetics is 30 times higher compared to age-matched controls. The factors responsible for its development, in addition to peripheral vascular disease, are microvascular disease, peripheral neuropathy with loss of both pain sensation and neurogenic inflammatory responses and secondary infection (Powers, 2001; Sheetz and King, 2002; Williams et al., 2004).

1.3.4 Ocular Complications of Diabetes

Diabetic patients start to develop ocular complications within ten years of the beginning of their diabetes (Henricsson et al., 2003). The longer the duration of diabetes and poor glycemic control increases the chances of having diabetic retinopathy (Williams et al., 2004). Nonproliferative and proliferative diabetic retinopathies are the two main categories of diabetic retinopathy. Nonproliferative diabetic retinopathy represents the earliest stage of retinal involvement by diabetes and is characterized by changes such as: microaneurysms, dot hemorrhages, exudates, and retinal edema. During this stage, the retinal capillaries leak proteins, lipids, and red cells into the retina. When this process occurs in the macula it interferes with the visual acuity; and causes

macular edema, which is the most common ocular complication of type 2 diabetes (Ciulla et al., 2003). Proliferative diabetic retinopathy involves the growth of new capillaries and fibrous tissue within the retina and into the vitreous chamber. Proliferative diabetic retinopathy is a consequence of small vessel occlusion that causes retinal hypoxia; which in turn stimulates new vessel growth. After 10 years of diabetes, half of all patients develop, at least some degree of retinopathy, and this percentage increases to more than 80% after 15 years of diabetes (Williams et al., 2004). Cataracts are also very common in diabetic patients. Cataracts are found in both diabetic and non-diabetic adults and tend to occur at a younger age in diabetic patients, particularly when glycemic control is poor. Cataracts are the main leading cause of visual impairment in diabetic patients (Klein et al., 1998b; Powers, 2001; Yanoff and Fine, 2002)

1.4 Epidemiology of diabetes

The prevalence of diabetes in the Canadian population varies between 2% and 2.7% (Canada, 1978). This number increases with age: 3% of people aged 35 to 64, and 10% of those aged 65 and over have been diagnosed with diabetes (Canada, 1999). Estimates from the Manitoba data base yielded rates of 0.8% in adults less than 45 years, 3.5% among those between 45 and 64 years of age, and 7.6% among those 65 and older (Young et al., 1991). The prevalence is higher in native Canadian populations. A study conducted in Northern Quebec revealed that 6.2% of the Cree population suffered from diabetes, with a prevalence rate of 20% in women over 50 years of age (Brassard et al., 1993).

Statistic Canada showed that diabetes is the seventh leading cause of death and is the first leading cause of end stage renal disease in Canada (Canada, 1999). Life expectancy is reduced by 5 to 10 years in diabetic patients compared to their normal counterparts. Moreover, the risk of having cardiovascular disease is increased by two to six times in diabetic patients compared with non-diabetics. However, studies suggested that ocular complications remain the most common manifestations of diabetes (Canada, 1999).

1.5 Epidemiology of ocular complications of diabetes

The prevalence of ocular complications increases with the duration of diabetes and the patient's age. Chronic hyperglycemia is responsible for the ocular complications of diabetes and improved glucose control reduces the risk of developing complications (Group, 1993; Group, 1998) . Cataract formation is a frequent cause of visual impairment in diabetic patients (Klein et al., 1995). The rates of cataract formation are significantly higher in diabetic patients compared to non-diabetic patients (21,9% vs. 14.1%, $p < 0,001$) (Canada, 1999). On the other hand, macular edema is the most common manifestation of type 2 diabetes and occurs in up to 18% of these patients over time. Its prevalence increases with the severity of the diabetes. Proliferative diabetic retinopathy can occur in both types of diabetes, but is more common in type 1 diabetes. It develops about 7-10 years after onset of symptoms, with a prevalence rate of 25% after 15 years of duration (Williams et al., 2004). Diabetic retinopathy remains the leading cause of blindness in diabetic patients between 20-64 years of age (Canada, 1999).

1.6 The eye

The eye is situated in the orbital cavity. It consists of three layers, the fibrous layer, the vascular-pigmented layer, and the nervous layer.

The fibrous layer is made up of the posterior opaque part, the sclera, and the anterior transparent part, the cornea. The sclera forms the posterior $5/6^{th}$ of the eye and is white in color. The transparent cornea forms the anterior $1/6^{th}$ of the eye (Figure 1.1).

The vascular-pigmented layer, also called the uveal tract, consists from back to front: the choroid, the ciliary body, and the iris, forming a continuous structure. The choroid is a thin, soft, brown coat lining the inner surface of the sclera and is extremely vascular. The choroid extends from the optic nerve to the ciliary body. The iris is a thin contractile, pigmented diaphragm with a central aperture; the pupil. The iris is suspended in the aqueous humor between the cornea and the lens (Figure 1.1).

The retina is the internal layer of the eye. The retina is a thin transparent membrane having a purplish-red color. It starts around the optic nerve and extends forward to become the epithelium of both the ciliary body and iris (Figure 1.1).

The eye contains two chambers: the anterior chamber and the posterior chamber. The anterior chamber of the eye is a small cavity lying behind the cornea and in front of the iris. It is filled with aqueous humor. The posterior chamber of the eye is a small slit like cavity. It is filled with aqueous humor and communicates with the anterior chamber through the pupil. The posterior chamber is formed in the front by the iris, peripherally by the ciliary body, and in the back by the lens and the ciliary ligaments.

The vitreous fills the eye behind the lens. It occupies about four-fifths of the eye and lies between the lens and the retina. The vitreous is a transparent gel having a denser cortex and more liquid center (Snell and Lemp, 1998).

1.7 The lens

The lens is a soft, elastic, transparent, biconvex structure situated behind the iris and the pupil and in front of the vitreous. The main functions of the lens are to maintain its own clarity, to refract light, and to provide accommodation. The lens has no blood supply or innervations after fetal development, and it depends entirely upon the aqueous humor to meet its metabolic requirements and to carry off its wastes (Figure 1.1). It is suspended in position by the ciliary ligaments that allow the lens to change its shape by performing the accommodation. Accommodation is the ability of the lens to change its dioptric power, to help us to see objects clearly when they are situated in front of the eye. The lens is composed of the capsule, the epithelium, and the fibres (Figure 1.2). The latter are formed from the cortex and the nucleus (Snell and Lemp, 1998).

1.7.1 The capsule

The capsule is a transparent elastic basement membrane that envelops the entire lens (Figure 1.2). In contrast to the other basement membranes in the body, the capsule is produced continuously throughout life by the underlying epithelium. The lens capsule is thickest in the anterior and posterior pre-equatorial zones and thinnest in the region of the central posterior pole, where it may be as thin as 2-4μm. The anterior lens capsule is

considerably thicker than the posterior capsule at birth and increases in thickness throughout life because of the underlying epithelium. The capsule is the thickest basement membrane in the body. When studied under light microscope the capsule appears dense and homogenous, while under electron microscope the lens capsule consists of parallel lamellae that are mainly composed from collagen IV and other matrix proteins. Type IV collagen is found only in basement membranes, and it is the only collagen which has been shown definitively to be present in basement membranes. In hematoxylin and eosin-stained sections, the capsule is pale, eosinophilic with a faint basophilic tinge, and slightly refractile. While in periodic acid- Schiff (PAS) stained sections, the capsule stains intensely. The lens capsule serves as a diffusion barrier and is freely permeable to low-molecular weight compounds, but restricts the movement of large colloidal particles. The capsule provides insertions to the ciliary ligaments allowing the lens to perform its own accommodation. The main function of the capsule is to mold the shape of the lens in response to the pull of ciliary ligaments during accommodation (Eagle Jr, 1996; Snell and Lemp, 1998).

1.7.2 The epithelium

The second layer of the lens is the epithelium, which represents a single layer of cuboidal cells lying behind the anterior capsule (Figure 1.2). These cells are metabolically active and can carry out all normal cell activities, including biosynthesis of DNA, RNA, proteins, and lipids, as well as generating ATP to meet the energy demands of the lens. The epithelium is present only under the anterior capsule of the lens and it is absent under the posterior capsule of the lens. The epithelial cells are

mitotic, with the greatest activity of premitotic DNA synthesis occurring in a ring around the anterior lens known as the germinative zone. The epithelial cuboidal cells elongate in size and change to columnar cells when they reach the equator of the lens where they start to loose their nucleus and transform into non-nucleated lens fibers (Figure 1.2). The division of the columnar cells in the equator continues throughout life, which is responsible for the continuous growth of the lens (Kuszak and Brown, 1994; Snell and Lemp, 1998).

