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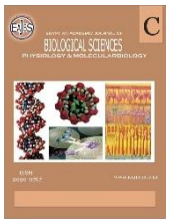

# Assessment of Serum Protein Carbonyls and Glycemic Biomarkers in Patients with Type 2 Diabetic Proliferative Retinopathy

**Zaid Najah Neamah[1] ; Omar .F. Abdul Rasheed[1]*and Alyaa Abode Kareem[2]**
[1]Department of Chemistry and Biochemistry University of AL-Nahrain.
[2]Ophthalmology Department medical faculty, Al Kufa University.
***E-mail:** *Biocem20@gmail.com* ; dr.omarabdalrasheed@nahrainuniv.edu.iq ; *alyaa.alajeeli@uokufa.edu.iq*

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PDR: Proliferative Diabetic RetinopathyPCs: protein carbonyls, T2DM: Type 2 Diabetes Mellitus, FBS: Fasting Blood Sugar, HbA1C: Glycated Hemoglobin.

**ABSTRACT**

**Purpose:** A case-control study was conducted to evaluate the role of protencarponyls and glycemic biomarkers in type two diabetic patients with proliferative diabetic retinopathy (PDR)**.Materials and Methods:** a case-control study includes a comparison between 3 groups, (40 healthy persons ) as a control compared with (40 type-2 diabetic patients without proliferative retinopathy) and (40 diabetic patients with proliferative retinopathy) serum PC were determined with ELISA while glycemic biomarkers FBS, HbA1c was determined by spectrophotometry method ( autoanlyser technique)**. Result**: PC serum level and FBS, HbA1c statistically significantly increased among the study groups, the highest level in PDR patients when compared with control groups and T2M Patients without RP. **Conclusion**: Protein carbonyls are increased significantly in both T2DM without PDR and PDR patients compared with healthy people ( control ).FBS and HbA1c decreased significantly in both T2DM without PDR and PDR patients compared with healthy people ( control ).

## INTRODUCTION

Diabetes mellitus DM is a set of metabolic diseases characterized by hyperglycemia, it has been classified mainly into two categories, type 1 and type 2 diabetes. It is associated with a wide range of microvascular and microvascular (retinopathy, neuropathy, nephropathy) complications(Association 2010), hyperglycemia plays an important role the in development of Diabetic retinopathy. The biochemical pathways associated with hyperglycemia-induced vascular damage include elevated glucose flux by means of the polyol pathway, Advanced glycation end-product accumulation, inflammation, as well as the activation of protein kinase C (hexosamine pathway)(Wang and Lo 2018) The overabundance of superoxide in the mitochondria induced by hyperglycemia leads to oxidative stress, which acts as a stressor, linking all these metabolic pathways. Oxidative stress gives rise to multiple early clinical hallmarks of Diabetic Retinopathy that include a thickened basement membrane, pericyte apoptosis, and mitochondrial dysfunction, which altogether result in Blood Retina Barrier breakdown (Duh, Sun *et al.*, 2017) Blood Retina Barrier caused impairment thickens retina, as well as increasing leukocytosis, which is an intravascular immune response and one of the early clinically recognizable pathologies of Diabetic Retinopathy. It causes the adherence of white blood cells (WBCs) to the endothelial cells lining the blood vessels that influence the plugging of capillaries and vascular leakage(Lechner, O'Leary *et al.*, 2017).

