

Impact of gene expression of NFE2L2 on serum superoxide dismutase and hemeoxygenase-1 levels in patients with type 2 diabetes and retinopathy

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Abstract. Diabetic retinopathy (DR) is a serious consequence of diabetes that is caused by reactive oxygen species (ROS) generated under conditions of oxidative stress. The nuclear factor erythroid-2-related factor 2 (NFE2L2) plays a critical endogenous protective role against oxidative stress, as it controls the expression of superoxide dismutase (SOD) and heme oxygenase-1 (HO-1) genes. The aim of the present study was to determine the gene expression level of NFE2L2 and its association with SOD and HO-1 serum levels among patients with type 2 diabetes and retinopathy. The present study included 90 patients diagnosed with type 2 diabetes mellitus (48 females and 42 males), aged 40 to 80 years. The patients were divided into two groups as follows: Group A included 60 patients diagnosed with diabetic retinopathy (DR) [among them were 29 patients with non-proliferative DR (NPDR) and 31 patients with proliferative DR (PDR)] and group B included 30 diabetic patients without any evidence of DR (DWR), considered as the control group to be enrolled in the study. The results revealed significantly lower serum levels of SOD and HO-1 among the patients with PDR and NPDR, compared with the patients in the DWR group. However, no significant differences were noted between the PDR and NPDR groups as regards the SOD and HO-1 levels. Of note, the gene expression of NFE2L2 was 3-fold higher in the DR groups compared with the DWR group. The gene expression of NFE2L2 also exhibited a significant negative correlation with the serum SOD and HO-1 levels in the DR groups. Furthermore, the decline in SOD and HO-1 activity in the DR groups indicated the consumption of antioxidant capacity for detoxifying ROS due to uncontrolled hyperglycemia, thus, increasing the expression of NFE2L2 to counteract the oxidative stress in DR. On the whole, the present study demonstrates that NFE2L2 expression may be a good indicator of the progression of DR.

Introduction

Diabetes mellitus (DM) is a disorder characterized by high blood glucose levels and varying degrees of dysfunction in the metabolism of proteins, lipid, and carbohydrates (1). Over the past two decades, the understanding and management of DM, including its causes, spread, prevention and therapy, have been firmly established (2). Damage to several biological systems, such as the blood vessels, eyes, heart and nerves, leads to diabetes-related complications and is associated with an elevated risk of developing multiple illnesses (3). A metabolic disease that affects a large number of individuals over time, is type 2 DM (T2DM) (2). Diabetic retinopathy (DR) is one of the microvascular complications associated with this disease globally, that may potentially harm the eyes, particularly in individuals between the ages of 20 and 65, resulting in visual impairment or loss (4). When the inner blood-retinal barrier weakens due to microvascular occlusion, one of the most prevalent secondary microvascular complications of diabetes is DR (4). DR may be classified into two forms as follows: Proliferative DR (PDR) and non-proliferative DR (NPDR) (5). The loss of pericytes from retinal capillaries to generate acellular capillaries, an increase in vascular permeability and the breakdown of the inner endothelium blood retinal barrier are the hallmarks of NPDR, an early stage of DR. Usually, there are no symptoms. PDR is an advanced stage in which the retina forms new, delicate and twisted blood vessels. These may result in retinal detachment, vitreous hemorrhage and fibrovascular epiretinal membranes, all of which are factors associated with loss of vision (5).

Hyperglycemia causes changes in metabolic processes, leading to the development of DR. The symptoms of diabetes result from several interrelated processes in its intricate pathophysiology, which include the generation of advanced glycation end products, the stimulation of the polyol pathway and protein kinase C, and the stimulation of the hexosamine pathway. Reactive oxygen species (ROS) activate and disrupt these pathways, leading to damage in the mitochondria and to an elevated death rate in capillary cells (6).

The nuclear factor erythroid-2-related factor 2 (NFE2L2; also known as Nrf2) expertly regulates redox homeostasis. This transcription factor belongs to the basic leucine zipper subfamily. Under normal conditions, Nrf2 is usually bound to its negative

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regulator, Keap1. However, when stress levels increase, this bond weakens and Nrf2 moves to the nucleus. In the nucleus, Nrf2 attaches to the antioxidant responsive element (ARE) and activates various genes. This activation leads to a wide range of activities, including detoxification, antioxidant activity, cellular redox homeostasis, glutathione homeostasis and mitochondrial biogenesis. Some of the genes activated by Nrf2 include superoxide dismutase (SOD) and heme oxygenase-1 (HO-1) (7).