1.7.3 The lens fibers

The third layer of the lens is composed of lens fibers, which in turn are composed of the nucleus in the central part of the lens and the cortex in the outer part of the lens. No cells are lost from the lens; as new fibers are laid down, they crowd and compact the previously formed fibers, with the oldest layers being the most central. The outermost fibers are the most recently formed and make up the cortex of the lens (Figure 1.2). The lens fibers constitute the main mass of the lens. During development, the lens fibers lose their nuclei and the cytoplasmic organelles and become specialized for the production of lens proteins, known as crystallins. There are three types of crystallins: alpha, beta, and gamma. They constitute up to 60% of the lens fiber mass (Kuszak and Brown, 1994; Snell and Lemp, 1998)

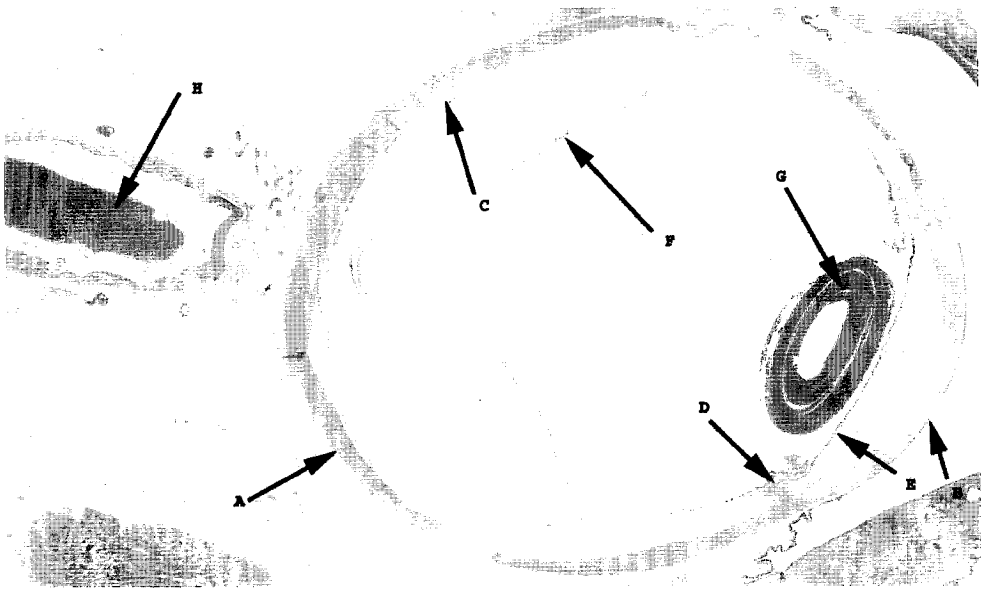

Figure 1.1: The anatomy of the eye**: A)** The sclera. **B)** The Cornea. **C)** The choroid. **D)** The ciliary body. **E)** The iris. **F)** The Retina. **G)** The lens**. H)** The optic nerve.

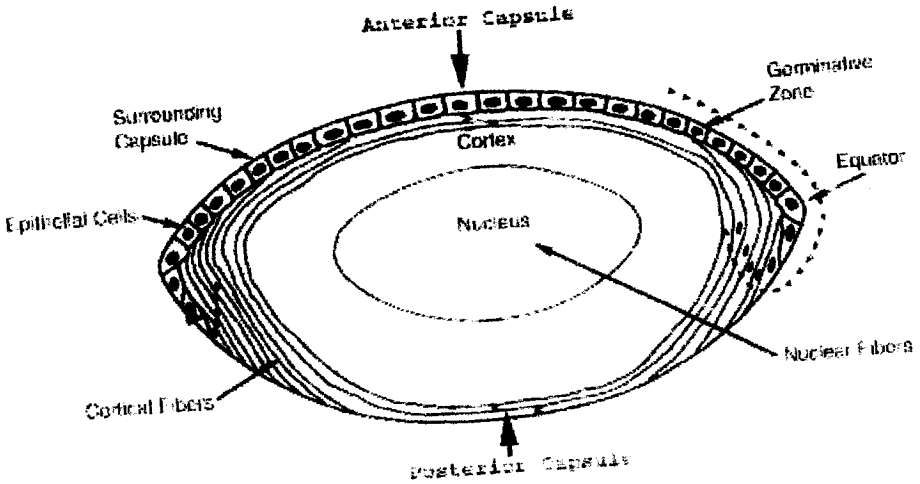

Figure 1.2: The anatomy of the lens.

1.8 What is cataract?

A cataract is the clouding of the lens of the eye. Normally, light passes through the clear lens and it is focuses onto the retina. However, as a result of the natural aging process, the lens gradually becomes cloudy. The cataract or cloudy lens blocks the passage of light through the eye and causes distorted or blurred vision. People who have cataracts complain of a decrease in vision that interferes with their daily life. With advancing age the risk of having cataract is increased. In cross-sectional studies, the prevalence of cataracts is 50% in people between ages of 65 and 74; these numbers increase to 70% in those over the age of 75. However, cataracts do have an increased incidence and a decreased age of onset in diabetic patients compared to non–diabetic patients (Di Benedetto et al., 1999).

The pathogenesis of age-related cataracts is multifactorial and is not completely understood. As the lens ages, it increases in weight and thickness and decreases in accommodative power. Other age related changes in the lens include decreased concentrations of glutathione and potassium, increased concentrations of sodium and calcium, and increased hydration. The three main types of age-related cataracts are nuclear, cortical, and posterior subcapsular cataracts (Klein et al., 1998b). In many patients, components of more than one type are usually present. The removal of cataract is the most common surgical procedure performed in those over the age of 65. Moreover, cataract is the leading cause of blindness worldwide, accounting for about 42 percent of all blindness, in spite of the availability of an effective surgical treatment. With increasing life expectancy, the number of cases of blindness from this disorder may double by the year 2010 (Harding, 1991; Klein et al., 1998b; Streeten, 1994).

1.9 Types of cataracts in diabetes

Diabetes can affect the clarity of the lens, its refractive index, and its accommodative amplitude. As the blood sugar level increases the glucose content in the aqueous humor increases. Since glucose from the aqueous humor enters the lens by diffusion, glucose content in the lens increases. Some of the glucose is converted by the enzyme aldose reductase to sorbitol, which remains in the lens unmetabolized (Harding, 1991).

Cataract is a common cause of visual impairment in diabetic patients. There are two types of cataract encountered in diabetic patients. The first one is called snowflake cataract or true diabetic cataract. It consists of bilateral, and widespread subcapsular lens changes of abrupt onset and acute progression, typically seen in young patients with uncontrolled juvenile diabetes (Santiago et al., 1997). In this type of cataract, multiple gray-white subcapsular opacities that have a snowflake appearance are initially seen in the superficial anterior and posterior lens cortex. Researchers believe that the underlying metabolic changes associated with the true diabetic cataract in humans are closely allied to the sorbitol cataract studied in experimental animals. Although true diabetic cataracts are rarely encountered in clinical practice today, any rapidly maturing bilateral cortical cataracts in a child or young adult should alert the clinician to the possibility of diabetes.

The second type of cataract is the senescent cataract, frequently observed in diabetic patients. The only difference is that this form of cataract develops at an earlier age and more often in diabetic patients compared to non-diabetics (Di Benedetto et al., 1999). The high risk of age-related cataracts in diabetic patients may be a result of the accumulation of sorbitol within the lens, subsequent hydration changes (Cheng, 2002),

and increased glycosylation of proteins in the diabetic lens (Agardh et al., 2000; Benson et al., 1988; Flynn et al., 2000; Johns, 1992; Yanoff and Fine, 2002).

1.10 Literature review

A review of the literature will be performed to cover the areas of research where scientists have tried to describe the relationship between the increasing thickness of the basement membrane of diabetic patients and the development of diabetic complications. We also believe that it is reasonable to introduce some of the studies where researchers have tried to link the degree of hyperglycemia with the advancement of complications of diabetes.

1.10.1 Basement membrane and microvascular complications of diabetes

Studies have shown that many complications associated with diabetes are the result of widespread abnormality in the thickness of the basement membrane in various human tissues and organs. The increased thickness is most evident in the capillaries of tissues, such as skin and retina. It may also be seen in non-vascular structures such as renal tubules and peripheral nerves (Danis et al., 1996; Hill and Williams, 2002; Ljubimov et al., 1996).

Several studies were performed in animal models to measure the thickness of the basement membrane in diabetic animals such as dogs, rats and mice (Daniele et al., 2000; Doi et al., 1989).

In dogs, Engerman reported diffuse thickening of capillary basement membrane of retina, renal glomerulus, and muscle. In contrast to other researchers findings Engerman mentioned that capillary basement membrane thickening did not reverse after glycemic control of 5 years (Engerman et al., 1993).

