Evaluation the Role of Malondialdehyde in Occurrence and Development of Diabetic Retinopathy Patients

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Abstract

Case control study was carried out in Salah-Addin city in private ophthalmology clinic from 1st November of 2019 to End february 2020. The study included 90 individuals including sixty diabetic patients (30 who had retinopathy and 30 without retinopathy) and 30 healthy individuals to evaluate the level of Malondialdehyde (MDA) in diabetic patients with and without retinopathy and healthy controls. All patients were aged between (51-82) years, with a negative history of antioxidant supplementation consumption. Blood samples were collected from each patients and controls to evaluate the levels of malondialdehyde and HbA1c by using immunofluorescence technique. The study showed that majority of diabetic retinopathy (DR) patients were females and the highest mean of age was recorded in diabetic retinopathy patients (60.76±7.25 year) compared with diabetic without retinopathy patients (58.31±9.19 year). The study displayed that the highest mean of MDA was recorded in DR patients as compared with diabetic without retinopathy patients (390.37±108.68 *vs* 336.97±94.95 ng/ml), although the result was non-significant (P: > 0.05). The study showed that the highest mean of MDA was in DR patients (390.37±108.68 ng/ml) followed by diabetic without retinopathy patients (336.97±94.95 ng/ml) and the lowest mean was in healthy individuals (293.32±42.51 ng/ml), (P: < 0.05). The study showed that, HbA1c was elevated significantly (P<0.05) in diabetic retinopathy patients (9.26±1.54) compared with diabetic without retinopathy patients (7.84±1.02). The present study displayed that the highest mean of duration of DM was recorded in diabetic retinopathy patients (13.48±6.00) compared with Diabetic without retinopathy patients (6.34±2.78), the result was significant (P:<0.05). The study showed negative correlation between HbA1c and BMI in DR patients; and no correlation between HbA1c and BMI in DM patients without retinopathy, The study showed negative correlation between MDA and HbA1c in DR patients and no correlation in DM patients without retinopathy.

Conclusion: Long duration of DM and the old age were risk factors for DR, poor control of was more disposed to develop retinopathy and oxidative stress is still higher in diabetic patients with retinopathy than patients without retinopathy.

Keywords: *Malondialdehyde; Diabetic retinopathy; Oxidative stress; HbA1c.*

Introduction

Diabetes mellitus (DM) is expected to affect around 550 million people all over the world according to global estimates of the prevalence of diabetes[(1)]. DM is characterized by constant hyperglycemia that damages various organs and manifests in macro vascular complications like premature atherosclerosis resulting in strokes, peripheral vascular disease, and myocardial infarctions and micro vascular complications such as nephropathy, neuropathy, and retinopathy[(2)]. Diabetic retinopathy (DR) is the number one cause of blindness in people between 27 and 75 years of age. Prevalence of DR is around 25% and 90% at 5 and 20 years, respectively, from diagnosis; it is calculated that 191 million people will be diagnosed with this micro-vascular complication by the year 2030[(3)]. Through the last three decades, extensive scientific reports have shown ROS to play an important role in DM complications such as diabetic neuropathy,

nephropathy, and retinopathy due to alterations on the biomechanisms involved in the instauration and progression of micro-vascular complications[4]. These three micro-vascular complications share high glucose levels as a starting point; such condition is necessary, but may not be enough to initiate the damage present in the peripheral nervous system (neuropathy), kidneys (nephropathy), and retinas (retinopathy) of diabetic patients[5,6]. Hyperglycemic states favor the activation of alternative pathways leading to reactive oxygen species (ROS) formation and augmented concentrations locally and in the rest of the body even at the point of surpassing the antioxidant capacity, a state known as oxidative stress affecting retinal integrity[7,8]. The study aim of this work was to evaluate the level of Malondialdehyde (MDA) in diabetic patients with and without retinopathy and healthy controls.