Extremely high blood pressure and blood glucose levels can easily harm the tiny blood vessels in the retina. NFE2L2 plays a protective role in the retina (8). The deterioration of the retinal pigment epithelium has been shown to occur in NFE2L2 knockout mice as they age, suggesting that a lack of NFE2L2 can cause retinal illness (9). Furthermore, during a shared time frame with angiogenesis, NFE2L2 protects the retina from hyperoxia-induced oxidative damage. Research using animals deficient in NFE2L2 has indicated that the protein protects the retina from damage caused by ischemia-reperfusion, indicating that the pharmacological induction of NFE2L2 may be a novel approach for the treatment of retinal illnesses, such as ischemia-reperfusion (10). The expression of NFE2L2 is increased in acute hyperglycemia and decreased in chronic hyperglycemia. The downregulation of NFE2L2 expression leads to microvascular changes that eventually lead to diabetes-related consequences (11). The expression of Nrf2 is regulated by interaction partners or post-translational modifications, which subsequently influence its stability and function (12).

The present study aimed to investigate the different forms of gene expression of NFE2L2 and their association with blood SOD and HO-1 levels in diabetic individuals with retinopathy.

Patients and methods

Study participants. A cohort of Iraqi individuals with T2DM diagnosed with the disease at least 5 years prior were included in the present observational case-control study. The participants included in the present study were patients from the Specialized Centre for Endocrinology and Diabetes and Ibn Al-Haitham Hospital of Ophthalmology in Baghdad, Iraq. The recruiting commenced in February, 2023 and was concluded in July, 2023. The research protocol was approved by the College of Pharmacy Scientific and Ethics Committee, University of Baghdad (REAFUBCP3112023A), Ibn Al Haitham Teaching Eye Hospital (EAC 6332 in February 6, 2023) and the Specialized Centre for Endocrinology and Diabetes (Registration no. 53664 on February 1, 2023). Moreover, a written informed consent was obtained from each participant. All participants were interviewed by the researchers and demographic data were obtained from them and recorded on a data collection sheet, including age, sex, the duration of disease, body weight and height (Table I).

A total of 102 participants initially participated in the study. Nevertheless, the blood samples from 12 patients were omitted from the study due to hemolysis. The remaining 90 patients were divided into the following groups: Group A consisted of 60 individuals who were diagnosed with T2DM and retinopathy. Their ages ranged from 40 to 80 years. Within this group, 29 patients had NPDR and 31 patients had PDR. Optical coherence tomography was used by an ophthalmologist to verify the presence and location of intra-retinal and

sub-retinal fluid, retinal hemorrhages and microaneurysms; the early treatment diabetic retinopathy study (ETDRS) criteria were used for the diagnosis of retinopathy (13). Group B consisted of 30 patients with T2DM without retinopathy (DWR), serving as the control group.

Inclusion and exclusion criteria. The inclusion criteria were as follows: Patients were selected to be previously diagnosed with T2DM according to the American Diabetic Association (ADA) diagnostic criteria (14). The age of the diabetic patients had to be between 40-80 years. The duration of DM in the patients need to be >5 years.

The following exclusion criteria were used: Patients with type 1, gestational DM, patients on insulin therapy, diabetic patients with cardiovascular, liver and renal diseases, acute bacterial and viral infection, autoimmune diseases and ocular diseases, diabetic patients using multivitamin supplements, and those with DR on anti-VEGF drugs were excluded from the study.

Specimen collection and handling. From each participant, 10 ml of venous blood were drawn by venipuncture. A total of 5 ml of the obtained blood sample was transferred into an EDTA blood collection tube (Ningbo Greetmed Medical Instruments Co., Ltd.) for HbA1c assay by modified enzymatic reagent for the *in vitro* determination of HbA1C in human blood; 250 μ l of the contents of the EDTA tube were transferred to a 750- μ l TRIzol Eppendorf tube (Shandong Leader Technology Co., Ltd.) and frozen at (-20°C) for RNA extraction and analysis. Following 30 min of coagulation, 5 ml of the remaining whole blood were transferred to a gel tube. The tube was then centrifuged at 1,008 x g for 10 min at room temperature to extract the serum. Some serum was utilized by the laboratory of the medical center (Specialized Centre for Endocrinology and Diabetes, Baghdad, Iraq) to determine fasting serum glucose (FSG) levels using an enzymatic colorimetric technique on the same day of sample collection. Aliquots of the remaining serum were stored in Eppendorf tubes and then frozen at -20°C until all samples were collected. Following this, SOD and HO-1 levels were measured using ELISA kits (MyBioSource; SOD kit cat. no. MBS005068 and HO-1 kit cat. no. MBS268886).

