# THE RELATIONSHIP BETWEEN DIABETES MELLITUS AND EXFOLIATION SYNDROME IN A UNITED STATES VETERANS POPULATION: A CASE-CONTROL STUDY

A thesis presented to the graduate faculty of The New England College of Optometry in partial fulfillment of the requirements for the degree of Master of Science

Sarah Dougherty Wood

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Sarah Dougherty Wood

This manuscript has been read and accepted by the Thesis Committee in satisfaction of the thesis requirement for the degree of Master of Science

4/27/10
Date

Barry Fisch, OD
Graduate Faculty Advisor

Louis R. Pasquale, MD

Anthony Cavallerano, OD

5/21/10
Date

Nancy J. Coletta, OD, PhD
Director of Graduate Studies

The New England College of Optometry

This work was presented at The World Glaucoma Congress, July 2009 and at the American Academy of Optometry Meeting, October 2009

**Abstract**

Purpose: Previous studies suggest an inverse relationship exists between diabetes mellitus and exfoliation syndrome. This study evaluated the relationship between diabetes mellitus and exfoliation syndrome while controlling for important covariates. In addition, glucose control, as measured by glycosylated hemoglobin (HbA1c) levels, was investigated to determine if it differed between the subset of diabetic patients with and without exfoliation syndrome.

Patients and Methods: This retrospective, case-control study included outpatients seen in Veterans Affairs (VA) Boston Healthcare System eye clinics. Exfoliation cases (n=328) and controls (n=328) were drawn from the same clinic and matched for age. For all subjects, diabetes status, gender, race, body mass index, and glaucoma status were ascertained. Among patients with diabetes mellitus, the five most recent HbA1c levels and type of diabetes control were collected.

Results: Diabetes mellitus was present in 96 (29.2%) cases and in 114 (34.8%) controls. In multivariate analysis, no statistically significant relationship between diabetes mellitus and exfoliation syndrome (OR = 0.77; 95% CI, 0.55-1.07) was identified. When glaucoma status

was added as a covariate, the results were essentially unchanged (OR = 0.81, 95% CI, 0.57-1.14). Adjusted mean HbA1c levels were similar in diabetic subjects with (6.85%; 95% CI, 6.66-7.04) and without (7.05%; 95% CI, 6.87-7.22) exfoliation syndrome (p=0.14).

Conclusion: In this predominately white male population, no statistically significant relationship between diabetes mellitus and exfoliation syndrome was observed. In addition, HbA1c levels did not vary among diabetics subjects based on exfoliation status.

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## Chapter 1: General Introduction

The prevalence of open-angle glaucoma worldwide in 2010 is approximately 45 million people and is expected to continue to increase (Quigley and Broman,2006). Glaucoma is a potentially blinding disorder which can result in diminished quality of life. It is a complex disease characterized by progressive optic nerve damage. Intraocular pressure (IOP) is often a critical contributory factor to the damage and the exact cause of elevated levels of IOP is not completely understood in the majority of glaucoma cases. The most clinically identifiable risk factor for elevated IOP and resultant open angle glaucoma is exfoliation syndrome (ES), also known as pseudoexfoliation syndrome (Ritch, Schlotzer-Schrehardt and Konstas,2003).

Clinically, the deposits of exfoliation syndrome are found in the eye. Electron microscopy and histological studies have shown that the deposits are also present in several tissues and organs throughout the body. The etiology of ES is not well understood, nor is its possible link to systemic disease. Speculation has been made that ES has a relationship with diabetes mellitus (DM), a common disease which also has systemic and ocular effects and has also been linked to open-angle glaucoma (Chopra, Varma, Francis, Wu, Torres and Azen,2008 ; Dielemans, de Jong, Stolk, Vingerling, Grobbee and Hofman,1996 ; Klein, Klein and Jensen,1994 ; Mitchell, Smith, Chey and Healey,1997a ; Pasquale, Kang, Manson, Willett, Rosner and Hankinson,2006). Previous studies have yielded conflicting results regarding the relationship between DM and ES and thus this relationship remains unclear. In this study, we explored the relationship between these two diseases via a retrospective case control study aimed to resolve the issue by including a large number of subjects, using a

careful study design, and included important covariates in the analysis. Chapter 2 provides a general overview of ES, a review of the studies looking at the relationship between ES and DM, speculation on the cause of their relationship, and the objectives of our study. Chapter 3 includes the designs and methods of the study. Chapter 4 reveals the results of the analysis. Finally, Chapter 5 contains the discussion, which includes an examination of the strengths and limitations of the study, future directions, and the conclusion.

## Chapter 2: Exfoliation Syndrome

### 2.1 ES, the eye, and glaucoma

ES is a generalized disorder of the extracellular matrix characterized by the production and accumulation of grayish-white, dandruff-like material throughout the anterior segment of the eye including the lens, lens capsule, iris, trabecular meshwork, and lens zonules. Several constituents of ES deposits have been identified but the exact composition and the cause of their formation remain unknown (Lemmela, Forsman, Sistonen, Eriksson, Forsius and Jarvela,2007). The deposits are known to contain a heterogeneous mixture of proteins such as non-collagenous basement membrane components (fibronectin, laminin) and epitopes of the elastic fiber system (microfibrils, tropoelastin, elastin)(Ritch, et al.,2003), most of which are normally present in the aqueous. The nature and diversity of ES deposits indicate their formation is undoubtedly a complex process. ES is perhaps part of a group of conditions called protein folding disorders, in which soluble proteins change their native

folding and form insoluble complexes that accumulate and aggregate in solution (Ovodenko, Rostagno, Neubert, Shetty, Thomas, Yang, Liebmann, Ghiso and Ritch,2007).

