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Assessment of Serum Levels of Advanced Oxidation Protein Products in Type 2 Diabetic Patients with and without Retinopathy Taking Different Antidiabetic Treatments

Sara Asaad Kadhim[*,1], Eman Saadi Saleh[2] and Aymen Dheaa Jaafer[3]

[1] Department of Clinical Pharmacy, College of Pharmacy, University of Baghdad, Baghdad, Iraq.
[2] Department of Clinical Laboratory Sciences, College of Pharmacy, University of Baghdad, Baghdad, Iraq.
[3] Ministry of Health and Environment, Ibn AL-Haitham Hospital for Ophthalmology, Baghdad, Iraq.

Abstract

Increased oxidative stress has a role in the development of diabetic retinopathy (DR). The aim of this study was to investigate the protein peroxidation role by measuring serum levels of advanced oxidation protein products (AOPP) in type 2 diabetic patients with or without diabetic retinopathy and comparing them to healthy subjects to see if circulating AOPP levels can be used as a detection biomarker for DR, and see which of the two widely used antidiabetic treatment groups had the most impact on this oxidative stress marker. The groups were divided into two subgroups: 1) Patients group included 70 type 2 diabetic patients of both gender (36 male, 34 female), 35 with diabetic retinopathy (DR) and 35 with no evidence of DR (NDR), 2) Control group included 20 healthy subjects (11 male, 9 female). advanced oxidation protein products levels were significantly higher in diabetic patients with (12.5±5.6 ng/ml) or without DR (5.1±4 ng/ml) when compared to those of controls (1.45 ± 0.8 ng/ml) ($p<0.05$). advanced oxidation protein products levels were higher in the late stage of DR compared to the early stage (14 ± 3.15 ng/ml) and (10 ± 2.13 ng/ml) respectively. Furthermore, Dipeptidyl peptidase-4 inhibitors (DPP-4 inhibitors) cause a better reduction in AOPP levels compared to Sulfonylureas (SUs) in the NDR group. In conclusion, increased protein oxidation may involve in the pathogenesis and severity of DR and the serum AOPP levels have the prospect to become a marker for the diagnosis of DR. Dipeptidyl peptidese-4 inhibitors were better in slowing the progression of the disease compared to SUs.

Keywords: Advanced oxidation protein products, Diabetic retinopathy, Oxidative stress, Dipeptidyl peptidese-4 inhibitors, Sulfonylureas.

تقييم مستويات المصل لمنتجات بروتين الأكسدة المتقدمة في عينة من مرضى السكري من النوع الثاني الذين يعانون من اعتلال الشبكية والذين يتلقون علاجات مختلفة من مضادات السكر

سارة اسعد كاظم [*،١] ، ايمان سعدي صالح [٢] و ايمن ضياء جعفر [٣]

[١] فرع الصيدلة السريرية ،كلية الصيدلة، جامعة بغداد، بغداد، العراق
[٢] فرع العلوم المختبرية السريرية ،كلية الصيدلة، جامعة بغداد، بغداد، العراق
[٣] وزارة الصحة والبيئة ، مستشفى ابن الهيثم لطب وجراحة العيون ، بغداد ، العراق

