VITAMIN D INSUFFICIENCY IN DIABETIC RETINOPATHY

(Running Head: Vitamin D and Diabetic Retinopathy)

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ABSTRACT

Objective: To assess the relationship between vitamin D status and diabetic retinopathy

Methods: A clinic-based, cross-sectional study was conducted at Emory University. A total of 221 subjects were classified into five groups based on diabetes status and retinopathy findings: no diabetes or ocular disease (n = 47), no diabetes with ocular disease (n = 51), diabetes with no background diabetic retinopathy (No BDR; n = 41), nonproliferative diabetic retinopathy (NPDR; n = 40), and proliferative diabetic retinopathy (PDR; n = 42). Key exclusion criteria included type 1 diabetes and those taking > 1000 IU vitamin D daily. Subjects underwent dilated fundoscopic examination and were tested for hemoglobin A_1c, serum creatinine, and 25-hydroxy-vitamin D (25(OH)D) between December 2009 and March 2010.

Results: Between the groups, there was no statistical difference in age, race, sex, or multivitamin use. Diabetic subjects had lower 25(OH)D levels than non-diabetic subjects (22.9 ng/ml versus 30.3 ng/ml, p<0.001). The mean 25(OH)D levels were as follows: No diabetes or ocular disease = 31.9 ng/ml, No diabetes with ocular disease = 28.8 ng/ml, No BDR = 24.3 ng/ml, NPDR = 23.6 ng/ml, PDR = 21.1 ng/ml. Univariate analysis of the 25(OH)D levels demonstrated statistical significance between the study groups, race, body mass index, multivitamin use, hemoglobin A_1c, serum creatinine, and estimated glomerular filtration rate. In a multivariate linear model with all potential confounders, only multivitamin use remained significant (p<0.001).

Conclusions: This study suggests that diabetic subjects, especially those with PDR, have lower 25(OH)D levels than those without diabetes.

INTRODUCTION

Diabetes mellitus continues to be a tremendous health burden in America. In 2007, the prevalence of diabetes was estimated to be 23.6 million people, or 7.8% of the population (1). The number of people diagnosed with diabetes is expected to increase to 48.3 million people by the year 2050 (2). Diabetes is also the leading cause of new blindness in patients 20 to 74 years of age (1). While it has been well established that intensive blood glucose control can lower the risk of microvascular complications from diabetes, the pathophysiology of retinopathy progression is not completely understood (3-5).

Vitamin D is essential for a vast number of physiologic processes and vitamin D insufficiency has reached pandemic proportions, with more than half the world's population at risk (6). Vitamin D insufficiency has been implicated in the development of diabetes and also correlated with an elevated risk of cardiovascular disease, cancer, and mortality (6-8). Additionally, vitamin D insufficiency has been associated with neurologic conditions, such as multiple sclerosis and Parkinson's disease (9-10).

Vitamin D may play a role in the pathogenesis of diabetic retinopathy through its effects on the immune system and on angiogenesis. Vitamin D exerts an anti-inflammatory effect by decreasing the proliferation of lymphocytes, natural killer cells, and several pro-inflammatory cytokines (11). Additionally, it has been shown that the active metabolite of vitamin D, calcitriol, is a potent inhibitor of retinal neovascularization in a mouse oxygen-induced ischemic retinopathy model (12). Given these associations, we sought to determine the relationship between vitamin D insufficiency and diabetic retinopathy.