Different studies support the idea that the morphological hallmark of diabetic microvascular disease is the generalized basement membrane thickening with the progressive increase in permeability of the vessels. A possible explanation for this phenomenon is the non-enzymatic glycolysation of the proteins of the basement membrane including type IV collagen, laminin and heparan sulfate proteoglycans (Kolbe et al., 1990; Mott et al., 1997; Ziyadeh, 1993).

Capillary basement membrane thickening is an ultra structural hallmark in diabetic patients and in diabetic animal models. Studies performed on transgenic diabetic mice compared to normal controls showed increased thickening of capillary basement membrane. Although the increased thickness of capillary basement membrane was not universal, the increasing thickness was statistically significant in the glomerular basement membrane, retinal capillary basement membrane, pulmonary alveolar basement membrane, and skeletal muscle capillary basement membrane from the thoracoabdominal diaphragm. While the increasing thickness of the capillary basement membrane in the pancreas, choroid and interventricular septum and left ventricle of the heart was not statistically significant (Carlson et al., 2003).

A study performed by a group of researchers in Poland to evaluate the morphological changes in human placentas of diabetic patients showed that there was a significant thickening of basal membranes of trophoblast. There were also structural abnormalities

in the perivascular space where they found proliferation of collagen in the terminal villi of placentas of women with hyperglycemia and fetal macrosomia. The intensity of these changes was related to the degree of hyperglycemia and affected fetal and neonatal well being (Pietryga et al., 2004) .

Until now the pathogenesis of diabetes-related increases in capillary basement membrane was poorly understood. Nevertheless, most theories regarding the pathogenesis of diabetic capillary basement membrane thickening begin with hyperglycemia as a major cause of microvascular damage, leading ultimately to basement membrane alterations (King and Banskota, 1994). Researchers confirmed that good glycemic control inhibits basement membrane thickening in a variety of tissues in diabetic patients; thus delaying the development of micro vascular complications that are associated with the basement membrane thickening (Engerman and Kern, 1987; King and Banskota, 1994; Peterson et al., 1980; Raskin et al., 1983).

1.10.2 Hyperglycemia and complications of diabetes

The risk of developing complications from diabetes increases with increasing concentrations of hyperglycemia. Reduction of hyperglycemia in DP reduces the risk of complications (Group, 1993; Group, 1998).

Stratton performed a prospective observational study, which included 4585 participants. The purpose of the study was to determine the relation between exposure to glycemia over time and the risk of developing macrovascular or microvascular complications in patients with type 2 diabetes. The results of the study showed that the incidence of clinical complications was significantly associated with hyperglycemia. Each 1%

reduction in mean glycosylated hemoglobin (HbA1c) levels was associated with reductions in risk of 21% for any end point complications related to diabetes, 21% of deaths related to diabetes, 14% of myocardial infarction, and 37% of microvascular complications including cataract. No threshold of hyperglycemia was observed for a substantive change in risk for any of the clinical outcomes examined. The lower the hyperglycemia, the lower the risk of developing complications. The data in this study showed that the rate of increasing risk of microvascular disease with hyperglycemia was greater than the rate of increasing risk of macrovascular disease (Stratton et al., 2000).

Corneal abnormalities are common in diabetic patients. These corneal abnormalities were recognized in the literature as "diabetic keratopathy". Histopathologically, the thickening of the corneal epithelial basement membrane and morphologic changes of the corneal epithelium and endothelium have been reported by many researchers (Schultz et al., 1984; Taylor and Kimsey, 1981; Tsubota et al., 1991).

Gekka recently found that the corneal epithelial barrier function was impaired in diabetic patients compared to non-diabetic patients. Moreover, diabetic patients with higher serum HbA1c levels were more predisposed to impaired corneal epithelial barrier function (Gekka et al., 2004) .

The nonenzymatic glycosylation of proteins is a process dependent on the prolonged exposure of proteins to a high concentration of glucose *in vivo* and *in vitro*.

Shin studied the nonenzymatic glycosylation of lens epithelial basement membranes of senile cataractous lenses in both diabetic and non-diabetic patients. The results of his study showed that diabetic patients have a two-fold increase in the amount of lens

epithelial basement membrane glycosylation compared to their non-diabetic counterparts (Shin et al., 1994).

1.10.3 Epithelial cells apoptosis and cataract formation

Researchers found that epithelial cells are also implicated in the pathogenesis of basement membrane thickening and cataract formation.

Several studies have shown that besides the basement membrane thickening, apoptosis (programmed cell death) of lens epithelial cells play an important role in the development of different types of cataract in humans and animals (Li et al., 1995).

Rehany found that there was focal degeneration of epithelial cells in the cornea and cytoplasmic accumulation of glycogen granules, besides the marked irregular thickening of the corneal epithelial basement membrane and abnormally spaced collagen formation mostly with type IV (Rehany et al., 2000). While Struck found that apoptosis of lens epithelial cells were implicated in the induction of cataract in diabetic patients (Struck et al., 2001).

Furthermore, studies performed to investigate the role of sugar induced cataracts in a rat model, confirmed that galactose induced apoptosis occurs in rat lens epithelial cells (Takamura et al., 2003).

1.11 Hypothesis

The following hypothesis were formed based on previous concepts

1-Diabetes causes increased thickening of anterior capsule basement membrane (ACBM) of human lens.

2-There is a correlation between the increasing thickness of ACBM and the duration of diabetes.

3-There is a correlation between the increasing thickness of ACBM and the severity of diabetes.

1.12 Objectives

To the best of our knowledge, this is the first study that measures the ACBM thickness in diabetic patients (DP) and non-diabetic patients (NDP).

To test the hypotheses, the following objectives were established:

1- Determine, for the first time, whether diabetes can cause thickening of the ACBM in the human eyes.

2- Compare the thickness of the ACBM in age-matched DP and NDP.

3- Correlate the ACBM thickness with the duration and the severity of the diabetes mellitus (DM).

CHAPTER 2

Materials and Methods

2.1 Materials

2.1.1 Tissue specimens

One hundred diabetic and one hundred non-diabetic anterior lens capsule specimens were collected in a time frame of two years extended from 2002 to 2004. All specimens were formalin-fixed, paraffin-embedded and stained with hematoxylin and eosin (H&E) and periodic acid-Schiff (PAS). The cases were obtained from the Henry C. Witelson Ophthalmic Pathology Laboratory and Registry at McGill University.

2.2 Methods

2.2.1 Tissue processing

All the specimens of DP and NDP were collected from routine phaco-emulsification cataract surgeries with capsulorexis (circular piece of anterior lens capsule). Two surgeons using the same equipments and techniques performed the cataract removal surgeries. All the specimens were formalin-fixed (10% formalin for 24 hours), and paraffin-embedded. Paraffin sections were cut at 5μm, mounted on commercially provided silanized slides (Surgipath, Snowcoat, X-tra), and dried overnight at 37 C, then stored at 60 C for at least 60 minutes.

2.2.2 Hematoxylin and eosin and periodic acid-Schiff staining

The prepared slides of ACBM of DP and NDP were stained with standard staining procedure of hematoxylin and eosin (H&E) and periodic acid-Schiff (PAS)

using (Surgipath) ready to use solutions for visual assessment and thickness measurement (Figures 2.1 and 2.2).

2.2.3 Measurement of anterior lens capsule basement membrane

The anterior lens capsule basement membranes of DP and NDP were reviewed under a light microscope at 400x magnification. The ACBM thickness was measured, in units (u), using a Carl-Zeis 444034 eyepiece. The ACBM thickness measurements were compared between DP and NDP in age-matched groups (Figures 2.1 and 2.2).

2.3 The groups of diabetic and non-diabetic patients

DP and NDP were divided into four groups according to their age: Group one (G1) ≤ 55 years old, group 2 (G2) 56-65 years old, group 3 (G3) 66-75 years old, and group 4 (G4) ≥ 76 years old. Moreover, DP were further subdivided into three different groups according to duration (D) of the disease, D1 ≤ 5 years, D2 6-10 years, and D3 ≥11 years, and also according to the severity of the disease: no diabetic retinopathy, non-proliferative diabetic retinopathy (NPDR), and proliferative diabetic retinopathy (PDR).

2.4 Statistical analysis

Statistical analysis was performed with Student's *t* test to compare the ACBM thickness between DP and NDP. The results are expressed as mean (M) ± standard deviation (SD). Linear regression analysis and Pearson correlation were used to assess the relationship between the increasing thickness of ACBM and age of DP and NDP.

The same regression analysis and Pearson correlation were used to assess the relationship between the increasing thickness of ACBM in DP with the duration and severity of the disease. Probability values of $p < 0.05$ were considered statistically significant.