الخلاصة

زيادة الاجهاد التأكسدي له دور في تطور اعتلال الشبكية السكري . كان الهدف من هذه الدراسة هو التحقق من دور بيروكسيد البروتين عن طريق قياس مستويات مصل منتجات بروتين الأكسدة المتقدمة في مرضى السكري من النوع الثاني المصابين أو غير المصابين باعتلال الشبكية السكري ومقارنتها بالاشخاص الاصحاء لمعرفة ما إذا كان من الممكن استخدام مستويات المصل لمنتجات بروتين الأكسدة المتقدمة كمؤشر بيولوجي للكشف عن اعتلال الشبكية السكري. ومعرفة أي من مجموعتي العلاج المضاد لمرض السكر المستخدمة على نطاق واسع كان لها التأثير الأكبر على علامة الإجهاد التأكسدي. تم تقسيم مجموعات الدراسة إلى مجموعتين: ١) ٧٠ من مرضى السكري من النوع الثاني (٣٦ ذكر ، ٣٤ أنثى) ، ٣٥ مصابًا باعتلال الشبكية السكري و ٣٥ مع عدم وجود دليل على اعتلال الشبكية، و ٢) اشخاص اصحاء (١١ ذكر ، ٩ إناث). كانت مستويات المصل لمنتجات بروتين الأكسدة المتقدمة أعلى في مرضى السكري مع (١٢,٥ ± ٥,٦ نانوغرام / مل) أو بدون اعتلال الشبكية (5.1±4 نانوغرام / مل) بالمقارنة مع الاشخاص الاصحاء (١,٤٥ ±٠,٨ نانوغرام / مل) ($p <0.05$). كانت مستويات المصل لمنتجات بروتين الأكسدة المتقدمة أعلى في المرحلة المتأخرة من المرض مقارنة بالمرحلة المبكرة (14 ±3.15 نانوغرام / مل) و (١٠ ±٢,١٣ نانوغرام / مل) على التوالي. علاوة على ذلك ، تسبب مثبطات انزيم الداي بيبتايديز-٤) انخفاضًا أفضل في مستويات المصل لمنتجات بروتين الأكسدة المتقدمة بالمقارنةمع السلفونيل يوريا) في مجموعة الاشخاص الغير مصابين باعتلال الشبكية. قد تنطوي زيادة أكسدة البروتين في التسبب في الإصابة وخطورة اعتلال الشبكية، ومن المحتمل أن تصبح هذه المستويات في المصل علامة لتشخيص مرض اعتلال الشبكية السكري . كانت مثبطات انزيم الداي بيبتايديز-٤ أفضل في إبطاء تقدم المرض مقارنة بالسلفونيل يوريا .

الكلمات المفتاحية : منتجات بروتين الأكسدة المتقدمة ، اعتلال الشبكية السكري ، الإجهاد التأكسدي ، السلفونيل يوريا و مثبطات انزيم الداي بيبتايديز-٤ .

[1]Corresponding author E-mail: saraasaad295@gmail.com
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Introduction

Diabetic retinopathy (DR) is one of diabetes' microvascular consequences that have sight-threatening repercussions for the eyes [1]. It is commonly believed that DR is the major cause of diabetes-related vision loss or impairment in adults aged 20 to 65 years around the world[2]. In 2030, the number of people affected by this condition will have risen to 191.0 million [2–4]. Diabetic retinopathy is caused by a sequence of changes in the retinal capillary basement membrane, increased permeability of the retinal vasculature, tissue hypoxia, and the production of various vasoactive chemicals, all of which contribute to neovascularization. Blood vessels start to appear on the retinal surface as the disease progresses. The composition of blood vessels "blood and extracellular fluids" flow out because of the fragility of these vessels, resulting in vitreous hemorrhage and retinal detachment. Proliferative diabetic retinopathy (PDR) is the name given to these pathophysiological changes, which might result in vision loss. Non-proliferative diabetic retinopathy (NPDR), on the other hand, is the early stage. Microaneurysms and tiny elongation of blood vessels of the retina, which are known as prefatory signs of DR, are the most common features of NPDR[5,6].

Oxidative stress is a condition that occurs when the formation and elimination of free radicals are out of equilibrium. In brief, any disturbance in the dynamic redox balance causes oxidative stress, which damages the cells of target organs like the retina, kidney, and heart [7,8]. The retina is susceptible to oxidative stress because it is constantly exposed to visible or UV light, which generates reactive oxygen species (ROS), and because the outer photoreceptor segment membranes contain a large amount of oxidized polyunsaturated fatty acids (PUFAs)[9]. In addition to the presence of PUFAs, Excessive oxygen is necessary for the retina's optical imaging function and energetic metabolism, and it is normally under the impact of increased oxygen pressure, which stimulates the generation of ROS [10].