Protein carbonyls are considered sensitive indices of severe oxidative injury and stress to proteins, cells and tissues in many diseases such as diabetes Although the presence of carbonyls is not necessarily indicative of protein oxidation, carbonyl content is the most general indicator and the most commonly used marker of protein oxidation in diabetes(Lechner, O'Leary *et al.*, 2017).Diabetic retinopathy (DR) involves microaneurysms or worse lesions affecting at least a single eye(Ansari, Tabasumma, *et al.*, 2022). It is one of the most pervasive secondary microvascular complications intrinsic in diabetes mellitus (DM), induced by leakage from the breakdown of the inner blood-retinal barrier and microvascular occlusion(Li, Tong *et al.*, 2023). DR has been classified as the most commonly occurring major secondary complication in individuals diagnosed with DM(Gomułka and Ruta 2023). It has also been classified as the most documented microvascular threat to diabetic patients(Ansari, Tabasumma, *et al.*, 2022). A lack of diagnosis or timely therapeutic intervention could result in visual impairment, partial blindness, and ophthalmic complications beyond these effects(Porta and Bandello 2002). DR can be differentiated into two major classes, namely, PDR and NPDR(Duh, Sun *et al.*, 2017, Shani, Eviatar *et al.*, 2018). PDR primarily begins with the abnormal growth of fibrous connective tissue on the retinal surface, whereas NPDR occurs due to lesions inside the retinal capillaries resulting from edema, hemorrhage, microaneurysms of the blood vessels, and/or capillary blockage (Duh, Sun et al. 2017, Ansari, Tabasumma, *et al.*, 2022). Risk factors of diabetic retinopathy include its duration, the presence of diabetic nephropathy, neuropathy, foot ulcer and amputation, along with hypertension, the level of cholesterol and triglyceride in the serum, fasting blood glucose, the level of HbA1c and the age of the patient(McNair, Christiansen *et al.*, 1978).

## MATERIALS AND METHODS

A case-control study involved 120 Clinical samples (120) that were collected from the 15$^{th}$ of April to the 25$^{th}$ of February 2023 from the hospital center of Alnajaf Govemoraate Iraq, this samples were divided into three subgroups (40) of healthy people as control, the ages included in the study between (30-60) years, samples d divided into three subgroups.

Group 1: 40 Healthy persons as a control group.

Group 2: 40 Diabetic patients without proliferative retinopathy.

Group 3: 40 Diabetic patients with proliferative retinopathy.

Ten milliliters of venous blood were collected from each patient, and stored in an EDTA tub 3 milliliters were for HbA1c estimation and 7 milliliters were placed in a gel tub and centrifuged to obtain serum ¸serum stored in frozen at 20 C and used to measured PCs and FBS by ELISA technique ( sandwich method ) and spectrophotometer.

**Statistical Studies:** The data was analyzed using SPSS, Version 26 for Windows. Numeric data were presented as mean ± SD. To calculate the individual p-value between normal and patients, Student's t-test was used, and Student's F test (ANOVA) was used to calculate the individual p-value between the control group and patient groups. A P-value of less than 0.05 is considered significant. ANOVA test is used to measure the difference between more than 2 means. If its p-value is significant (< 0.05), then we don't know where the significance is and between which couple groups, so we used one of the post hoc tests to explore the location of significance. to appear as a pair and take each 2 groups together and test the difference between their means. Finally, we can see in-depth the real significant difference.

## RESULTS

A total of 120 individuals were enrolled in the study, distributed among three groups; control, type 2 DM without PDR and type 2 DM with PDR, with 40 participants for each. There was no statistical significance in sex distribution among the groups (p-value>0.05) as in Table 1 and Figure 1 .

**Table 1:** Sex distribution of the study population.

| Characteristic | | | Status | | | Total | P value |
|---|---|---|---|---|---|---|---|
| | | | Control | T2DM without PDR | T2DM with PDR | | |
| Gender | Female | No. | 19 | 19 | 15 | 53 | 0.582 |
| | | % | 47.5% | 47.5% | 37.5% | 44.2% | |
| | Male | No. | 21 | 21 | 25 | 67 | |
| | | % | 52.5% | 52.5% | 62.5% | 55.8% | |
| Total | | No. | 40 | 40 | 40 | 120 | |
| | | % | 100.0% | 100.0% | 100.0% | 100.0% | |