METHODS

The Emory University School of Medicine Institutional Review Board approved this study and all work was conducted in accordance with the Health Insurance Portability and Accountability Act regulations. This research followed the tenets of the Declaration of Helsinki. A clinic-based cross sectional study was designed at the Emory Eye Center. All patients were enrolled between December 16, 2009 and March 21, 2010 to minimize seasonal bias. All patients who were seen in the retina, glaucoma, cornea, and comprehensive ophthalmology clinics during the enrollment period were considered potential study subjects. These patients were screened by the study investigators to determine their diabetes status, age, race, and sex. Patients underwent routine ophthalmic examination, including dilated fundoscopy, and were asked to participate in the study if they were able to be matched into a study group based on their demographics. Attempts were made to keep the study groups equally matched according to age (within 10 years), race, and sex. For example, if a 50-year-old Caucasian man was enrolled in the proliferative diabetic retinopathy (PDR) group, we tried to match a Caucasian man between 45 and 55 years of age in each of the other four groups. Towards the end of the enrollment period, subjects were enrolled even if they did not have a corresponding match in the other groups. Study subjects provided informed consent prior to participation in the study, and once enrolled, completed a medical history questionnaire and had their blood tested for hemoglobin A_1c, serum creatinine, and 25-hydroxy vitamin D (25(OH)D).

A total of 221 subjects were divided into five distinct groups based on their diabetes status and retinopathy findings. The first group consisted of subjects without diabetes or any ocular disease. Subjects in the second group also lacked diabetes but had some other form of ocular disease, such as uveitis or macular degeneration. The no background diabetic retinopathy

(No BDR) group consisted of subjects with type 2 diabetes but no evidence of diabetic

retinopathy, such as microaneurysms, cotton-wool spots, intraretinal hemorrhages, or macular

edema. Subjects in the nonproliferative diabetic retinopathy (NPDR) group had evidence of

retinopathy, such as microaneurysms, cotton-wool spots, intraretinal hemorrhages, or macular

edema, but no evidence of retinal or iris neovascularization. The PDR group consisted of

subjects with neovascularization on the optic disc, retina, or iris, with or without vitreous

hemorrhage or prior panretinal photocoagulation. When the diabetic retinopathy was

asymmetric, the subject was assigned to the group corresponding to the eye with the worse

retinopathy findings.

Subjects were excluded if they had type 1 diabetes, were younger than 18 or older than

90 years of age. Subjects were also excluded if they were taking vitamin D analogues,

multivitamins containing more than 1000 IU of vitamin D per day, or any medications that could

alter vitamin D metabolism, such as rifampin, phenytoin, or phenobarbital. Subjects with prior

diseases that suggested baseline alterations in vitamin D and calcium metabolism, such as

hyperparathyroidism or hypoparathyroidism, or recent nephrolithiasis were also excluded.

Patients who were cognitively impaired or unable to provide written informed consent were

excluded as well.

Data collected in the medical history questionnaire included demographic variables, such

as age, sex, height, weight, and race (self-assigned as white, black, or Asian). The presence of

hypertension or macrovascular disease was also recorded. Subjects were considered to have

macrovascular disease if they had ever been diagnosed with a myocardial infarction,

cerebrovascular accident, if they had undergone coronary artery bypass grafting surgery or a

cardiac stenting procedure. Additionally, the duration of diabetes, which was defined as the

interval between their diagnosis of type 2 diabetes and the time of their enrollment into this study, and insulin usage was recorded for each diabetic patient. Subjects were also asked to report their usage of daily multivitamins. A phone interview was subsequently performed with those individuals who were taking multivitamins to ascertain the exact dosage of vitamin D contained in their multivitamin.

Demographic variables were categorized as following: age was grouped by decades of life (18 to 39 years, 40 to 49 years, 50 to 59 years, 60 to 69 years, 70 to 90 years); and body-mass index (BMI) was grouped as well (less than 25.0 kg/m^2, 25.1 to 29.9 kg/m^2, 30.0 to 34.9 kg/m^2, and greater than 35 kg/m^2). For those subjects with diabetes, duration of diabetes was categorized into groups (less than 10 years, 10.1 to 14.9 years, 15.0 to 19.9 years, 20.0 to 24.9 years, and greater than 25 years). Laboratory variables were grouped as following: hemoglobin A_1c (less than 6.0, 6.0 to 6.9, 7.0 to 7.9, 8.0 to 8.9, 9.0 to 9.9, greater than 10.0), and serum creatinine (less than 1.0 mg/dl, 1.0 to 1.49 mg/dl, 1.50 to 1.99 mg/dl, and greater than 2.0 mg/dl). The estimated glomerular filtration rate (GFR) was calculated using the Modification of Diet in Renal Disease formula. The results for estimated GFR were grouped according to the chronic kidney disease staging criteria (greater than 90 $ml/min/1.73m^2$, 60 to 89 $ml/min/1.73m^2$, 30 to 59 $ml/min/1.73m^2$, 15 to 29 $ml/min/1.73m^2$, and less than 15 $ml/min/1.73m^2$).