The deposits of ES have the potential to raise IOP and increase the risk of glaucoma. In a normal, healthy eye, aqueous humor is produced in the ciliary body. The aqueous bathes the anterior segment structures and then drains out of the eye into episcleral veins via the trabecular meshwork, Schlemms canal, and uveoscleral outflow. If disruption in the drainage of aqueous occurs, it backs up and the intraocular pressure rises. In ES, the deposits and associated pigment block the outflow and damage the critical endothelial pumping cells lining the trabecular meshwork. Once the intraocular pressure increases, there is an increased risk of glaucomatous optic neuropathy. In one study found that the 15-year risk of ES syndrome conversion to ES glaucoma was 44% (Jeng, Karger, Hodge, Burke, Johnson and Good,2007). Another study found the risk of elevated IOP in ES after 5 years was 5.3% and 15.4% after 10 years (Henry, Krupin, Schmitt, Lauffer, Miller, Ewing and Scheie,1987). Thus, the risk for elevated IOP and furthermore, the risk of glaucoma, is significant for eyes with ES. In general, exfoliative glaucoma tends to have higher intraocular pressures at diagnosis and progresses more rapidly when compared to primary open angle glaucoma (POAG) making it a more aggressive form of glaucoma (Leske, Heijl, Hussein, Bengtsson, Hyman and Komaroff,2003). ES has a high ocular burden with other possible complications such as: angle closure glaucoma, cataracts, cataract surgery complications, such as vitreous loss and capsular rupture, neovascularization of the iris, central retinal vein occlusions, and possibly pseudouveitis and corneal decompensation (Naumann, Schlotzer-Schrehardt and Kuchle,1998 ; Ritch and Schlotzer-Schrehardt,2001).

## 2.2 Age relevance and ES

Exfoliation syndrome is an age-related condition with the mean age of 76 years at diagnosis, found in one 15-year incidence study (Karger, Jeng, Johnson, Hodge and Good,2003). The prevalence of ES continues to increase with age. In a study in Iceland, 2.6% of those age 50-59 years had ES, where up to 40% in those over the age of 80 had this condition (Arnarsson, Damji, Sverrisson, Sasaki and Jonasson,2007).

## 2.3 Distribution of ES

Prevalence ranges will vary widely with different geographic populations. Eskimos have virtually no ES whereas in the Navajo Indians the prevalence may be as high as 38% (Faulkner,1971). Generally, ES is found to have widespread geographic distribution (Aasved,1969) with particularly high prevalence in Scandinavian countries (Jonasson,2007). For example, in those over age 60, ES has about a 25% prevalence in Iceland and over 20% in Finland. In some countries, ES accounts for the majority of glaucoma cases (Ritch,1994).

## 2.4 LOXL1 Gene

Due to the geographic distribution of cases, a hereditary component to the development of ES has historically been generally accepted but a specific gene variant had not been identified until recently. Thorleifsson, et al discovered single nucleotide

polymorphisms (SNPs) in the LOXL1 (lysyl oxidase-like one) gene present in 99% of an Icelandic and Swedish population with ES (Thorleifsson, Magnusson, Sulem, Walters, Gudbjartsson, Stefansson, Jonsson, Jonasdottir, Stefansdottir, Masson, Hardarson, Petursson, Arnarsson, Motallebipour, Wallerman, Wadelius, Gulcher, Thorsteinsdottir, Kong, Jonasson and Stefansson,2007). Subsequently, similar results have been found in numerous populations throughout the world (Challa,2009). LOXL1 is a protein which catalyzes the extracellular cross-linking of tropoelastin which is laid down on a microfibril scaffold. The final result is the formation of mature elastin polymer (Liu, Zhao, Gao, Pawlyk, Starcher, Spencer, Yanagisawa, Zuo and Li,2004). It remains unclear exactly how the SNPs in LOXL1 trigger the accumulation of fibrillar material in the eye. This discovery was a breakthrough in the understanding of ES. However, the high prevalence of LOXL1 gene variants was present in up to 88% of controls in these studies. This suggests that other unknown factors, perhaps environmental and/or genetic, must also contribute to the ocular manifestations of ES (Challa,2009).

**2.5 ES and the systemic implications**

Exfoliation deposits are present not only in the eye, but have also been identified in the connective tissue of organs such as the lungs, skin, heart, kidney, gall bladder, and cerebral meninges as seen by electron microscopy (Schlotzer-Schrehardt and Naumann,2006). This implies that ocular manifestations of ES may be just part of a generalized systemic biological process. Numerous studies have attempted to determine if these deposits have systemic consequences and the results are controversial and inconclusive.

ES has been possibly linked to an increased risk of medical conditions, most notably abdominal aortic aneurysms, sensory neural hearing loss (Shaban and Asfour,2004), and cardiovascular disease (Mitchell, Wang and Smith,1997b).

It has been suggested that ES may increase morbidity mainly due to increased risk of systemic vascular disease (Tarkkanen,2008) and perhaps due to elastin abnormalities in blood vessel walls (Mitchell, et al.,1997b). Risk of increased vascular disease is also supported by the discovery of elevated plasma, aqueous humor, and tear fluid homocysteine levels in ES subjects in several studies (Leibovitch, Kurtz, Shemesh, Goldstein, Sela, Lazar and Loewenstein,2003 ; Puustjarvi, Blomster, Kontkanen, Punnonen and Terasvirta,2004 ; Roedl, Bleich, Reulbach, Rejdak, Kornhuber, Kruse, Schlotzer-Schrehardt and Junemann,2007 ; Vessani, Ritch, Liebmann and Jofe,2003). Increased homocysteine levels are thought to be linked to systemic vascular disease (Arnesen, Refsum, Bonaa, Ueland, Forde and Nordrehaug,1995). In addition, evidence of ischemia due to decreased vascular perfusion has been found in ES. Specifically, studies show specifically reduced ocular, retrobulbar, cerebral, and carotid blood flow (Ritch,2008). Examples of ischemia in an ES eye are the presence of neovascularization of the iris and the risk of central retinal vein occlusion (Naumann, et al.,1998). Tarkkanen (Tarkkanen,2008) reviewed the literature on ES and vascular disease, specifically arterial hypertension, ischemic heart disease, aneurysms of the abdominal aorta, and diabetes mellitus. He concluded that currently no molecular genetics findings link ES and vascular diseases and that more large-scale, randomized clinical studies need to be conducted to further understand these relationships. Clarification of such associations would have clinical relevance because patients who present with ES may

need to be referred for systemic testing if they are at increased risk of other health complications.