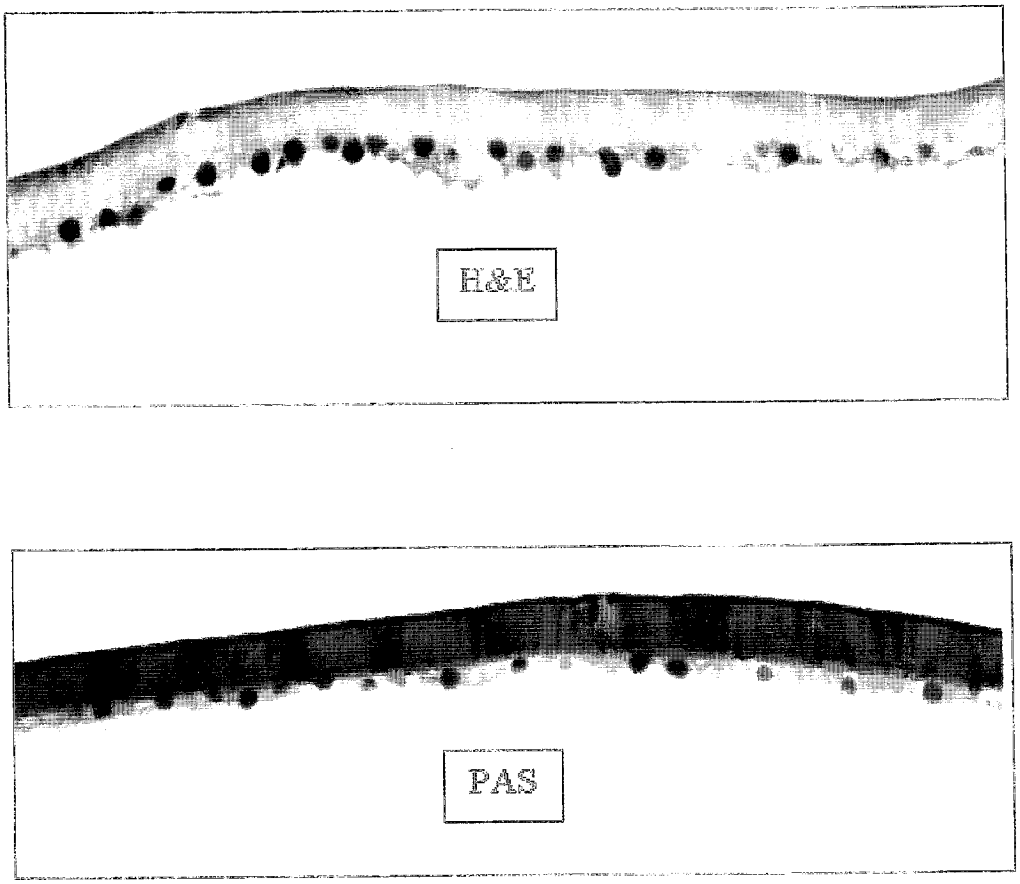

Figure 2.1: Anterior capsule basement membrane of non-diabetic patient (NDP) stained with hematoxylin and eosin (H&E x400) and periodic acid-Schiff (PAS x400).

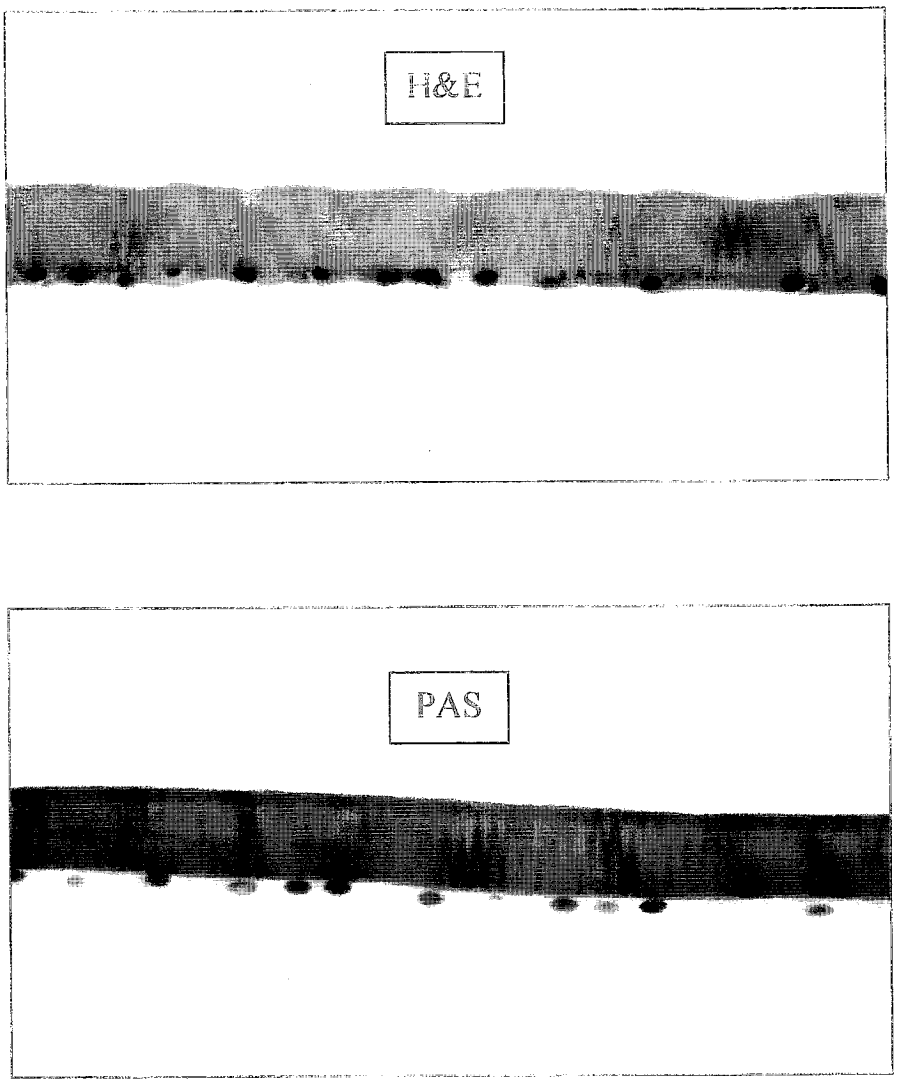

Figure 2.2: Anterior capsule basement membrane of diabetic patient (DP) stained with hematoxylin and eosin (H&E x400) and periodic acid-Schiff (PAS x400)

CHAPTER 3

Results

3.1 Patient characteristics

Patient's characteristics of both DP and NDP were presented in (Table 3.1). The group of DP was representative of type 2 diabetes. The age distribution of both DP and NDP was the same. Diabetic patients widely ranged in duration of diabetes and the severity of diabetic retinopathy.

3.1.1 Diabetic and non-diabetic patients

The total number of cases was 200, 100 for DP and 100 for NDP in aged matched groups. The mean age was 73.29±8.57 for DP and NDP. The sex distribution (male/female ratio) for DP was 39/61 and for NDP was 42/58. The eye distribution (right eye/left eye) for DP was 54/46 and for NDP 43/57. The mean thickness of the ACBM was 11.18±1.56u for DP, and 9.24±0.9u for NDP (Table 3.1) (Figure 3.1). There was a statistically significant difference in the thickness of ACBM between DP and NDP, $p < 0.001$. In both DP and NDP, the ACBM thickness increased with patient age (Figure 3.2).

