Diabetic Retinopathies and their associated factors; a study in a tertiary care hospital in Karachi Pakistan

Muhammad Tahir Rizwan Khan

Supervisor: Prof. Akhtar Hussain

The Faculty of Medicine, Institute of Health & Society, Department of Community Medicine, University of Oslo

Co-Supervisor: Prof. Abdur Rasheed Khokar

Professor & Head Departmemt of Ophthalmology, Unit II, Dow University Hospital

Dow University of Health Sciences

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Author: Muhammad Tahir Rizwan Khan

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Trykk: Reprosentralen, Universitetet i Oslo

Dedication

To my dear wife Dr. Syeda Quratulaen Tahir

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Abbreviations

BMI: Body Mass Index

CI: Confidence Interval

CSME: Clinically Significant Macular Edema

DCCT: Diabetes Control and Complication Trial

DKA: Diabetic Ketoacidosis

DM : Diabetes Mellitus

DME: Diabetic Macular Edema

DR: Diabetic Retinopathy

DUH: Dow University Hospital

DUHS: Dow University of Health Sciences

FBG: Fasting Blood Glucose

HbA1c: Glycosylated Hemoglobin

HOMA IR: Homeostasis Model of Assessment - Insulin Resistance.

IR: Insulin Resistance

IRMA: Intra Retinal Microvasculature Abnormalities

MI: Multiple Imputations

NIDE: National Institute of Diabetes & Endocrinology

NPDR: Non-Proliferative Diabetic Retinopathy

OR: Odds Ratio

PDR: Proliferative Diabetic Retinopathy

RBG: Random Blood Glucose

ROS: Reactive Oxygen Species

SBP: Systolic Blood Pressure

T2DM: Type 2 Diabetes Mellitus

UK: United Kingdom

UKPDS: United Kingdom Prospective Diabetes Study

USA: United States of America

USD: United States Dollar

VB: Venous Beading

WESDR: Wisconsin Epidemiologic Study of Diabetic Retinopathy

WHR: Waist Hip Ratio

Abstract

Background:

Diabetic retinopathy, the leading cause of blindness among people aged 20 to 65 years in developed countries, is also a major sight threatening complication of Diabetes Mellitus in the developing world. It is estimated that almost all diabetics eventually suffer from any form of Diabetic Retinopathy. Pakistan, a developing nation, has a high burden of Diabetes with a prevalence of 6.7% and has nonetheless a high presence of Diabetic Retinopathy which is responsible for 0.5% of all acquired blindness in the country. Previous studies from the country report the frequency of Diabetic Retinopathy as high as 55%. However, none of these studies used fundus camera for the detection of Diabetic Retinopathy which is the gold standard procedure.

Objectives:

To study the frequency of Diabetic Retinopathy and Diabetic Macular Edema in Type 1 & Type 2 Diabetes Mellitus patients using fundus photography and factors associated with them in a tertiary care hospital in Karachi, Pakistan

Methods:

A retrospective cross-sectional analysis of diabetic patients' records undergoing fundus photography between May 2010 & December 2013 at Dow University Hospital was done.

A total of 1167 diabetic patients were identified for the study. Data was retrieved from hospital records using Medical Record numbers of patients who underwent fundus photography. Patients were identified as having (1) No Diabetic Retinopathy (No DR), (2) Mild Non-Proliferative Diabetic Retinopathy (Mild NPDR), (3) Moderate Non-Proliferative Diabetic Retinopathy (Moderate NPDR), (4) Severe Non-Proliferative Diabetic Retinopathy (Severe NPDR) or (5) Proliferative Diabetic Retinopathy (PDR). Presence of Diabetic Macular Edema was identified separately as (6) retinopathy patients without Diabetic Macular Edema (No DME), (7) patients with Diabetic Macular Edema but no Clinically Significant Macular Edema (DME only) and (8) patients with Clinically Significant Macular Edema (CSME).

Statistical analyses were performed using IBM SPSS v 20 for Windows. The records revealed 36% cases with missing data in 20 variables. The data was missing at random therefore

Multiple Imputations was employed to handle missing values. The variables retained their characteristics and there was no statistically significant difference in the mean values of all 20 variables before and after multiple imputations. Therefore, we used all 1167 patients' data for analyses (using pooled values after multiple imputations). Means and corresponding 95% confidence intervals were calculated for continuous variables while frequencies and percentages were computed for categorical variables. One-Way ANOVA and Pearson's Chi-Squared test were used to identify statistically significant difference in these variables for all categories of retinopathy. Binary Logistic Regression was employed to calculate Crude and Adjusted Odds Ratios with 95% confidence intervals for the factors associated with Diabetic Retinopathy and Diabetic Macular Edema.

Results:

A total of 853(73.1%) were found to have Diabetic Retinopathy with Mild Non Proliferative Diabetic Retinopathy in 395 (34%), Moderate Non Proliferative Diabetic Retinopathy in 321 (27.5%), Severe Non Proliferative Diabetic Retinopathy in 45 (3.9%) and Proliferative Diabetic Retinopathy in 92 (7.9%) patients. Diabetic Macular Edema was present in 214 (25.1%) of these 853 patients while Clinically Significant Macular Edema was present in 130 (15.2%). Ninety two (11%) patients had Clinically Significant Macular Edema in both eyes.

Univariate analyses using Binary Logistic Regression revealed that Fasting Blood Glucose, Random Blood Glucose, Glycosylated Hemoglobin, Duration of Diabetes Mellitus, presence of Hypertension, Bod Mass Index, Urinary Micro Albumin excretion Waist Circumference and Waist-Hip Ratio were positively associated with any form of Retinopathy in diabetics, whereas High Density Lipoprotein levels were negatively associated with Diabetic Retinopathy. However, on multivariable analyses, only Fasting Blood Glucose, Glycosylated Hemoglobin, Duration of Diabetes, presence of hypertension, High Density Lipoprotein levels and Urinary Micro Albumin excretion retained their statistically significant associations.

For Diabetic Macular Edema, Univariate analyses showed Glycosylated Hemoglobin, presence of Hypertension and Lower levels of High Density Lipoprotein levels were significantly associated. However, in multivariable model, only HbA1c and presence of Hypertension retained their statistically significant association with Diabetic Macular Edema.

Conclusion:

Prolonged hyperglycemia, lower levels of High Density Lipoproteins and Urinary Microalbumin excretion are associated with Diabetic Retinopathy. Whereas, progression of Diabetic Macular Edema is associated with hyperglycemia and presence of Hypertension.

Key Words:

Diabetes Mellitus, Diabetic Retinopathy, Diabetic Macular Edema, Clinically Significant Macular Edema

Chapter 1: Introduction

1.1 BACKGROUND

1.1.1 Diabetes Mellitus & its complications; the Dreaded Pandemic:

The turn of the 21st century saw the drastic increase in the prevalence of Diabetes Mellitus (DM), mainly type 2. In the year 2000, the global prevalence of Diabetes Mellitus (both type I &II) was estimated to be 2.8%, however, based on the reported data and trends from around the world, it is estimated that 4.4% of the world's population will have Diabetes Mellitus in the year 2030.[1] This projected increase in the prevalence means there will be 195 million new cases of Diabetes Mellitus in just 30 years (increasing from 171 Million diabetics in 2000 to 366 Million in 2030). These new cases are more than the total number of Diabetics in the year 2000 alone. The tragedy doesn't end here; the epidemiologic transition through which most of the developing countries are going through means that majority of the burden of diabetics will be in these countries. According to the International Diabetes Federation (IDF), by the year 2030, 9 of the top 10 countries with highest projected Diabetes burden will include nations like China, India, Brazil, Bangladesh, Mexico, Rusian Federation, Egypt and Pakistan. These nine countries (tenth being USA) are developing economies and will bear 333.6 Million of 366 Million cases of Diabetes Mellitus in 2030.[2] These projections implicate that about 91% of the world's Diabetes burden will be in aforementioned 9 developing countries by 2030. These projections are nothing but horrifying.

Much of the burden of DM on healthcare systems is attributed to the complications associated with it. This is evident by the fact that in 2007 the global health expenditure on preventing and treating Diabetes and its complications was 232 Billion US Dollars. This is projected to exceed 302 Billion USD by year 2025.[3] Tragic enough, just 20% of this money is spent on diabetics in the low and middle income countries where more than 80 percent of the diabetics live. For comparison, in 2010, annual expenditure for Diabetes patients in the USA was 7,383 USD per patient, while this reduced to just 24 USD per patient when it comes to Pakistan. The monetary burden of DM complications can be imagined by the fact that the health expenditure is three times higher on patients with macro vascular complications with type 2 DM than those without complications.

With increased longevity of patients with DM and increasing prevalence, the complication of diabetes mellitus pose a great threat to the overall health situation in the world. Although, better appreciation of the risk factors and early detection of the complications have lowered the burden of these complications in developed countries but this is unfortunately not true for the developing countries where sub optimal care and lack of proper implementation of screening protocols is adding up to the burden of DM complications. [4]

Although, Cardiovascular disease (CVD) is the leading macrovascular complication of T2DM and approximately half of the patients with T2DM die of cardiovascular causes, the microvascular complications are the major cause of social and financial burden of Diabetes. [5] These complications include Retinopathy, Nephropathy, Neuropathy and small vessel vasculopathy causing lower limb amputation.

1.1.2 Diabetic Retinopathy; the earliest complication:

Diabetic Retinopathy (DR) is the earliest and often underestimated complication of Diabetes Mellitus. It is a chronic progressive, sight threatening complication of DM affecting the retinal microvasculature associated with prolonged hyperglycemia. The importance of DR can be estimated by the fact that the knowledge of the cutoffs of plasma glucose levels for diagnosing diabetes mellitus are based on two sets of information and Plasma Glucose Level associated with Retinopathy is one of them.

Global estimates report that, around 34.6% of all diabetics suffer from any form of retinopathy, and it is the leading cause of acquired blindness among adults of working age in industrialized countries [6, 7]. Globally, it accounts for 5% of all cases of blindness. [8] This situation becomes worse when it comes to non-industrialized countries where screening protocols are not strictly observed.

1.1.3 Pathophysiology &Natural history of Diabetic Retinopathy

The natural history of diabetic retinopathy is affected by the effect of increased bllod glucose due to DM on the retinal capillaries. It progresses from Non-Proliferative form, characterized by increased vascular permeability causing few microaneurysms in Mild NPDR to more microaneurysms and blot haemorrhages in Moderate NPDR to venous beading in Severe form causing vascular closure. As a consequence new but abnormal & fragile blood vessels are formed on the retina and posterior surface of Vitreous.

Diabetic Macular Edema (DME), which is the thickening of the macula due to more permeable vessels, affecting central vision can develop at any stage of DR. it is sometimes described as a complication of Diabetic Retinopathy.[9] Clinically Significant Macular Edema (CSME) is the more severe form requiring immediate intervention.

1.1.4 Diagnosis & Classification:

According to the Early Treatment Diabetic Retinopathy Study (ETDRS) the gold standard for detecting Diabetic Retinopathy consists of 30-degree stereoscopic photography of seven standard fields on color film. [10] ETDRS is in turn a modification of Diabetic Retinopathy Study (DRS) [11] and the original Airlie House Classification. [10] This classification is used as a full disease classification and it is not for population based screening.

The classification of DR is based on the presence or absence of abnormal new vessels as Proliferative Diabetic Retinopathy (PDR) or Non- Proliferative Diabetic Retinopathy (NPDR) respectively. Non-Proliferaive DR is further divided into Mild, Moderate & Severe forms based on the number of microaneurysms seen on fundoscopy.