Patients and Method

Case control study was carried out in Salah-Addin city, a private ophthalmology clinic from 1st November of 2019 to End february 2020. The study included 90 individuals including sixty diabetic patients (30 who had retinopathy and 30 without retinopathy) and 30 healthy individuals. The information about patients in this study was retrieved from patient's itself. The diabetic patients (with and without) retinopathy were diagnosed by analysis RBS and HbA1c and fundoscopical examination by the ophthalmologist. All patients were aged between (51-82) years, with a negative history of antioxidant supplementation consumption, and their weight were (64–120)kg, with BMI range (19.56 - 49.94) . The criteria of exclusion include non-diabetic and malignant disease . The results of the patients groups were compared with healthy individuals nearly comparable age and BMI. About five milliliters of blood were collected from the antecubital vein of patients and controls in plain tubes without any anticoagulant at room temperature for 10-15 minutes and allowed to clot. The tube then were centrifuged (3000 rpm) for 15min. The clear serum was pipetted into clear dry Eppendorf's and stored at (-20^{o}C) until used for the various investigations. The levels of malondialdehyde, HbA1c were measured by using immunofluorescence technique.

Results

The study displayed that the highest mean of MDA was recorded in DR patients as compared with Diabetic without retinopathy patients (390.37±108.68 *vs* 336.97±94.95 ng/ml), although the result was non-significant (P: > 0.05). as shown in the Table 1.

Table 1: Level of MDA in diabetic patients (with and without retinopathy).

MDA(ng/ml)					
Group	**Mean**	**SD**	**Max**	**Min**	**P value**
Diabetic retinopathy	390.37	108.68	548.56	197.76	NS
Diabetic without retinopathy	336.97	94.95	113.36	94.95	

In the current study a significant difference between DR patients and healthy individuals regarding mean of MDA (390.37±108.68 *vs* 293.32±42.51 ng/ml), (P: < 0.05), as shown in the Table 2.

Table 2: Level of MDA in DR patients and healthy individuals.

MDA(ng/ml)					
Group	**Mean**	**SD**	**Max**	**Min**	**P value**
Diabetic retinopathy	390.37	108.68	548.56	197.76	< 0.05
Healthy individuals	293.32	42.51	386.56	211.76	

The study showed that the highest mean of MDA was recorded in diabetic without retinopathy patients (336.97±94.95 ng/ml) as compared with healthy individuals (293.32±42.51 ng/ml), the result was significant (P: <0.05). Figure 1.