Table 3.1 Characteristics of DP and NDP

	DP	NDP
N	100	100
Age (years)	73.29 ± 8.57	73.29 ± 8.57
Sex (M/F)	39/61	42/58
Eye R/L	54/46	43/57
ACBM thickness (u)	11.18 ± 1,56	9.24 ± 0.91

Data are means ± SD

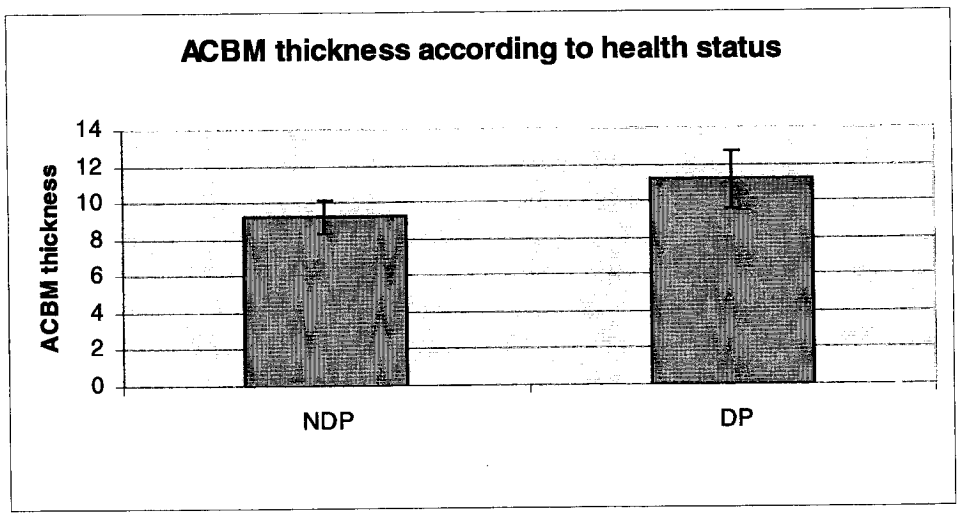

Figure 3.1 The graph shows the mean thickness of anterior capsule basement membrane (ACBM) of non-diabetic patients (NDP) and diabetic patients (DP). The mean ACBM thickness of NDP and DP were 9.24±0.9u and 11.18±1.56u, respectively. The student's *t* test was $p < 0{,}001$.

3.1.1.1 Diabetic and non-diabetic patients of G1 (≤ 55y)

For G1 the total number of cases was 4, 2 for DP and 2 for NDP in age matched groups. The mean age for DP and NDP was 52±50. The sex distribution (male/female ratio) for DP was 2/0 and for NDP was 2/0. The eye distribution (right eye/left eye) for DP was 1/1 and for NDP was 2/0. The mean thickness of ACBM was 10.0±00u for DP and 8.50±0.71u for NDP (Table 3.2). There was not a statistically significant difference in the thickness of ACBM between DP and NDP, $p = 0.95$ (Figure 3.2).

3.1.1.2 Diabetic and non-diabetic patients of G2 (56-65y)

For G2 the total number of cases was 34, 17 for DP and 17 for NDP in aged matched groups. The mean age for DP and NDP was 60.71±2.68. The sex distribution (male/female ratio) for DP was 4/13 and for NDP was 8/9. The eye distribution (right eye/left eye) for DP was 10/7 and for NDP was 8/9. The mean thickness of ACBM was 10.71±1.79u for DP and 8.94±0.89u for NDP (Table3.3). There was a statistically significant difference in the thickness of ACBM between DP and NDP, $p = 0.001$ (Figure 3.2).

Table 3.2 Characteristics of DP and NDP of G1

	DP	NDP
N	2	2
Age (years)	52.50 ± 0.70	52.50 ± 0.70
Sex (M/F)	2/0	2/0
Eye R/L	1/1	2/0
ACBM thickness (u)	10.00±0.00	8.50 ± 0.70

Data are means ± SD

Table 3.3 Characteristics of DP and NDP of G2

	DP	NDP
N	17	17
Age (years)	60.71± 2.68	60.71± 2.68
Sex (M/F)	4/13	8/9
Eye R/L	10/7	8/9
ACBM thickness (u)	10.71 ± 1.79	8.94 ± 0.89

Data are means ± SD

3.1.1.3 Diabetic and non-diabetic patients of G3 (66-75y)

For G3 the total number of cases was 68, 34 for DP and 34 for NDP in aged matched group. The mean age for DP and NDP was 71.26±2.50. The sex distribution (male/female ratio) for DP was 14/20 and for NDP was 13/21. The eye distribution (right eye/left eye) for DP was 20/14 and for NDP was 15/19. The mean thickness of ACBM was 11.29±1.50u for DP and 8.88±0.68u for NDP (Table 3.4). There was a statistically significant difference in the thickness of ACBM between DP and NDP, $p <$ 0.001 (Figure 3.2).

3.1.1.4 Diabetic and non-diabetic patients of G4 (≥76y)

For G4 the total number of cases was 92, 46 for DP and 46 for NDP in aged matched group. The mean age for DP and NDP was 80.50±4.25. The sex distribution (male/female ratio) for DP was 18/28 and for NDP was19/27. The eye distribution (right eye/left eye) for DP was 23/23 and for NDP was 18/28.The mean thickness of ACBM was 11.33±1.53u for DP and 9.63±0.92u for NDP (Table 3.5). There was a statistically significant difference in the thickness of ACBM between DP and NDP, $p < 0.001$ (Figure 3.2).

Table 3.4 Characteristics of DP and NDP of G3

	DP	NDP
N	34	34
Age (years)	71.26± 2.50	71.26± 2.50
Sex (M/F)	14/20	13/21
Eye R/L	20/14	15/19
ACBM thickness (u)	11.29 ± 1.50	8.88 ± 0.68

Data are means ± SD

Table 3.5 Characteristics of DP and NDP of G4

	DP	NDP
N	46	46
Age (years)	80.50± 4.25	80.50± 4.25
Sex (M/F)	18/28	19/27
Eye R/L	23/23	18/28
ACBM thickness (u)	11.33 ± 1.53	9.63 ± 0.92

Data are means ± SD

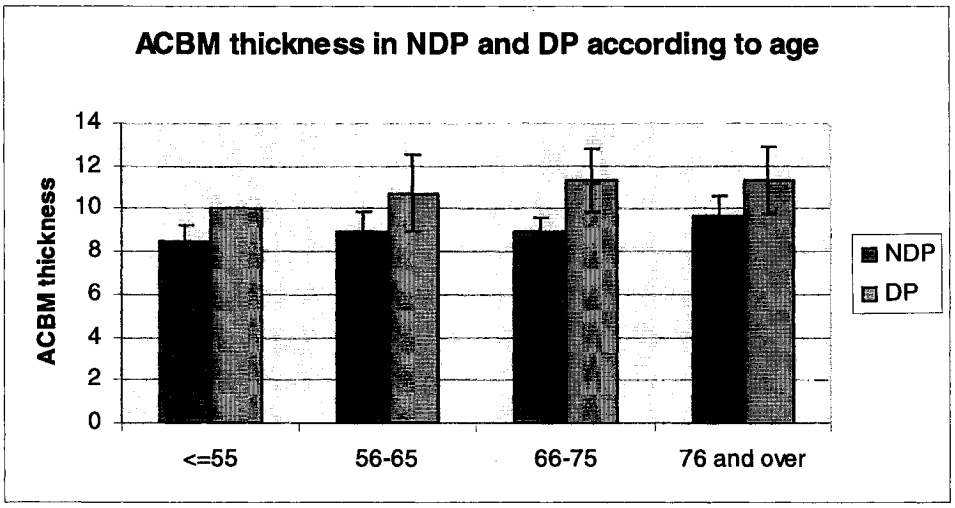

Figure 3.2:The graphs show the changes in the mean thickness of anterior capsule basement membrane (ACBM) of non-diabetic patients (NDP) and diabetic patients (DP) according to age. The mean ACBM thickness of NDP and DP, of G1 ≤ 55 years old were 8.50±0.71u and 10.0±00u, of G2 56-65 years old were 8.94±0.89u and 10.71±1.79u, of G3 66-75 years old were 8.88±0.68u and 11.29±1.50u, and of G4 ≥ 76 were 9.63±0.92u and 11.33±1.53u, respectively.

3.2 Diabetic patients' characteristics according to the duration of the disease

3.2.1 Diabetic patients of D1 (≤ 5 years)

For D1 the total number of cases was 48. The mean age was 72.5±9.59. The sex distribution (male/female ratio) was 22/26. The eye distribution (right eye/left eye) was 24/24. The mean thickness was 10.98±1.65u (Figure3.3) (Table 3.6).

3.2.2 Diabetic patients of D2 (6-10 years)

For D2 the total number of cases was 19. The mean age was 74.16±7.91. The sex distribution (male/female ratio) was 4/15. The eye distribution (right eye/left eye) was 8/11. The mean thickness was 11.37±1.50u (Figure3.3) (Table 3.6).

3.2.3 Diabetic patients of D3 (≥ 11 years)

For D3 the total number of cases was 33. The mean age was 73.97±7.42. The sex distribution (male/female ratio) was 13/20. The eye distribution (right eye/left eye) was 22/11. The mean thickness was 11.36±1.40u (Figure3.3) (Table 3.6).