Diabetic Retinopathy affecting the Macula lutea, area responsible for central vision, is separately defined as Diabetic Maculopathy. ETDRS defined Clinically Significant Macular Edema (CSME) as retinal thickening and/or hard exudates within 500 µm of the fovea, or when there is a zone of edema that is larger than the papilla at a distance of one papillary diameter. [12] CSME is a serious condition requiring urgent intervention as it can be present while the patient can still see well however the vision is acutely endangered by the retinopathy. Another aspect of Diabetic Maculopathy is ischemic in which capillary network is occluded and confers a poor prognosis for visual acuity as this condition is still not treatable. [13] Diabetic Maculopathy can also present as the combination of both edematous and ischemic types.

1.1.5 Delay in diagnosis & ScreeningProtocols:

Current screening protocols recommend that T1DM patients should be examined by ophthalmologist for dilated eye examination within 5 years of diagnosis. [14] However, studies suggest that some proportion of T2DM patients had already developed DR when they are diagnosed therefore these patients should have an initial dilated eye examination at the time of their diagnosis of diabetes.

However, this is not in practice in the developing parts of the world. For example a study [15] from Pakistan showed that 15% of T2DM patients had already developed some form of Retinopathy when they were first diagnosed with Diabetes. similarly, Mahar PS and associates report that om their community based study, 2.18% of newly diagnosed T2DM patients had retinopathy. [16]

Looker HC et al [17] reported that 19.3 % of patients were found to have any form of retinopathy immediately after diagnosis of T2DM. Similarly a study from Inida reports that 5% of newly diagnosed diabetics had DR. [18] The UKPDS reports that retinopathy was present at the time of diagnosis of T2DM in 39% of men and 35% of women.[19]

Where these study points towards the delay in diagnosis of T2DM, the use of direct ophthalmoscopy to detect DR raises the question that whether its an underestimation of the true burden. The gold standard to detect DR is Indirect Ophthalmoscopy using Fundus Camera. [10] Despite this, very few studies used indirect fundusphotography to diagnose DR due to its high cost.

1.2 EPIDEMIOLOGY OF DIABETIC RETINOPATHY

1.2.1 Global Burden:

In many countries Diabetic retinopathy is the commonest cause of preventable blindness among individuals of working age group. In the US, the prevalence of DR is reported as high as 28% while that of Diabetic Macular Edema as 3%. [20] In other developed countries the tally is similar, ranging between 17 & 45% for DR while that for DME between 3 & 13%. [21, 22] However, a recent meta-analysis considering studies between 1980 to 2008 reports that the overall all risk of any form of DR is 35% while that of PDR is 7.2% and for DME, 7.5%. [23]

1.2.2 Diabetic Retinopathy in South Asia

Recently, Asia has emerged as a major Diabetes burden bearer in the world. Countries like China, India, Bangladesh and Pakistan have, not only high prevalence of Diabetes, but also of its complications.

Though DR is not the main cause of blindness, nonetheless its prevalence is far more than that in the developed world. The poor control of diabetes and lifestyle related risk factors in these countries pose a great threat.

Literature published from China report that DR is present in 37 to 43% of diabetics while DME is present in 2.6 to 3.5% of these patients. [24, 25] Studies from India report the prevalence to be ranging between 17.6% & 28.2% . [18, 26] similarly, While Ahmed KR [27] reports that in Bangladesh 50.6% of diabetics develop DR within 15 years of onset of DM.

1.2.3. Situation in Pakistan

With a national diabetic prevalence of 6.7%, Pakistan is one of the high burden countries. [2] However, there have been no nationwide survey of the complications attributed to diabetes mellitus. Many small studies, differing in study settings and methods have reported a great deal of variation in the prevalence of DR in the country. A population based screening study in subjects over 30 years reported that DR was seen in 27.43% of Diabetics. [16] Similarly, another community based screening study reported DR to be present in 15.7% of the diabetics. However a hospital based study from Quetta city reports the frequency of DR to be 54.6%. [28]

Another considerable issue with past studies is the usage of non-uniform diagnosing tools for DR. to the best of of our knowledge, no study used fundus camera for the indentification of DR which is the gold standard. [12]

1.3 FACTORS ASSOIATED WITH DIABETIC RETINOPATHY

Many studies have been carried out to acquire an in-depth understanding of the factors associated with Diabetic Retinopathy. The most prominent of these are the Wisconsin Epidemiologic Study of Diabetic Retinopathy (WESDR). This study started in 1979 was aimed to describe the frequency and incidence of diabetic complications. The study resulted in more than 210 reports adding great to the present knowledge of DR. [29-33] A clinical trial run between 1977 & 1997, the United Kingdom Prospective Diabetes Study (UKPDS) and its post trial studies helped further in understanding Diabetic Retinopathy and other complications of Diabetes Mellitus. [34-37]

1.3.1 Blood Glucose Levels/HbA1c:

Hyperglycemia is by far the most important factor associated with diabetic retinopathy. High blood glucose level is the key factor in the pathogenesis of DR in many ways. During the last decade of 20th century and the first decade of 21st century a number o studies and trials gave a greater understanding of how high blood glucose levels caused the microvascular complications of Diabetes Mellitus. [38-41] Sparing the complex bio-chemical mechanism in production of Reactive Oxygen Species (ROS), it is now clear that high blood glucose levels adversely affects the functional retinal vasculature causing impaired retinal blood flow, leakage of capillaries, increased leaukocyte & monocyte adhesion and capillary closure causing localized hypoxia (leading to neovascularization in PDR).

Long standing high glucose levels are associated with the worse forms of DR. Although the easiest way to measure blood glucose is the Random (Post Prandial) and Fasting Glucose levels espeicaially after the introduction of portable glucose monitoring devices, however, the better picture is given by the glycosylated hemoglobin or the HbA1c. The Wisconsin Epidemiologic Study of Diabetic Retinopathy showed that the relative risk of developing Proliferative Diabetic Retinopathy increases to 2.64 (95% CI, 2.18-3.20) times when the HbA1c is between 12.1 to 19.5% and chances of developing Macular Edema is 3.37 times (95% CI, 2.12-5.34).[30] Lauritzen T et al showed in a study among T1DM patients that near normal glucose levels over a period of 1 year (maintained by Insulin) significantly stopped the progression of DR. [42] Many studies around the World have shown the association of HbA1c with the progression of DR with Odds Ratio ranging from 1.38-4.53. [43-50]

FBG: Komamoto study reports that based on insulin treatment among typoe 2 diabetics, the threshold of FBG for onset and progression of microvascular complications of diabetes such as Retinopathy is <6.0 mmol/L (110mg/dL). That of HbA1c is <6.5% and that of 2h postprandial blood glucose is <9.9mmol/L (<180 mg/dl).[51]

Another trial, the DCCT [52, 53] demonstrated that the risk of progression of DR can be reduced by 54% and development of Severe NPDR or PDR can be reduced by 47% by intense blood glucose control. Similarly, the trial also reported that the need for laser surgery can be reduced by 56% and the risk of DME can be reduced by 23%.

RBG

1.3.2 Hypertension:

The epidemiological association between DR and Hypertension has long been known. The effect of elevated blood pressure on ratina is evident from Hypertensive Retinopathy. However, HTN is not just a risk marker for DR. The combination of HTN and uncontrolledblood glucose levels has been shown to increase the risk of DR many fold. (REF) UKPDS showed that good control of blood pressure can reduce the risk of development as well as progression of diabetic eye complications. [34, 54]

Systemic hypertension further worsens the already impaired retinal due to hyperglycemia causing by susceptibility to capillaries. However, blood pressure levels are associated with the progression of retinopathy in diabetics even in the absence of systemic hypertension. A study among T1DM patients showed that even within normal range, higher blood pressure levels are associated with the progression of retinopathy. [55]

1.3.3 Duration of DM:

The duration of Diabetes Melitus has been shown to be associated with both the incidence of DR and also the progression of it. It might not be an independent risk factor for DR since longer exposure to hyperglycemia and other factors might be the main causing factors behind. Nevertheless, duration of DM is reported to be positively associated with the incidence and progression of DR in both types of DM by majority of researchers. [32, 50, 56, 57]

1.3.5 Urinary Micro Albumin:

The association of overt albuminuria, with micro and macro vascular complication of diabetes mellitus has been known for quite some time. However, microalbuminuria has now been reported as independently associated with the presence of diabetic retinopathy in several studies. [58-60] The Wisconsin Epidemiologic Study of Diabetic Retinopathy showed that Microalbuminuria can be used as a marker for the risk of PDR. [59] Suvage S et al [58] in their study on T2DM patients found that Urinary Albmin Excretion was not only positively associated with DR but with Neuropathy & Cardiovascular Diseases as well. Similar study from China [45] also found out that 24hour urinary albumin excretion was greater in those diabetic who had developed retinopathy.

However, a cross sectional study on Australian T2DM patients reported however that urinary albumin to creatnine ratio was significantly higher in those with DR. [44]

1.3.6 Serum Cholesterol:

Several studies have pointed towards the association of serum cholesterols with diabetic retinopathy. In Early Treatment Diabetic Retinopathy Study, the authors showed a relationship of total cholesterol & LDL with hard retinal exudates. [61] Klein BEK et al showed in the Wisconsin Epidemiologic Study of Diabetic Retinopathy that the ratio of total to HDL cholesterol was positively associated with the progression of diabetic retinopathy. [62]

1.3.7 Type of Diabetes Mellitus:

Possibly due to early onset of type 1 diabetes mellitus, retinopathy is usually seen earlier and more in these patients. According to Fong DS et al [63], all patients with type 2 DM suffer from DR within first twenty years of the onset of DM while over 60% of T2DM patients suffer with DR during this time. This might be due to relatively aggressive need of glycemic control in T1DM patients which is not always obtained. However, sight-threatening retinopathy in these patients is rare before puberty. WESDR [32] findings support the aggressive progression of DR in T1DM patients as they reported the prevalence of DR after 5 years of diagnosis as 17% while this was raised to 97.5% when they have had T1DM for 15 years or more. Moreover, though progression of DR is slower in T2DM, almost all patients suffer from some degree of DR in their life.

1.3.8 Age:

Age has a unique association with the incidence & prevalence of DiabeticRetinopathy. WESDR reports that the risk of DR increases 3.2 times for post-menarchal T1DM patients. [64] Espeially in the case of Proliferative DR, pre-pubertal age seems to attribute a protective effect. Though not clearly understood, many factors have been suspected to play a role in this association like increased growth hormone, increased insulin growth factor, poorer glycemic control and increased sex hormones like testosterone. Another study reports that the odds of having Daibetic retinopathy is raised to 6.1 times when comparing pre & post-pubescent patients. [65] WESDR however reports that in the T2DM patients, the age had no or very little effect on the incidence of retinopathy and no patient older that 80 years of age was found to *develop* proliferative DR. [64]

However, Holl RW and associates [66] are of the opinion that the apparent lack of microvascular complications especially Retinopathy in children (prepubescent) is due to the lack of routine diagnosis of these complications in the diabetic children.

1.3.9 Smoking:

Some researchers have reported smoking to be positively associated with the progression of DR among diabetic patients. [67-70]it is reported that cigarette smoking increases the risk of diabetic complication probably by increasing insulin resistance. [70]smoking has been shown to increase the risk of microvascular complications among T1DM patients to a greater extent while macrovascular complications in T2DM patients.

UKPDS 50[37] however reports that *not smoking* was positively associated with both DR development and progression. The authors of this study suspects the possible pharmacologic effects of nicotine to be responsible for this inverse association.

