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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Objective

The aim of 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.

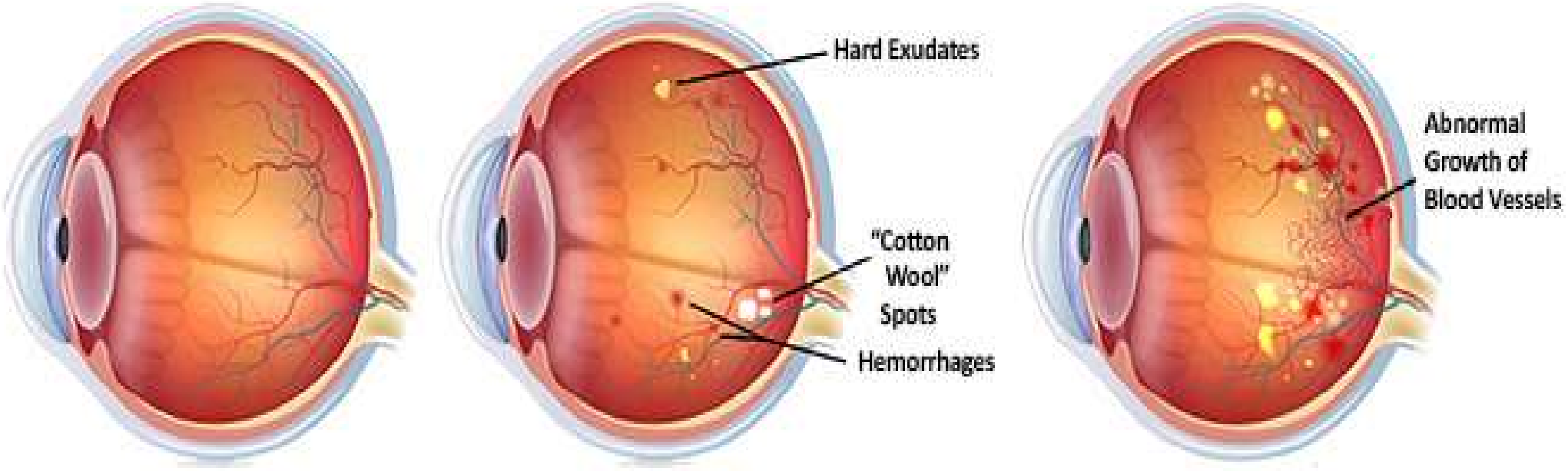
Diabetic Retinopathy

Diabetic retinopathy (DR) is a serious microvascular complication of diabetes mellitus that specifically affects the retina, leading to progressive vision impairment and potential blindness.

It is currently the leading cause of preventable blindness among adults aged 20 to 74 years worldwide, significantly impacting the quality of life and imposing a substantial economic burden on healthcare systems.

Approximately one-third of diabetic patients develop diabetic retinopathy, with prevalence increasing with duration of diabetes and level of glycaemic control. Globally, it affects over 100 million individuals, and the incidence continues to rise parallel to the increasing prevalence of diabetes.