### 2.5.1 DM and ES

There are several parallels between DM and ES. DM and ES are both systemic disorders that have an ocular effect, involve ischemia, possible increased risk of cardiovascular disease, and are associated with open-angle glaucoma. Interestingly, in some studies specifically involving DM and ES, instead of finding a positive association between DM with ES, the studies have found that DM is actually *inversely* related to ES (Jonas and Grundler,1998 ; Konstas, Tsatsos, Kardasopoulos, Bufidis and Maskaleris,1998 ; Psilas, Stefaniotou and Aspiotis,1991 ; Shingleton, Heltzer and O'Donoghue,2003 ; Tarkkanen, Reunanen and Kivela,2008). These results have not been supported in other studies including those in which no associations, neither positive or negative, were found (Brajkovic, Kalauz-Surac, Ercegovic, A, Susic and Buric,2001 ; Citirik, Acaroglu, Batman, Yildiran and Zilelioglu,2007 ; Miyazaki, Kubota, Kubo, Kiyohara, Iida, Nose and Ishibashi,2005).

## 2.6 Review of the current literature on DM and ES

### 2.6.1 Inverse relationship studies

Konstas, et al examined the pre-operative characteristics, including systemic disorders, of Greek patients with EG and POAG who were undergoing filtration surgery. In the 26 POAG subjects, they found 19.2% had DM. In the 74 EG subjects, 5.4% had diabetes ($p<0.05$) (Konstas, et al.,1998). Konstas recognized that this finding could be a result of

POAG being positively associated with DM rather than DM being negatively associated with ES. Additionally, the mean age of the EG group was statistically higher, yet there was no adjustment for age in the analysis.

Tarkkanen et al reviewed the records of 519 patients in Finland who were seeking free medication for glaucoma, either POAG or EG. The prevalence of systemic vascular disease was noted in these subjects. In the 344 subjects with POAG, 10% had DM while 5% of the 155 EG subjects had DM (p=0.047) (Tarkkanen, et al.,2008), which was significant even when age and gender adjustments were made. Again, just as in the Konstas study, the Tarkkanen study lacks a true control group because of the positive association between POAG and DM (Chopra, et al.,2008 ; Dielemans, et al.,1996 ; Klein, et al.,1994 ; Mitchell, et al.,1997a ; Pasquale, et al.,2006). As a result, the relationship of DM and EG remains unclear.

Jonas and Grundler's study sought to determine the prevalence of DM and arterial hypertension in primary and secondary open-angle glaucomas. In their study, they had 85 EG subjects, 529 POAG subjects, and 660 controls, with balanced gender pools. They found diabetes in 8.2% subjects with ES glaucoma and in 16.5% of age-matched controls without POAG, which just met statistical significance (p=0.05). There was a non-significant difference in DM prevalence between the EG and the age-matched POAG group (8.2% vs. 15.2%, p=0.10) (Jonas and Grundler,1998). Jonas and Grundler suggest further studies with larger numbers of subjects be conducted to clarify the DM and EG relationship.

Psilas and Stefaniotou prospectively examined subjects from Northwest Greece in a normal control group (n=489) and a diabetic group (n=325). The diabetic group was split

into three subgroups: 1) no diabetic retinopathy 2) non-proliferative diabetic retinopathy and 3) proliferative retinopathy. They found ES in 11% of the collective group of diabetics and 23.7% of controls, a difference which was statistically significant ($p<0.001$). The prevalence of ES seemed to decrease as the subjects had more retinopathy: 20.6% with no retinopathy, 6.2% with non-proliferative retinopathy, and 3.2% with proliferative retinopathy (Psilas, et al.,1991). They concluded that individuals with diabetic retinopathy exhibit significantly lower frequency of ES, when compared to those without diabetes or in diabetics without retinopathy. They did not attribute these findings to age or gender.

Shingleton et al performed a retrospective analysis on patients who underwent cataract surgery. There were 297 patients with ES and 427 without ES in the study. The study examined the prevalence of vascular disease in these patients, among several other characteristics. They found less DM in patients with ES. They found the prevalence of DM in non-ES subjects was 11% (47/409) and 5% (18/364) ES subjects. This difference was statistically significant ($p<0.01$) (Shingleton, et al.,2003). The mean age of the ES group was 5 years older than the non-ES group. The non-ES group was 59.7% women and the ES group was 70.0% women.

In summary, two of these studies (Konstas and Tarkkanen) did not have adequate control groups. Several of these studies had fairly small numbers of exfoliation subjects. All of these studies, except the Psilas study, involved only EG subjects, no ES subjects. Including only EG could potentially confound the results because EG subjects are likely to have had ES for a longer period of time. Also, not including ES in general could eliminate those with a milder form of the exfoliation syndrome.

### 2.6.2 No relationship studies

Brajkovic et al, examined 646 patients in an ophthalmology clinic in Croatia, 161 (25%) of whom had ES in one or both eyes. They noted the prevalence of diabetes and found DM in 20% of subjects with unilateral ES (n=60), 17% of patients with bilateral ES (n=101), and 22% of controls (n=485) had diabetes. This trend of less diabetes in ES was not statistically significant (p=0.49), so they concluded there was no relationship (Brajkovic, et al.,2001). The ES subjects were older and were not age matched in the analysis.

Citirik et al performed a case-control study in Turkey. The goal was to explore the relationship between ES and CAD. They matched for age and gender and found 11 diabetics in the ES group (n=40) and 15 diabetics in the non-ES group (n=60), (p=0.78), an insignificant result.

Miyazaki et al performed a cross-sectional, population-based study in Japan. They examined 50 ES patients and 1414 controls and found no significant difference in the presence of DM between the two groups (age-adjusted odd ratio of 0.57, 95% CI 0.20-1.62). Additionally, they found no statistical difference in the body mass index (BMI) between the two groups (OR=0.98, 98% CI 0.90-1.08). This study excluded patients who had a history of bilateral cataract surgery.

In summary, all three of these studies have fairly small numbers of subjects, particularly small numbers of ES subjects thus strong conclusions are difficult to make.

### 2.6.3 Speculation of the reason for the inverse relationship between DM and ES

While several studies found an inverse relationship between DM and ES, an explanation for these results is not clear. However, Tarkkanen speculated that a biological basis for an inverse relationship between DM and ES may involve a biochemical alteration in aqueous humor biochemistry. He suggested that glycation of critical basement membrane components prevent the accumulation of exfoliation material (Tarkkanen,2008). In general, glycation is the non-enzymatic bonding of a sugar molecule to a protein or lipid molecule. In diabetes, particular attention is paid to the glycation of the protein hemoglobin, specifically hemoglobin A1c. There is a two- to three-fold increase in glycated hemoglobin A1c in the red blood cells of diabetics and these levels reflect the blood sugar control over about 120 days, the life span of a red blood cell. This hemoglobin glycation serves as a reflection of the glycation of other biological proteins (Bunn, Gabbay and Gallop,1978). Tarkkanen's hypothesis is intriguing because the discovery of a glycation product that prevents accumulation of exfoliation material could lead to new pharmacological strategies to prevent the development of ES.