1.3.10 Insulin Use:

Since intensive glycemic control by Insulin Treatment has shown to delay or event prevent the progression of diabetic retinopathy Therefore Serum Insulin level is inversely propotional to the severity of DR especially in T2DM patients. [19, 71] In T1DM patients, WESDR reports that better glycemic control using better dosing of insulin proves to be a protective factor against DR incidence & progression. These findings are also similar to those found by other study groups like the Krov Collabortaion Study Group.[72] The Komamoto Eye Study in T2DM patients reported that group of patients receiving multiple insulin injections to attain better glycemic control had less cumulative worsening of Retinopathy compared to the group receiving conventional insulin injection therapy. [51]

However, The Los Angeles Latino Eye Study reports that being on insulin treatment has greater Odds of having DR (OR 3.2). [57]

1.3.11 Anthropometry:

Anthropometry has been indicated in several studies for risk factor assessment for DR. Dirani M et al reported in their study that higher BMI is associated with risk of any form of DR aming type 2 DM patients and that Obese people hve 6.5 times more risk of having DR. the authors also reports that Neck circumference and waist circumference are also associated with any form of dr while BMI and neck circumference were positively associated with the

progression of DR.[73] Similary, a recent study from Croatia, reported that, besides other factors, higher BMI was positively associated with the progression of DR. [74] especially in Asian population, BMI has been frequently reported in studies to be associated with DR. [75, 76]

EURODIAB study explained that waist-hip ratio and fasting triglyceride levels which are markers of Insulin Resistance are positively associated with DR amiong type 1 DM patients.[60]

1.4 PROBLEM STATEMENT:

There is considerable variation in the prevalence of DR reported in the studies from Pakistan. In addition, the methods to detect DR in diabetic patients used in these studies are not uniform. To the best of our knowledge, no study from the country used fundus photography to identify DR. instead, direct ophthalmoscopy was used which is very much operator dependant hence, an underestimation of the problem can be suspected.

1.5 PRESENT STUDY:

Present study was designed to study the frequency of retinopathy including macular edema in diabetic patients using fundus photography, which is the gold standard tool for the detection of DR. There is evidence that use of Fundus Camera to diagnose and detect grading of Diabetic Retinopathy can significantly detect more cases of diabetic retinopathy especially milder forms then direct fundoscopy. [10, 77] This study used *Topcon TRC.50EX® retinal camera with Nikon D5000® 12.3 megapixel digital camera* for detection of DR I diabetic patients. This study also looked into the factors associated with Diabetic Retinopathy and Diabetic Macular Edema.

1.5 Objectives

General Objective:

To study the frequency of Diabetic Retinopathies using fundus photography and factors associated with them among patients with Diabetes Mellitus in a tertiary care hospital in Karachi, Pakistan

Specific Objectives:

1. To find out the frequency of Diabetic Retinopathy among type1 and type2 diabetes mellitus patients
2. To find out the frequency of Diabetic Macular Edema among type1 & type2 diabetes mellitus patients
3. To study the factors associated with Diabetic Retinopathy among type 1 & type 2 diabetes mellitus patients
4. To study the factors associated with Diabetic Macular Edema among type 1 & type 2 diabetes mellitus patients

Chapter 2: Patients & Methods

2.1 Research Design:

Cross Sectional; A retrospective analysis of hospital records were done on diabetic patients who underwent fundus photography.

2.2 Study Setting:

Department of Ophthalmology & National Institute of Diabetes & Endocrinology, Dow University Hospital. Dow University Hospital is a large, government run, tertiary care hospital in Karachi, the largest city of Pakistan (with a population of over 20 Million). The hospital has a total monthly patient turn over of around 100,000 patients. Department of Ophthalmology & National Institute of Diabetes & Endocrinology combined, however have a monthly patients turnover of approximately 40,000 patients.

2.3 Duration of Study:

August 2013 to December 2013

2.4 Sample Size:

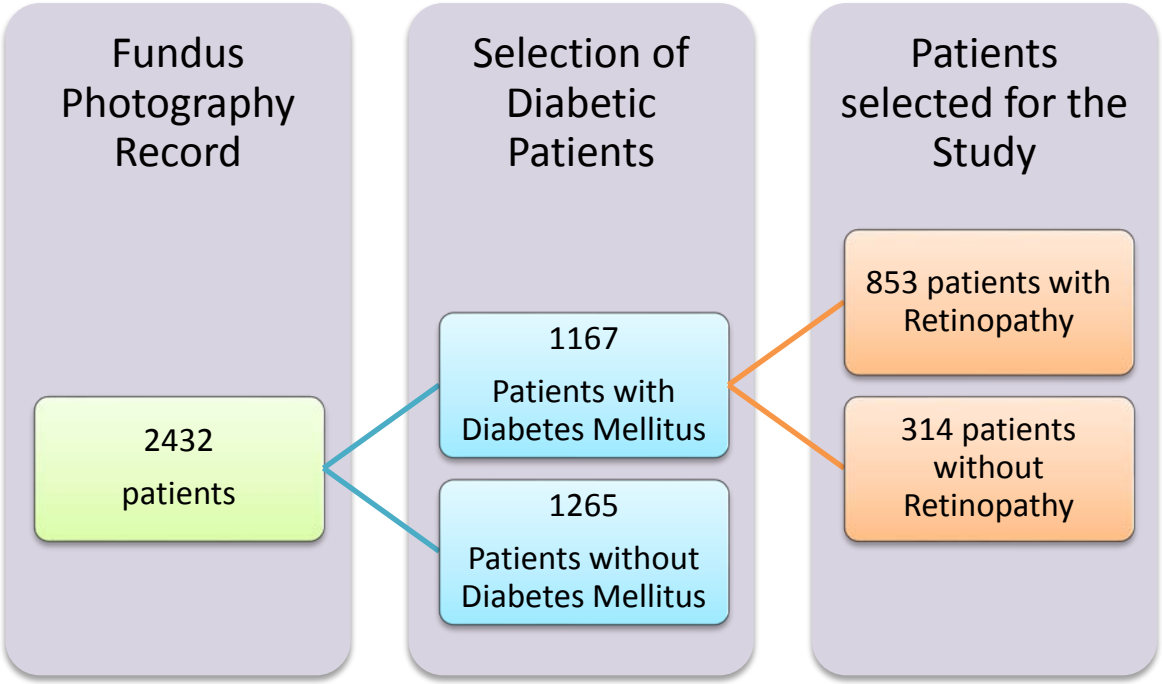
Using OpenEpi sample size calculator, keeping the frequency of Diabetic Retinopathy among T2DM patients in Karachi as 27.43% as reported by Mahar PS et al [16], and Confidence Level at 95%, the sample size was calculated to be **157** at maximum error of ± 7% with estimate.

Sample size was calculated using the proportion of DR among T2DM patients which yielded larger sample size compared to that calculated with T1DM reported by WESDR [30](n=95). Therefore, larger sample size was considered for this study

2.5 Sample Selection

Data records for all the patients who had gone through fundus photography and were previously diagnosed as having Diabetes Mellitus of either type were taken into the study. Figure 1 shows the procedure of sample selection.

Figure 1 Selection of study subjects from Department of Ophthalmology, Dow University Hospital



2.6 Operational Definitions

1. Diabetic Retinopathy:

Diabetic Retinopathy was identified on the basis of history of Diabetes Mellitus (both type 1 & 2) and findings of fundus photography using *Topcon TRC.50EX® retinal camera with Nikon D5000® 12.3 megapixel digital camera* as follows: [78]

2. Non-Proliferative Diabetic Retinopathy (NPDR):

NPDR was diagnosed and graded by the presence of microaneurysm without any formation of abnormal new blood vessels.

(i) **Mild NPDR:** Atleast one of the following:

a) Microaneurysms

b) Dot/blot hemorrhages

(ii) **Moderate NPDR:** Marked Hemorrhages, Cotton Wool spots, Venous Beeding (VB), Intra Retinal Microvasculature Abnormalities (IRMA) to mild degree

(iii) **Severe NPDR:** Marked Hemorrhages in all four quadrants, Venous Bleeding (VB) in 2 or more quadrants, Marked Intra Retinal Microvasculature Abnormalities (IRMA) in one quadrant

3. Proliferative Diabetic Retinopathy (PDR):

Proliferative Diabetic retinopathy was diagnosed by the presence of abnormal new blood vessels on or around the optic disc.

4. Diabetic Macular Edema:

Retinal thickening within 2 disc diameters of the center of the macula, resulting from the leakage of plasma constituents into the surrounding retina.

5. Clinically Significant Macular Edema (CSME):

CSME was identified when one of the following was present:

a) Retinal thickening at or within 500 microns or 1/3 disc diameter of center of macula.
b) Hard exudates at or within 500 microns of the center of the macula with adjacent retinal thickening.
c) Retinal thickening GREATER than 1 disc diameter in size which is within 1 disc diameter from the center of the macula

6. Grading of Diabetic Retinopathy:

In case the findings in both eyes of the same patient differed, the patient was graded according to more severe form as per Early Treatment Diabetic Retinopathy Study. [10]

7. Type of Diabetes:

Type of Diabetes Mellitus was identified from the medical records. These patients were already diagnosed as having either type 1 or 2 DM at NIDE.

2.7 Methods:

A Retrospective cross-sectional analyses was performed for the identification of patients suffering from Diabetic Retinopathy. From May 2010 (when the facilty of Fundus Photography was initiated at the department) till December 2013, Two thousand four hundred and thirty two patients underwent Fundus Photography for both Anterior & Posterior chambers of the eye after pupil dilatation using 1% tropicamide Eye drops. *Topcon TRC.50EX® retinal camera with Nikon D5000® 12.3 megapixel digital camera* was used for fundus photography for both eyes during this period.

Of these 2432, 1167 were suffering from Diabetes Mellitus (either type I or II). File records were accessed using medical record numbers of these patients present in the records of

fundus photographs and information on the variables were collected. For Patients with more than one retinal photographs (on different dates) the earliest one was considered.

Selection of Variables:

For a total of 1167 diabetic patients, variables were selected for sociodemographic, clinical and biochemical characteristics. The list of the variables is as follows:

Age, Gender, Education, Marital Status, Family History of Diabetes, Type of Diabetes, Smoking Status, Duration of Diabetes, Height, Weight, Systolic Blood Pressure, Diastolic Blood Pressure, Hip circumference, Waist circumference, Type of Medication for Diabetes Mellitus

Random Blood Glucose (RBG), Fasting Blood Glucose (FBG), Glycosylated Hemoglobin (HbA1c), Urea, Creatinine, Sodium, Potasium, Bicarbonate, Total Cholesterol, Total Triglycerides, High Density Lipids, Low Density Lipids, Urinary Microalbumin,

Data Collection tools:

All biochemical parameters and anthropometric measurements were collected in Dow Lab (DUH) and NIDE respectvely. Height was measured as standing height using standard height scale for adults at NIDE. Weight of the patients were recorded using analogue weighing scale. Blood Pressure readings were measured using mercurial sphygmomanometer. Waist circumference was measured using standard cm/inch tape at midway between bottom of ribs & top of hip bone. Similarly, Hip circumference was measured at the widest point. Lipid profile, was assessed by enzymatic calorimetric test. Fasting and random blood glucose was estimated using the automatic biochemical analyzer (Hitachi 902) which uses the photometric technique of glucose estimation at DOWLAB. HbA1c was analyzed using automated analyser (Sysmex). Urinary Micro Albuminuria was determined by using semi-quantitavie dry immunological screening strip.

Ethical considerations:

The study was approved by Regional Committee for Medical and Health Research Ethics (REK) Norway and Institutional Review Board (IRB) Dow University of Health Sciences, Karachi, Pakistan.

Statistical Analyses:

Statistical analyses were performed using IBM SPSS v 20 for Windows (SPSS Inc. Chicago, Illinois).