Classification of Diabetic Retinopathy

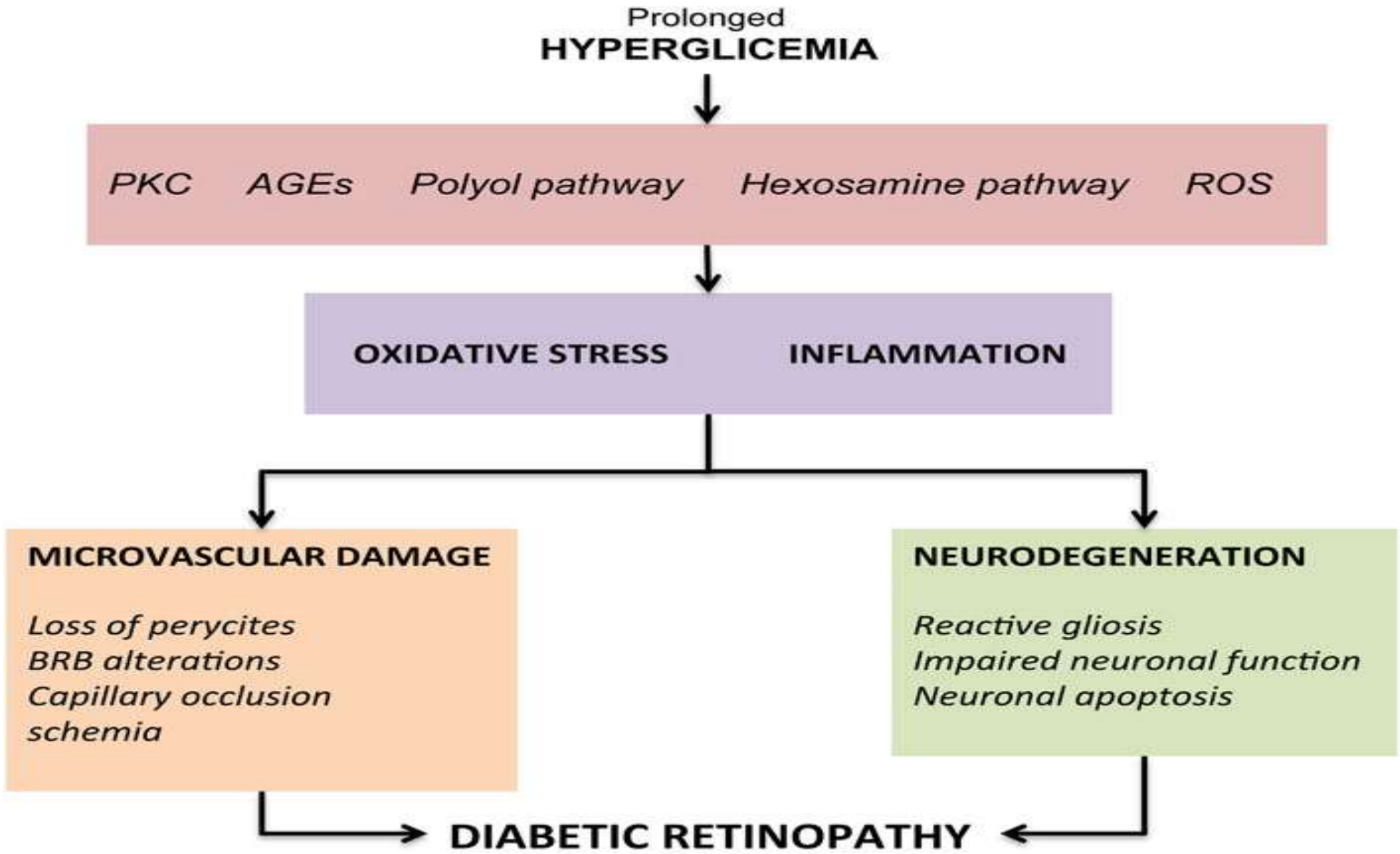


Normal eye

Nonproliferative diabetic retinopathy (NPDR)