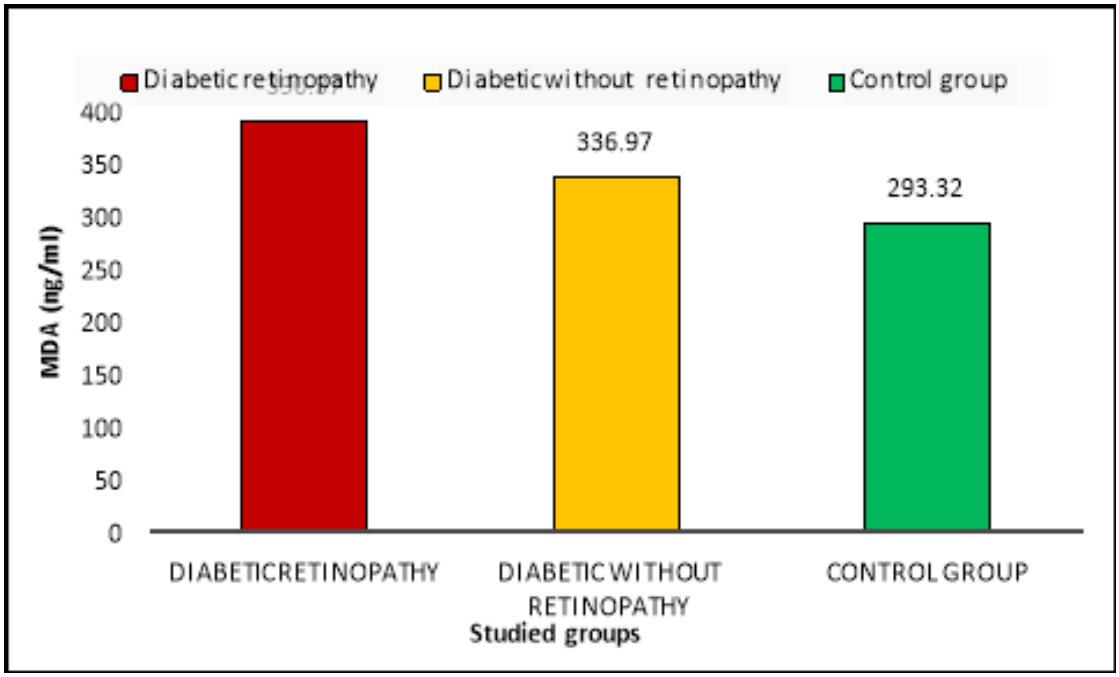

Figure 1: Mean of malondialdehyde (MDA) in the studied groups

The study showed that, HbA1c was elevated significantly (P<0.05) in diabetic retinopathy patients (9.26±1.54) compared with diabetic without retinopathy patients (7.84±1.02), as shown in the Figure 2.

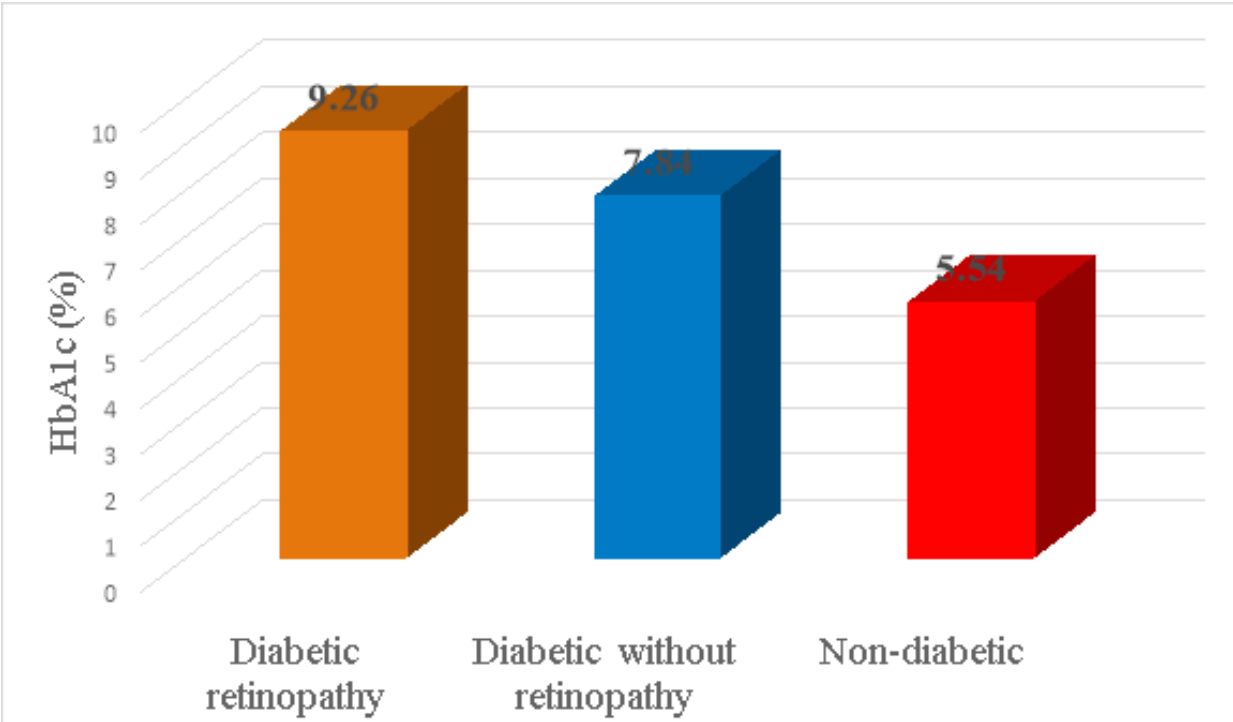

Figure 2: Means of HbA1c levels in the studied groups

The present study displayed that the highest mean of duration of DM was recorded in diabetic retinopathy patients (13.48±6.00) compared with Diabetic without retinopathy patients (6.34±2.78), the result was significant (P:<0.05). As shown in the Table 3.