Handling of Missing Data; Multiple Imputations:

Missing data identification analyses was done on 20 variables to identify at least 0.01% of data missing to be selected for Multiple Imputation using IBM SPSS v 20 for Windows (SPSS Inc. Chicago, Illinois). The analyses yielded that 90% values were found missing while only 745 (63.83%) of the cases were complete. The percentages of missing data for all 20 variables are shown in Figures 2 & 3 and Table 1 shows the pattern of missing data. The missing data pattern showed that the data was "Missing at Random" (Figure 3 showing isolated islands of missing data suggesting the 'missing at random pattern') therefore Markov Chain Monte Carlo method was used to impute the missing data. A total of five iterations were done to generate the pooled results for the missing values.

After Multiple Imputations for missing variables, the values with their mean and standard deviations alongwith their comparison with non-imputed data (analyses including missing values) are shown in Table 2. There was no statistically significant difference observed in the values before MI & Pooled Values after MI. Therefore all descriptive results for 1167 patients are shown using pooled values (after MI).

Figure 2 Percentages of missing data according to Variables, Cases and Values for all patients (n=1167)

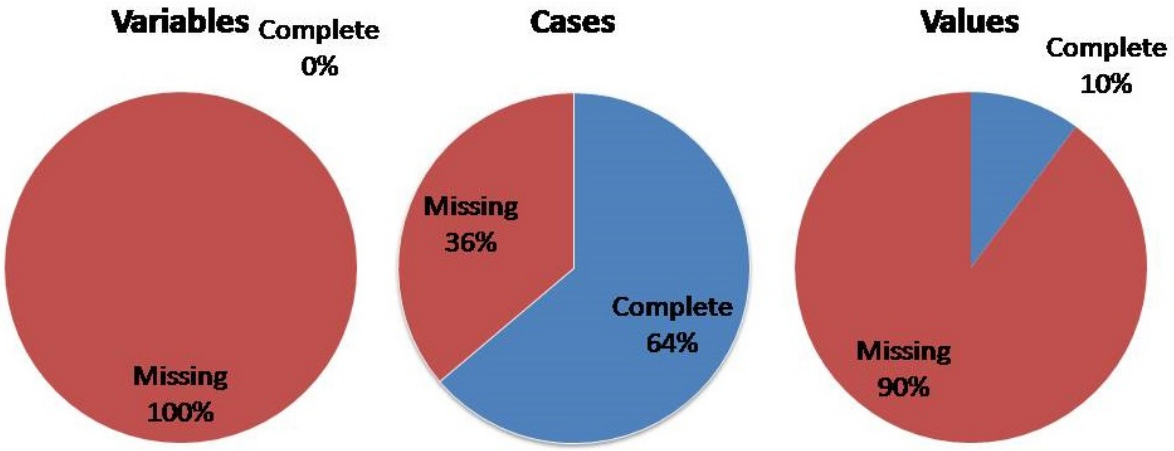


Figure 3 Missing values pattern showing data 'missing at random' for 18 selected variables of all patients (n=1167)

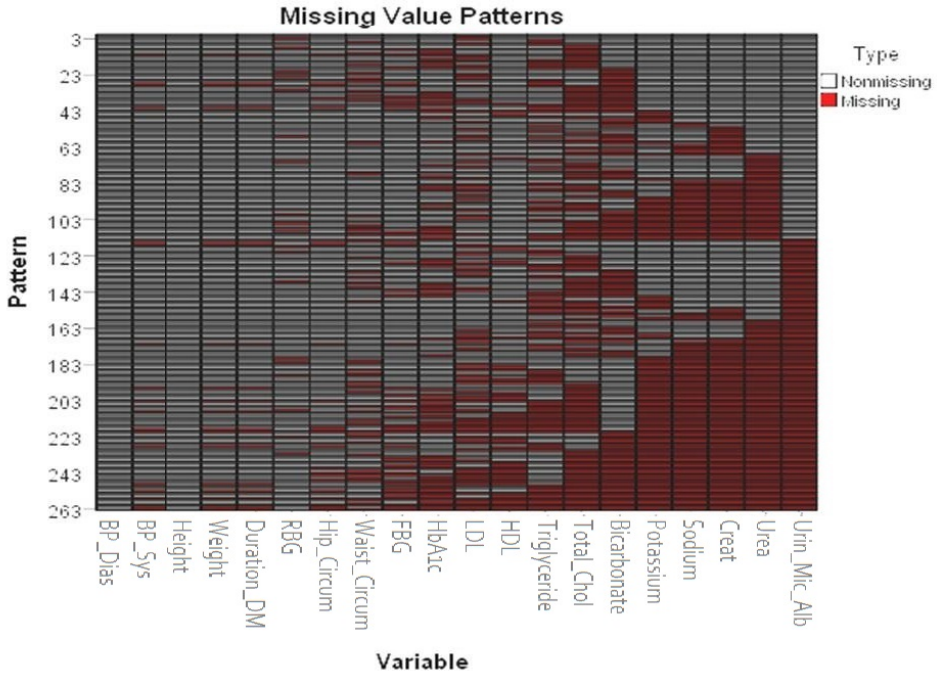


Table 1 The variables and corresponding percentages of missing data (in order of highest missing percentage first)

S. No.	Variable	Valid Values	Missing
1	Urinary Micro Albumin	512	56
2	Urea	591	49
3	Creatinine	598	49
4	Sodium	598	49
5	Potassium	598	49
6	Bicarbonate	598	49
7	Total Serum Cholesterol	598	49
8	Triglycerides	689	41
9	HDL	689	41
10	LDL	689	41
11	HbA1c	712	39
12	FBG	751	35
13	Waist Circumference	751	35
14	Hip Circumference	765	35
15	RBG	872	25
16	Duration of Diabetes	1089	7
17	Weight	1121	4
18	Height	1121	4
19	BP Systole	1160	1
20	BP Diastole	1160	1

Table 2 Comparison of Imputed & Non-Imputed Data (n=1167)

	Mean (SD) Before MI	Pooled Mean after MI	P-value
FBG mmol/L	9.89 (3.97)	9.86 (4.03)	0.881
RBG mmol/L	13.38 (4.97)	13.38(5.09)	0.993
HbA1c %	9.6 3 (3.39)	9.63 (3.39)	0.999
Duration of Diabetes years	15.08 (7.43)	15.08 (7.43)	0.999
Total Serum Cholesterol mmol/L	4.78 (1.25)	4.85 (1.28)	0.243
Total Triglyceride mmol/L	1.98(1.05)	1.97 (1.06)	0.861
HDL mmol/L	1.04 (0.40)	1.05 (0.41)	0.832
LDL mmol/L	2.88 (0.94)	2.88 (0.98)	0.829
Urea mmol/L	9.89 (4.96)	9.71 (5.01)	0.313
Creatinine µmol/L	85.75 (75.14)	84.86(74.25)	0.860
Sodium mmol/L	139.99 (5.64)	140.01 (5.83)	0.670
Potassium mmol/L	4.76 (0.4)	4.75 (0.5)	0.771
Bicarbonate mmol/L	24.23 (4.2)	24.13 (4.2)	0.607
Urine Micro Albumin µg/min	66.92 (88.22)	70.81 (90.91)	0.446
Weight Kg	69.47 (14.01)	69.45 (14.05)	0.960
Height m	1.61 (0.10)	1.61 (0.11)	0.898
BP Systole mmHg	127.45 (19.09)	127.58 (19.17)	0.869
BP Diastole mmHg	80.27 (9.74)	80.23 (9.78)	0.934
Waist cm	100.08 (11.69)	99.99 (11.69)	0.869
Hip cm	103.95 (10.25)	103.65 (10.24)	0.541

Descriptive Statistics:

The data on all 1167 patients after Multiple Imputation for missing values and descriptive results were generated. Frequencies and percentages of Diabetic Retinopathy, Diabetic Macular Edema & Clinically Significant Macular Edema was calculated. Mean and 95% CI for continuous variables like bio-chemical values were calculated. Similarly, frequencies and percentages of categorical variables were computed.

Inferential Statistics:

T-Test & One Way ANOVA was employed to see the difference between continuous variables of patients with and without DR & DME. Tukey's Post Hoc test was carried out to

identify statistically significant differences between different groups of DR. Variables with statistically significant difference (p-value <0.05) were included in Univariate analyses calculating Odds Ratios with 95% CI using Binary Logistic Regression. Variables maintaining their statistical significance were included in Multivariable analyses to calculate the adjusted Odds Ratios.

Chapter 3: Results

From a total of 1167 diabetic patients who underwent fundus photography, 703 (60%) were male while 464 (40 %) were female. The mean age of the patients was 52.56 years (95% CI 51.96- 53.17). Twenty nine (2.4%) of the patients had T1DM while 1138 (97.6%) patients had T2DM.

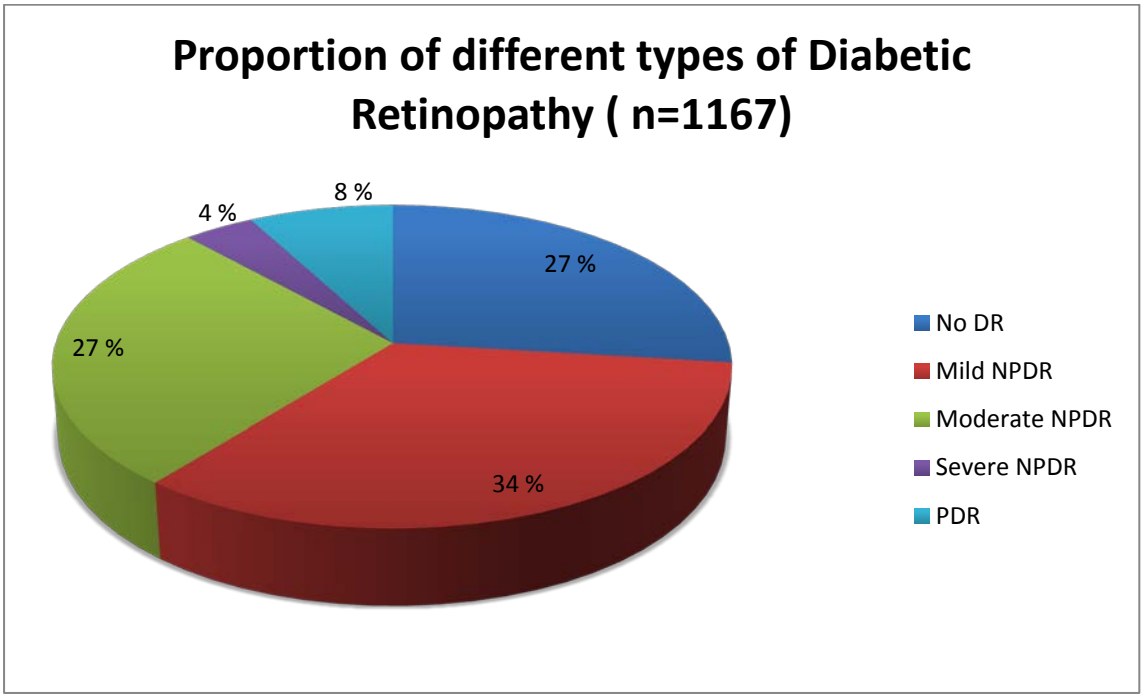
3.1 Frequency of Diabetic Retinopathy and Diabetic Macular Edema

A total of 853 (73.1%) of 1167 patients were found to have Diabetic Retinopathy. Frequency of different forms of Diabetic Retinopathy is as follows.

Three hundred and ninety five (33.8%) patients were graded as having Mild NPDR, 321 (27.5%) Moderate NPDR, 45 (3.9%) Severe NPDR whereas 92 (7.9%) were graded as having PDR. Figure 4 shows the proportions of different types of DR.