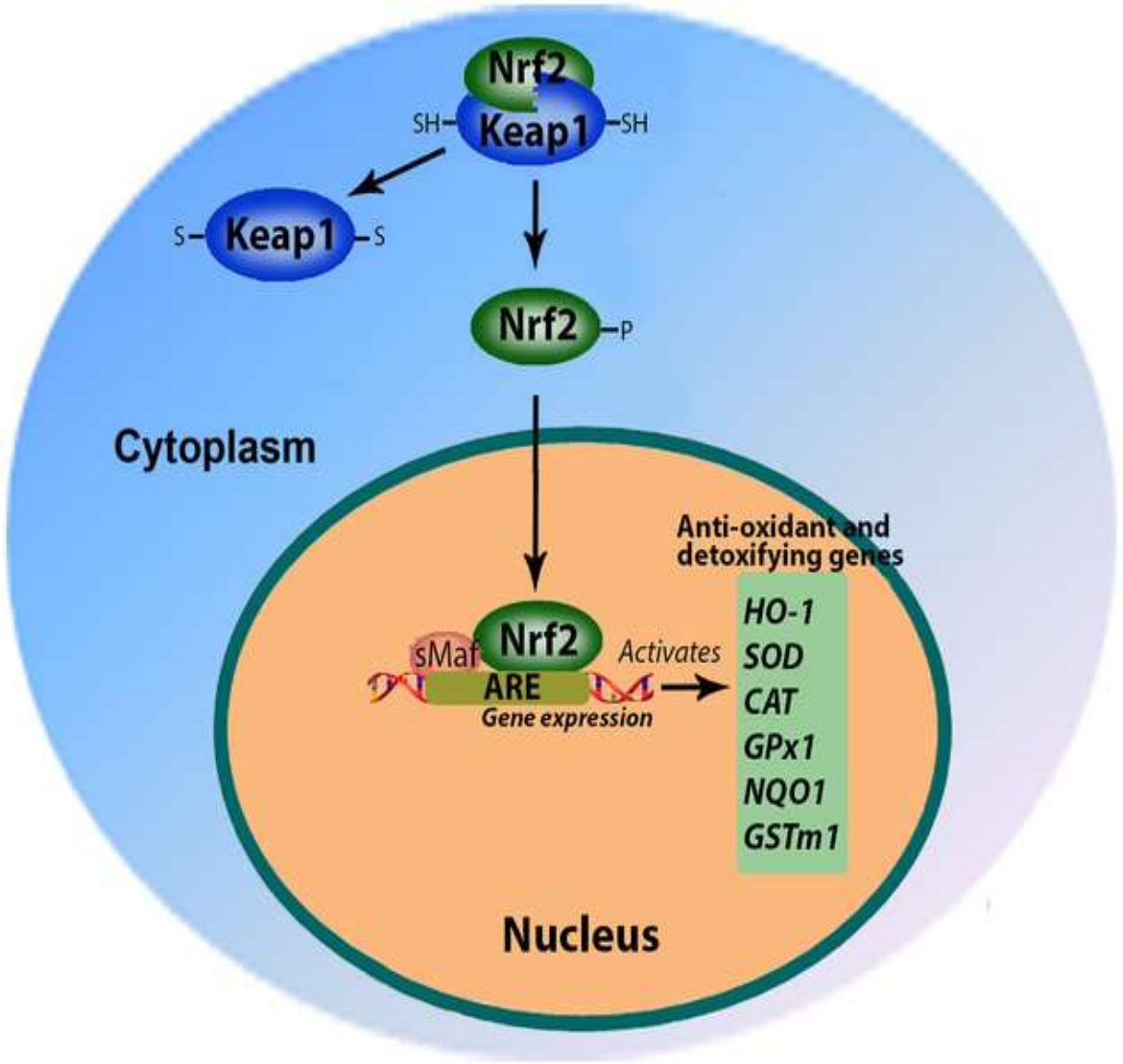
Proliferative diabetic retinopathy (PDR)

Pathophysiology



Nuclear Factor Erythroid 2-Related Factor 2

- Nuclear Factor Erythroid 2-Related Factor 2 (NFE2L2 or NRF2) is a critical transcription factor involved in cellular defense mechanisms, particularly against oxidative stress and inflammation. It regulates the expression of various antioxidant and cytoprotective genes.
- NFE2L2 primarily acts through the Antioxidant Response Element (ARE) pathway. Under basal conditions, NFE2L2 is bound by Kelch-like ECH-associated protein 1 (KEAP1), which targets it for degradation. Oxidative or electrophilic stress disrupts this binding, allowing NRF2 to translocate into the nucleus and activate ARE-driven gene transcription.