Table 3: Mean of duration of DM in diabetic retinopathy patients and Diabetic without retinopathy patients.

Duration of DM (year)					
Group	**Mean**	**SD**	**Max**	**Min**	**P value**
Diabetic retinopathy	13.48	6.00	30.00	5.00	< 0.05
Diabetic without retinopathy	6.34	2.78	10.00	0.80	

The study showed negative correlation between HbA1c and BMI in DR patients (r: - 0.21) and no correlation between HbA1c and BMI in DM patients without retinopathy (r: -0.04), Figure 4 (A & B).

The study showed negative correlation between MDA and HbA1c in DR patients and no correlation in DM patients without retinopathy (r: -0.35 and 0.04 respectively), Figure 4(B & C).

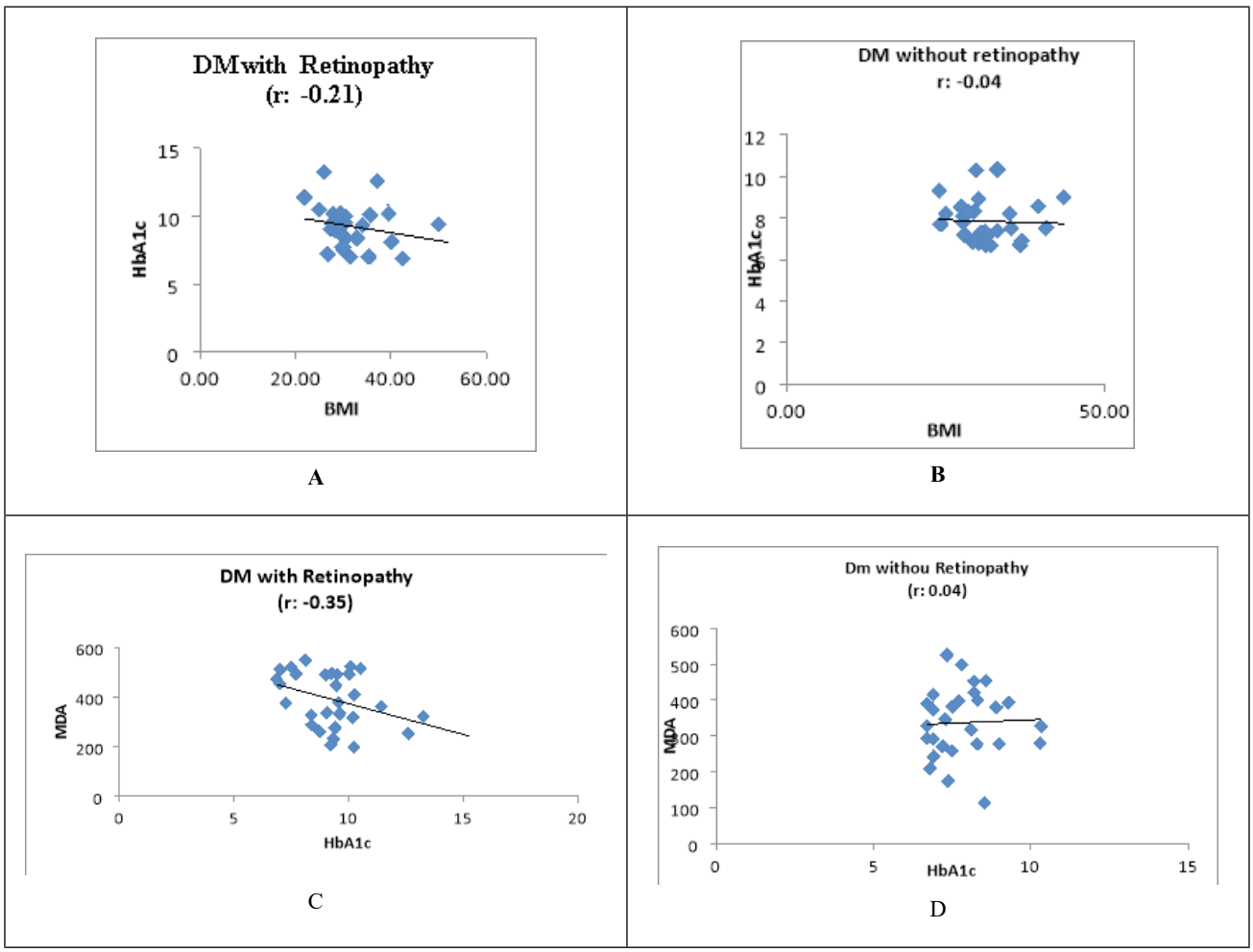

Figure 4: Correlation between A- HbA1c and BMI in DR patients;B- HbA1c and BMI in DM patient without retinopathy;C- MDA and HbA1c in DR patients;D- MDA and HbA1c in DM patients without DR

Discussion

The study showed that majority of diabetic retinopathy (DR) patients were females (23 of 29 (79.31%)) compared by 17 (58.62%) females of DM group without retinopathy and 14(48.28%) of healthy individuals were females.

The study showed that the highest mean of age was recorded in diabetic retinopathy patients compared with

diabetic without retinopathy patients although the result was non-significant (P: > 0.05). The highest mean of age was recorded in diabetic retinopathy patients compared with healthy individuals, the result was significant (P<0.05). The study revealed that the highest mean of age was recorded in diabetic without retinopathy patients as compared with healthy individuals, although the result was non-significant (P: > 0.05).

This finding agree with study done by Rajab[9] . The mean age was 50 years old ranging from 25-67 years, with 65% between the ages of 50-65 years old and the standard deviation was (± 10.95) and the retinopathy rate was highest in the age group 50-59 years (37%). Also, Abebe *et al* [10] reported that Diabetic patients aged ≥65 years are less likely to have poor glycemic control compared to other age groups similar to another study done here in Gondar and in USA, which indicated that elderly