Analysis of gene expression. RNA was extracted from all samples using a pre-made solution, namely TRIzol® LS reagent (Guangzhou Dongsheng Biotech Co., Ltd.), following the manufacturer's protocol. The concentration and purity of the extracted RNA were assessed using a Nanodrop spectrophotometer (Thermo Fisher Scientific, Inc.) to determine the quality of the samples for later analysis using reverse transcription-quantitative PCR (RT-qPCR). By using an EasyScript® One-Step gDNA Removal and cDNA Synthesis SuperMix kit, total RNA were reverse transcribed to complementary DNA (cDNA) and stored for expression analysis. The NFE2L2 gene expression levels were determined using RT-qPCR. Alpha DNA Ltd. created and produced the primer sequences for the NFE2L2 gene (Table II), which were then freeze-dried and kept at a temperature of -20°C. The GAPDH housekeeping gene was used as an internal control to determine the Δ Ct value (Table II). To normalize the quantities

Table I. Demographic data for patients in the PDR, NPDR and DWR groups.

Characteristic	NPDR (n=29)	PDR (n=31)	DWR (n=30)	P-value
Age (years), mean ± SD	57.59±8.382 ^a	56.23±8.601 ^a	51.77±8.529 ^b	0.02
BMI, mean ± SD	29.9847±5.15348	28.0697±3.06455	29.4913±4.94645	0.2
Duration of diabetes (years), mean ± SD	13.48±5.865 ^a	13.23±5.290 ^a	9.60 ±6.333 ^b	0.01
Sex, n (%)				0.1
Male	15 (51.7%)	17 (54.8%)	10 (33.3%)	
Female	14 (48.3%)	14 (45.2%)	20 (66.7%)	
Smoking status, n (%)				0.04
Yes	4 (13.8%)	8 (25.8%)	1 (3.3%)	
No	25 (86.2%)	23 (74.2%)	29 (96.7%)	

Means followed by different superscript letters (a, b) indicate statistically significant differences according to Duncan's multiple range comparisons; means followed by the same superscript letters indicate no significant difference. For normally distributed data, one-way analysis of variance (ANOVA) was utilized for continuous variables. When the latter test results were significant, a post-hoc analysis was performed using Duncan's multiple range test. Fisher's exact or Chi-squared tests were utilized to measure the group differences between categorical variables. PDR, proliferative diabetic retinopathy; NPDR, non-proliferative diabetic retinopathy; DWR, diabetic patients without retinopathy; BMI, body mass index.

Table II. Primers sequence for used for the analysis of the gene expression of GAPDH and NFE2L2.

Gene	Primer sequence (5'→3' direction)	Primer size (bp)	Product size (bp)	Temperature (°C)
NFE2L2				
Forward	ACCCTTGTCACCATCTCAGG	20	134	52
Reverse	AGCGGCTTGAATGTTTGTCT	20		
GAPDH				
Forward	GAAATCCCATCACCATCTTCCAGG	24	160	58
Reverse	GAGCCCCAGCCTTCTCCATG	20		

NFE2L2, nuclear factor erythroid-2-related factor 2.

Table III. Conditions used for the RT-qPCR analysis of GAPDH and NFE2L2 genes.

Step	Temperature	Time	No. of cycles
Initial denaturation	94°C	5 Min	1
Denaturation	94°C	10 Sec	
Annealing	52°C (NFE2L2) 58°C (GAPDH)	15 Sec	40
Extension	72°C	20 Sec	
Final extension	72°C	5 Min	1

NFE2L2, nuclear factor erythroid-2-related factor 2.

of mRNA that are produced by the NFE2L2 gene, the levels of the internal control gene GAPDH were amplified and analyzed. A smart cycler real-time PCR System was used. The components of TransStart® Top Green qPCR Super Mix kits TransGen Biotech Co., Ltd. were used to measure the threshold cycle (Cq), which allowed for the determination of the fold change and the levels of gene expression, as shown in Table III.

Calculation of gene expression. The determination of fold differences in the quantitative expression of mature RNAs was accomplished using the relative cycle threshold (2- $\Delta\Delta Cq$) methodology (15). The real-time cycler software was used in order to establish a threshold cycle (Cq) for each sample. The Cq values for the housekeeping gene, GAPDH, and the target gene, NFE2L2, being tested in the patients and controls were documented.