- The NFE2L2 plays a critical endogenous protective role against oxidative stress, as it controls the expression of superoxide dismutase (SOD) and hemeoxygenase-1 (HO-1) genes.
- The SOD activity was decreased in type 2 DM with complication due to auto-oxidation of glucose and more H_2O_2 production. SOD convert superoxide ion to hydrogen peroxide and formation of water by catalase enzyme .
- Heme oxygenase-1 (HO-1) is a NFE2L2-regulated gene that plays a critical role in the prevention of vascular inflammation. It is the inducible isoform of HO, responsible for the oxidative cleavage of heme groups leading to the generation of biliverdin, carbon monoxide, and release of ferrous iron.
- Retinal vascular endothelium may be protected by HO-1 and SOD in diabetic situations, This cytoprotective function of HO-1 and SOD seem to be transient, since it is impaired with extended exposure to high glucose

- 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 .
- The expression of Nrf2 is regulated by interaction partners or post-translational modifications, which subsequently influence its stability and function

Subjects and Methods

Study Design

- A cross-sectional study was carried out on a group of Iraqi type 2 diabetic patients, whom were diagnosed with type 2 DM at least 5 years ago.
- The patients enrolled in this study were selected from those attending *Ibn Al-Haitham Ophthalmology Teaching Hospital* and the *Specialized Center for Endocrinology and Diabetes* in Baghdad, Iraq.