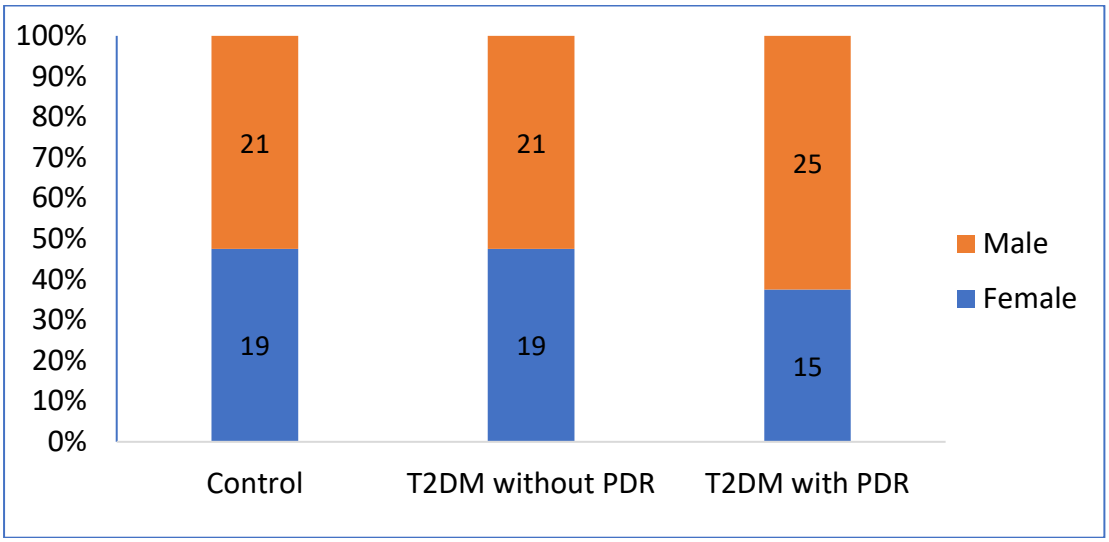


**Fig. 1:** Sex distribution among the study population

The mean age of the study population was 49 years (± 8.9 SD) and varied among the groups significantly as it was 44.9 (±9.6 SD) in control whereas 48.4 (±8.7 SD) in T2DM without PDR and 53.7 (±6.1 SD) in T2DM with PDR (p-value < 0.001) as inTable 2 .

**Table 2:** Age distribution of the study population by ANOVA and post hoc analysis.

| Age | No. | Mean | Std. Deviation | P value | Post hoc analysis | | p value |
|---|---|---|---|---|---|---|---|
| Control | 40 | 44.9 | 9.6 | <0.001 | Control | T2DM without PDR | 0.144 |
| T2DM without PDR | 40 | 48.4 | 8.7 | | Control | T2DM with PDR | <0.001 |
| T2DM with PDR | 40 | 53.7 | 6.1 | | T2DM without PDR | T2DM with PDR | 0.015 |
| Total | 120 | 49 | 8.9 | | | | |

The mean duration of the disease was 3.3 years (±0.7 SD) in T2DM without PDR and 8.6 years (±2.6 SD) in T2DM with PDR (p-value < 0.001). The mean value of HBA1C was 4.8 % (±0.4 SD) in control while it was 7.3 % (±0.5 SD) in T2DM without PDR and 9.3 % (±0.6 SD) in T2DM with PDR (p-value < 0.001). This distribution was also significant within different groups by post hoc analysis. The studied group also differs significantly by FBS means as it was 97.1 mg/dl (±6.1 SD) in control while it was 128.1 mg/dl (±10.6 SD) in T2DM without PDR and 187.5 mg/dl (±37.7 SD) in T2DM with PDR (p value < 0.001) as in Table 3.

**Table 3:** distribution of disease duration, HBA1C and FBS among studied groups by ANAOVA and Post hoc analysis.

| | | No. | Mean | Std. Deviation | P value | Post hoc analysis | | P value |
|---|---|---|---|---|---|---|---|---|
| Duration of Diabetes | Control | 40 | 0 | 0 | <0.001 | Control | T2DM without PDR | <0.001 |
| | T2DM without PDR | 40 | 3.3 | 0.7 | | Control | T2DM with PDR | <0.001 |
| | T2DM with PDR | 40 | 8.1 | 2.6 | | T2DM without PDR | T2DM with PDR | <0.001 |
| | Total | 120 | 3.8 | 3.6 | | | | |
| HbA1c | Control | 40 | 4.8 | 0.4 | <0.001 | Control | T2DM without PDR | <0.001 |
| | T2DM without PDR | 40 | 7.3 | 0.5 | | Control | T2DM with PDR | <0.001 |
| | T2DM with PDR | 40 | 9.3 | 0.6 | | T2DM without PDR | T2DM with PDR | <0.001 |
| | Total | 120 | 7.2 | 1.8 | | | | |
| F.B.S | Control | 40 | 97.1 | 6.1 | <0.001 | Control | T2DM without PDR | <0.001 |
| | T2DM without PDR | 40 | 128.1 | 10.6 | | Control | T2DM with PDR | <0.001 |
| | T2DM with PDR | 40 | 187.5 | 37.7 | | T2DM without PDR | T2DM with PDR | <0.001 |
| | Total | 120 | 137.5 | 43.9 | | | | |

**Determination of studied biomarker level and their association with basic information among groups.**

Among controls, the mean value of Protein carbonyls was 33.8 (±7.2 SD); 67.3 (±8.4 SD) in T2DM without PDR and 97.2 (±10.7 SD) in T2DM with PDR (p-value < 0.001) as Table 4 shows.