For the laboratory testing, the serum creatinine was assayed via high performance liquid chromatography, which had been standardized using isotope dilution mass spectroscopy. Hemoglobin A_1c levels were also measured using high performance liquid chromatography. The 25(OH)D levels were measured from serum samples via an automated competitive immunoassay detected by chemiluminescence (IDS iSYS assay; Scottsdale, AZ). The interassay coefficients of variation for the IDS iSYS assay at 6.5 ng/ml, 27.3 ng/ml, and 64.9 ng/ml were 15.9%, 12.4%,

and 9.8%, respectively. The intra-assay coefficients of variation at 10.9 ng/ml, 40.4 ng/ml, and 69.2 ng/ml were 3.5%, 5.0%, and 3.4%, respectively. The Emory University laboratory is certified by the Clinical Laboratory Improvement Amendments (CLIA), and also participates in Vitamin D External Quality Assessment Scheme (DEQAS), which is an international organization that aims to ensure the analytical reliability of 25(OH)D assays.

Statistical Considerations

The means and percentages for patient characteristics were compared across study groups using one-way analysis of variance (ANOVA) and the chi-square test. Unadjusted comparisons of mean 25(OH)D were made across levels of patient characteristics using one-way ANOVA. Two-way ANOVA was done to compare mean 25(OH)D levels across study groups while adjusting for multivitamin use using least squares means. Tukey's multiple comparison procedure was used to test pair-wise comparisons of mean 25(OH)D between levels of the patient characteristics in the adjusted and unadjusted analyses.

Vitamin D was also analyzed as a dichotomous variable with patients being labeled as vitamin D sufficient (25(OH)D $\geq$ 30.0 ng/ml) or vitamin D insufficient (25(OH)D < 30.0 ng/ml) (13). The percentages of subjects with vitamin D insufficiency were compared across the various patient characteristics using the chi-square test.

A multiple linear regression model was used to assess the statistical significance of the 25(OH)D levels amongst the study groups and those using multivitamins. A second model was used to determine the significance of all of the patient characteristics found to be significant in the univariate analyses. Statistical calculations were performed using SAS and statistical significance was set as a two sided p-value of 0.05. The mean and standard deviation are denoted as mean $\pm$ standard deviation.

In the design phase of the study, the power of the study was calculated based on comparing the mean 25(OH)D level between two groups using the independent group's t test. The two-sided probability of a Type I error was set to 0.005 (0.05/10) to provide a Bonferroni adjustment to account for the 10 pair-wise comparisons that could be made among the five groups. Based on the work of Pepper et al and personal experience, the standard deviation for the 25(OH)D levels was assumed to be 5.0 ng/ml (14). With 40 patients per group, the difference between the means of two groups that could be detected with 80% power was 4.2 ng/ml.

RESULTS

Subject characteristics

Table 1 shows the univariate analysis across the study groups. There was no statistical difference between the study groups with regards to age, race, sex, or multivitamin use. However, there was a statistically significant difference in the study groups with regards to BMI ($p = 0.003$), hemoglobin A_1c ($p < 0.001$), hypertension ($p < 0.0001$) and macrovascular disease ($p = 0.002$). Amongst those with diabetes, there was also a statistically significant difference in the duration of diabetes ($p < 0.001$), and insulin usage ($p < 0.001$).