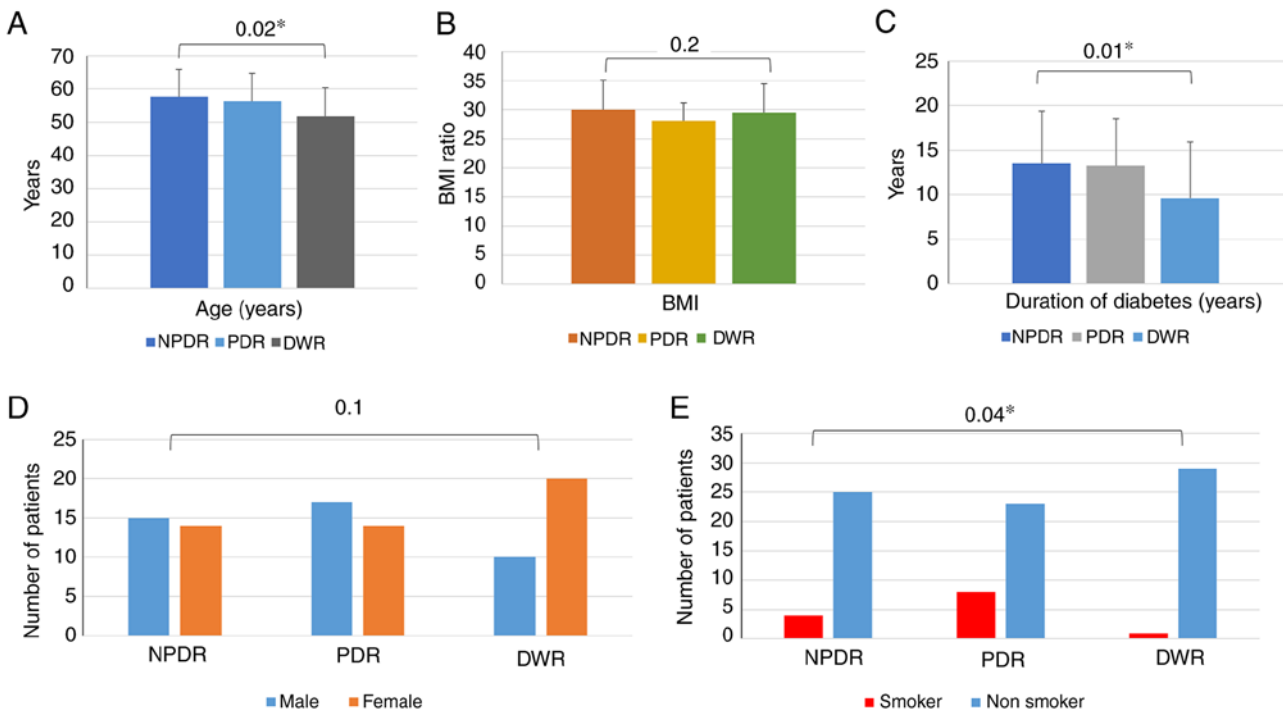


Figure 1. Demographic data of patients with type 2 diabetes with two stages of retinopathy (PDR and NPDR) and DWR in terms of (A) age, (B) BMI, (C) duration of diabetes, (D) sex and (E) smoking status. Fisher's exact or Chi-squared tests were utilized to measure the group differences between categorical variables. While ANOVA was used for continuous variables. * $P < 0.05$, denotes a statistically significant difference. PDR, proliferative diabetic retinopathy; NPDR, non-proliferative diabetic retinopathy; DWR, diabetic patients without retinopathy; BMI, body mass index.

Statistical analysis. Data were analyzed using SPSS version 25 software (IBM Corp.). The Shapiro-Wilk test was used to test the normality of the results. Continuous variables are expressed as the mean \pm SD, while numbers and frequencies were used for presenting categorical data. For normally distributed data, one-way analysis of variance (ANOVA) was utilized for more than two groups for continuous variables. When the latter test results were significant, a post-hoc analysis was performed with Duncan's multiple range test. Fisher's exact or Chi-squared tests were utilized to measure the group differences between categorical variables. Pearson's correlation analysis was performed and the correlation coefficient (R) was used to calculate the correlation between parameters. Receiver operation characteristic curve (ROC curve) analysis was also used. A P-value < 0.05 was considered to indicate a statistically significant difference.

Results

Demographical data of the two groups of patients with T2DM with retinopathy and in those without retinopathy. The differences between the PDR, NPDR and DWR groups were analyzed. Statistically significant differences were found for age, the duration of diabetes and smoking status between these groups, whereas the mean values of BMI and sex did not exhibit any significant differences between the groups (Fig. 1).

Biochemical characteristics of patients with DM with and without retinopathy. Although the PDR group exhibited elevated levels of FSG compared with the NPDR and DWR groups, there

were no significant differences among the three groups. The results of the analysis of HbA1c revealed significantly higher levels in the PDR group than in the NPDR and DWR groups. Conversely, the HbA1c levels exhibited no significant difference between the NPDR and DWR groups (Table IV).