Indirect evidence suggests that oxidative stress has a role in the pathogenesis of DR. Retinal vasculature and surrounding tissue can be damaged by an excessive accumulation of reactive oxygen species, leading to DR. Activation of the protein kinase C (PKC) pathway, polyol pathway flux, activation of the hexosamine pathway, as well as intracellular development of advanced glycation endproducts (AGEs), have all been linked to oxidative damage caused by high blood glucose levels[11,12]. In DR, oxidative stress caused by hyperglycemia results in mitochondrial defects, cellular death, inflammation, lipid peroxidation, as well as structural and functional changes (including microcirculatory abnormalities and neurodegeneration). Many biomarkers have been identified to detect oxidative stress. These markers include either lipid peroxidation products, protein oxidation products, or DNA damage products such as (acrolein, malondialdehyde (MDA), conjugated dienes), (AOPP, protein carbonyl), and 8-hydroxy-2' -deoxyguanosine (8-OHdG) respectively[13].

Advanced oxidation protein products (AOPP) are formed by the effect of chlorinated oxidants, primarily hypochlorous acid and chloramines (made by myeloperoxidase in active neutrophils), as a result of oxidative stress [14]. In structure and biologic function, AOPPs are similar to advanced glycation end-products (AGEs), and their level will be elevated in patients with renal failure, atherosclerosis, and diabetes [15,16]. In diabetes, AOPP is generated as a result of increased glycoxidation processes, dynamic redox imbalance, and inflammation. The procedure for detecting AOPP levels is simple and quick, and it can also be utilized as a marker for diagnosing and monitoring diabetes complications [17].

In addition to lifestyle modifications," the American Diabetes Association " recommends metformin and sulfonylurea in combination with metformin to achieve tight glycemic control in patients with type 2 diabetes [18]. Adherence is compromised and long-term treatment outcomes may be negatively impacted by hypoglycemia and weight gain caused by SU.

Incretin-based medication is recommended in the management of individuals with type 2 diabetes, according to the "American Association of Clinical Endocrinologists/American College of Endocrinology" guidelines issued in 2009 [19]. Sitagliptin, Vildagliptin, and Saxagliptin are three DPP-4 inhibitors that are used to treat type 2 diabetic patients. They preferentially inhibit the enzyme DPP-4, boosting the action of incretins such as GLP-1 and GIP. They also improve 24-hour blood glucose fluctuation and manage blood glucose levels. Vildagliptin appears to lower glycated hemoglobin levels and glucose fluctuations [20,21]. HbA1c levels were also reduced with Linagliptin and Alogliptin [22,23]. Furthermore, oxidative stress markers are expected to be affected by suppressing postprandial blood glucose rises. Measuring one of these oxidative stress markers is thought to be important in predicting diabetic retinopathy in its early stages since current treatment focuses on late-stage DR when vision has already been compromised and based on the results, the antihyperglycemic agent that has a better effect on this biomarker and thus on the progression of DR could be known. In this study, serum levels of AOPP were measured in diabetic patients that have or have not developed retinopathy and compared the results with healthy

controls to correlate them with the severity of DR and to find out whether serum AOPP levels can be used as a biomarker for diagnosing diabetic retinopathy at early stages. Furthermore, This study aimed to evaluate the effect of some antidiabetic treatments on this serum biomarker.

Materials and Methods

This case-control study was conducted from November 2021 to March 2022 at the Ibn Al-Haitham Hospital of Ophthalmology and a Specialized Center for Endocrinology and Diabetes, and it was approved by the Ethical Committee of the College of Pharmacy/University of Baghdad before the start of the study, following the Declaration of Helsinki[24]. After the participants were given information about the study's goal, they gave their informed consent. Plasma samples were taken from type 2 diabetic patients with diabetic retinopathy, without diabetic retinopathy, and healthy controls. All of the patients had diabetes duration for at least 5 years and were given either DPP4 inhibitors or SUs plus metformin as treatment given by their endocrinologist. Healthy controls were age-matched people who didn't have any signs of diabetes or ocular hemorrhages, neovascularization, or exudation. Previous intraocular surgery, other neovascular disorders in the eye such as occlusion of the central retinal vein (CRVO) and age-related macular degeneration, a history of any inflammation in the eye, glaucoma, clinically significant hepatic, pulmonary or cardiac insufficiency, autoimmune disorders such as type 1 diabetes, pregnancy, or lactation, regular use of antioxidants, and non-steroidal anti-inflammatory drugs were all considered exclusion criteria.