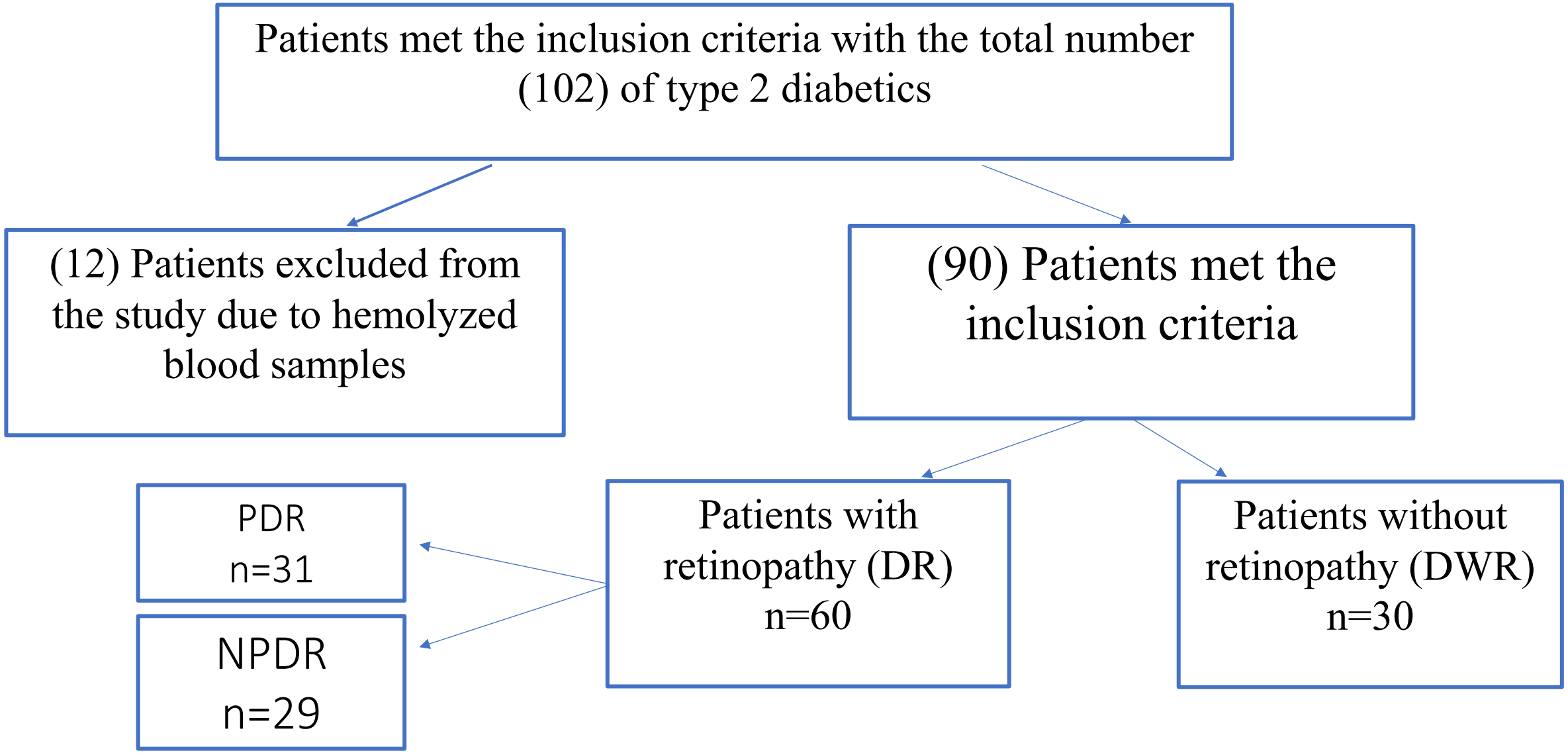
Inclusion criteria

1. Diabetic patients previously diagnosed with type 2 diabetes mellitus according to ADA diagnostic criteria ·
2. Age between (40-80) years
3. Duration of DM ≥ 5 years