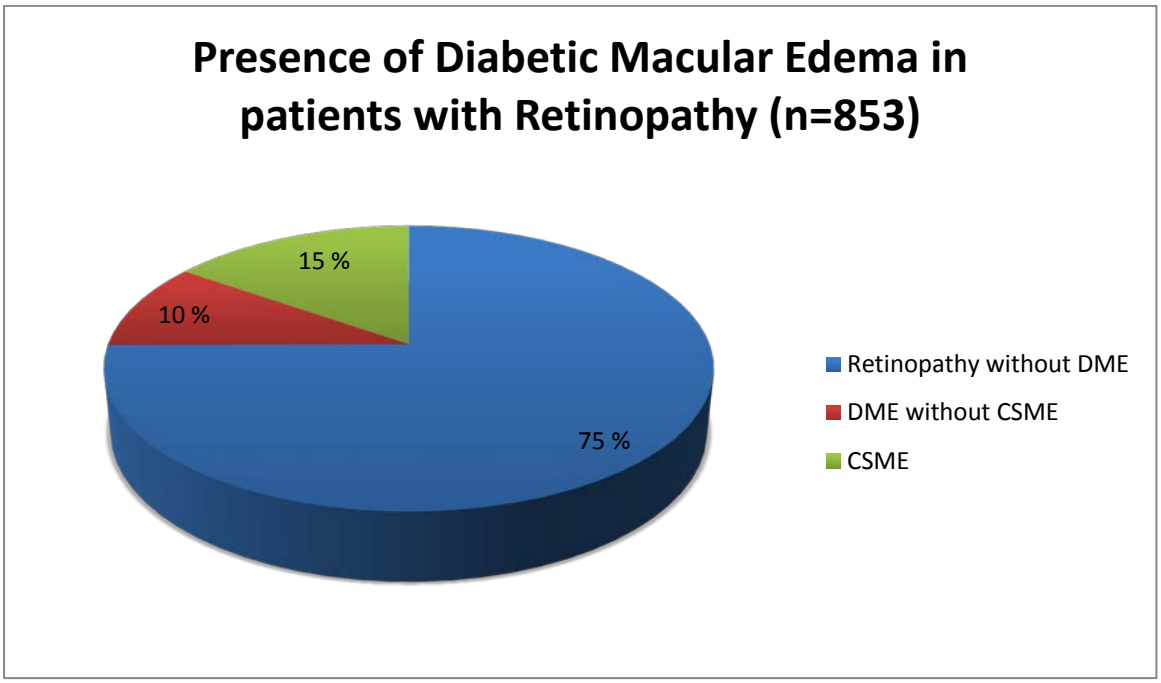
A total of 214 (25.1%) of 853 were found to have Diabetic Macular Edema. Whereas, Clinically Significant Macular Edema (CSME) was found in 130 (15.2%) cases. Figure 5 shows the proportion of DME & CSME in patients with DR. There were a total of 92 patients who had CSME in both eyes.

Figure 4 Distribution of Diabetic Retinopathy according to severity



DR: Diabetic Retinopathy, NPDR: Non-Proliferative Diabetic Retinopathy, PDR: Proliferative Diabetic Retinopathy

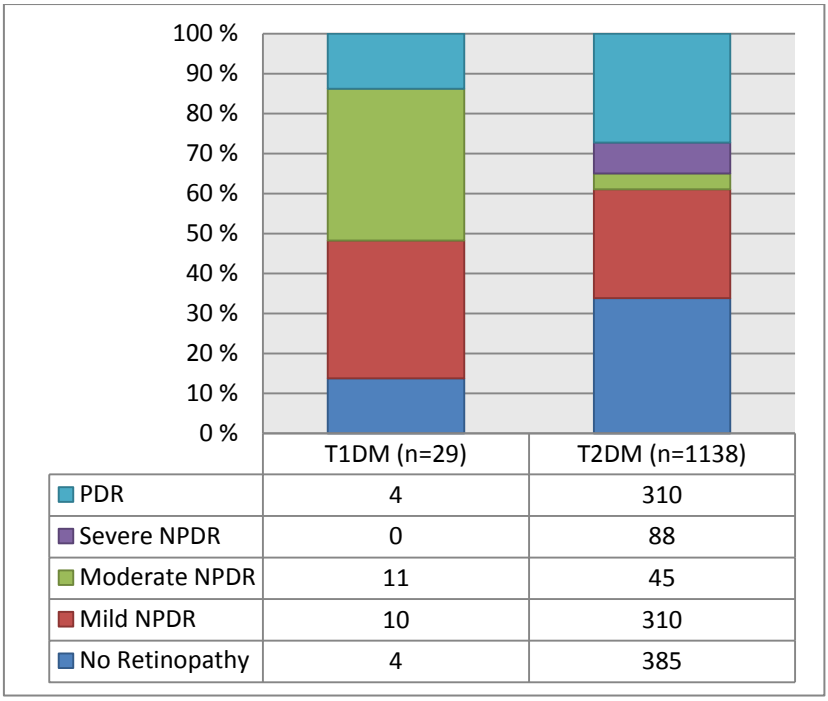
Figure 5 Distribution of Diabetic Macular Edema and Clinically Significant Macular Edema in Retinopathy patients



DME: Diabetic Macular Edema, CSME: Clinically Significant Macular Edema

In regards to type of diabetes, 25 of 29 (86.2%) patients with T1DM had Diabetic retinopathy. Whereas 828 of 1138 (72.8%) T2DM patients had any form of Diabetic Retinopathy. Figure 6 shows the distribution of different grading of Diabetic Retinopathy according to the type of DR.

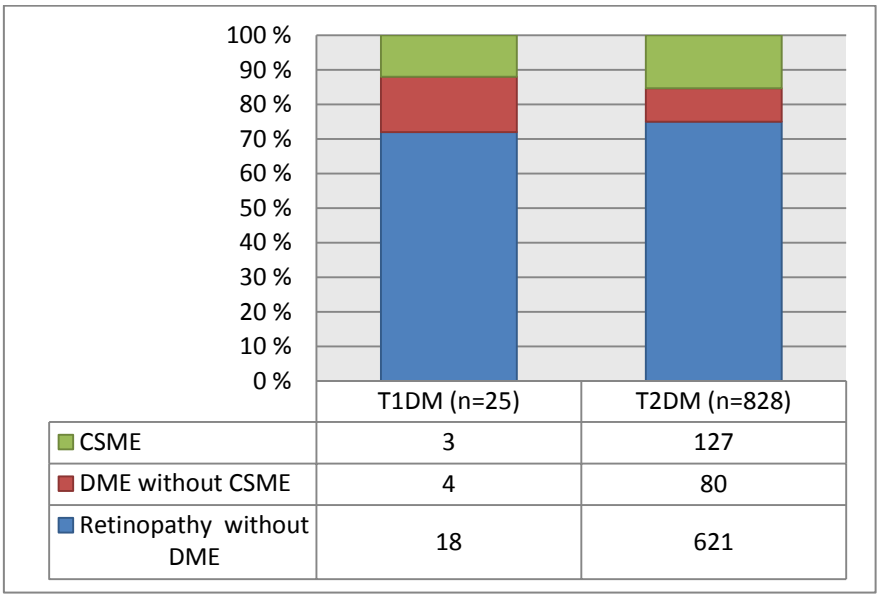
Figure 6 Distribution of different forms of Diabetic Retinopathy according to type of Diabetes



	T1DM (n=29)	T2DM (n=1138)
PDR	4	310
Severe NPDR	0	88
Moderate NPDR	11	45
Mild NPDR	10	310
No Retinopathy	4	385

There were 7 T1DM patients with Diabetic Macular Edema (DME) while 207 with T2DM had macular edema. There was no statistically significant difference found in the presence of Diabetic Macular Edema according to the type of Diabetes.

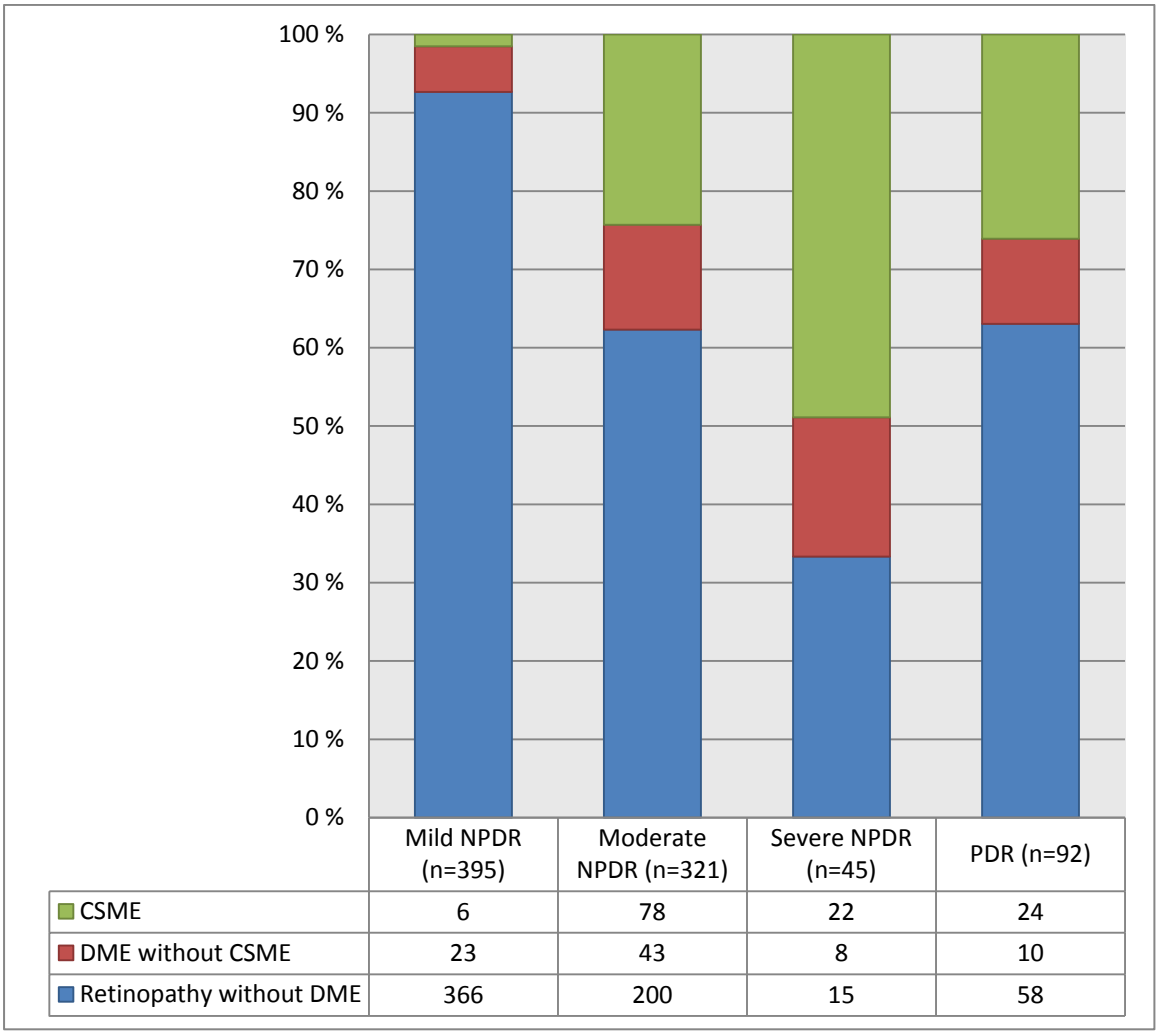
Figure 7 Distribution of Diabetic Macular Edema and Clinically Significant Macular Edema according to type of Diabetes



	T1DM (n=25)	T2DM (n=828)
CSME	3	127
DME without CSME	4	80
Retinopathy without DME	18	621

Diabetic Macular Edema and Clinically Significant Macular was found to be present in all 4 grades of retinopathy, that is, mild, moderate and severe forms of Non Proliferative Diabetic Retinopathy and Proliferative Diabetic Retinopathy. The distribution of DME and CSME according to different grades of retinopathy is shown in Figure 8.