Another motivation to continue exploring the relationship between diabetes and exfoliation syndrome comes from a study by R. Lee which addresses the molecular pathophysiology of exfoliation glaucoma. He believes that the leaky iris vessels in ES allow for abnormal proteins (or normal proteins which are not normally present) to accumulate in the aqueous and bind to normal anterior segment proteins to form the ES deposits. Some of these proteins include hemoglobins and, in his proteonomic studies, he found a spatial separation within the anterior chamber of the types of hemoglobin in exfoliation patients.

Hemoglobin A1 (HA1, made of 2α and 2β chains) was present in the aqueous humor and hemoglobin A2 (HA2, made of 2α and 2δ chains) was found on the lens capsule. This implies the HA2 precipitates out of the aqueous while the HA1 does not, even though HA2 only accounts for 3% of the body's hemoglobin and HA1 accounts for the other 97%. To relate this to diabetes, HA1c (one specific subset of HA1) is the form measured to assess blood sugar control. Even though they are not measured clinically, HA2 also becomes more glycosylated with an increase in blood sugar levels in diabetes (Steinberg and Adams,1991). Could more glycosylation of HA2 prevent the HA2 from precipitating out of the aqueous, thus allowing for DM to be protective against ES? In the previous inverse relationship studies, none examined blood sugar levels to compare the diabetics with and without ES. Lee's article motivated this study to include HbA1c levels in the analysis (Lee,2008).

Another motivator to examine blood sugars was the Psilas study which concluded that the presence of ES makes the possibility of diabetic retinopathy rather unusual. It is known that tighter blood sugar control decreases the risk of diabetic retinopathy (Stratton, Kohner, Aldington, Turner, Holman, Manley and Matthews,2001). Perhaps the diabetics with tighter blood sugar control have decreased risk of retinopathy but increased risk of ES because they lack the glycation which is "protective" against ES. This would make it appear, indirectly via blood sugars, that ES and diabetic retinopathy are inversely related, as seen in the Psilas study (Psilas, et al.,1991). To test these ideas, recording of HbA1c levels in all diabetics in our study were included and analyzed.

### 2.7 Objectives of the study

Understanding how systemic diseases, such as DM, might relate to ES may provide insight into why exfoliation material accumulates in the eye and perhaps may suggest new therapeutic targets for the condition. After a review of the current literature, there appeared to be a need to further clarify the relationship between DM and ES in a large, case-control study. The primary objective of this study was to assess this relationship with adjustments for age, race, body mass index, lens status, presence of glaucoma, and type of diabetes control. Based on previous literature findings, we hypothesized that DM and ES were inversely related. The secondary objective of this study was to assess whether recent glycosylated hemoglobin (HbA1c) levels were different in diabetics patients with and without ES. Due to Tarkkanen's proposed mechanism of alteration of glycation in ES and the findings by Lee and Psilas et al, we speculated that higher levels of glycation, thus higher HbA1c levels, could be protective against ES. Therefore, higher blood sugar levels would be found in the controls.

## Chapter 3: Design and Methods

A retrospective, case-control study was performed among subjects examined at Boston Veterans Administration (VABHCS) eye clinics between January 2003 and December 2007. The Boston VA Institutional Review Board and Research and Development Committee approved the study protocol.

### 3.1 Inclusion and Exclusion criteria

All cases and controls were required to have had a comprehensive eye examination, including dilation. For ES cases, eye record review had to include documentation of the presence of exfoliation material on the lens capsule and/or pupillary ruff (bilateral or unilateral). These findings had to be confirmed by at least two independent examiners. Clinically, ES deposits can be difficult to detect even with careful observation and dilation, therefore, agreement between examiners helped increase the confidence of the documented diagnosis. Cases could have ES or EG. Controls were allowed to have the diagnosis of glaucoma, but not EG. Glaucoma status was determined by diagnosis in the patient chart and the use of pharmacologic or surgical management. For the purposes of this study, glaucoma suspects were categorized as non-glaucoma. Controls were excluded if medical record review indicated they had a history of cataract surgery without documentation of a pre-operative slit lamp examination, which would allow for detection of exfoliation material. All subjects were required to have a primary care appointment within one year of being identified as a case or control, including an evaluation for diabetes.

#### 3.1.1 Alteration of exclusion criteria for controls

The original exclusion criteria for the control group were: 1) no signs of exfoliation syndrome 2) documentation of a pre-operative slit lamp examination in the records if cataract surgery had been performed in either eye 3) no glaucoma present, of *any* type. The

rationale for the third requirement was due to the possible link between POAG and DM (Chopra, et al.,2008 ; Dielemans, et al.,1996 ; Klein, et al.,1994 ; Mitchell, et al.,1997a ; Pasquale, et al.,2006). In this study, if controls were allowed to have POAG and there is a positive association between POAG and DM, the results may reveal more DM in the control group which may falsely appear as less DM in ES. It would then be impossible to differentiate whether this was truly because DM and POAG are linked or because ES and DM are inversely related or both. In the data collection, the original control group (n=328) was collected as stated above. The results were as follows: 100 diabetics in the control group and 96 diabetics in the case group. Conditional logistic regression was performed with exfoliation status as the outcome variable, and age, BMI, DM status, gender, and race as covariates. None of the covariates were statistically significant including DM status (OR 0.93, 95% CI 0.67-1.32).

Upon re-examination of the controls criteria, the decision was made to allow the control population to be more reflective of a typical patient in an eye clinic, therefore, the control group was reconstituted, allowing for any type of glaucoma, except for exfoliative glaucoma. The controls were matched to the original case group from the same clinic and same day, if possible. To avoid the potential bias in our results regarding the link between POAG and DM, glaucoma status was added as a covariate in the logistic regression model.