Table 3.6 Characteristics of DP according to the duration of the DM

	D1	D2	D3
N	48	19	33
Age (years)	72.5 ± 9.59	74.16 ± 7.91	73.97 ± 7.42
Sex (M/F)	22/26	4/15	13/20
Eye R/L	24/24	8/11	22/11
ACBM thickness (u)	10.98 ± 1.65	11.37 ± 1.5	11.36 ± 1.4

Data are means ± SD

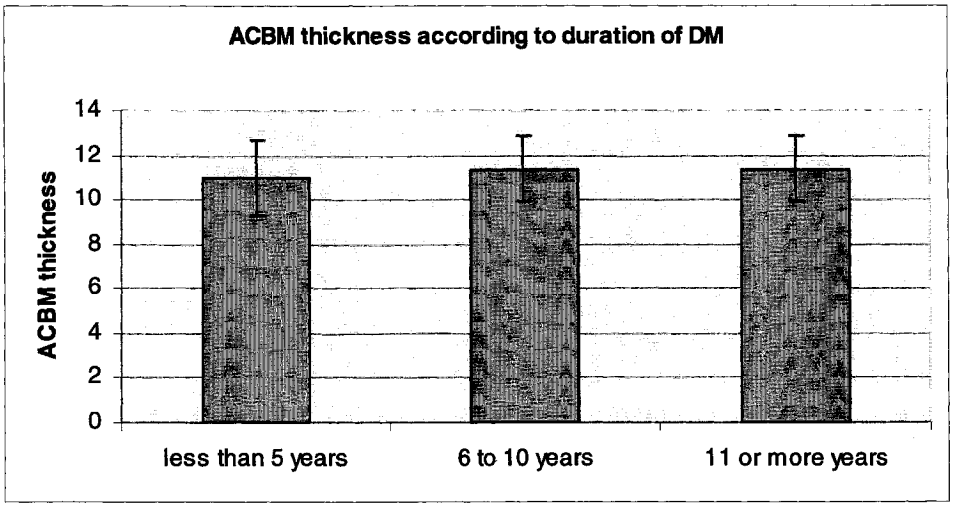

Figure 3.3: The graph shows the mean thickness changes of anterior capsule basement membrane (ACBM) of diabetic patients (DP) according to the duration of diabetes mellitus (DM). In D1 patients (DM < 5 years), the mean thickness of ACBM was 10.98±1.65u. In D2 patients (DM between 6-10 years) and in D3 patients (DM ≥ 11 years) the ACBM thickness were 11.37±1.50u and 11.36±1.40u, respectively.

3.3 Diabetic patients' characteristics according to the severity of the disease

3.3.1 Diabetic patients with no retinopathy

The total number of cases was 73. The mean age was 73.33±8.92. The sex distribution (male/female ratio) was 27/46. The eye distribution (right eye/left eye) was 38/35. The mean thickness was 11.14±1.61u (Figure 3.4) (Table 3.7).

3.3.2 Diabetic patients with non-proliferative diabetic retinopathy (NPDR)

The total number of cases was 27. The mean age was 73.19±7.69. The sex distribution (male/female ratio) was 12/15. The eye distribution (right eye/left eye) was 16/11. The mean thickness was 11.30±1.43u (Figure 3.4) (Table 3.7).

3.3.3 Diabetic patients with proliferative diabetic retinopathy (PDR)

The total number of cases with proliferative diabetic retinopathy was zero.

Table 3.7 Characteristics of DP according to the severity of the DM

	No Retinopathy	**NPDR**
N	73	27
Age (years)	73.33 ± 8.92	73.19 ± 7.69
Sex (M/F)	27/46	12/15
Eye R/L	38/35	16/11
ACBM thickness (u)	11.14 ± 1.61	11.30 ± 1.43

Data are means ± SD

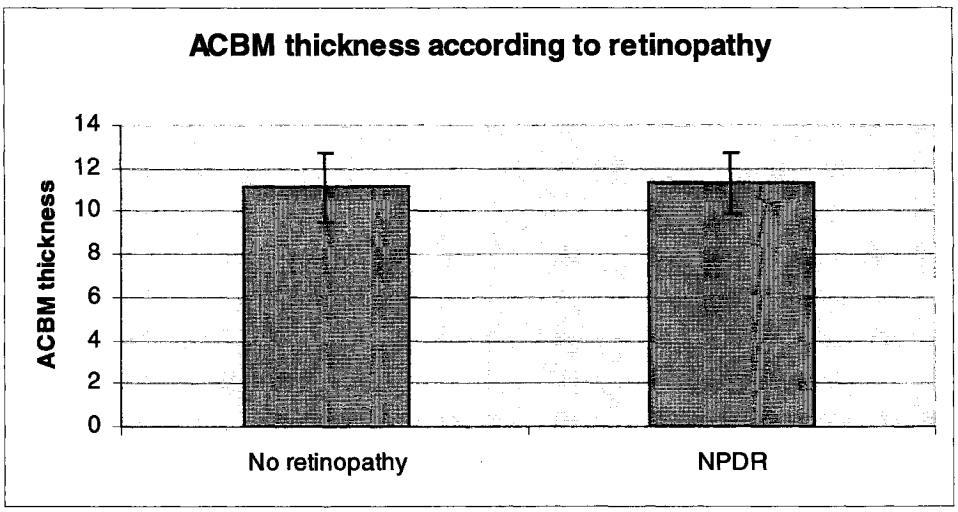

Figure 3.4: The graph shows the mean thickness changes of anterior capsule basement membrane (ACBM) of diabetic patients (DP) according to the severity of diabetic retinopathy. The mean thickness of ACBM of DP without retinopathy and with non-proliferative diabetic retinopathy (NPDR) were 11.14±1.61u and 11.30±1.43u, respectively.

3.4 The relationship between the age and the increasing thickness of ACBM of both DP and NDP

The ACBM thickness increased with increasing age in both DP and NDP. The p value was not significant in DP, $p = 0.08$. On the other hand it was significant in NDP, $p < 0.001$. The correlation coefficient of ACBM of DP was Rsq=0.029, and of NDP was Rsq=0.156 (Figure 3.5).

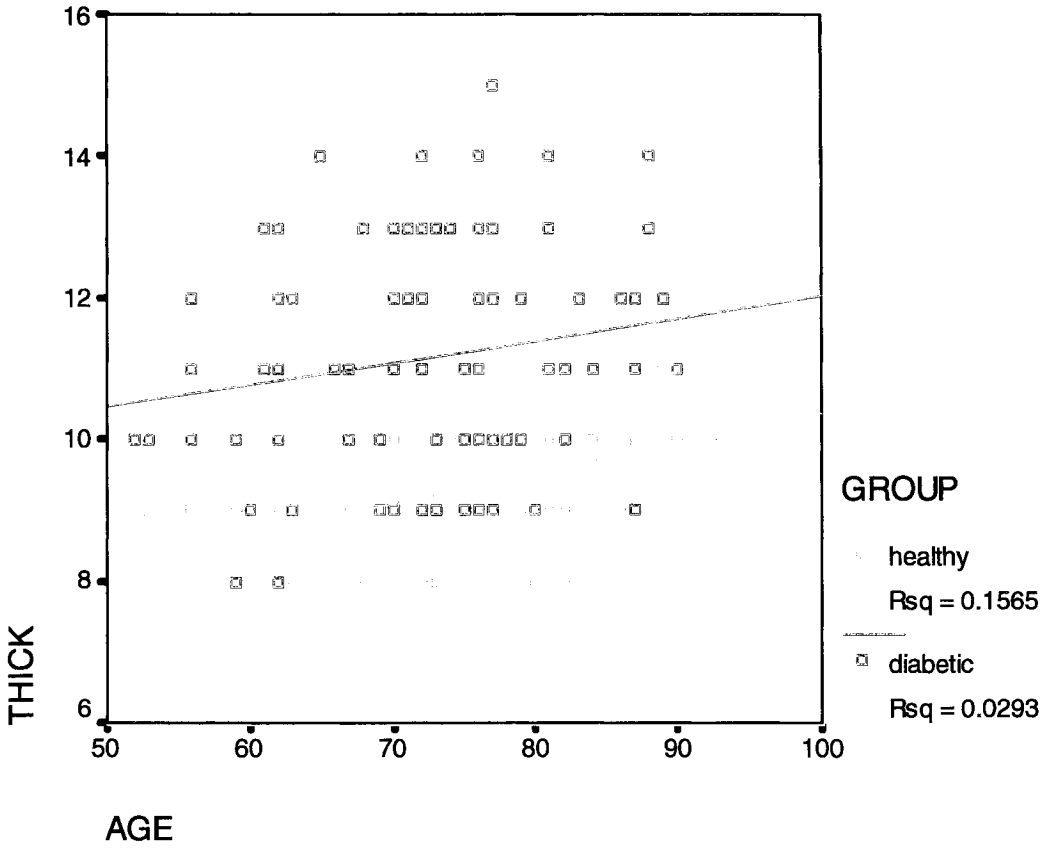

Figure 3.5: The plot displays the increasing thickness of anterior capsule basement membrane (ACBM) of non-diabetic patients (NDP) and diabetic patients (DP) according to age.