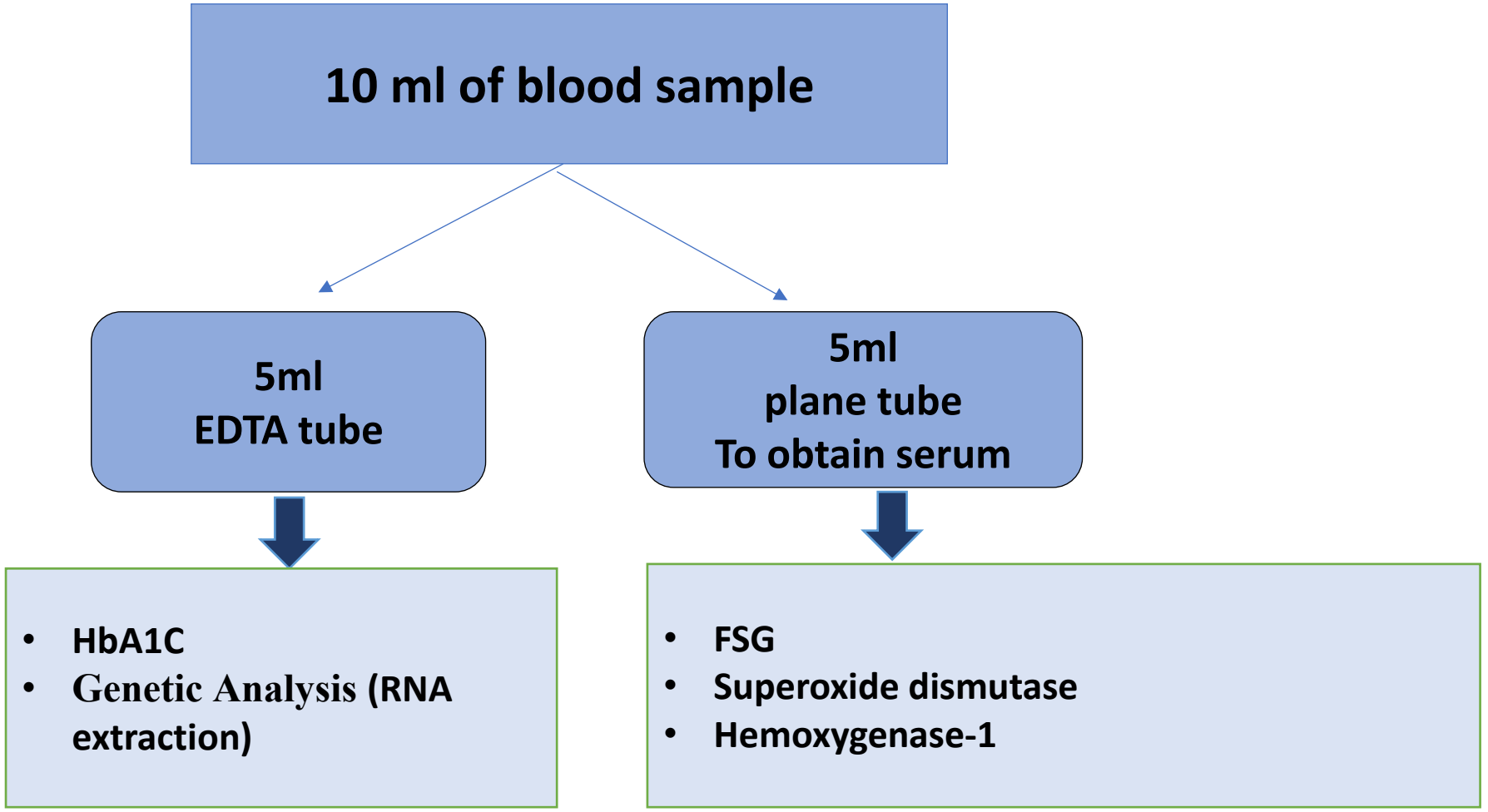
Exclusion criteria

1. Type 1,Gestational diabetes mellitus and Type 2 diabetic patients on insulin therapy
2. Diabetic patients with cardiovascular, liver and renal diseases, acute bacterial and viral infection, autoimmune diseases and ocular diseases
3. Diabetics using multivitamin supplements
4. Patients with Diabetic retinopathy on antiVEGF drugs