people had better diabetes control. The retinopathies rate increased with age until the age of 70 years; however, the small number of people with diabetes in this age group limits our ability to interpret the finding. Some studies reported a significant association between DR and age. Aging, high blood glucose and high blood pressure cause microvascular destruction of blood vessels which increase with time of exposure so aging and duration of diabetes is main risk factors of diabetic retinopathy [11,12].

On the other hand the current study disagree with study done by Goyal *et al* [13] showed the mean age of patients with DR was significantly higher (P < 0.001) in comparison to patients without DR.

Gender was not identified as a risk factor in our study, which agrees with a study conducted by Rajab[9] demonstrated that women had significantly higher rate of DR than men. Other studies have suggested insignificant differences in DR by sex, which disagree with current study. Vinícius *et al* [14] reported that Mean age of group case was 59.5 years with a slight female predominance, however gender, age and were not associated with higher chances of DR. Also Tawfeeq indicated [8] that, there was no significant differences between the rate of males and females in this study (P>0.5) which was disagreed with our finding.

The study displayed that the highest mean of BMI was recorded in diabetic retinopathy patients compared with control group and diabetic without retinopathy patients, although the result was non-significant. In agreement with current study's findings, Maghbooli et al [15] & Wang et al [16] demonstrated that insignificant association between BMI and DR .Also, Dipayan et al[17] reported that no significant relationship between DR and BMI.

Additionally, Zhou et al [18] demonstrated that elevated BMI did not increase the risk of DR . However, since being overweight and obesity are risk factors for multiple diseases, it is still imperative to maintain a healthy weight. Obesity has also been observed to have detrimental effects on multiple eye diseases such as glaucoma, late age-related maculopathy, and cataracts[19].