### 3.2 Sample size determination

To determine the sample size, type one error of 0.05, power of 0.8, and a DM prevalence, based on the Jonas and Gundler study, of 8% in the ES population and 16% in the controls (Jonas and Grundler,1998) were used. This yielded a sample size of 228 cases and 228 controls. As the study progressed, it was clear that the study population had a higher burden of DM than had been assumed for the power calculation. Furthermore, the possibility was entertained that the presumed protective effect of DM might be more modest than originally anticipated; thus, the sample size was recalculated assuming a prevalence of DM of 23% in cases and 30% in controls yielding 328 in each group. This study was adequately powered to detect a 25% alteration in risk of ES among subjects with DM; a more modest effect would be undetected in this study.

### 3.3 Data collection

The VA computerized patient record system (CPRS) was used to generate a list of cases who received a diagnosis of ES (ICD-9-CM 366.11) or EG (ICD-9-CM 365.52) during the study period. The list of potential cases generated was randomized using the random number generator on Excel (Microsoft) which assigned each case a number and then these numbers were sorted into numerical order. The list was used from the top down until the adequate number of eligible cases was attained. If the case met the inclusion criteria, one control was drawn from the same clinic where the cases was identified, and on the same day, whenever possible. Controls were matched to cases 1:1 on the basis of age (in 5-year age intervals). Age matching was important since the prevalence of ES is closely tied to age. The

gender and self-declared race (white, black, Hispanic or other) of cases and controls were recorded but matching was not done on these parameters.

For all subjects, the presence or absence of DM was confirmed by any of the following: a diagnosis of DM entered on their problem list (ICD-9-CM 250.00), use of medications to treat DM (either insulin and/or an oral hypoglycemic agent), or reference to the diagnosis in a clinic note from their last primary care physician appointment. The methodology of identifying DM cases by (1) at least 2 coded outpatient visits for DM or (2) prescription for a DM medication has been validated within the VA with a very high degree of accuracy. A study published in 2004 found the best criterion for diabetes diagnosis in the VA was a prescription for a diabetes medication in the current year and/or 2+ diabetes codes from inpatient and/or outpatient visit over a 24-month period. This definition had high sensitivity (93%) and specificity (98%) when compared to patient self-report (Miller, Safford and Pogach,2004).

Diabetes could have been controlled by diet/exercise and/or medications. Subjects could have had type 1 or type 2 diabetes mellitus, although based on our patient population, the vast majority would likely to have had type 2. If the subject had DM, up to five of the most recent HbA1c results were noted. Body mass index (BMI) data was collected at the date closest to being identified as a case or control on all subjects. Due to the fact that BMI is positively linked to DM (Colditz, Willett, Stampfer, Manson, Hennekens, Arky and Speizer,1990), we gathered BMI data on all subjects so that if there was a difference between the two groups, we could control for it to reduce potential confounding. Initially, the

duration of diabetes for each subject was to be collected. However, this information was often not available and/or not reliable in the record, therefore, was not included in this study.

The necessary information for each case/control was found in the electronic patient record and recorded on a paper data intake form (see appendix A). This data was then transferred to an Excel spreadsheet for statistical analysis. Each subject was given a study number and no identifiable patient health information was included on the spreadsheet. The paper forms were kept in a locked drawer in the principle investigator's office. All HIPPA protocols were followed.

### 3.4 Data analysis

The mean age and BMI, gender, race, and glaucoma status for cases and controls were compared using unpaired t-tests and chi-squared tests where appropriate. Conditional logistic regression models assessed the impact of DM on ES while controlling for key covariates (independent variables) with ES status as the outcome (dependent) variable, (statpages.org). Secondary analysis was performed with ANCOVA (SPSS 17.0 for Windows) to compare the mean HbA1c of the two DM groups: those with and those without ES. The conventional p value of $<0.05$ was required for statistical significance.

## Chapter 4: Results

A total of 656 subjects were included in the study (328 ES cases and 328 age matched controls). The mean age of cases (79.7 ± 7.5 years), ranging from 52-94, and controls (79.8

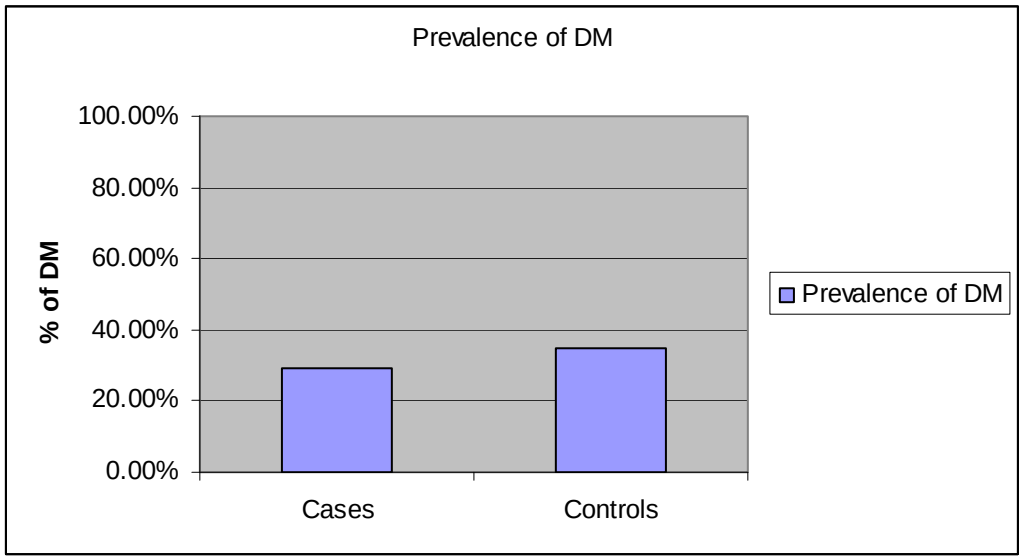
± 7.5 years), ranging from 54-94, were almost identical (p = 0.86). The majority of the subjects were white, non-Hispanic males, reflecting the demographics of our clinic population. The mean BMI of the two groups was similar: 27.5 ± 5.6 kg/$m^2$ in cases versus 27.3 ± 4.9 kg/$m^2$ in controls (p = 0.59). Glaucoma was present in 123 (37.5%) of the cases and 56 (17%) of the controls. These results are summarized in Table 1. Subjects with history of cataract surgery included 140 of the cases and 95 of the controls.