Distribution of Patients Included in The Study



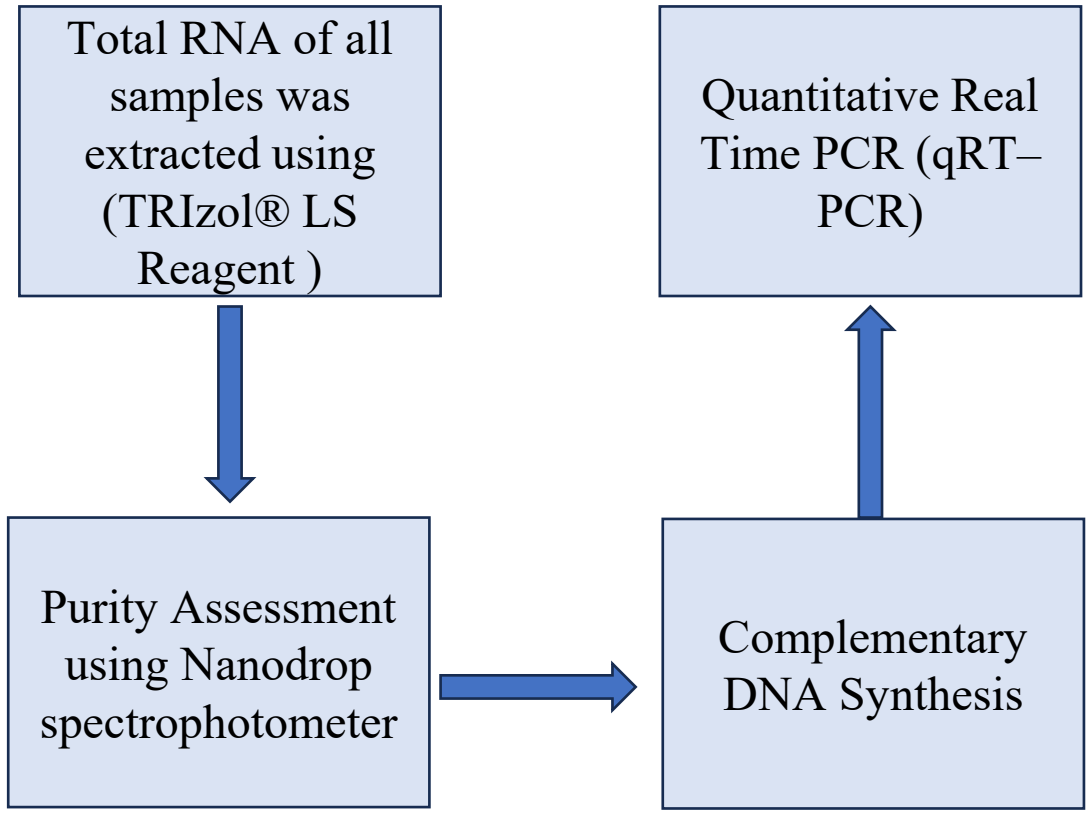
Specimen collection and handling



RNA extraction

Primer	Sequence (5′→3′ direction)	primer size bp	Product size bp	Ta °C
NFE2L2 (Gene Expression)				
Forward	ACCCTTGTCACCATCTCAGG	20	134	52
Reverse	AGCGGCTTGAATGTTTGTCT	20		
***GAPDH*- Glyceraldehyde 3-phosphate dehydrogenase**				
Forward	GAAATCCCATCACCATCTTCCAGG	24	160	58
Reverse	GAGCCCCAGCCTTCTCCATG	20		

RNA extraction



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	40
Annealing	52˚C *(NFE2L2)* 58˚C (GAPDH)	15 sec	
Extension	72˚C	20 sec	
Final extension	72˚C	5 min	1

Results and Discussion

Demographic Data for DR and DWR Groups

Characteristics		DR (N=60)	DWR (N=30)	P-value
Age (years) Mean ± SD		56.88±8.451	51.77±8.529	0.008**
BMI (Kg/m2) Mean ± SD		28.9952±4.27908	29.4913 ±4.94645	0.6
Duration of DM (years) Mean ± SD		13.35 ±5.529	9.60 ±6.333	0.005**
Gender N (%)	Male	32 (53.3%)	10 (33.3 %)	0.07
	Female	28 (46.7%)	20 (66.7 %)	
Smoking N (%)	Yes	12 (20.0%)	1 (3.3%)	0.03*
	No	48 (80.0%)	29 (96.7%)	