Criteria recommended by the "World Health Organization"[25] was used for the diagnoses of type 2 diabetes mellitus and retinopathy was diagnosed by an independent ophthalmologist using ophthalmoscopy, fundus photography (FP), or optical coherence tomography (OCT) according to the finding in Early Treatment Diabetic Retinopathy Study (ETDRS)[26]. DR was categorized into non-proliferative diabetic retinopathy (NPDR), or proliferative diabetic retinopathy (PDR) by using the worldwide clinical DR severity scale[27]. The clinical laboratory reported fasting blood glucose (FBG), glycated hemoglobin (HbA1c), lipid profile, urea, and creatinine. A patient with hypertension was classified as having arterial blood pressure of more than 140/90 mm Hg at rest or being on antihypertensive medication. Body mass index was calculated using height and weight measurements. A data collection sheet was used to capture prior histories, personal characteristic behaviors, diabetes duration, and antidiabetic treatment from all individuals.

Collection of blood samples

Blood samples of ten milliliters (ml) were taken from the antecubital vein. Two milliliters of blood were transferred to an ethylene diamine tetraacetic acid (EDTA) tube and stored at (+2 to +8 Cº) for HbA1c assay within one week. The remaining 8 ml of blood was transferred to a gel tube and allowed to coagulate for 30 minutes before being centrifuged for 10 minutes at 3000 rpm to get the serum. On the day of collection, a portion of the serum was used by the hospital's laboratory to evaluate fasting serum glucose (FSG), urea, creatinine, and lipid profile. The residual serum was kept in Eppendorf tubes and kept frozen at (–20Cº) until AOPP levels were determined.

Measurement of AOPP levels

Commercial enzyme-linked immunosorbent assay (ELISA) kits (MyBioSource, USA) were used to measure AOPP levels in the plasma. It is a "sandwich enzyme immunoassay" that allows AOPP detection within a range of 0.156-25 ng/ml. The procedures were carried out following the manufacturer's recommendations. Variations across and within assays were 12.0% and 8.0 percent, respectively.

Other biochemical parameters

Fasting blood glucose was measured using an enzymatic colorimetric method[28], HBA1C was measured using the D-10™ hemoglobin testing system, which depends on the chromatographic separation of analytes[28], lipid profile, and urea were measured using an enzymatic hydrolysis method[29,30], and creatinine was assessed by alkaline hydrolysis method[30].

Statistical analysis

Statistical Package for the Social Science (SPSS, IBM, USA version 25) was used to conduct statistical analysis. The Kolmogorov–Smirnov test was used to examine the data distribution. Data were provided as the median, interquartile range, and frequencies, depending on their distribution. When multiple comparisons were assigned, the Kruskal–Wallis H test was employed; when just two comparisons were allocated, the Mann–Whitney U-test was used. To compare categorical variables, a Chi-square test was used. The relation between AOPP and the study variables was determined using the Spearman correlation test. The optimal AOPP level "cutoff score" for distinguishing patients from non-diabetic controls was determined using a receiver-operating characteristic analysis (ROC). Youden index J was used to establish the best AOPP cut-off points for detecting DR. The area under the curve (AUC) with 95 percent confidence intervals, as well as sensitivity and specificity, were used to assess diagnostic accuracy. A two-tailed P-value of less than 0.05 was considered statistically significant.

Results

Table 1 summarizes the Sociodemographic and clinical characteristics of the individuals. Plasma samples were drawn from seventy type 2 diabetic patients (35 DR and 35 NDR), as well as 20 non-diabetic controls. There were 14 NPDR patients and 21 PDR patients in the DR group.