Vitamin D analysis

Overall, subjects with diabetes (n = 123) had lower 25(OH)D levels than those without diabetes (n = 98) (22.9 ± 10.4 ng/ml versus 30.3 ± 13.7 ng/ml respectively, $p < 0.001$). Additionally, black subjects had lower 25(OH)D levels compared to white subjects (23.7 ng/ml versus 29.2 ng/ml, $p = 0.001$). Figure 1 shows the 25(OH)D levels for the five study groups. Table 2 shows the univariate analyses of the mean 25(OH)D levels and of those with vitamin D insufficiency. This analysis revealed a statistically significant difference in mean 25(OH)D

levels according to the study groups (p < 0.001), race (p = 0.033), BMI (p = 0.010), multivitamin use (p < 0.001), hemoglobin A$_1$c (p = 0.003), serum creatinine (p = 0.030), and estimated GFR (p = 0.047). When analyzing vitamin D as a dichotomous variable, it was found that approximately two-thirds (65.2%) of the 221 study subjects were vitamin D insufficient. There was a significant difference in the percentage of patients with vitamin D insufficiency amongst the study groups (p = 0.048). The univariate analysis also showed subjects who were taking multivitamins were less likely to experience vitamin D insufficiency (p < 0.001).

Because the univariate analysis of the mean 25(OH)D levels showed a significant difference between 25(OH)D levels amongst the study groups (p < 0.001), pair-wise comparisons were done to determine which groups were significantly different. There was a significant difference between the group with no diabetes or ocular disease and each of the three diabetic groups (No BDR group, NPDR group, and PDR group). There was also a significant difference between the no diabetes with ocular disease group and the PDR group. Because multivitamin use appeared to affect the 25(OH)D levels in the univariate analysis, a model was then fit that included both study group and multivitamin use. Both variables were found to be statistically significant (study group, p = 0.003; multivitamin use, p < 0.001). Therefore, the pair-wise comparisons among study groups were adjusted for multivitamin use (Table 3). The differences between the groups were the same in the adjusted pair-wise comparisons as the unadjusted pair-wise comparisons.

A multivariate linear regression model was created to compare all potential confounders (study group, BMI, hemoglobin A$_1$c, serum creatinine, estimated GFR, and multivitamin use). Only multivitamin use remained statistically significant (p < 0.001).

DISCUSSION

There were two purposes of this cross-sectional study. The first was to assess the relationship between vitamin D status and diabetic retinopathy and the second was to establish baseline data from which a larger prospective clinical study could be designed. It was found that patients with type 2 diabetes, particularly those with PDR, had lower vitamin D levels than those without diabetes. Moreover, there was a higher percentage of subjects with vitamin D insufficiency in the diabetic retinopathy groups. Multivitamin usage had a significant impact on the vitamin D levels of the study subjects.

There is evidence to suggest that vitamin D may play a role in the pathogenesis of diabetic retinopathy through its effects on the immune system. Inflammatory cytokines, such as TNF-α, TNF-β, IL-6, and plasminogen activator inhibitor-1 are upregulated in patients with type 2 diabetes, and it has been shown that vitamin D decreases the production of several pro-inflammatory cytokines, such as IL-2, IL-6, IL-8, IL-12, and TNF-α (11). Vitamin D also exerts an anti-inflammatory effect by decreasing the proliferation of helper T-cells, cytotoxic T-cells and natural killer cells (11). A recent study found that vitamin D deficiency was associated with vascular endothelial dysfunction in middle aged and elderly adults. The authors concluded that this dysfunction was related to increased vascular endothelial cell expression of the pro-inflammatory transcription factor, nuclear factor κB (15).

Vitamin D may also contribute to diabetic retinopathy via angiogenesis mechanisms. Albert and colleagues have shown that the active metabolite of vitamin D, calcitriol, was a potent inhibitor of retinal neovascularization *in vivo* (12). This study also found that calcitriol inhibits retinal endothelial cell capillary morphogenesis *in vitro* (12). Furthermore, calcitriol downregulates hypoxia-inducible factor-1 (HIF-1) transcriptional activity, as well as HIF-1

target genes, such as vascular endothelial growth factor (VEGF) (16). As several of the complications in diabetic retinopathy, such as macular edema and neovascularization, are driven by VEGF production (17-19), vitamin D could exert its positive effect via calcitriol mediated VEGF reduction.