**Table 1 Characteristics of exfoliation syndrome cases and controls**

| | No exfoliation, n=328 | Exfoliation syndrome, n=328 | p-value |
|---|---|---|---|
| Mean age in years ± SD | 79.8 ± 7.5 | 79.7 ± 7.5 | 0.86 |
| Gender | 7 females<br>321 males | 22 females<br>306 males | 0.008 |
| Ethnicity | | | 0.0007 |
| White/non-Hispanic | 299 | 322 | |
| Black | 22 | 6 | |
| White/Hispanic | 2 | 0 | |
| Other | 5 | 0 | |
| Mean Body Mass Index ($kg/m^2$), ± SD | 27.3 ± 4.9 | 27.5 ± 5.6 | 0.59 |
| Glaucoma | 56 (17.0%) | 123 (37.5%) | <0.0001 |

SD= standard deviation

**Figure 1 Prevalence of Diabetes (in %) in cases and controls**




### 4.1 Primary Objective Results

DM was present in 96 out of 328 (29.2%) cases and in 114 out of 328 (34.8%) control subjects (Figure 1). Conditional logistic regression modeling with covariates of age, race, gender, and BMI, but not glaucoma status, demonstrated a non-statistically significant relationship between DM and ES (OR= 0.77; 95% CI, 0.55-1.07) (Table 2). The only significant covariate was gender with females less likely than males to have ES (OR 0.30, 95% CI 0.13-0.72). Only 4.4% (29/628) of all the subjects were female.

**Table 2 The relation between diabetes mellitus and exfoliation syndrome in a multivariate model with ES status as the outcome variable**

| Covariate | Odds Ratio, (95% CI) |
|---|---|
| Age (years) | 1.00 (0.98 – 1.02) |
| Body Mass Index ($kg/m^2$) | 1.01 (0.98 – 1.05) |
| Gender* | 0.30 (0.13 – 0.72) |
| Race** | 1.16 (0.66 – 2.05) |
| Diabetes status | 0.77 (0.55 – 1.07) |

*Males served as the reference group; **White/non-Hispanics served as the reference group

Due to the complex relations between POAG and DM, the data was examined to determine if the presence of glaucoma influenced the prevalence of DM differently in the cases and controls. The prevalence of DM in glaucoma subjects was analyzed. In the control group, 21/56 (37.5%) of the glaucoma subjects had DM, while 28/123 (22.8%) of the glaucoma subjects in the case group had DM. Thus, the case group subjects with glaucoma were less likely to have DM than those in the control group (Table 3)(Table 4).

**Table 3 Distribution of glaucoma and diabetes in the control group**

| Controls (n=328) | +glaucoma | -glaucoma |
|---|---|---|
| +dm | 21 | 93 |
| -dm | 35 | 179 |

**Table 4 Distribution of glaucoma and diabetes in the case group**

| Cases (n=328) | +glaucoma | -glaucoma |
|---|---|---|
| +dm | 28 | 68 |
| -dm | 95 | 137 |

As a result of these findings and to avoid potential bias as previously discussed, glaucoma status was added as a covariate to the multivariate model. The result revealed that the relationship between DM and ES essentially remained unchanged (OR = 0.81, 95% CI, 0.57-1.14)(Table 5) and was not significant.

**Table 5 The relation between diabetes mellitus and exfoliation syndrome in a multivariate model, glaucoma added as a covariate**

| Covariate | Odds Ratio, (95% CI) |
|---|---|
| Age (years) | 0.99 (0.97 - 1.02) |
| Body Mass Index ($kg/m^2$) | 1.02 (0.98 -1.05) |
| Gender* | 0.29 (0.12 - 0.69) |
| Race** | 1.37 (0.75 - 2.49) |
| Glaucoma status | 3.00 (2.07 - 4.36) |
| DM status | 0.81 (0.57 - 1.14) |

*Males served as the reference group; **White/non-Hispanics served as the reference group

Another logistic regression model was created to isolate the relationship between DM and ES excluding all subjects with glaucoma. With the covariates of age, BMI, gender, and race, the association between DM and ES in subjects without glaucoma was essentially null (OR=0.92; 95% CI, 0.62-1.37)(Table 6).

**Table 6 The relation between diabetes mellitus and exfoliation syndrome in a multivariate model without glaucoma subjects in cases or controls**

| Covariate | Odds Ratio, (95% CI) |
|---|---|
| Age (years) | 1.00 (0.97 - 1.02) |
| Body Mass Index ($kg/m^2$) | 1.03 (0.99 - 1.07) |
| Gender* | 0.28 (0.11 - 0.74) |
| Race** | 1.32 (0.59 - 2.93) |
| Diabetes status | 0.92 (0.62 - 1.37) |

*Males served as the reference group; **White/non-Hispanics served as the reference group

The original model showed that female gender was inversely associated with ES (OR= 0.30, 95% CI, 0.13-0.72). Logistic regression was repeated after removing females (who accounted for only 4% of the study population) and the relationship between DM and ES was similar to the model reported in Table 2 (OR= 0.76, 95% CI, 0.54-1.07)(Table 7). No conclusion can be made about females and ES from our small study numbers.

**Table 7 The relation between diabetes mellitus and exfoliation syndrome in a multivariate model, excluding females**

| Covariate | Odds Ratio, (95% CI) |
|---|---|
| Age (years) | 0.99 (0.97 - 1.02) |
| Body Mass Index ($kg/m^2$) | 1.01 (0.98 - 1.05) |
| Race | 1.36 (0.74 - 2.47) |
| Glaucoma status | 2.97 (2.05 - 4.30) |
| Diabetes status | 0.80 (0.57 - 1.14) |

Because ES is an age-related disorder, one more analysis was done on the prevalence of DM in the cases and controls based on 10-year age ranges. The data is displayed in Table 8, which shows no trend in the distribution of DM based on age in either group.