Figure 8 Distribution of Diabetic Macular Edema and Clinnically Significant Macular Edema according to different grades of retinopathy



	Mild NPDR (n=395)	Moderate NPDR (n=321)	Severe NPDR (n=45)	PDR (n=92)
CSME	6	78	22	24
DME without CSME	23	43	8	10
Retinopathy without DME	366	200	15	58

3.2 Sociodemographic characteristics

The sociodemographic charactistics including Age, Gender, Marital status, Education, Smoking habit, and characteristics related to medical and family history are shown in tables 3 and 4 for different grades of DR and DME respectively.

Table 3 Sociodemographic and past medical and family hostory according to different grades of retinopathy (n=1167)

		No DR n=314	Mild NPDR N=395	Moderate NPDR n=321	Severe NPDR n=45	PDR n=92
Diabetes Type	T1DM T2DM	4 310	10 385	11 310	0 45	4 88
Gender	Male Female	190 124	227 168	205 116	24 21	57 35
Age	<30 year >30 years	16 298	9 386	10 311	0 45	4 88
Marital Status	Ever Married Never Married	298 16	388 7	313 8	45 0	90 2
Education	Illiterate Can Read Upto 5 years Middle (8 years) Upto 10 Years Graduation Post Graduation	54 32 52 48 42 54 29	72 57 67 59 33 71 36	54 32 55 51 43 56 30	3 4 8 11 14 5 2	7 18 10 14 23 17 3
Smoking	Ever Smoked Never Smoked	96 218	104 291	67 254	4 41	10 82
HTN	Present Absent	183 131	300 95	239 85	36 9	69 23
On Insulin Treatment	Yes No	26 278	32 363	31 29	2 43	14 74
Family History of DM	Positive Negative Unknown	162 124 28	189 183 23	195 106 20	23 19 3	46 42 4
Family History of HTN	Positive Negative Unknown	116 152 46	168 137 90	169 95 57	26 11 8	46 27 19
Family History of CVD	Positive Negative Unknown	103 128 83	176 122 97	155 104 62	15 27 3	24 29 39

Table 4 Sociodemographic and past medical and family history according to presence of Diabetic Macular Edema and Clinincallly SIgnificant Macular Edema

		No DME (only DR) N=639	DME (without CSME) N= 84	CSME N=130
Diabetes Type	T1DM T2DM	18 621	4 80	3 127
Gender	Male Female	349 290	48 36	122 14
Age	<30 year >30 years	17 622	4 80	2 128
Marital Status	Ever Married Never Married	624 15	83 1	130 0
Education	Illiterate Can Read Upto 5 years Middle (8 years) Upto 10 Years Graduation Post Graduation	78 94 103 147 110 86 21	3 11 14 18 27 6 5	55 6 23 25 3 16 2
Smoking	Ever Smoked Never Smoked	105 534	51 33	29 101
HTN	Present Absent	541 98	51 33	52 78
On Insulin Treatment	Yes No	41 598	15 69	23 107
Family History of DM	Positive Negative Unknown	340 207 92	59 18 7	64 45 21
Family History of HTN	Positive Negative Unknown	312 223 104	30 36 18	67 11 52
Family History of CVD	Positive Negative Unknown	224 244 171	68 12 4	78 26 26

3.3 Biochemical characteristics

The distribution of mean values with 95% CI of biochemical parameters according to different grades of diabetic retinopathy is shown in table 5. Similarly, mean values with corresponding 95% confidence intervals for these variables in 853 DR patients according to the presence of DME and CSME is shown in table 6.

Table 5 Biochemical parameters (Mean & 95% CI) according to different grades of retinopathy (n=1167)

Mean (95% CI)	No DR n=314	Mild NPDR N=395	Moderate NPDR n=321	Severe NPDR n=45	PDR n=92
FBG mmol/L	8.03 (7.70-8.37)	10.38 (9.94-10.82)	9.70 (9.10-10.31)	10.79 (9.71-11.86)	10.74 (9.64-11.83)
RBG mmol/L	11.68 (11.10-12.26)	14.72 (14.16- 15.28)	13.97 (13.35-14.60)	14.62 (13.14-16.16)	15.66 (14.68-16.63)
HbA1c %	7.84 (7.55-8.13)	9.59 (9.38-9.80)	9.79 (9.56-10.03)	10.23 (9.70-10.76)	10.49 (10.07-10.91)
DM Duration Years	4.39 (3.72-5.05)	13.29 (12.74-13.84)	17.99 (17.47-18.52)	19.26 (17.60-20.93)	17.89 (16.93-18.85)
Age Years	51.41 (49.11-53.70)	51.38 (50.48-52.28)	50.11 (48.09-52.13)	53.29 (50.91-55.67)	53.76 (52.3-55.18)
Urea mmol/L	10.26 (9.33-11.20)	9.58 (9.13-10.02)	9.98 (9.08-10.87)	11.43 (10.76-12.10)	11.64 (11.16-12.11)
Creatinine µmol/L	82.21 (77.79-87.51)	54.80 (52.15-56.57)	78.67 (71.60-85.78)	90.16 (85.01-95.31)	114.03 (102.54-126.41)
Sodium mmol/L	140.09 (139.00-141.19)	141.37 (140.69-142.04)	140.67 (139.91-141.44)	141.40 (139.64-143.15)	138.26 (136.26-140.21)
Potassium mmol/L	4.84 (4.72-4.96)	4.50 (4.40-4.59)	4.70 (4.58-4.82)	4.74 (4.28-5.19)	4.15 (3.84-4.46)
Chloride mmol/L	103.93 (102.82-105.04)	101.35 (100.42-102.28)	100.65 (100.12-101.18)	104.40 (101.63-107.16)	102.28 (100.67-103.89)
BiCarbonate mmol/L	31.58 (26.78-36.37)	25.35 (24.80-25.90)	22.79 (22.00-23.57)	23.28 (20.89-25.66)	21.94 (20.30-23.58)
Urine Micro Albumin µg/min	30.30 (23.79-36.81)	80.53 (67.49-93.57)	87.63 (72.15-103.11)	93.11 (74.33-111.88)	96.89 (79.65-114.14)
Height m	1.63 (1.62-1.64)	1.60 (1.59-1.61)	1.62 (1.60-1.63)	1.57 (1.54-1.59)	1.66 (1.64-1.68)
Weight Kg	71.10 (69.02-73.18)	71.29 (69.96-72.62)	68.98 (67.30-70.66)	63.51 (59.74-67.28)	86.31 (82.85-89.77)
BMI Kg/m^2	26.09 (25.62-26.57)	27.81 (27.32-28.30)	26.65 (26.13-27.17)	26.23 (24.89-27.56)	31.50 (29.93-33.06)
Waist cm	97.20 (95.41-98.99)	97.65 (96.68-98.62)	99.05 (97.69-100.40)	93.12 (88.91-97.33)	105.58 (103.75-107.42)
HIP cm	105.29 (104.01-106.57)	104.07 (103.05-105.09)	104.38 (102.96-105.80)	98.35 (94.77-101.93)	105.44 (105.03-105.84)
Waist-Hip Ratio	0.93 (0.92-0.94)	0.94 (0.93-0.95)	0.95 (0.94-0.96)	0.95 (0.93-0.98)	1.01 (0.99-1.03)
BP Systole mmHg	124.54 (122.33-126.75)	123.58 (121.43-125.73)	126.74 (124.12-129.37)	126.32 (117.08-135.56)	129.56 (127.35-131.77)
BP Diastole mmHg	80.00 (18.77-81.22)	77.87 (76.82-78.92)	79.33 (77.94-80.71)	77.29 (74.18-80.40)	81.13 (82.11-86.14)
Total Serum Cholesterol mmol/L	5.22 (5.08-5.37)	6.60 (6.31-6.89)	6.83 (6.58-7.09)	7.58 (7.25-7.90)	7.29 (6.79-7.79)
Total Serum Triglyceride mmol/L	1.69 (1.64-1.73)	1.71 (1.63-1.78)	1.73 (1.65-1.81)	2.06 (1.48-2.64)	2.15 (1.67-2.63)
LDL mmol/L	3.26 (3.19-3.34)	3.18 (3.05-3.31)	3.19 (3.07-3.31)	3.26 (3.01-3.51)	3.27 (3.03-3.51)
HDL mmol/L	1.01 (0.96-1.02)	0.89 (0.85-0.93)	0.86 (0.83-0.89)	0.78 (0.76-0.80)	0.79 (0.76-0.83)

BMI: Body Mass Index, BP: Blood Pressure, CSME: Clinically Significant Macular Edema, FBG: Fasting Blood Glucose, DM: Diabetes Mellitus, DME: Diabetic Macular Edema, DR: Diabetic retinopathy, HbA1c: Glycosylated Hemoglobin, HDL: High Density Lipoprotein, RBG: Random Blood Glucose, LDL: Low Denisty Lipoprotein

Table 6 Biochemical parameters (Mean & 95% CI) according to the presence of Diabetic Macular Edema and Clinically SIgnificant Macular Edema in patients with retinopathy (n=853)

	No DME (only DR) N=639	DME (without CSME) N= 84	CSME N=130
FBG mmol/L	7.09 (6.01-8.18)	9.13 (8.02-10.26)	10.07 (9.10-11.04)
RBG mmol/L	12.19 (10.12-13.26)	13.61 (11.47-15.75	14.97 (13.35-15.60)
HbA1c %	8.6 (7.1-9.0)	9.6 (9.2-9.9)	9.7 (9.3-10.1)
DM Duration Years	14.7 (11.5-18.3)	15.8 (14.7-16.9)	16.1 (14.8-18.7)
Age Years	52.1 (49.8-53.3)	53.7 (52.2-55.3)	53.6 (48.5-58.8)
Urea mmol/L	10.26 (9.33-11.20)	11.58 (9.13-12.99)	10.98 (9.08-11.87)
Creatinine µmol/L	75.05 (72.79-77.51)	75.80 (62.15-.57)	78.01 (71.60-85.78)
Sodium mmol/L	141.02 (139.58-142.47)	141.61 (139.69-142.53)	141.33 (138.46-143.19)
Potassium mmol/L	4.31 (4.04-4.73)	4.55 (4.44-4.66)	4.61 (4.32-4.85)
Chloride mmol/L	101.67 (100.99-104.04)	101.89 (100.57-102.64)	101.99 (100.39-102.58)
BiCarbonate mmol/L	24.08 (23.69-30.56)	24.22 (24.18-24.24)	23.88 (22.60-24.36)
Urine Micro Albumin µg/min	85.94 (70.23-98.64)	87.60 (68.77-101.42)	87.26 (71.51-104.82)
Height M	1.62 (1.61-1.63)	1.61 (1.58-1.71)	1.62 (1.59-1.64)
Weight Kg	72.32 (68.51-75.99)	71.44 (66.10-70.78)	75.52 (66.57-79.48)
BMI Kg/m^2	27.53 (25.95-29.02)	28.01 (25.94-30.51)	28.57 (25.66-29.42)
Waist Cm	99.01 (97.46-101.22)	100.15 (96.30-102.51)	100.64 (98.32-101.96)
Hip cm	104.11 (103.77-106.45)	104.44 (103.50-105.06)	104.17 (101.62-106.63)
Waist-Hip Ratio	0.97 (0.91-1.06)	0.99 (0.91-1.02)	0.99 (0.92-1.04)
BP Systole mmHg	125 (124-126)	125 (122-128)	125 (122-129)
BP Diastole mmHg	79 (76-80)	78 (75-81)	79 (77-81)
Total Serum Cholesterol mmol/L	6.22 (5.12-6.97)	6.62 (6.33-6.79)	6.86 (6.47-7.20)
Total Serum Triglyceride mmol/L	1.69 (1.64-1.74)	1.70 (1.63-1.78)	1.71 (1.64-1.78)
LDL mmol/L	3.15 (3.10-3.21)	3.19 (3.15-3.23)	3.19 (3.07-3.31)
HDL mmol/L	0.98 (0.96-1.01)	0.88 (0.85-0.91)	0.85 (0.80-0.92)

BMI: Body Mass Index, BP: Blood Pressure, CSME: Clinically Significant Macular Edema, FBG: Fasting Blood Glucose, DM: Diabetes Mellitus, DME: Diabetic Macular Edema, DR: Diabetic retinopathy, HbA1c: Glycosylated Hemoglobin, HDL: High Density Lipoprotein, RBG: Random Blood Glucose, LDL: Low Denisty Lipoprotein

3.4 Factors associated with Diabetic Retinopathy and Diabetic Macular Edema

Regarding the comparison of facors associated with DR, One Way ANOVA revealed statistically significant difference between the mean values of FBG, RBG, HbA1c, Urinary Micro Albumin levels, Weight, BMI, Total Serum Cholesterol, Total Serum Triglycerides and HDL among No Diabetic Retinopathy, Mild NPDR, Moderate NPDR, Severe NPDR and PDR groups. The comparison of mean values and corresponding p-values are shown in table 7.