Vitamin D may also play a protective role through its effects on glycemic control and hypertension, both significant risk factors for the development and progression of diabetic retinopathy (3-5,20). There is evidence to suggest that vitamin D plays a role in normal insulin secretion in response to glucose (11,21,22). Additionally, vitamin D and calcium citrate supplementation in patients with impaired fasting glucose leads to a reduction in insulin resistance and an attenuation of the rise in fasting glucose levels (23). Vitamin D deficiency has also been implicated in the development of hypertension as several studies have shown an inverse association between vitamin D levels and blood pressure (24,25). A large prospective cohort study found that vitamin D deficiency was associated with 6.1-fold and 2.7-fold increased relative risk of developing hypertension in men and women, respectively (26). Interestingly, the current study did not show an association between hypertension and vitamin D status. It is possible that the sample size of this study was not sufficient to detect such an association. Nonetheless, it is possible that treating vitamin D insufficiency may lead to an improvement in blood sugar and blood pressure control, which could ultimately slow the progression of retinopathy.

This is the largest study to date that has been designed to assess the relationship between vitamin D status and retinopathy in subjects with type 2 diabetes mellitus. The only other study designed to evaluate this relationship consisted of 66 diabetic patients and 20 normal controls (27). Concurrent with this study, mean serum 25(OH)D levels were higher in nondiabetic

patients than those with diabetes (60.6 versus 31.0 nmol/L, p<0.001). However, differences in demographic variables, such as race or BMI were not addressed, and multivitamin use was not reported. Obesity has been shown to affect vitamin D levels, possibly through vitamin D sequestration in fat deposits (28). The current study found a statistically significant association between vitamin D concentration and BMI (p = 0.010), but this association fell out of significance in the multivariate model. It is possible that with a larger subset of patients, this would have remained a significant factor. Furthermore, because multivitamin use can influence vitamin D levels, it will be important to account for this in future studies. In order to improve our understanding of vitamin D deficiency and diabetic retinopathy, a larger prospective study will be needed. The data derived from this study points to a potential relationship between vitamin D deficiency and proliferative retinopathy and can be used in the design of larger studies. Certainly any study examining the relationship between vitamin D and retinopathy prospectively will take years to examine.

There are several limitations to the current study. First, the cross sectional design of this study limits the ability to assess causality. For example, it is not possible to determine, from this study, if the vitamin D insufficiency leads to diabetic retinopathy or if diabetic retinopathy leads to vitamin D insufficiency. Second, only one time point was recorded for the subjects in this study. It would be valuable to follow these patients with serial fundoscopic examinations and blood testing. Third, there is possible selection bias as this is not a study of consecutive patients seen at our institution. While dietary intake and outdoor exposure data were not collected, these limitations would not be expected to have a large effect on the results. Heaney and colleagues have shown that the amount of daily vitamin D obtained from dietary sources have small effects on serum 25(OH)D levels (29). Additionally, effects from sunlight exposure were minimized in

this study as all subjects were enrolled over a three month period in winter. Finally, the overall mean standard deviation of 25(OH)D in this study was 11.9 ng/ml. Using this data, the sample size only provided the opportunity to detect a 9.4 ng/ml difference between two groups. This limited our ability to detect smaller differences between the groups, but can be used to calculate the sample sizes for future studies.

CONCLUSION

In conclusion, this study showed that subjects with type 2 diabetes mellitus, especially those with PDR, had lower vitamin D levels and were more likely to be vitamin D insufficient than patients without diabetes. The use of multivitamins was also somewhat protective against vitamin D insufficiency. While this is the largest study to date designed to assess this relationship, larger studies are needed to confirm these findings. Additionally, studies are needed to assess whether the treatment of vitamin D insufficiency will slow the progression of diabetic retinopathy.