Table 1. Sociodemographic and clinical characteristics of the participants

Variables	Type 2 DM N=70	Control N=20	P-value
Age (years)	48 ± 8.25	45 ± 14	0.93
Gender (m/f)	36/34	11/9	0.78
BMI (kg/m^2)	29.35± 4	28.5 ± 4	0.628
Smoking (n)	18	8	0.214
Hypertensive (n)	47	……..	……..
Retinopathy Yes/No	35/35	……..	…….
Stage NPDR	14	……..	…….
PDR	21		
NDR	35		
Treatment SU/DPP4	34/36	……	…….

n, numbers; BMI, body mass index; f, female; m, male; NPDR,non-proliferative diabetic retinopathy; PDR, proliferative diabetic retinopathy; NDR, without diabetic retinopathy; SU, Sulfonylureas; DPP4, Dipeptidyl peptidase-4 inhibitors; Continuous variables presented as median ± interquartile range and discrete variables as numbers, * Significant when p<0.05.

Biochemical characteristics of the participants

Serum levels of FBG, HbA1c%, TC, TG, LDL, s.creatinine, and urea were significantly higher, and serum levels of HDL were significantly lower in T2DM patients compared to non-diabetic controls (P<0.05) as shown in Table 2.

Table 2. Biochemical characteristics of the participants

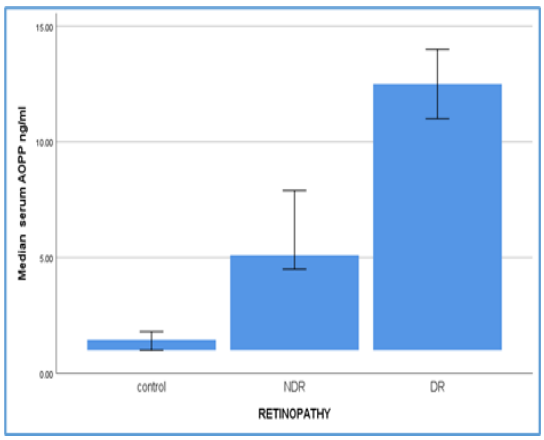
Variables	TYPE 2 DM n=70	Control n=20	P-value
FBG(mg/dl)	194 ± 73	89.5 ± 9.5	0.000*
HbA1c**%**	8.3 ± 1.1	5 ± 0.2	0.000*
TC(mg/dl)	198 ± 70.75	150 ± 16.75	0.000*
LDL(mg/dl)	158±7	149±0.4	0.000*
HDL(mg/dl)	33 ± 7	34.5 ± 7	0.038*
TG(mg/dl)	190 ± 34	90 ± 11.75	0.000*
S.cr(mg/dl)	1.1 ± 0.6	0.6 ± 0.2	0.000*
urea(mg/dl)	43 ± 14.25	23 ± 9.5	0.000*

FBG, fasting blood glucose; HbA1c, glycated hemoglobin; TC, total cholesterol; LDL, low-density lipoproteins; HDL, high-density lipoproteins; TG, triglyceride; S.cr ., serum creatinine; Continuous variables presented as median± interquartile range (IQR), * Significant when p<0.05, Mann–Whitney U-test.

Serum levels of AOPP in the studied groups

A statistically significant difference existed in AOPP levels in the plasma among DR, NDR, and healthy control groups (p≤0.05, Kruskal–Wallis H test). The levels of AOPP in the DR group (median=12.5±5.6 ng/ml; p≤0.05, Mann–Whitney U-test) were considerably higher than those in the NDR groups (5.14 ng/ml; p≤0.05, Mann–Whitney U-test) and non-diabetic controls (1.45±0.8 ng/ml; p≤0.05, Mann–Whitney U-test), as shown in Figure 1a. These findings revealed that increased serum AOPP levels are linked to the existence of DR or its development. Furthermore, we investigated the relationship between AOPP levels and the severity

of DR. AOPP levels in the plasma were higher in PDR patients (14 ± 3.15 ng/ml) than in NPDR patients (10 ± 2.13 ng/ml ; P<0.05, Mann–Whitney U-test; Figure 1b). These results find out that increased serum AOPP levels are associated with the severity of DR or its progression.