Post Hoc (Tukey's) test revealed that mean value of FBG for No DR group was significantly less than mean FBG of all forms of DR.

Mean HbA1c in patients with all forms of diabetic retinopathy (Mild NPDR, Moderate NPDR, Severe NPDR or PDR) was significantly greater than that in Patients without Retinopathy.

There was no statistically significant difference found between the Mean Urinary Micro Albumin levels of patients without DR (No DR) group and that in Mild NPDR (=0.951) while it was significantly different from all other categories (p-values, 0.008 vs Moderate NPDR, 0.001 vs Severe NPDR and <0.001 vs PDR). Whereas, mean MICRAL level in patients with Mild NPDR was also found to be statistically significantly lesser than those in Moderate NPDR, Severe NPDR and PDR, p-values 0.046, 0.004, <0.001 respectively.

Mean weight of patients with PDR (70.25 kg) was significantly more than the mean weight of patients without any retinopathy (81.14 Kg) p-value <0.001, or patients with Mild NPDR (.

BMI, the mean BMI of patients with PDR (29.53 Kg/m^2) was significantly greater than the mean BMI of patients without any retinopathy (26.53 kg/m^2) p-value <0.001. Similarly, the patients with PDR had greater BMI compared to all other categories of DR (Mild NPDR 26.57 Kg/m2, Moderate NPDR 26.57 Kg/m2, Severe NPDR 26.75 Kg/m2) with p-values <0.001, <0.001 and 0.004 respectively.

Total Cholesterol was significantly lower than patients with No DR compared to patients with any form of Retinopathy, however there was no significant difference between different groups of DR.

Triglycerides in NO DR groups was significantly lower than those with Severe NPDR and PDR (p-values 0.031 and <0.001 respectively). Similarly triglyceride levels in PDR group was significantly more than all categories (No DR, Mild, Moderate and Severe NPDR) except Severe NPDR.HDL in No DR group was significantly higher than all categories of DR with p values <0.001 in all categories.

Similarly for categorical variables pearson's chi-squared test revealed that Hypertension and family history of CVD was more in patients with diabetic retinopathy of any form as compared to patients without DR (p-value <0.001 and 0.004 respectively). No statistically significant difference was observed among the number of patients using Insulin across different grades of DR.

Table 7 Variables with mean and 95% confidence intervales showing statistically significant difference across different grades of retinopathy (n=1167)

Variables Mean (95%CI)	No DR n=314	Mild NPDR N=395	Moderate NPDR n=321	Severe NPDR n=45	PDR n=92	p-value*
FBG mmol/L	8.03 (7.70-8.37)	10.38 (9.94-10.82)	9.70 (9.10-10.31)	10.79 (9.71-11.86)	10.74 (9.64-11.83)	<0.001
RBG mmol/L	11.68 (11.10-12.26)	14.72 (14.16-15.28)	13.97 (13.35-14.60)	14.62 (13.14-16.16)	15.66 (14.68-16.63)	0.01
HbA1c %	7.9 (7.6-8.2)	9.6 (9.2-9.9)	9.7 (9.3-10.1)	10.2 (9.4-10.7)	10.4 (9.5-11.4)	<0.001
DM Duration Years	3.9 (3.5-4.3)	13.8 (12.7-14.9)	17.8 (16.8-18.7)	19.5 (15.9-22.9)	17.8 (15.6-20.0)	<0.001
Age Years	51.1 (49.8-52.3)	53.7 (52.2-55.3)	50.6 (48.5-52.8)	53.9 (48.3-59.4)	54.7 (50.9-58.4)	0.045
Urine Micro Albumin µg/min	36.94 (32.23-41.64)	64.60 (48.77-80.42)	88.26 (70.60-105.91)	107.75 (63.23-152.27)	99.65 (60.28-139.03)	<0.001
Weight Kg	70.25 (68.51-71.99)	68.44 (66.10-70.78)	69.52 (66.57-72.48)	66.33 (58.53-74.13)	81.14 (75.47-88.81)	0.01
BMI Kg/m^2	26.53 (25.95-27.11)	26.57 (25.94-27.51)	26.57 (25.56-27.49)	26.75 (24.20-29.29)	29.53 (26.97-32.02)	0.02
Waist cm	97.81 (96.36-99.27)	98.15 (96.30-100.01)	98.64 (96.32-100.96)	96.57 (87.82-105.31)	105.71 (101.58-109.84)	0.03
HIP cm	105.13 (103.60-106.63)	104.49 (102.50-106.46)	104.13 (101.62-106.63-)	101.30 (94.07-108.53)	105.50 (99.14-111.85)	0.01
Waist-Hip Ratio	0.93 (0.92-0.94)	0.95 (0.93-0.97)	0.95 (0.93-0.97)	0.96 (0.92-1.00)	1.01 (0.71-1.31)	0.01
Total Serum Cholesterol mmol/L	5.22 (5.08-5.37)	6.60 (6.31-6.89)	6.83 (6.58-7.09)	7.58 (7.25-7.90)	7.29 (6.79-7.79)	<0.001
Total Serum Triglyceride mmol/L	1.69 (1.64-1.73)	1.71 (1.63-1.78)	1.73 (1.65-1.81)	2.06 (1.48-2.64)	2.15 (1.67-2.63)	<0.001
HDL mmol/L	1.01 (0.96-1.02)	0.89 (0.85-0.93)	0.86 (0.83-0.89)	0.78 (0.76-0.80)	0.79 (0.76-0.83)	<0.001

BMI: Body Mass Index, BP: Blood Pressure, CSME: Clinically Significant Macular Edema, FBG: Fasting Blood Glucose, DM: Diabetes Mellitus, DME: Diabetic Macular Edema, DR: Diabetic retinopathy, HbA1c: Glycosylated Hemoglobin, HDL: High Density Lipoprotein, RBG: Random Blood Glucose, LDL: Low Denisty Lipoprotein

*p-values were calculated using One-Way ANOVA

Similarly, regarding the factors associated with DME, One-Way ANOVA showed that mean values for FBG, HbA1c, Duration of diabetes, age, weight, BMI, waist, waist-hip ratio, total serum cholesterol and HDL were significantly different across the DR patients without DME, with DME and with CSME. The comparison of these variables with mean and 95% CIs and corresponding p-values are shown in table 8.

Table 8 Variables with mean and 95% confidence intervales showing statistically significant difference according to presence of Diabetic Macular Edema

	No DME (only DR) N=639	**DME (without CSME) N= 84**	**CSME N=130**	**p-value***
FBG mmol/L	7.09 (6.01-8.18)	9.13 (8.02-10.26)	10.07 (9.10-11.04)	0.002
HbA1c %	8.6 (7.1-9.0)	9.6 (9.2-9.9)	9.7 (9.3-10.1)	<0.001
DM Duration Years	14.7 (11.5-18.3)	15.8 (14.7-16.9)	16.1 (14.8-18.7)	0.012
Age Years	52.1 (49.8-53.3)	53.7 (52.2-55.3)	53.6 (48.5-58.8)	0.045
Weight Kg	72.32 (68.51-75.99)	71.44 (66.10-70.78)	75.52 (66.57-79.48)	0.018
BMI Kg/m^2	27.53 (25.95-29.02)	28.01 (25.94-30.51)	28.57 (25.66-29.42)	0.029
Waist Cm	99.01 (97.46-101.22)	100.15 (96.30-102.51)	100.64 (98.32-101.96)	0.034
Waist-Hip Ratio	0.97 (0.91-1.06)	0.99 (0.91-1.02)	0.99 (0.92-1.04)	0.017
Total Serum Cholesterol mmol/L	6.22 (5.12-6.97)	6.62 (6.33-6.79)	6.86 (6.47-7.20)	0.021
HDL mmol/L	0.98 (0.96-1.01)	0.88 (0.85-0.91)	0.85 (0.80-0.92)	<0.001

BMI: Body Mass Index, BP: Blood Pressure, CSME: Clinically Significant Macular Edema, FBG: Fasting Blood Glucose, DM: Diabetes Mellitus, DME: Diabetic Macular Edema, DR: Diabetic retinopathy, HbA1c: Glycosylated Hemoglobin, HDL: High Density Lipoprotein, RBG: Random Blood Glucose, LDL: Low Denisty Lipoprotein

*p-values were calculated using One Way ANOVA

Chi-squared test revealed that only presence of hypertension significantly differed among groups of patients with DR only, with DME and wth CSME with p-value <0.001.

3.4.1 Univariate Analyses; Logistic Regression

To see the association of variables showing significant diffeence in One-Way ANOVA and Chi-squared tests with DR, Unadjusted (Crude) Odds Ratios with corresponding 95% CIs were calculated for the variables showing statistically significant differences using Binary Logistic Regression for FBG, RBG, HbA1c, Duration of Diabetes, Age, Urinary Micro Albumin, Weight, BMI, Waist circumference, Hip circumference, Waist-Hip Ratio, Total Cholesterol, Triglycerides, HDL and presence of Hypertension. Table 9 shows the Crude Odds Ratios with corresponding 95% CIs for these variables. FBG, RBG, HbA1c, Duration of Diabetes, presence of Hypertension, BMI, HDL, Urinary Micro Albumin excretion, Waist and Waist Hip Ratio retained significant association with Diabetic Retinopathy. Whereas, Weight, Total Cholesterol, Triglyceride, Hip circumference and Age were unable to show significant association with DR. Table 9 shows Crude ORs with 95% CIs for these variables.

Table 9 Univariate Analyses showing Crude Odds Ratios with 95% Conficence Intervales for the factors associated with Diabetic Retinopathy (n=1167)

	No DR (n=314)	**DR (n=853)**	**P-value**	**Crude OR**	**95% CI**
FBG					
<7 mmol/L	72	126	0.002	R	--
>7 mmol/L	242	727		1.72	1.34-2.95
RBG					
<11 mmol/L	85	138	0.048	R	--
>11 mmol/L	229	715		1.92	1.01-2.92
HbA1c					
< 7%	81	62	<0.001	R	--
≥7.1%	233	791		4.43	2.67-7.01
DM Duration					
<5 years	222	316	<0.001	R	--
> 5 years	92	537		4.10	3.02-5.79
HTN					
Absent	131	209	0.003	R	--
Present	183	644		2.20	1.89-2.57
BMI					
<30 kg/m^2	232	421	<0.001	R	--
>30 kg/m^2	82	432		2.80	1.04-8.51
HDL					
<1 mmol/L	188	643	<0.001	R	--
>1 mmol/L	126	210		0.48	0.03-0.89
Urinary Micro Albumin					
<100 mg/dl	140	24	<0.001	1.01	--
>100 mg/dl	174	829			1.01-1.02
Waist circumference[a]					
<90 cm	78	142	<0.001	1.64	--
>90 cm	236	711			1.08-2.61
WHR[b]					
<0.95	154	232	0.003	2.34	--
>0.95	160	530			1.00-4.70

a: IDF criteria, b: WHO criteria for WHR

Similarly, to calculate Unadjusted (Crude) Odds Ratios to see the association of FBG, HbA1c, Duration of Diabetes, Age, Weight, BMI, Total Cholesterol, HDL, Waist circumference, WHR and Hypertension with DME, Binary Logistic Regression revealed that only HbA1c, presence of Hypertension and HDL were significantly associated with DME while FBG, Duration of Diabetes, Age, BMI, Weight, Total Cholesterol, Waist & WHR failed to show any significant association. Table 10 shows the Crude ORs with 95% CIs for these variables.