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Figure 1: 25(OH)D levels for the five study groups. The upper limit of each box represents the 75th percentile while the lower limit represents the 25th percentile. The dotted lines represent the 95th and 5th percentile, and the numerical value is the median for each group.

25-(OH) Vitamin D Level (ng/mL)

No DM or Ocular Disease — 28.8
No DM with Ocular Disease — 24.7
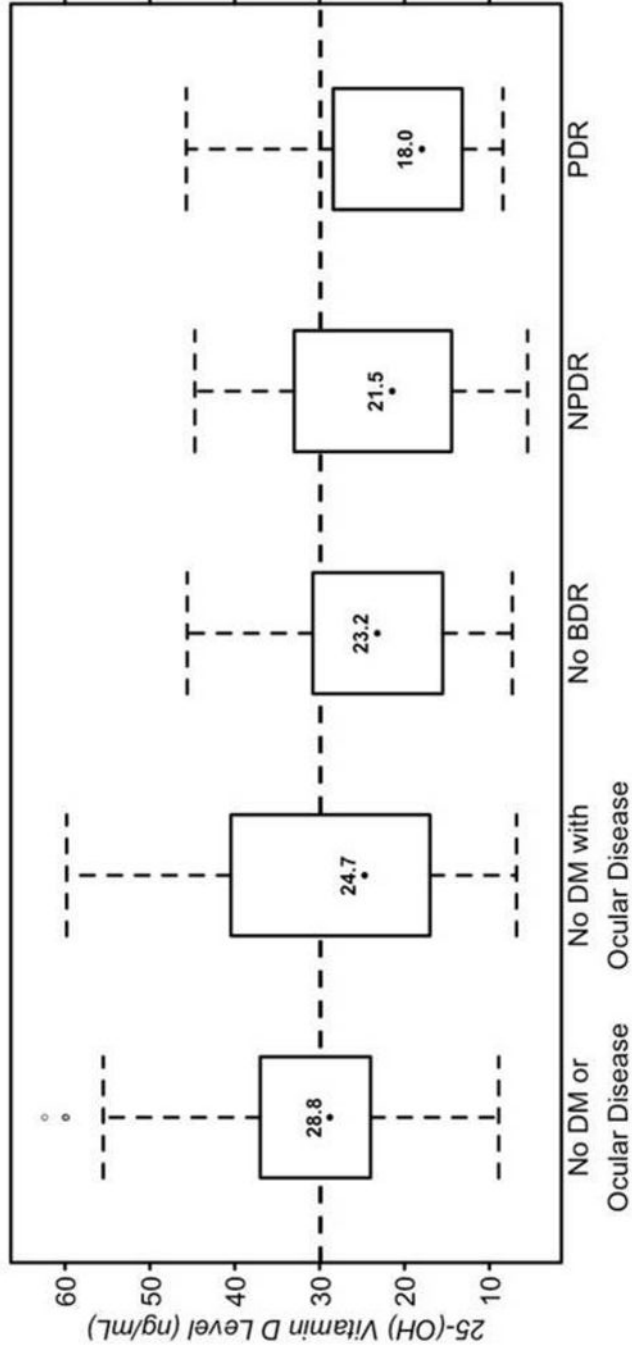No BDR — 23.2
NPDR — 21.5
PDR — 18.0

Table 1: Clinical characteristics of the five study groups.