In addition, significantly lower serum levels of SOD and HO-1 were observed in PDR and NPDR groups, compared with the DWR group. However, there were no significant differences between the PDR and NPDR groups as regards the SOD and HO-1 levels (Table IV).

Comparison of gene expression of NFE2L2 among the different groups. The amplification plots of GAPDH and NFE2L2 mRNA expression were determined as a Cq value (Fig. 2). A lower Cq value indicated the presence of larger copies of the target, whereas a higher Cq value indicated the presence of smaller copies. As regards gene expression, high Cq values indicate low expression, and low Cq values indicates a high expression (Fig. 2).

The mean Cq value of GAPDH, the housekeeping gene used in the present study, and the mean Cq value of the NFE2L2 gene are presented in Table V. The gene expression of NFE2L2 was 3-fold higher in the DR groups than in the DWR group (Table V). In addition, a significant increase in the folds of gene expression was observed in the DR groups compared with the DWR group (Fig. 3).

Analysis of NFE2L2 gene expression using a ROC curve. The validity of the NFE2L2 fold of expression as a marker for the diagnosis of retinopathy was assessed using ROC curves. The results revealed that the fold of gene expression was a good indicator for retinopathy in patients with T2DM,

Table IV. Biochemical characteristics of diabetic patients with two stages of retinopathy and without retinopathy.

Marker	PDR (n=31), mean ± SD	NPDR (n=29), mean ± SD	DWR (n=30), mean ± SD	P-value
FSG mg/dl	221.61±71.307	193.07±72.896	204.90±72.844	0.3
HbA1C	9.69±1.724 ^a	8.23±1.733 ^b	8.361.736 ^b	0.002
HO-1 (ng/ml)	4.295±0.609 ^b	4.259±0.656 ^b	7.697±0.921 ^a	0.0001
SOD (U/ml)	70.799±20.313 ^b	68.271±22.740 ^b	246.013±50.619 ^a	0.0001

Means that are accompanied by distinct letters exhibit significant differences as per ANOVA with Duncan's multiple range comparisons. Conversely, means that are accompanied by the same letters do not exhibit significant differences. SD, standard deviation; PDR, proliferative diabetic retinopathy; NPDR, non-proliferative diabetic retinopathy; DWR, diabetic patients without retinopathy; HO-1, heme oxygenase-1; SOD, superoxide dismutase; FSG, fasting serum glucose; ng, nanogram; mg, milligram; dl, deciliters; ml, milliliter. P-values <0.01 indicate highly significant differences and P-values <0.001 very highly significant differences.

Table V. Fold of NFE2L2 expression as per the 2^{-ΔΔCq} method.

Groups	Mean Cq of NFE2L2	Mean Cq of GAPDH	ΔΔCq (mean Cq of NFE2L2)	2 ^{-ΔΔCq}	Experimental group/control group	Fold of gene expression
PDR (n=31)	25.97	13.98	11.99	0.000246	0.000246/0.000076	3.2
NDPR (n=29)	26.24	14.20	12.03	0.000239	0.000239/0.000076	3.1
DWR (n=30)	27	13.30	13.67	0.000076	0.000076/0.000076	1.00

NFE2L2, nuclear factor erythroid-2-related factor 2; NPDR, non-proliferative diabetic retinopathy; PDR, proliferative diabetic retinopathy; DWR, diabetes without retinopathy; Cq, cycle threshold.

Table VI. Receiver operating characteristic curve analysis of NFE2L2 fold of gene expression.

Parameter	AUC	Explanation	P-value	Best cut off	Sensitivity (%)	Specificity (%)
Fold	0.80	Very good	0.001	2.5928	70	87

NFE2L2, nuclear factor erythroid-2-related factor 2; AUC, area under the curve.

with an area under the ROC curve of 0.8, a sensitivity of 70% and a specificity of 87% (Fig. 4 and Table VI).

Correlation of fold for gene expression with the studied biomarkers. Correlation analysis revealed that NFE2L2 gene expression was significantly high, with a decrease in the of SOD and HO-1 serum levels, thus indicating a negative correlation between NFE2L2 gene expression, and SOD and HO-1 serum levels (Table VII).