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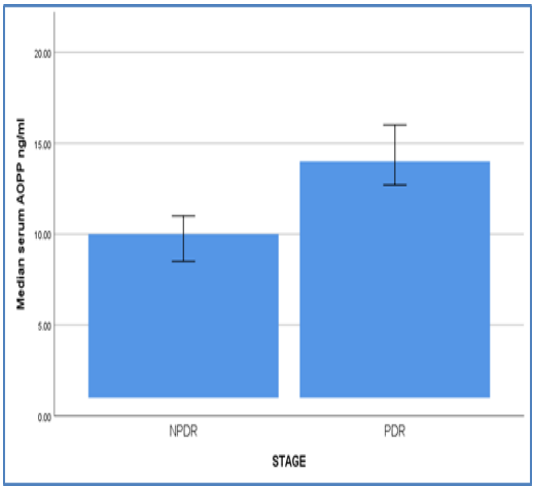

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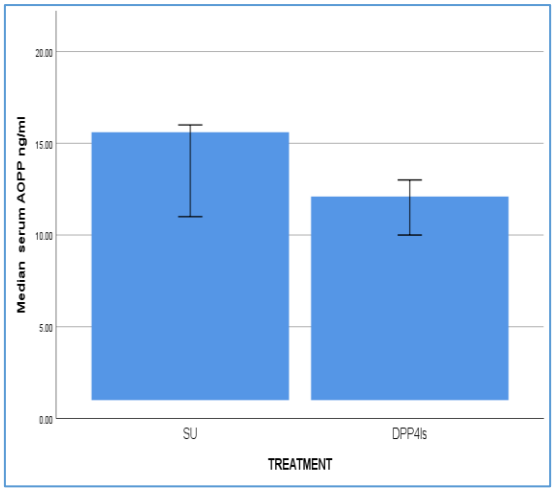
Figure 1. Comparison of serum AOPP levels in the studied groups. (a) AOPP levels in the plasma of healthy subjects (controls), with diabetic retinopathy (DR), and diabetic patients without retinopathy (NDR). (b) AOPP levels in patients with different stages of DR. Data were analyzed with Kruskal–Wallis H test and Mann–Whitney U-test.

There are no statistically significant differences in AOPP levels among DR patients taking either SU or DPP4 (P>0.05, Mann–Whitney U-test; figure 2a). in the other hand AOPP levels were significantly lower in NDR patients taking DPP4 compared to those taking SU (P<0.05, Mann–Whitney U-test; figure 2b). This means in those without retinopathy DPP-4 inhibitors may delay the incidence or slow the progression of DR.



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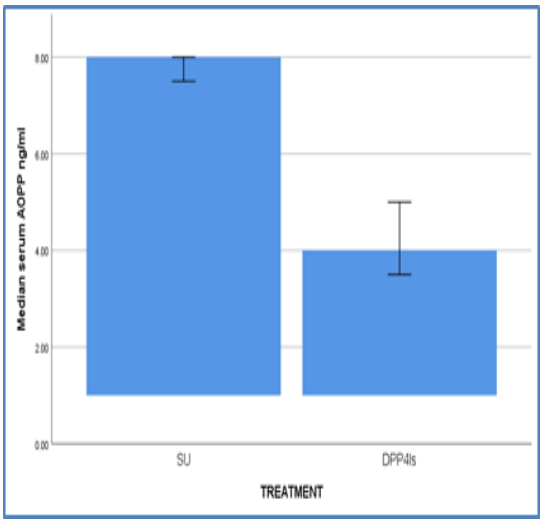

b

Figure 2. Comparison of serum AOPP levels in diabetic patients treated with SU or DPP-4 inhibitors. (a) AOPP levels in the serum of the DR group. (b) AOPP levels in the NDR group. Data were analyzed with Mann–Whitney U-test.

Correlation of AOPP with the study variables.

Correlation studies of serum AOPP with the studied variables of the pooled data are shown in table 3. Serum AOPP has a positive correlation with FBS, HbA1c, TC, TG, S.CR, and urea p<0.05, While, there was no correlation between serum AOPP with HDL, and LDL (P>0.05).

Table 3. Correlation of serum AOPP level with the other variables

Variables	R-value	P-value
FBG	0.60	0.000*
HbA1c	0.713	0.000*
T.C	0.655	0.000*
LDL	0.09	0.34
HDL	-0.128	0.228
TG	0.70	0.000*
S.cr	0.71	0.000*
Urea	0.783	0.000*

FBG, fasting blood glucose; HbA1c, glycated hemoglobin; TC, total cholesterol; LDL, low-density lipoproteins; HDL, high-density lipoproteins; TG, triglyceride; S.Cr, serum creatinine;* significant when the p-value of spearman correlation was <0.05.