	No Diabetes or Ocular Disease (n = 47)	No Diabetes with Ocular Disease (n = 51)	No BDR Group (n = 41)	NPDR Group (n = 40)	PDR Group (n = 42)	p value
Age (years)	62.0 ± 11.6	59.8 ± 13.3	62.4 ± 11.3	68.3 ± 10.0	59.8 ± 12.0	0.797
Race: Black	25 (53%)	23 (45%)	21 (51%)	22 (55%)	22 (52%)	0.977
Sex: Male	25 (53%)	23 (45%)	21 (51%)	21 (53%)	21(50%)	0.938
Body Mass Index (kg/m^2)	27.9 ± 6.1	28.7 ± 8.0	31.7 ± 9.8	31.2 ± 6.7	33.1 ± 6.7	**0.003**
Multivitamin Usage	26 (55%)	22 (43%)	20 (49%)	19 (48%)	15 (36%)	0.439
Mean Daily Dose Vitamin D (IU)	312.8 ± 341.8	207.8 ± 284.8	200 ± 265.5	282..5 ± 371.3	228.6 ± 354.3	0.700
Duration of Diabetes (years)[*]	-	-	7.4 ± 7.8	18.9 ± 11.1	22.0 ± 10.5	**< 0.001**
Hemoglobin A_1c	5.8 ± 0.5	5.8 ± 0.3	7.5 ± 2.0	7.4 ± 1.2	8.1 ± 1.9	**< 0.001**
Insulin Usage[*]	-	-	10 (24%)	29 (73%)	31 (74%)	**< 0.001**
Macrovascular Disease	9 (19%)	4 (8%)	6 (15%)	13 (33%)	19 (45%)	**0.002**
Hypertension	32 (68%)	23 (45%)	27 (66%)	38 (95%)	39 (93%)	**< 0.001**
Serum Creatinine (mg/dL)	0.95 ± 0.02	1.02 ± 0.87	0.92 ± 0.28	1.89 ± 1.87	2.38 ± 2.70	**< 0.001**
Estimated GFR† (mL/min/1.73m^2)	80.98 ± 18.70	84.75 ± 26.43	89.30 ± 30.07	59.00 ± 31.88	48.62 ± 29.61	**< 0.001**

IU: international units;

[*] Only those patients with diabetes were included in this analysis

† Estimated glomerular filtration rate as calculated by the MDRD formula

Table 2: The analysis of the mean 25(OH)D levels and of those with vitamin D insufficiency.

	Mean 25(OH)D Level	p value	Vitamin D Insufficiency (n = 144)	p value
Study Group		**< 0.001**		**0.048**
No DM or Ocular Disease (n= 47)	31.9 ± 12.9		26 (55%)	
No DM with Ocular Disease (n= 51)	28.8 ± 14.3		28 (55%)	
No BDR Group (n= 41)	24.3 ± 10.3		28 (68%)	
NPDR Group (n= 40)	23.6 ± 10.3		28 (70%)	
PDR Group (n= 42)	21.1 ± 10.5		34 (81%)	
Age (years)		0.306		0.241
Younger than 40 (n= 7)	29.4 ± 12.1		4 (57%)	
40 to 49 (n= 30)	22.1 ± 13.0		24 (80%)	
50 to 59 (n= 57)	25.5 ± 12.0		40 (70%)	
60 to 69 (n= 65)	26.9 ± 11.8		40 (62%)	
Older than 70 (n= 62)	27.6 ± 13.3		36 (58%)	
Race		**0.033**		**0.048**
Black (n= 113)	23.7 ± 12.5		81 (72%)	
White (n= 102)	29.2 ± 11.7		58 (57%)	
Asian (n= 6)	22.0 ± 14.3		5 (83%)	
Sex		0.639		0.450
Male (n= 111)	25.7 ± 12.0		75 (68%)	
Female (n= 110)	26.6 ± 13.0		69 (63%)	
Body Mass Index (kg/m^2)		**0.010**		**0.045**
Less than 25.0 (n= 48)	30.1 ± 14.4		25 (52%)	
25.1 to 29.9 (n= 84)	27.0 ± 11.9		52 (62%)	
30.0 to 34.9 (n= 43)	25.0 ± 11.7		32 (74%)	
Greater than 35.0 (n= 46)	21.8 ± 10.8		35 (76%)	
Daily Multivitamin Usage		**< 0.001**		**<0.001**
Yes (n= 102)	31.1 ± 11.1		45 (44%)	
No (n= 119)	22.0 ± 12.1		99 (83%)	
Duration of Diabetes (years)[*]		0.463		0.656
Less than 10.0 (n= 42)	23.2 ± 9.8		31 (74%)	
10.0 to 14.9 (n= 15)	26.5 ± 7.6		11 (73%)	
15.0 to 19.9 (n= 14)	19.5 ± 10.0		12 (86%)	
20.0 to 24.9 (n= 18)	23.5 ± 11.4		11 (61%)	
Greater than 25 (n=3 4)	22.2 ± 11.6		25 (28%)	
Hemoglobin A$_1$c		**0.003**		0.094
Less than 6.0 (n= 78)	30.3 ± 13.6		43 (55%)	
6.0 to 6.9 (n= 66)	26.1 ± 11.9		43 (65%)	
7.0 to 7.9 (n= 39)	21.7 ± 9.5		32 (82%)	
8.0 to 8.9 (n= 17)	20.8 ± 11.4		13 (76%)	
9.0 to 9.9 (n= 13)	23.7 ± 11.5		8 (62%)	
Greater than 10.0 (n= 8)	24.0 ± 12.2		5 (63%)	