**Table 8- Prevalence of DM in the cases and controls stratified by age**

| Age range | Controls (n=114) | Cases (n=96) |
|---|---|---|
| 50-59 | 1/3=33.3% | 0/3=0% |
| 60-69 | 12/29=41.4% | 11/29=38.0% |
| 70-79 | 41/104=39.4% | 31/104=29.8% |
| 80-89 | 54/178=30.3% | 52/178=29.2% |
| 90+ | 6/14=42.9% | 2/14=14.3% |

### 4.2 Secondary objective analysis

In the subset of subjects with DM, we compared the mean HbA1c between those with and without ES. Table 9 summarizes the characteristics of subjects with DM in cases and controls. A non-significant difference between the subgroups in terms of age, gender, BMI, and type of DM control was observed. The average unadjusted mean HbA1c for the ES group was significantly higher than the non-ES group (p=0.05); however adjustment for age, race, gender, BMI, and type of DM control revealed the difference in HbA1c was no longer significant (7.04% vs. 6.85%)(p=0.14)(Figure 2, Table 10). After removing non-white subjects (due to their small number in the study population) the difference in adjusted mean HbA1c values remained insignificant: 6.79% (95% CI: 6.61 - 6.96%) for cases and 6.96% (95% CI: 6.79 - 7.12%) for controls (p=0.18) (Table 11). These HbA1c levels represent excellent control, on average, in our subject population.

**Table 9: Characteristics of subjects with diabetes mellitus in cases and controls**

| | Diabetes Mellitus without Exfoliation Syndrome, n=114 | Diabetes Mellitus with Exfoliation Syndrome, n=96 | p value |
|---|---|---|---|
| Mean Age in years ± SD | 79.0 ± 7.6 | 79.2 ± 7.5 | 0.87 |
| Mean Body Mass Index ($kg/m^2$) ± SD | 28.7 ± 5.4 | 28.9 ± 5.6 | 0.80 |
| Race | | | 0.004 |
| White/non-Hispanic (%) | 99 (86.8%) | 95 (99.0%) | |
| Black (%) | 14 (12.3%) | 1 (1.0%) | |
| Other (%) | 1 (0.9%) | 0 | |
| Gender | | | 0.15 |
| male (%) | 112 (98.2%) | 90 (93.8%) | |
| female (%) | 2 (1.8%) | 6 (6.3%) | |
| Type of Diabetes Mellitus control | | | 0.08 |
| oral (%) | 52 (45.6%) | 59 (61.5%) | |
| insulin and oral (%) | 13 (11.4%) | 4 (4.2%) | |
| diet/exercise (%) | 27 (23.7%) | 18 (18.8%) | |
| insulin only (%) | 22(19.3%) | 15(15.6%) | |

**Figure 2 Hemoglobin A1c results in diabetics with and without ES**

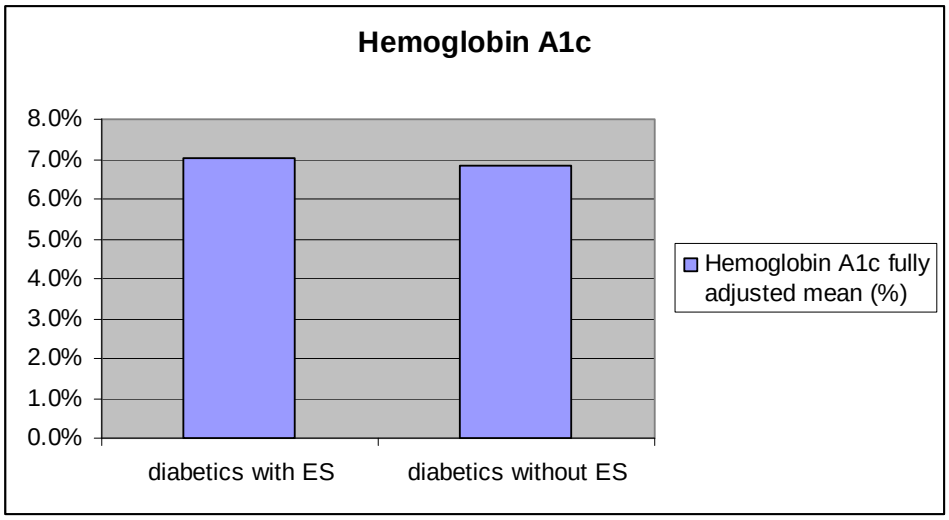



**Table 10 Hemoglobin A1c results**

| Hemoglobin A1c* (%) | Diabetics with ES, n=96 | Diabetics without ES, n=114 | p value |
|---|---|---|---|
| Unadjusted mean ± SD | 7.08% ± 0.96 | 6.80% ± 1.04 | 0.05 |
| Fully adjusted mean %** | 7.04% (95% CI: 6.87-7.22) | 6.85% (95% CI: 6.66-7.04) | 0.14 |

* The mean of the past 5 available HbA1c values were used for each participant.

**ANCOVA was used to adjust the mean HbA1c for age, race, gender, BMI, and type of diabetes mellitus control, SD = standard deviation

**Table 11 Hemoglobin A1c results with non-whites removed**

| Hemoglobin A1c (%) | Diabetics with ES, white, n=95 | Diabetics without ES, white, n=99 | p value |
|---|---|---|---|
| Adjusted mean | 6.79% (95% CI: 6.61 - 6.96%) | 6.96% (95% CI: 6.79 - 7.12%) | 0.18 |

## Chapter 5: Discussion

This large case-control study found no statistically significant association between DM and ES. The results were not significantly different when further controlled for glaucoma status. Additionally, no difference was observed in mean HbA1c among DM subjects stratified by ES status.

### 5.1 Strengths of the study

Our result may differ from other studies, which found an inverse relationship, due to several aspects of our study design. First, the present study accounted for complex relations between POAG and DM. Our control group could have POAG but in the logistic regression, glaucoma was included as a covariate. In two prior studies (Konstas, et al.,1998), (Tarkkanen, et al.,2008) the prevalence of DM in POAG subjects was higher when compared

to EG subjects. Since DM may be a risk factor for POAG (Chopra, et al.,2008 ; Dielemans, et al.,1996 ; Klein, et al.,1994 ; Mitchell, et al.,1997a ; Pasquale, et al.,2006) such study designs could mask the true real relationship, if any, between DM and ES.

Second, cases and controls in our study required a pre-cataract surgery slit lamp examination with post-dilation iris and anterior capsule findings documented. This was critical to avoid misclassification of cases for controls. Patients with DM (Saxena, Mitchell and Rochtchina,2004) and ES (Puska and Tarkkanen,2001) develop cataracts earlier than those without these conditions. After cataract surgery, exfoliation material may not be clinically detectable due to the removal of the anterior capsule. If preoperative slit lamp findings are not sought in pseudophakic patients, cases may look like controls. If a diabetic with ES, who has increased the risk of cataract, has cataract surgery and is then enrolled in the study, he/she may now be classified as a diabetic without ES, thus contributing to the false appearance of an inverse relationship between DM and ES. Possible misclassification was minimized with our study design, whereas the lens status was not addressed in some of the prior studies (Brajkovic, et al.,2001 ; Jonas and Grundler,1998 ; Psilas, et al.,1991 ; Tarkkanen, et al.,2008).