Discussion

Previous research has demonstrated that the transcription factor NFE2L2 plays a crucial protective role against DR by regulating ARE-antioxidant genes (16,17). This suggests that NFE2L2 may be a promising therapeutic target for DR. The present study observed the increased expression of the NFE2L2 gene among patients with DR. In addition, its expression negatively correlated with the downstream antioxidant enzymes, SOD and HO-1. A number of studies analyzing the expression of the NFE2L2 gene have demonstrated similar results. The

study conducted by Sun *et al* (18) revealed the increased of expression Nrf2 in the retina of rats with DM, suggesting the beginning of an endogenous oxidative stress system in these animals. It was discovered that rat retinal tissues under DM conditions had an abnormally high oxidative stress marker, with considerably lower levels of SOD and such a diabetic status can further induce the upregulation of Nrf2 expression (18). In the study by Cao *et al* (19), retinal ganglion cells exhibited an increased apoptosis and ROS content under high glucose induction, suppressed cellular anti-oxidation indices such as SOD, an increased expression of NFE2L2, and a decreased expression of the negative regulatory protein for Nrf2, Keap1. In another study by Xu *et al* (10), retinal tissues from mice with DM exhibited a significantly increased Nrf2 expression and nuclear translocation, together with potentiated peroxidation products. Based on these findings, it appears that the antioxidation potency of the body is still lacking, with abnormally high ROS concentrations, even if the oxidative stress mechanism has been started.

HO-1, a crucial activator of NFE2L2/ARE-dependent signaling, has the ability to protect retinal neurons and vascular endothelial cells from damage caused by DR. HO-1