Insulin Usage[*]		0.387		0.748
Yes (n= 70)	22.2 ± 10.5		52 (74%)	
No (n= 53)	23.9 ± 10.2		38 (72%)	
Macrovascular Disease		0.162		0.207
Yes (n= 51)	24.0 ± 10.2		37 (73%)	
No (n=170)	26.8 ± 13.0		107 (63%)	
Hypertension		0.753		0.414
Yes (n= 159)	26.4 ± 13.0		101 (64%)	
No (n= 62)	25.8 ± 11.1		43 (69%)	
Serum Creatinine (mg/dL)		**0.030**		**0.024**
Less than 1.0 (n= 112)	27.2 ± 13.1		71 (63%)	
1.0 to 1.49 (n= 69)	26.5 ± 12.3		43 (62%)	
1.50 to 1.99 (n= 16)	28.0 ± 12.6		8 (50%)	
Greater than 2.0 (n= 24)	19.1 ± 7.5		22 (92%)	
Estimated GFR		**0.047**		**0.050**
Less than 15 (n= 8)	20.5 ± 7.6		7 (88%)	
15 to 29 (n= 13)	18.6 ± 7.8		12 (92%)	
30 to 59 (n= 47)	26.9 ± 12.3		26 (55%)	
60 to 89 (n= 95)	28.1 ± 12.8		26 (58%)	
Greater than 90 (n= 58)	24.9 ± 12.7		41 (71%)	

* Only those patients with diabetes were included in this analysis

Table 3: The pair-wise analysis of the differences in mean 25(OH)D levels between the study groups when controlling for multivitamin use

Group Comparison	Difference between mean 25(OH)D levels (ng/ml)	95% Confidence Limits		p-value
No DM or Ocular Disease vs. No DM with Ocular Disease	2.06	-4.18	8.30	0.893
No DM or Ocular Disease vs. No BDR	7.07	0.50	13.65	**0.028**
No DM or Ocular Disease vs. NPDR	7.65	1.03	14.27	**0.015**
No DM or Ocular Disease vs. PDR	9.15	2.57	15.73	**0.002**
No DM with Ocular Disease vs. No BDR	5.01	-1.44	11.46	0.208
No DM with Ocular Disease vs. NPDR	5.59	-0.91	12.08	0.129
No DM with Ocular Disease vs. PDR	7.09	0.68	13.50	**0.022**
No BDR vs. NPDR	0.57	-6.26	7.41	0.999
No BDR vs. PDR	2.08	-4.69	8.85	0.916
NPDR vs. PDR	1.51	-5.30	8.31	0.974

No BDR: no background diabetic retinopathy; NPDR: nonproliferative diabetic retinopathy; PDR: proliferative diabetic retinopathy