3.5 The relationship between increasing thickness of ACBM of DP and the duration of diabetes

The ACBM thickness increased with increasing duration of diabetes mellitus (DM), but was not statistically significant. The correlation coefficient of ACBM of DP of D1 (DM ≤ 5 years) was Rsq=0.035, of D2 (DM 6-10 years) was Rsq=0.050, and of D3 (DM ≥ 11 years) was Rsq=0.005 (Figure 3.6).

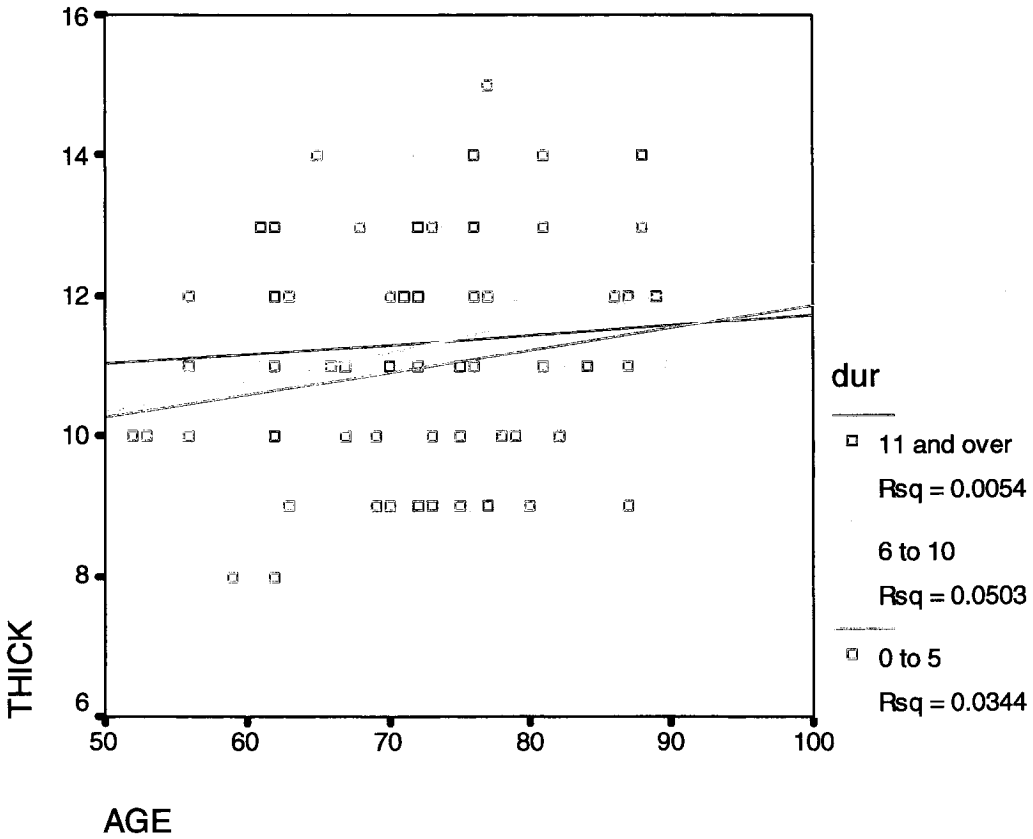

Figure 3.6: The plot displays the increasing thickness of anterior capsule basement membrane (ACBM) of diabetic patients (DP) according to the duration of diabetes mellitus (DM).

3.6 The relationship between increasing thickness of ACBM of DP and the severity of diabetes

The ACBM thickness increased with increasing severity of diabetic retinopathy, but was not statistically significant. The correlation coefficient of ACBM of DP without retinopathy was Rsq=0.026, and of ACBM of DP with NPDR was Rsq=0.041 (Figure 3.7).

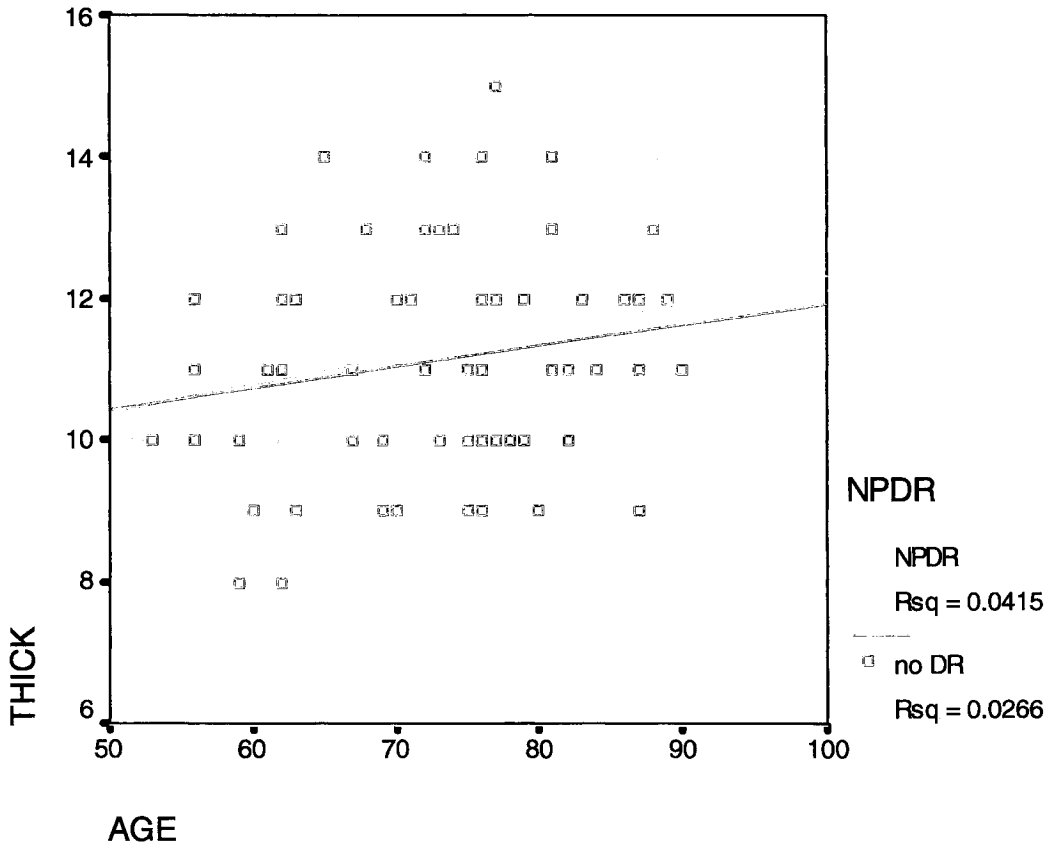

Figure 3.7: The plot displays the increasing thickness of anterior capsule basement membrane (ACBM) of diabetic patients (DP) according to the severity of diabetes.

CHAPTER 4

Discussion

4.1 Introductory remarks

Previous studies measured the thickness of the lens capsule in humans and animals, but did not compare the thickness between DP and NDP. To the best of our knowledge this is the first study that measures the ACBM thickness in DP and NDP. The reason for this study was to determine, for the first time, whether diabetes could cause thickening of ACBM of the lens and correlate the findings with the duration and the severity of the disease.

4.2 The relationship between diabetes and increasing thickness of ACBM

Increasing thickness of the glomerular basement membrane occurs soon after the onset of diabetes and gradually increases with the clinical manifestations of diabetic nephropathy (Obineche et al., 2001). In addition to the increased thickness of the glomerular basement membrane, the basement membrane underlying the proximal tubular epithelial cells shows similar abnormalities when diabetic nephropathy develops. A thickened tubular basement membrane is part of diabetic tubulo-interstitial disease, and represents a better indicator of diabetic nephropathy than glomerular basement membrane thickening (Phillips et al., 2001; Trachtman et al., 2002).

On the other hand, changes in the vascular basement membranes of retinal vessels in diabetic patients with diabetic retinopathy may develop along with or without the development of diabetic nephropathy. As in the kidney, the histopathological changes of diabetic retinopathy include the thickening of the retinal capillary basement membranes,

loss of intramural pericytes and the formation of aberrant neovascularization, which in fact, if remained untreated, leads to blindness (Chavers et al., 1994; Kim et al., 1998).

The first objective of this thesis was to investigate and compare the ACBM thickness between DP and NDP. Our study confirmed that ACBM thickening is indeed a major morphological feature in DP. Our data showed that the rate of thickening in the ACBM of DP varies in different samples of ACBM. Anterior capsule basement membrane thickening was not universal, and some of ACBM samples in DP were not significantly different from those in control groups. However, most of ACBM in DP showed increased thickening compared to their normal counterparts. Our data showed that the mean thickness of ACBM in DP (11.18±1.56u) was significantly thicker compared to the mean thickness of ACBM in NDP (9.24±0.91u). The computed, student *t*-test was $p << 0.001$.