A further strength of this study was that ES status was chosen as the outcome variable in the regression model instead of DM status to avoid confounding introduced by differential duration of DM and ES. DM and ES have very different average ages of onset. The mean age of onset of type 2 DM in one US population-based study was 46 years (Koopman, Mainous, Diaz and Geesey,2005). The mean age at diagnosis of ES in a 15-year incidence study was 76 years (Karger, et al.,2003). This was reflected in the present study in which the

mean age of the ES subjects was 79.7 years. More importantly, DM is a risk factor for pre-mature mortality. The life expectancy of a patient with DM after age 50 is decreased compared to patients without DM: shorter by an average of 7.5 years in men and 8.2 years in women (Franco, Steyerberg, Hu, Mackenbach and Nusselder,2007). Thus, with DM as the outcome, patients with DM may not live long enough to manifest ES giving the false impression of an inverse relation between ES and DM associated with under-reporting the incidence of ES.

This study was the first to include HbA1c levels in the analysis of DM in ES. Tarkkanen speculated that a biological basis for an inverse relation between DM and ES may exist and involve aqueous humor biochemical alteration in patients with DM. He suggested that glycation of critical basement membrane components might prevent the accumulation of exfoliation material (Tarkkanen,2008). Obviously, our case control study cannot directly test this biological hypothesis. However, if glycation of certain ocular basement membrane macromolecules in DM truly prevents the accumulation of exfoliation material, one would expect that patients with DM who develop ES would have lower average HbA1c levels because they would have less glycation products in general. Our study detected no statistical difference in the average of five HbA1c levels in diabetics with and without ES. Therefore, our findings are not consistent with Tarkkanen's proposed mechanism. Based on this study's results, significant blood sugar level differences cannot explain the decreased prevalence of diabetic retinopathy in ES in the Psilas study or the spatial separation of HA1 and HA2 in the aqueous of ES subjects, as found in by Lee (Lee,2008 ; Psilas, et al.,1991).

### 5.2 Study Limitations

A potential limitation of this study is its retrospective nature, which restricted us from collecting potentially useful data regarding duration of diabetes and the HbA1c at time of onset of ES. In addition, both the cases and controls had a higher burden of DM compared to the general veterans population (16% self reported in males using VA services) (Reiber, Koepsell, Maynard, Haas and Boyko,2004). This can be explained by the fact that subjects were gathered from eye clinic visits where patients with DM were referred by their primary care doctors for annual dilated eye examinations to screen for diabetic retinopathy. However, this increase in DM prevalence should have affected both the cases and controls equally, and therefore, should not bias the study. Also, while the study sample was large, the number of females and non-white participants was limited reflecting our VA patient demographics. Therefore, our results may not be applicable to the general population.

### 5.3 Future directions

The understanding of the complex relationships between ES and systemic diseases is a subject which needs further exploration. In the future, clarification could lead to a better understanding of why exfoliation deposits occur and could perhaps suggest new treatment options. Specifically regarding DM and ES, there is room for additional prospective, large, clinical studies to further explore the questions examined in this manuscript. Additional data could be collected such as the duration of DM and the presence or absence of diabetic

retinopathy. A subject pool which is more balanced in regard to gender and race would be helpful to produce a more generalizable conclusion.

### 5.4 Conclusion

In summary, our case-control study found no statistical significant relationship between DM and ES in this predominately male, white population with a high burden of DM. In subjects with DM, no difference was found in HbA1c levels based on ES status.

**Appendix A**

DATA INTAKE FORM

Relationship Between DM and ES Study (DMX)

Patient ID #_____

DEMOGRAPHICS

2) Last name_____ 3) First name_____

4) Last 4_____ 5) DOB (mm/d/yr) ___/___/___

6) Age at time of eye exam _____ 7) Gender ___ (1: male, 2: female)

8) Race ____ (1: White, 2: Black, 3: Hispanic, 4: other)

SYSTEMIC HEALTH

14) Diabetes present? _____ (1: no; 2: yes)

15) Diabetes confirmed by _____ (1: meds, 2: pcp note, 3: cover sheet dx) N/A

16) PCP exam to evaluate for DM? _____ (1: no, 2: yes) (within 1 year of eye exam)

17) Duration of DM _____ (years) N/A

18) HbA1c a) ____ b) ____ c) ____ d) ____ e) ____ N/A

19) DM control type ____ (1: insulin, 2: oral, 3: both, 4: diet/exercise) N/A

20) BMI _____

OCULAR HEALTH

9) Exfoliation present (DFE required)? ____ (1: no, 2: yes)

10 a) Date of first exam when confirmed (mm/d/yr): ___/___/___ N/A

10 b) Date of second exam when confirmed (mm/d/yr): ___/___/___ N/A

11) Cataract surgery? _____ (1: no, 2: yes, 3: N/A)

12) Date of pre-op SL w/ no evidence of ES (mm/d/yr) ___/___/___ N/A

13) Glaucoma present? _____ (1: no, 2: yes)

For ES subjects, exam date: _____ Clinic:_____

Comments: _____

**KEY to Data intake form**:

-DOB- date of birth

-DFE- dilated fundus exam

-ES- exfoliation syndrome

-DM- diabetes

-SL- slit lamp

-PCP- primary care physician

-BMI- body mass index

-HA1c- hemoglobin A1c, last 5 readings, if available

-Patient id#- A- ES case, B- control

-Diabetes medicines available at the VA:

Insulin: Regular, Aspart (Novolog), NPH, Glargine (Lantus)

Oral: Glipizide, Glipizide SA, Glyburide, Glyburide/Metformin combination, metformin,

Metformin SA, pioglitazone, rosiglitazone

Non-VA formulary: exenatide (Byetta), sitagliptin (Januvia)

-Exclusion criteria: controls with ES, controls with h/o cataract surgery and no slit lamp findings pre-op to r/o ES material

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