Potential of AOPP levels as an indicator for de tection of retinopathy.

The ROC analysis was done to determine whether circulating AOPP levels can be used as an indicator for the detection of DR. The area under the ROC curve (AUC) was used to estimate total accuracy, and the appropriate cut-off value of AOPP was established using the "Youden index J". ROC curve showed that the optimum diagnostic cutoff for AOPP was 8.25ng/ml, with an AUC of 0.99 (95% CI, 0.977-1.0; P<0.05). This corresponds to a sensitivity of 100%, and specificity of 82% as shown in table 4 and figure 4. These results give an idea that the circulating AOPP level has the prospect to become a biomarker for the detection of DR.

Table 4. Receiver operating characteristic curve and AUC analysis of AOPP for retinopathy

Variable	AUC	95%CI Of AUC	P-Value	Optimal cut-Off	SN	SP
AOPP	**0.990**	**0.977-1.000**	**0.000**	**8.25**	**1.0**	**0.82**

AUC, the area under the curve; CI, confidence interval; SN, sensitivity; SP, specificity; AOPP, advanced oxidation protein products.

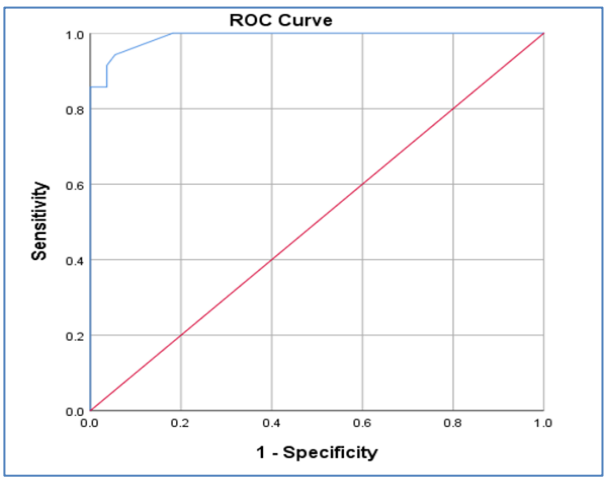

Figure 4. ROC analysis of plasma AOPP levels for DR diagnosis. The ROC curve was drawn with the data of the studied groups. Each point on this curve represents the sensitivity vs (1-specificity) "false-positive results", corresponding to the cut-off value. ROC, receiver operating-characteristic curve.

Discussion

Advanced oxidation protein products, an oxidative stress marker, were discovered for the first time in 1996 [31]; proteins were vulnerable to free radical and oxidant assaults, which resulted in structural and functional changes, resulting in endothelial dysfunction. AOPPs are thought to be both indicators for prooxidant-antioxidant imbalances and mediators of inflammation since they activate mononuclear phagocytes and act as cytokine-like mediators between neutrophils and monocytes[32]. AOPP has recently been identified as a key marker for detecting the effects of oxidative stress on proteins. This is due to their early occurrence, greater stability and dependability, and lengthy lifespan[33].

Oxidized albumin (aggregates or fragments), fibrinogen, and lipoproteins are the components of AOPP. The first step in this transformation is oxidative stress, specifically the myeloperoxidase/H2O2/halide system. Mild levels of AOPP production persist throughout life and increase with age. The AOPP is eliminated via the liver and spleen[33].

The present study found that when compared to non-diabetic controls, all diabetes patients had considerably higher levels of AOPP. Furthermore; AOPPs were significantly higher in DR patients compared to those without retinopathy which could be explained by increasing evidence in both experimental and clinical studies suggesting that there is a relationship between increasing glucose levels, oxidative stress, and diabetic retinopathy.

The findings of this study were consistent with those of other investigations. For example, Baskol et al. investigated the protein peroxidation role by measuring serum levels of advanced oxidation protein products (AOPP) in diabetic patients with or

without retinopathy (DR) and compared the results to non-diabetic control subjects; their findings revealed that AOPP levels were significantly higher in diabetic patients with or without DR when compared to controls[34].