Moreover, our data showed that the ACBM thickness was increased with increasing age in both DP and NDP. These findings confirmed the results of other studies where they found that lens capsule thickness increases throughout the life (Eagle Jr, 1996). However, in this particular study it was found that the increasing thickness of ACBM was more significant in DP compared to NDP.

The second objective of this thesis was to correlate the ACBM thickness of DP with the duration of diabetes. The thickness of ACBM in DP was increased with increasing duration of the disease. Although the mean thickness of ACBM in DP that have had the disease for less than five years was (10.98±1.65u) compared to mean thickness of ACBM in DP that have had the disease for more than ten years was (11.36±1.4u) was not statistically significant. It has been found that there was a weak correlation between

the increasing thickness of ACBM of DP and the duration of the disease. The correlation coefficient for D1, D2, and D3 groups were Rsq = 0.035, Rsq= 0.050, and Rsq = 0.005 respectively. This was consistent with other studies, which found that the increasing thickness of glomerular basement membrane was the earliest finding of diabetic patients before developing the renal failure (Obineche et al., 2001; Phillips et al., 2001).

The third objective of this thesis was to correlate the ACBM thickness in DP with the severity of diabetic retinopathy. The thickness of ACBM in DP was increased with increased severity of diabetic retinopathy. The mean thickness of ACBM was 11.14±1.61u in DP that did not develop the retinopathy yet, while it was 11.30±1.43u in DP that already developed NPDR. Since we did not have any case of proliferative diabetic retinopathy (PDR) in our population sample we could not compare the ACBM thickness between DP with PDR and DP without retinopathy. It was noted that ACBM thickness was increased with increasing severity of retinopathy. The increasing thickness of ACBM in DP according to the severity of retinopathy was not statistically significant. On the other hand it was found that there was a weak correlation between the increasing thickness of ACBM in DP and the severity of diabetic retinopathy. The correlation was stronger in the ACBM of DP with NPDR (Rsq= 0.041) compared to the ACBM of DP without retinopathy (Rsq=0.026).

4.3 The relationship between HbA1c levels, ACBM thickness and cataract formation

It is already known that cataracts have an increased incidence and a decreased age of onset in diabetic patients compared to healthy people (Di Benedetto et al., 1999;

Klein et al., 1995). Several previous studies have attempted to understand the underlying mechanism by using different types of animals such as rats, mice and dogs for experimental models (Cheng, 2002; Hegde et al., 2003; Takamura et al., 2003).

Until now the mechanisms of diabetic cataract formation were not well understood. It seems that the induction of cataracts in diabetic patients can be related to multiple biochemical effects such as oxidative stress and advanced glycation end products (Franke et al., 2003; Hegde et al., 2003). These factors have been well documented in the literature (Boscia et al., 2000; Delcourt et al., 2003; Zoric, 2003). Moreover, studies have shown that increased glycosylated hemoglobin levels were associated with increased risk of nuclear and cortical cataracts in diabetic patients compared to non-diabetic patients. For those diabetic patients a 1% difference in glycosylated hemoglobin was associated with an average of 15% increase in risk of having nuclear cataract and 12% increase in risk of having cortical cataract (Klein et al., 1998a).

Little is known about the role of the lens capsule in the induction of cataract in diabetics and non-diabetics patients. As we know the lens capsule is the thickest basement membrane in the body (Grant M E, 1982). The capsule is the first barrier that permits the nutrients to enter the lens, and allows the waste products to leave the lens. This means that if the normal physiology of the lens capsule has been changed due to diabetes, the overall metabolism of the lens will be affected. On the other hand, we already know from previous studies that the development of diabetic microvascular disease is associated with the thickening of the basement membrane in different tissues and organs (Carlson et al., 2003; Danis et al., 1996). We also know that poor glycemic control in diabetic patients is one of the factors that can accelerate the cataract formation (Di

Benedetto et al., 1999; Shin et al., 1994). Our preliminary results with thirty cases of ACBM of DP showed that there is a direct relationship between the increasing levels of glycosylated hemoglobin (HgA1c) and increased thickness of ACBM (Bakalian S, 2004). The results of our present study have confirmed that the ACBM in DP is significantly thicker compared to NDP (Table 3.1) (Figure3.1). Moreover, a positive linear relationship has been found between the increasing thickness of the ACBM in accordance to the duration and the severity of diabetes (Figure 3.6) (Figure 3.7). Taking all this into consideration we believe that the increasing thickness of ACBM in DP could play an important role in the induction and acceleration of cataract in DP compared to NDP. Although the results of current research do not explain the structural changes of the observed increase in ACBM of DP compared to NDP, they do shed some light on several explanations that could be possible for the mentioned results. One of the possible mechanisms could be due to the over expression of collagens in the lens capsule.

4.4 The relationship between collagen IV expression, ACBM thickness and cataract formation

Collagen type IV is the main component of the basement membrane. It consists of six genetically distinct chains. It has been proposed that basement membrane heparin sulfate proteoglycan synthesis was decreased in diabetic state and that basement membrane thickening might occur as a result of collagen and laminin hyper secretion (Mohan and Spiro, 1986). In support of this observation, an increased synthesis of type IV collagen in the glomerular basement membrane of diabetic animals has been reported (Grant et al., 1976). In order to evaluate the expression of collagen III and collagen IV in

the ACBM of DP and NDP, we performed another study. The immunohistochemical analysis of eighty ACBM specimens from DP and NDP showed that collagen IV was the main component of the ACBM in DP and NDP. Moreover, data found in our study proved that the increasing thickness of ACBM in DP was due to over expression of collagen IV (Bakalian S, 2003). These findings agreed with the results of other studies where they found that the distribution of collagen varied quantitatively and qualitatively with the age and the formation of cataract (Hatae et al., 1993; Kawarazaki, 1996; Kelley et al., 2002).

4.5 Implications

Anterior capsule basement membrane thickness measurement following cataract surgery may serve as a useful prognostic marker for the development of ocular complications such as diabetic retinopathy and cataract. The results of this current thesis have shed some light on the possible involvement of the lens capsule in the formation of cataracts of DP compared to NDP. We believe that by studying the structural changes of the lens capsule of both DP and NDP we will be able to answer more definitive questions about the role of the lens capsule in the induction of cataracts. Consequently, this will help us search for ways to slow or prevent the progression of cataracts in DP and NDP.

4.6 Future studies

Diabetes is a major concern and a big health issue due to the increasing number of people with type 2 diabetes all over the world and especially in North America

including Canada (American Diabetes Association, 1998; Amos et al., 1997; Canada, 1999). This study showed that ACBM is thicker in DP compared to NDP. The increasing thickness of ACBM in DP was dependent on the duration and the severity of the disease. This study has revealed a relationship between the increasing thickness of the ACBM and the increasing risk of developing cataract in DP. Further research and studies will be needed to elucidate the exact role of the ACBM in cataract formation.

In the future studies with more samples could be done to confirm the results of our previous study where the thickness of ACBM in DP was correlated with HgA1c levels. HgA1c represents the average of blood glucose levels in the blood of the DP for the past 3 months. HgA1c is commonly used to assess long-term blood glucose control in DP. It has been shown that HbA1c value is useful to predict the risk of developing the ocular complications in DP.

Further in vivo studies with the aid of new anterior optical coherence tomography (OCT) will be helpful to determine the role of ACBM thickness in assessing and quantifying the risk of developing cataracts in diabetic and non-diabetic patients. With this technique it will be possible to compare the ACBM thickness with or without cataracts in diabetic and non-diabetic patients. This will allow us to assess the effectiveness of measuring the ACBM thickness to use it as a prognostic marker to evaluate the risk of developing the ocular complications. On the other hand, it will confirm the recent findings of our study.

4.7 Summary

This is the first study in humans that measures the ACBM thickness in DP and NDP. The distribution of DP and NDP according to their age was the same in this study.

This was done on purpose to minimize the bias. The results of this study showed that age plays an independent role in the increasing thickness of ACBM regardless of diabetes. On the other hand it was found that diabetes accelerates the increasing thickness of ACBM. Moreover, the results of this study indicate that the duration and the severity of diabetes are also important factors that play a significant role in the increasing thickness of ACBM of DP.

4.8 Conclusion

This thesis provides evidence that the ACBM is significantly thicker in DP compared to NDP. Moreover, the thickness of the ACBM in DP correlates with the age of the patients as well as the duration and the severity of the disease. Furthermore the increasing thickness of ACBM plays a role in the induction and acceleration of cataracts in diabetic patients.

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