BMI has been implicated both for positive and negative relation. Joel et al [20] & Rooney et al [21] showed that an inverse association between obesity and incident DR and also between BMI and DR. As well as, Zahra et al [22] & Jun et al [23] demonstrated that BMI had inverse relationship with DR, so Elevated BMI may confer a protective effect on DR through many ways. First, increased C-peptide levels were found in higher BMI individuals,[58] which could reduce the risk of DR. Moreover, a higher BMI may be a reflection of better glycemic control or shorter diabetes duration. Obese individuals were also more vulnerable to suffer from comorbid conditions; consequently, aggressive treatments have been taken, and reduced the development of DR[24].

Meta-analysis of prospective cohort studies done Wei Zhuby et al [25] suggest that obesity was a risk factor for DR. A higher BMI may have adverse effects on DR. First, an elevated BMI is often correlated with hypertension and dyslipidemia, both of which are risk factors for DR. Additionally, hyperleptinemia in obese individuals may increase blood pressure and oxidative stress levels, which may partly be responsible for the development of DR. Moreover, higher vascular endothelial growth factor levels were observed in obese individuals, which has been shown to be involved in the pathogenesis of proliferative DR[26].

In the current study, the highest mean of MDA were recorded among diabetic retinopathy patients compared with diabetic without retinopathy patients and control group. These findings were close to that reported Dharmveer *et al*[27] indicated a highly significantly increased MDA levels, in diabetic retinopathy patients with respect to controls. Also agreed with study done by

. Shaikh *et al* [28] indicated Serum MDA was significantly elevated in diabetic retinopathy patients compared with control group.

Additionally, Chatterjee *et al*[29], *Vlatka et al* [30] and Manish *et al* [31] indicated a highly significantly increased MDA levels, in diabetic retinopathy patients with respect to diabetic without retinopathy patients, point towards a role of free radicals in causation of diabetic complications like retinopathy. Nair *et al* [32] & Al-Duais *et al* [33] revealed signifcantly higher MDA in diabetics compared to control and similarly in diabetic retinopathy compared to those without DR. Also, Asmat *et al* [34] & Dos Santos *et al* [35] reported that Oxidative stress play a vital role in the pathogenesis of diabetes, oxidative stress harmfully affects the insulin activity through several interacting pathways and generating ROS. These could deteriorate the islets β-cells of the pancreas resulting in the reduced release of insulin. In addition, Free radical formation by non-enzymatic glycation of proteins, glucose oxidation and increased lipid peroxidation leads to damage of enzymes, cellular injury machinery, changes in the cell membrane and increased insulin resistance which are risk for diabetes. Fonseca *et al* [36] MDA is a marker of lipid peroxidation which reacts with cell membrane phospholipids. The elevated level of MDA is found in different pathological diseases such as diabetes, cardiovascular diseases, renal disease, neurodegenerative disorders and cancer so that it is a good biomarker of oxidative stress and tissue damage. Hadeel *et al* [37] revealed significantly higher MDA in diabetics compared to control and similarly in diabetic retinopathy compared to those without DR, and MDA can use for the prognosis of DR, in addition, malondialdehyde may be independent predictor of diabetes and DR.

Chatziralli *et al* [38] and Manish *et al* [39] showed that, serum MDA has been found to be significantly associated with the severity of DR in patients with *type 2* insulin-dependent DM. Marcino *et al* [40] reported that increased MDA is associated with oxidative stress and poor antioxidant defense, which promotes the progression of DR to its proliferative form. María *et al* [41] reported that retinal microvascular complications are closely related to the severity of oxidative stress, as expressed as increased level of MDA among DR patients. Olvera *et al* [42] reported that the exact mechanism by which the oxidative stress contributes to diabetic complications remains unclear, but all biochemical alterations due to DM lead to anatomical and functional impairment in the retinal microvascular network, such as changes in blood flow in the retina, disruption of the blood-retina-barrier and consequently capillary occlusion and ischemia.