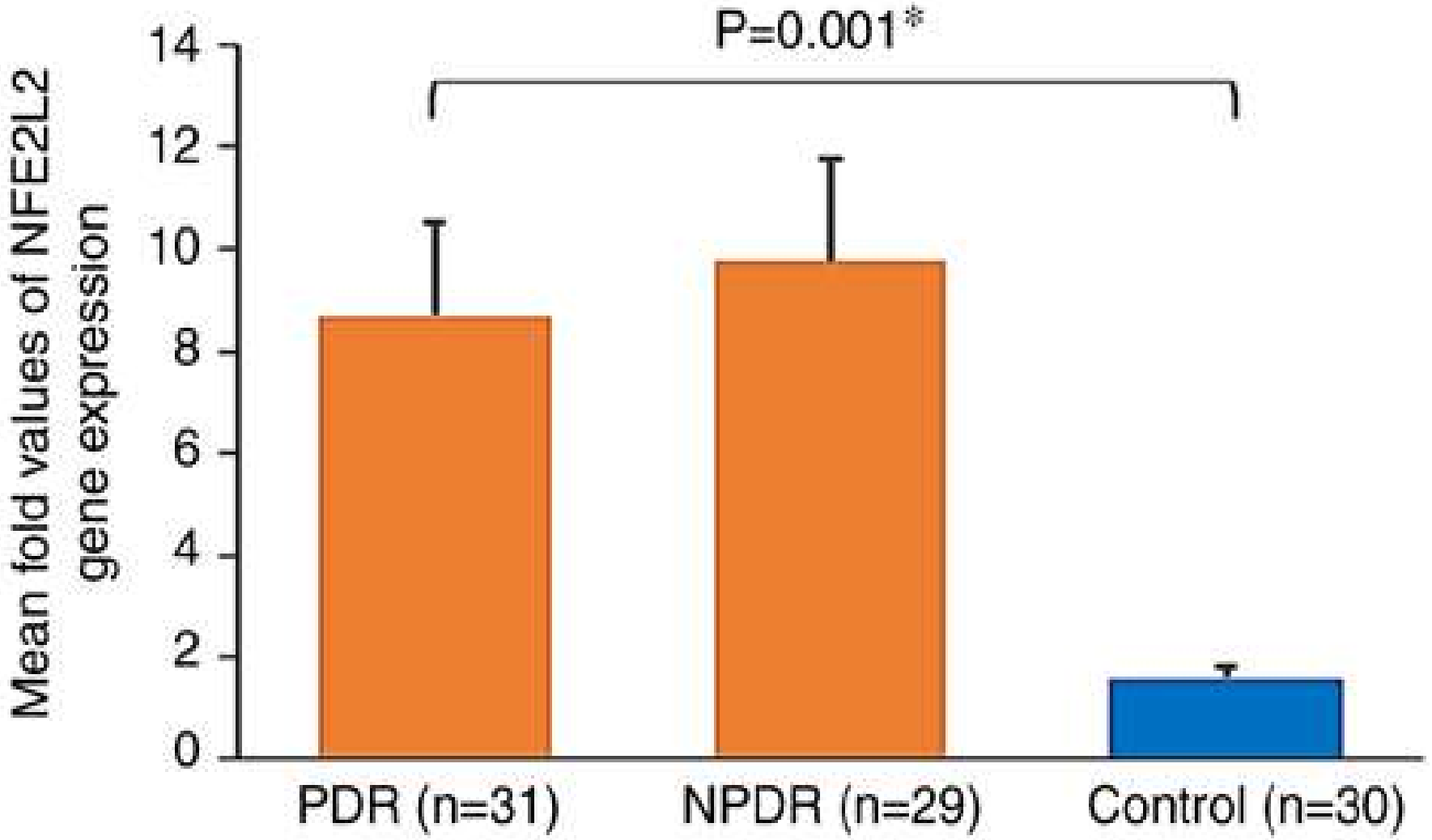
Biochemical characteristics of diabetic patients with two stages of retinopathy and without retinopathy

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**

Fold of NFE2L2 expression Depending on $2^{-\Delta CT}$ Method

Groups	Means Ct of NFE2L2	Means Ct of GAPDH	ΔCt (Means Ct of NFE2L2)	$2^{-\Delta Ct}$	Experime-ntal group/ Control group	Fold of gene expre-ssion
PDR	25.97	13.98	11.99	0.000246	0.000246/ 0.000076	3.2
NDPR	26.24	14.20	12.03	0.000239	0.000239/ 0.000076	3.1
DWR	27	13.30	13.67	0.000076	0.000076/ 0.000076	1.00

Mean folds of NFE2L2 Gene Expression of Different Groups by $2^{-\Delta\Delta Ct}$



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**

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-κB, allows it to be induced by inflammatory stimuli. A high basal activity of NFE2L2 has been attributed to the constitutive NF-κB-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.

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

Conclusions

1- The serum SOD and HO-1 levels were significantly lower in the DR groups than in the DWR group.

2- The expression of the NFE2L2 gene was increased by 3-fold in diabetic patients with retinopathy.

3- 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.


Thank You