Table 10 Univariate Analyses showing Crude Odds Ratios with 95% Conficence Intervales for the factors associated with Diabetic Macular Edema (n=853)

	No DME N=639	DME N= 214	P-value	Crude OR	95% CI
HbA1c < 7% >7%	 54 585	 12 202	0.041	 R 2.28	 -- 1.58-6.07
HTN Absent Present	 164 475	 45 169	0.035	 R 1.29	 -- 1.12-3.45
HDL <1 mmol/L >1 mmol/L	 483 156	 160 54	0.021	 R 0.79	 -- 0.54-0.81

a: IDF criteria, cb: WHO criteria for WHR

3.4.2 Multivariable Analyses:

Multivariable Analyses by putting all significant variables from Univariate Analyses in single regression model showed that only FBG (Adjusted OR 1.02, 95% CI 1.01-1.03), HbA1c (Adjusted OR 2.20, 95%CI 1.36-3.54), Duration of diabetes (Adjusted OR 2.14, 95%CI 1.63-2.81), presence of Hypertension (Adjusted OR 1.10, 95%CI 1.01-1.29) and Urinary Mircro Albumin excretion (Adjusted OR 1.02, 95%CI 1.01-1.03) were postively associated with any form of DR with Adjusted ORs.Whereas HDL was negatively associated with DR with Adjusted OR 0.85 and 95% CI 0.76-0.95.

Table 11 Multivariable Analyses showing Adjusted Odds Ratios with 95% Conficence Intervales for the factors associated with Diabetic Retinopathy (n=1167)

	No DR (n=314)	DR (n=853)	p-value	Adjusted OR	95% CI
FBG* <6 mmol/L >6 mmol/L	72 242	126 727	0.04	R 1.02	-- 1.01-1.03
HbA1c * < 7% ≥7.1%	81 233	62 791	<0.001	R 2.20	-- 1.36-3.54
DM Duration* <5 years > 5 years	222 92	316 537	<0.001	R 2.14	-- 1.63-2.81
HTN Absent Present	131 183	209 644	0.04	R 1.10	-- 1.01-1.29
Urinary Micro Albumin <100 mg/dl >100 mg/dl	140 174	24 829	<0.001	R 1.02	-- 1.01-1.03
HDL <1 mmol/L >1 mmol/L	188 126	643 210	0.023	R 0.85	-- 0.76-0.95

Moreover, for DME, multivariable analyses showed that out of 4 variables only HbA1c and Hypertension retained their association with DME with Adjusted ORs 1.87 (95% CI 1.06-3.88) & 1.55 (95% CI 1.12-4.57) respectively. Table 12 shows the adjusted ORs with 95% CI for the factors associated with DME.

Table 12 Multivariable Analyses showing Adjusted Odds Ratios with 95% Conficence Intervales for the factors associated with Diabetic Macular Edema (n=853)

	No DME N=639	**DME N= 214**	**p-value**	**Adjusted OR**	**95% CI**
HbA1c **< 7%** **≥7.1%**	54 585	12 202	0.011	R 1.87	-- 1.06-3.88
HTN **Absent** **Present**	164 475	45 169	0.042	R 1.55	1.12-4.57

Regarding the association of DME with different grades of DR, Severe NPDR was found to be positively associated with DME with OR 8.56 when compared with patients with Mild NPDR.Whereas, the Odds of finding DME was 3 times more in patients with PDR compared to Mild NPDR. Table 13 shows the association of DME with different grades of retinopathy with corresponding ORs and 95%CI.

Table 13 Association of DIabetic Macular Edema with different grades of retinopathy (n=853)

	No DME N=639	**DME N= 214**	**p-value**	**Odds Ratio**	**95% CI**
Mild NPDR	366	29	--	R	--
Moderate NPDR	200	121	0.06	1.08	0.96-1.21
Severe NPDR	15	30	<0.001	8.56	1.92-15.04
PDR	58	34	0.014	3.02	1.22-5.03

Chapter 4: Discussion

This study was conducted to examine the frequency of diabetic retinopathies using fundus photography as detection tool and factors associated with them in a tertiary care hospital in Karachi, Pakistan. To date, there are no studies published that used fundus photography to detect Diabetic Retinopathy (DR) and Diabetic Macular Edema (DME) as the primary tool in such patients in Pakistan.

The population of interest was diabetic patients with either type 1 or 2 Diabetes Mellitus at the National Institute of Diabetes & Endocrinology, Dow University Hospital, Karachi Pakistan. These patients were referred to the department of Ophthalmology, in the same hospital for fundus photography between May 2010 and December 2013.

We found a high frequency of DR (73.1%) among those who underwent fundus photography. This is more than the frequencies reported by previous studies from Pakistan and abroad. In a community based study by Mahar P.S. and associates [16] DR was found to be present in 27.4% of diabetics. This study was however conducted in population based eye camps in Karachi. Moreover, a hospital based pilot study from the same city revealed a similar 26% frequency of DR among diabetics.[79]

A study from India reported that 26.2% of the diabetic patients suffered from any form of retinopathy. [80] He B.B. and associates, in their study on Chinese patients, found that DR was present in 30% of the type 2 diabetics. [45] Similarly another study from Oman conducted on both T1DM & T2DM patients, reported DR prevalence to be 42.4%. [56]. Varma R. et al [57] & Tapp R.J. et al [44] reported DR prevalence to be 46% & 15.3% in US and Australian patients respectively.

We found that the frequency of DR in our study was greater than all these national and international studies. The reason for this high frequency of DR can, firstly, be due to the use of highly sensitive fundus photography technique employed to diagnose DR. Secondly, we considered both T1DM & T2DM patients whereas all but one of these studies enrolled only T2DM patients. This might have an incremental effect on our findings as studies suggest that T1DM patients develop any form of DR earlier. Thirdly, our study was conducted in hospital setting, where the department of Ophthalmology and the National Institute of Diabetes and

Endocrinology are present in the same premises, which might have influenced only more severe cases of diabetes to be examined for fundus photography.

Published literature from around the globe reports many factors associated with retinopathy among diabetics. Our study took into account the basic socio-demographic and biochemical factors and looked for their association with the presence of DR in both T1DM & T2DM patients. When these factors were taken individually, FBG, RBG, HbA1c, Duration of Diabetes, Presence of Hypertension, Weight, BMI, Total Cholesterol, Triglycerides, HDL Waist Circumference, Hip Circumference and Waist-Hip Ratio was found to be associated with DR. However, multivariable analyses to calculate adjusted ORs revealed only FBG (Adjusted OR 1.02; 95% CI, 1.01-1.03), HbA1c (Adjusted OR 2.20; 95% CI, 1.36-3.54), Duration of Diabetes (Adjusted OR 2.14; 95% CI, 1.63-2.81), Hypertension (Adjusted OR 1.10; 95% CI, 1.01-1.29), Urinary Micro Albumin excretion (Adjusted OR 1.02; 95% CI, 1.01-1.03) and HDL (Adjusted OR 0.85; 95% CI, 0.76-0.95) to be associated with DR. These findings are in accordance with the previously published national and international reports.

We however, did not find any significant association with factors like BMI, WHR, Total Cholesterol with retinopathy among diabetics in the final regression model. This is in contrast to the findings of some researchers [29, 60, 62]

Diabetic Macular Edema, which is considered further a complication of DR itself is often asymptomatic. Hence underestimation of the condition is always a possibility. In our study, we found that Diabetic Macular Edema was present in 25.1% of patients with Diabetic Retinopathy. Whereas, CSME was present in atleast one eye in 15% of DR patients while 11% of patients had CSME in both eyes. To the best of our knowledge, only one study from Pakistan has reported the frequency of DME among diabetics. Qayyum A. et al [28] in their study from Quetta, Pakistan reports that DME was present in 33% of the patients with DR. likewise, in a study from USA, Lopes de Faria J.M. and associates [81]report that DME was present in 70% of the patients suffering from Diabetic Retinopathy.

The relatively high frequency of CSME in these patients point towards the possible late acknowledgement of the condition and further exaggerated by poor risk factors control.

Regarding the factors associated with Diabetic Macular Edema, HbA1c, Hypertension and lower HDL values were independently associated. However, multivariable analyses to calculated adjusted ORs left only HbA1c (Adjusted OR 1.87; 95%CI, 1.06-3.88) and Hypertension (Adjusted OR 1.55; 95%CI, 1.12-4.57) to be positively associated with Diabetic Macular Edema. We also found that the Odds of finding DME in patients with Severe NPDR was 8.56 time more compared to Mild NPDR. Whereas the odds of finding DME in patients with PDR was 3 times more than in patients with Mild NPDR.

No local study discussed the factors associated with DME. Lopez de Faria et al reported in their study that high BP, Presence of CVD and Proliferative Diabetic Retinopathy was positively associated with DME. [81]

4.2 Strenghts

This study used the gold standard procedure, fundus photography, for the detection of retinopathies among diabetic patients. It is also, to the best of our knowledge, the first study from Pakistan which studied the frequencies of Diabetic retinopathy and Diabetic Macular Edema and the factors associated with them in both type 1 and typ 2 diabetes mellitus patients. The study was conducted in a large tertiary care hospital in the city of Karachi which is the largest city of Pakistan with a multi-ethnic population of 20 Million people. Therefore, though the findings of this study cannot be generalized over the entire population of Pakistan, it points towards the national trends regarding the burden of and factors associated with Diabetic Retinopathy in Pakistan. Furthermore, large sample size was included in the study to have optimal power for statistical analyses. Missing data was dealt with Multiple Imputations, an advanced and reliable statistical technique to avoid bias to the maximum.

4.3 Limitations

A number of limitations were present in the study. Firstly, retrospect ive analysis was done on the data which was clinically oriented and not specifically collected for research. Secondly, a possibility of overestimation of the frequency of DR cannot be ignored due to possible selection bias, as the fundus photography is an expensive procedure and usually more severe cases of diabetic patients are refered for it. Furthermore, high proportion of missing data

could be a source of bias even though missing values were imputed using statistical techniques.

4.4 Implications

The high frequency of DR & DME found in our study imply that there may be a general lack of appreciation of the risk factors associated with the complicatons of diabetes melilitus in the general population. Furthermore, general lack of data on DME suggests that in health care providers there might be a lack of acknowledgement of the sight threatening complication such as DME as a major health issue.

4.5 Conclusion

We observed a higher frequency of Diabetic Retinopathy and Diabetic Macular Edema with the use of undus photography as a detection tool compared to previous studies. Our findings suggest that poor glycemic control along with the presence of hypertension are the main roleplayers in the progression of retinopathies in diabetic patients.

4.6 Recommendations

Based on the findings of our research, strict glycemic control is warranted to apprehend the microvascular complications in the eye. Steps towards increasing awareness regarding risk factors and their prevention in diabetic patients is indicated. Moreover, further studies are recommended using cohort designs to quantify the risks of the factors associated with DR & DME.

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