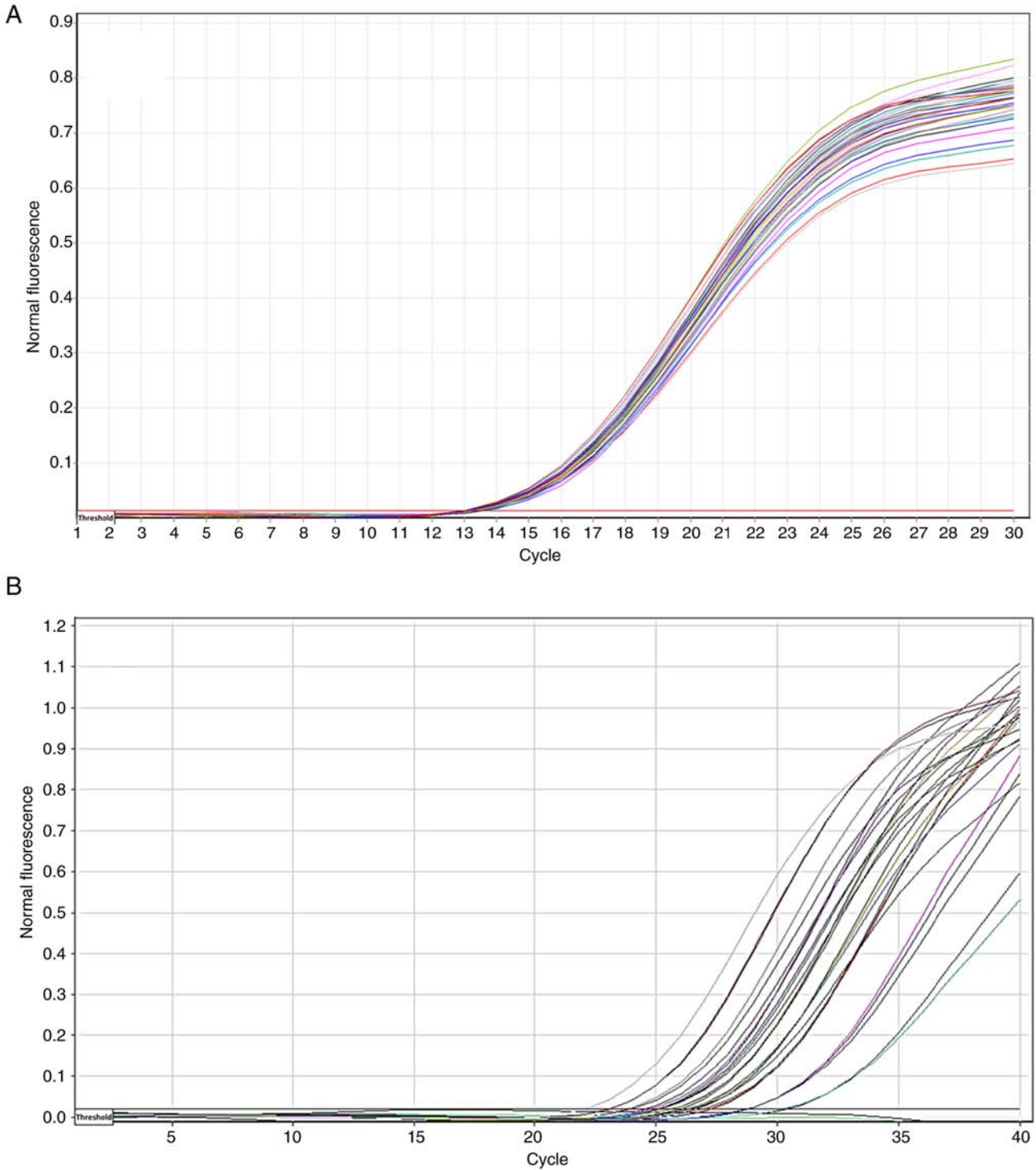


Figure 2. (A) The amplification plot of GAPDH expression using qPCR samples that covered all research groups. (B) The amplification plot of nuclear factor erythroid-2-related factor 2 mRNA expression for all research groups.

has marked anti-inflammatory, antioxidant and antiproliferative properties. Retinal pigment epithelial cells, microglia and neurons all contain HO-1 (20).

Compared with the diabetic group without retinopathy, the HO-1 level in the DR group was markedly lower, according to previous studies (21,22). These results corroborate the findings of the present study, demonstrating that HO-1 influences the development of DR, particularly the malfunction and death of retinal endothelial cells (21,22).

As a catalyst for the conversion of superoxide to hydrogen peroxide, SOD is responsible for its elimination. Hydrogen peroxide retains some active, but is less so than superoxide; it plays a crucial role in typical cellular signaling. It is possible to further break down hydrogen peroxide to water by using catalase or peroxidases. The retina of diabetic mice exhibits a reduced SOD activity (23). One way to prevent the development of acellular retinal capillaries in diabetes is to overexpress SOD in transgenic mice. As a result, it

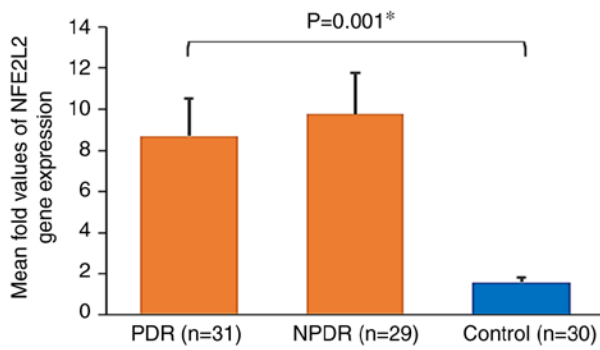


Figure 3. Mean fold values of nuclear factor erythroid-2-related factor 2 gene expression in the different groups, determined using the $2^{-\Delta\Delta Cq}$ method. * $P < 0.05$, denotes a statistically significant difference.

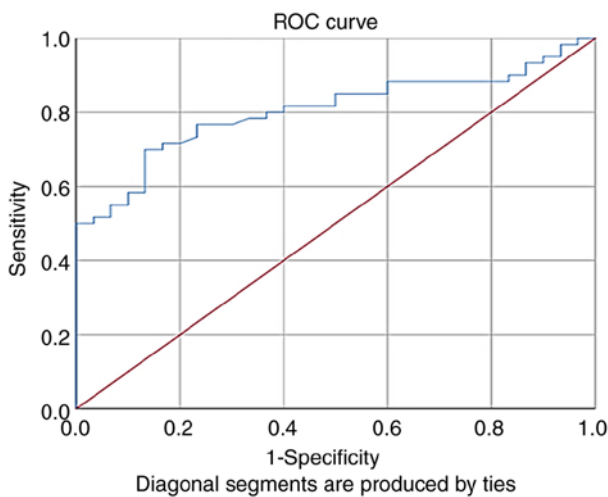


Figure 4. Receiver operating characteristic curve of nuclear factor erythroid-2-related factor 2 fold of gene expression.

keeps electron transport chain complex III active, inhibits the increase in retinal superoxide caused by diabetes, and prevents the mitochondria from becoming more permeable. This suggests that the restoration of SOD activity exerts a protective effect (23).

Consistent with previous studies (24,25), the present study demonstrated that high HbA1c levels increased the risk of retinopathy (in the PDR and NPDR groups) and that stringent blood glucose control lowers both the risk and severity of retinopathy.

In the case of diabetic microvascular issues, glycemic control is crucial. There is a 37% decrease in the risk of developing microvascular complications for every 1% decrease in the revised mean of HbA1c (26).