**Table 4:** distribution of Protein carbonyls among studied groups by ANOVA and post hok analysis.

| | | No. | Mean | Std. Deviation | P value | Post hoc analysis | | P value |
|---|---|---|---|---|---|---|---|---|
| Protein carbonyls | Control | 40 | 33.8 | 7.2 | <0.001 | Control | T2DM without PDR | <0.001 |
| | T2DM without PDR | 40 | 67.3 | 8.4 | | Control | T2DM with PDR | <0.001 |
| | T2DM with PDR | 40 | 97.2 | 10.7 | | T2DM without PDR | T2DM with PDR | <0.001 |
| | Total | 120 | 66.1 | 27.4 | | | | |

**Correlation of Protein Carbonyls With Demographic Characteristics And Basic Lab Tests Among T2DM Without PDR Patients:**

The linear regression analysis (Table 5) reveals that there is a significant correlation between the level of Protein carbonyls and HbA1c level (R2 =0.138, B coefficient= 6.7, p value= 0.018) and this accounts for each point increase in HbA1c, there was about 7 points increase in Protein carbonyls value and this model explains about 13% of the noticed correlation (Fig. 2).

**Table 4:** Distribution of Protein carbonyls and HIF-1a among studied groups by ANOVA analysis.

| Model Selecting only cases for which status = T2DM without PDR | | Unstandardized Coefficients | | Standardized Coefficients | t | P value | 95% Confidence Interval for B | |
|---|---|---|---|---|---|---|---|---|
| | | B | Std. Error | Beta | | | Lower Bound | Upper Bound |
| Dependent Variable: protein carbonyls | age | -0.301 | 0.149 | -0.312 | -2.024 | 0.050 | -0.603 | 0.000 |
| | Duration of Diabetes | -0.931 | 1.982 | -0.076 | -.470 | 0.641 | -4.943 | 3.081 |
| | F.B.S | 0.111 | 0.127 | 0.139 | 0.867 | 0.391 | -0.148 | 0.369 |
| | HbA1c | 6.711 | 2.726 | 0.371 | 2.462 | 0.018 | 1.192 | 12.230 |