Another study done by Pan et al. aimed to examine the relationship between oxidative stress and diabetic retinopathy, as well as to detect oxidative stress markers in type 2 diabetes mellitus patients with or without retinopathy; the results showed a statistically significant increase in AOPP levels in DR patients compared to NDR and non-diabetic controls [13].

Brzovi-ari et al. discovered the vitreous and blood oxidative stress indicators were linked in patients with type 2 diabetes who had acquired proliferative diabetic retinopathy, with serum AOPP levels considerably higher ($p<0.05$) in PDR patients than in NDED patients in non-diabetic eye disease (NDED) patients [35].

In the present study, there was a statistically significant reduction in AOPP levels in NDR patients taking DPP-4 inhibitors compared to those taking SU. On the other hand, there were no significant differences in AOPP levels among the DR group taking either DPP-1inhibitors or SU.

Dipeptidyl peptidese-4 inhibitors have been designed to prevent incretins (such as GIP and GLP-1) from being broken down, hence extending their effect. These incretins may be able to decrease the generation of reactive oxygen species (ROS) as well as plasminogen activator inhibitor -1 (PAI-1) Through the cAMP pathway[36].

Chung et al., who reviewed the medical records of patients with type 2 diabetes and diabetic retinopathy and investigated the effects of DPP-4 inhibitors on the progression of diabetic retinopathy in patients with type 2 diabetes based on the diabetic retinopathy severity scale, found that DPP-4 inhibitors slowed the progression of diabetic retinopathy in patients with type 2 diabetes. Independent of glycemic control, treatment with DPP-4 inhibitors significantly reduced the progression of diabetic retinopathy in patients when compared to treatment with other oral diabetes drugs [37]. Kolaczynski et al. investigated the efficacy of Vildagliptin versus Sulfonylurea on diabetic retinopathy in their retrospective cohort study utilizing a large sample from the German electronic medical record database. In this clinical scenario, treatment with Vildagliptin was linked with a considerably reduced incidence of retinopathy than treatment with the sulfonylurea group [38].

Clinical research on the effects of DPP-4 inhibitors on diabetic retinopathy is currently scarce. According to existing research, using this class in diabetic patients has been reported to improve vascular homeostasis and maybe restore early diabetic retinopathy-related hemodynamic anomalies[39].

Depending on the ideal “cut-off” score of AOPP obtained from the ROC curve, we attempted to quantify the prospect of circulating AOPP levels to serve as a biomarker for DR. The results showed that AOPP had a 99% probability of correctly distinguishing DR samples from control samples, with a sensitivity of 100% and a specificity of 82%. These results suggest that the measurement of plasma AOPP levels has the potential to be a biomarker for DR.

This study had some limitations. First, because this is a case-control study, further longitudinal clinical investigations are needed to evaluate whether greater circulating AOPP levels make people more prone to DR or diabetic vascular problems, or whether assessing AOPP levels can help with early diagnosis and prognosis. Second, there were a small number of participants thus, a considerably more long-term, large-scale study must be conducted. Third, lifestyle elements such as diet and exercise were not taken into account. Fourth, the majority of clinical trials of DPP-4 inhibitors as monotherapy or in combination with metformin have lasted 26 weeks. As a result, it's unclear whether a full therapeutic response has been obtained. Finally, we did not investigate whether AOPP levels in the vitreous, are connected to circulating levels.

Conclusion

Increased protein oxidation (reflected by increased serum AOPP level) might be an important event in the pathogenesis of diabetic retinopathy and we can use it as a biomarker for the early detection of DR. In addition, the suppression of AOPP formation or the development of oxidative stress by effective glycemic control with the use of DPP-4 inhibitors can be regarded a potential target for therapeutic intervention in sight-threatening DR.

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None

Conflicts of Interest

The authors have no conflict of interests.

Ethics Statements

It was approved by the Ethical Committee of the College of Pharmacy/University of Baghdad before the start of the study

Author Contribution

The first author did the practical work and result analysis. The second author supervised the whole work. The third author helped with samples collection.

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