Consistent with the findings of the present study, Hou *et al* (27) demonstrated that hyperglycemia induces the presence of ROS, such as superoxide and hydrogen peroxide, in the body and is caused by the auto-oxidation of glucose, lipid peroxidation and protein glycation. This leads to a decrease in SOD activity and an increase in malondialdehyde levels in diabetic individuals with retinopathy compared to those without retinopathy (27). Diabetic individuals with retinopathy exhibit a significant decrease

Table VII. Pearson's correlation analysis between serum biomarker levels included in the present study and fold change.

Parameter	HO-1	SOD
Fold change		
R value	-0.357	-0.364
P-value	0.001	0.0001

HO-1, heme oxygenase-1; SOD, superoxide dismutase.

in SOD activity, suggesting a deficiency in antioxidants. This deficiency directly leads to the production of ROS (28). The function and activity of NFE2L2 may be hindered by a number of factors, which lead to an increased expression of the NRF2L2 gene without an increased ARE-target gene expression (SOD and HO-1). First, the transcriptional regulation in which the NFE2L2 promoter contains a binding site for NF- κ B, allows it to be induced by inflammatory stimuli. A high basal activity of NFE2L2 has been attributed to the constitutive NF- κ B-mediated upregulation of the NFE2L2 gene. Second, post-transcriptional regulation also plays a role: MicroRNAs are endogenous single-stranded, non-coding RNAs with an average of 22 nucleotides in length that repress gene expression by sequence-specific binding with mRNA molecules and subsequent inhibition of protein translation and destabilization of mRNA. Third, as regards the regulation of the Nrf2 transcriptional activation of its target genes, gene transcription profiles have revealed that not all genes in the vicinity of NFE2LE are transcriptionally regulated by NFE2L2 binding. The regulation of NFE2L2 activity is not limited to the control of its abundance, but can also be modulated by the availability of its binding partners. Fourth, the as regards the regulation of Nrf2 protein stability, NFE2L2 possesses seven conserved NRF2-ECH homology (Neh) domains with different functions to control NFE2L2 transcriptional activity. Neh6 domain contains two redox-independent degrons that bind to E3 ubiquitin ligase β -transducin repeat-containing protein, which mediates NFE2L2 degradation in oxidatively stressed cells. The Neh7 domain mediates interaction with retinoic X receptor alpha, which represses NFE2L2 activity. These domains modulate NFE2L2 stability and transcriptional activation of its target genes at multiple levels, including transcriptional and post-transcriptional and post-translational regulation in response to various insults. Thus, the aforementioned possible factor may alter the activity of NFE2L2 at the levels of transcription, translation, post-translational modifications, nuclear translocation, and binding to the promoters of regulated genes (29).

The present study had certain limitations which should be mentioned. One was that it only included two diabetic endocrine centers in the city of Baghdad. Furthermore, the measurement of NFE2L2 in vitreous fluid was not possible in the present study. In addition, the present study was not able to accommodate a sufficient amount of time for follow-up between pre- and post-treatment groups of diabetic retinopathy patients owing to capacity constraints. However, one

of the primary limitations of the present study was the small sample size. The limited number of participants may reduce the generalizability of the results, making it difficult to apply the findings to a broader population. The limited financing has restricted the authors' ability to obtain more kits to assess the aforementioned markers. Future studies with larger sample sizes are thus required to validate these findings and enhance their applicability.

In conclusion, the present study demonstrated that the serum SOD and HO-1 levels were significantly lower in the DR groups than in the DWR group. The expression of the NFE2L2 gene was increased by 3-fold in diabetic patients with retinopathy. The correlation analysis revealed a negative correlation between the fold change and serum SOD and HO-1 levels in the DR groups. The decline in SOD and HO-1 levels in the DR groups indicated the consumption of antioxidant capacity in detoxifying ROS due to uncontrolled hyperglycemia, thus increasing the expression of NFE2L2 to counteract the oxidative stress conditions in DR.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

SHM was involved in the conception and design of the study, in the literature search, clinical and data analysis, statistical analysis, and in the preparation and manuscript reviewing of the manuscript. SHA was involved in the conception and design of the study, in data analysis, and in the preparation and manuscript reviewing of the manuscript. SHM and SHA confirm the authenticity of all the raw data. Both authors have read and approved the final manuscript. SHM and SHA confirm the authenticity of all the raw data.

Ethics approval and consent to participate

Prior to sample collection, a statement of patient by written informed consent to participate in the study as specified in the Declaration of Helsinki was sought from each patient. Administrative ethical approval was granted by the University of Baghdad/College of Pharmacy Ethics Committee (registered under REAFUBCP3112023A), Ibn Al Haitham Teaching Eye Hospital (EAC 6332 in February 6, 2023) and the Specialized Centre for Endocrinology and Diabetes (Registration no. 53664 on February 1, 2023).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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