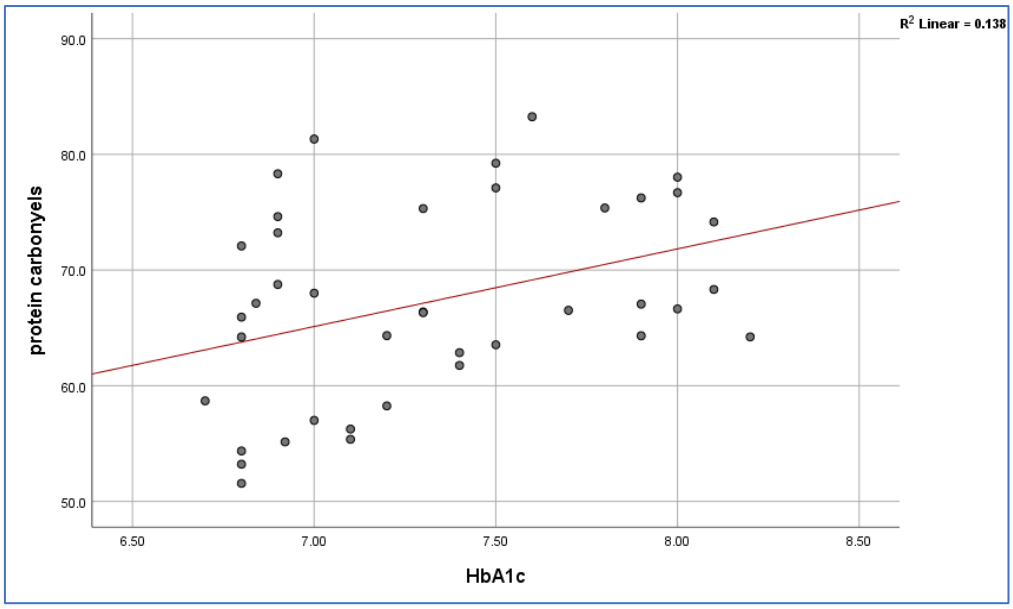


**Fig. 2:** correlation of Protein carbonyls with HbA1c among T2DM without PDR patients

## DISCUSSION

This study aimed to estimate protein carbonyl levels in three groups (control, T2DM without PDR, and T2M with PDR, according to the statistical analysis there is no conservable significance between sex among the groups of the study, no remarkable significance with regard to age and other two groups except T2DM patient with PDR showing higher mean of age, this means patient with PDR older than healthy people. In the case of the duration of diabetes, there are clearly significant between the 2 patient groups, logically on the assumption that the complication of diabetes is possible during the progression of the disease.

**Biochemical Results**:

Glycaemic biomarkers (FBS, HbA1c) indicate statistically significant among the study group, FBS means it was (97.1 mg/dl ±6.1 SD) in control while it was 128.1 mg/dl (±10.6 SD) in T2DM without PDR and 187.5 mg/dl (±37.7 SD) in T2DM with PDR (p-value < 0.001) while The mean value of HBA1C was 4.8 % (±0.4 SD) in control while it was 7.3 % (±0.5 SD) in T2DM without PDR and 9.3 % (±0.6 SD) in T2DM with PDR (p-value < 0.001). the obvious mechanism that explains this association suggests that hyperglycemia stimulates the synthesis of diacyleglecerol (DAG ) in vascular cells leads to promote activation of Protein Kinase C isozymes ( PKC) isozymes, especially PKC-b, leads to convert phosphorylate proteins that possess endothelial function and neovascularization, These changes activate intracellular signaling proteins such as PKC, PKB, AGE, and MAPK which are finally resulting in pathological induction of transcription factors

such as NF-κB and AP-1, the previous study that conducted in Malaysia by (Safi, Qvist, *et al.*, 2014) agrees with this proposition. Other studies describe the role of hyperglycemia in the progression of DR, the metabolic abnormalities of diabetes induce the overproduction of mitochondrial superoxide in vascular endothelial cells (ECs), which subsequently leads to increased flux through the polyol pathway, the production of advanced glycation end-products (AGEs), upregulation of the receptor for AGEs and its activating ligands, activation of the protein kinase C pathway, and overactivity of the hexosamine pathway. These pathways elevate the levels of intracellular reactive oxygen species and cause irreversible cell damage through epigenetic changes, such as histone modifications, DNA methylation, and non-coding RNAs. Consistent with this concept of "metabolic/hyperglycemic memory, glycemic re-entry after transplantation of pancreatic islet cells to STZ-induced diabetic mice fails to heal retinal microvascular damage. These findings might explain the effects of early glycemic control on the future development of Similar research results, and assess the effect of the glycemic factors in the development of DR and other complications, A Wisconsin study that deals with the epidemiology of Diabetic retinopathy has explained the relationship of hypoglycemia with progression of PDR and NPDR(Klein, Klein *et al.,* 1989, Nathan 1996). HbA1c is regarded as an essential indicator of glycemic control in diabetic retinopathy because it determines glucose level for a duration of 2-3 months, moreover, patients with DR possess a high level of HbA1c. This information is supported by a study by the Australian Diabetes Society(Özmen, Güçlü et al. 2007), The American Diabetes Association (ADA) suggested the maximum level of HbA1C must be( < 7%) to reject the development of micro and microvascular complications (Hinnen, Nielsen *et al.,* 2006).

In addition, this study dealt with estimating the level of protein carbonyls, this marker indicated strong significance among the groups of the study, the mean of Protein carbonyls was 33.8 (±7.2 SD); 67.3 (±8.4 SD) in T2DM without PDR and 97.2 (±10.7 SD) in T2DM with PDR (p-value < 0.001), elevated of protein carbonyls level is attributed to the effect of hyperglycemia lead to occurring the oxidative stress which regarded as an important factor to the development of a diabetic complication, due to imbalance between ROS levels and antioxidant resulting defect of the biological system and tissue damage (Tarr, Kaul, *et al.,* 2013).

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