The current study showed that, HbA1c was elevated significantly ($P<0.05$) in diabetic retinopathy patients compared with control group and diabetic without retinopathy patients. In agreement with the current results, study done by Dharmveer *et al* [27] showed that increased HbA1c levels in diabetic retinopathy patients as compared to control healthy group and the results were statistically significant . As well as, Namir *et al* [43] showed that significant increase of HbA1c in diabetic retinopathy patients as compared to control group.

Manish *et al* [39] showed that HbA1c significantly increase in diabetic retinopathy patients compared to diabetic without retinopathy patients. Also agree with study done by Ojoye *et al* [44] showed the mean value of HbA1c for Type 2 diabetics were significantly higher ($p< 0.05$) when compared with that of the control group. Goyal *et al* [13] showed that the % of HbA1C measured in DR patients was significantly higher than non-DR patients evidencing the fact that long term poor control of blood sugar levels had adverse effect on retina (8.16 ± 0.52 vs. 7.04 ± 0.32).

Additionally, Xing *et al* [45] revealed that HbA1c were higher in diabetic retinopathy than in diabetic non retinopathy patients and HbA1c were associated with increased risk of diabetic retinopathy. A longitudinal observation study in Southeast Sweden done by Maria *et al* [46] reported that Long-term weighted mean HbA1c, measured from diagnosis, is closely associated with the development of severe complications in type 1 diabetes. Keeping HbA1c below 7.6% (60 mmol/mol) as a treatment target seems to prevent proliferative retinopathy for up to 20 years. As well as, retrospective cohort study done by Ki-Ho *et al* [47] showed that the mean HbA1c levels was higher in the diabetic retinopathy progressors than in the diabetic retinopathy non-progressors . The mean HbA1c was a significant predictor for DR progression independent of the duration of diabetes . In agreement with the current study Bhasker *et al* [48] reported that the HbA1c was found to be higher in diabetics without retinopathy as compared to controls ($p<0.05$) and the highest value was seen in the mild NPDR group . Study done by Nam H *et al* [49] showed that HbA1c cutoff of (6.6 %) and (6.9 %) best detected the presence of any diabetic retinopathy and moderate/severer retinopathy, respectively. Also study

done by Khalid *et al* [50] showing that HbA1c was raised in patients having diabetic retinopathy (p<0.001) and patients who have uncontrolled diabetes (high HbA1c levels) have 66.61% chance of developing the diabetic retinopathy, so HbA1c is a good indicator of glycemic control as it can help diabetic individuals in deterrence of microvascular complications especially DR.

A study done by Lokesh *et al* [12] reported that lower frequency of DR in patients with lower HbA1c group and increase in frequency of DR as the HbA1c increases . As well as, A study done by Leske et al[51], in Barbodose eye study, they found that every 1% increase in HbA1C from baseline was associated with a >2-fold risk of DR, up to 4 years of follow up which was correlating with the present study in telling the linear relationship of HbA1c levels with the development of DR.

The present study displayed that the highest mean of duration of DM was recorded in diabetic retinopathy patients (mean±SD) (13.48±6.00) compared with Diabetic without retinopathy patients (mean±SD) (6.34±2.78), the result was significant (P:<0.05). These findings were close to that reported Goyal *et al* [13] showed that a significant difference was observed in the mean duration of diabetes (12 ± 5 vs. 8 ± 5 years, (P<0.05). Additionally, study done by Yan Liu *et al* [11] showed that patients were getting less likely to suffer from DR every 10 years after 60 years of age, while no difference was found before age 60. Ojoye *et al* [44] reported that the increasing duration of the diabetes mellitus further depresses the antioxidative system. As the disease condition progresses, antioxidative parameters SOD and GPx shows significantly decreasing with increasing years of illness, with the decrease most evident in those affected for the period of 16-20years. This could be the result of increased production of ROS and also increased glycation of the enzymes.

Conflict of Interest: None

Source of Findings: None

Ethical Clearance: None

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