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Diabetic retinopathy and its relation to serum brain-derived neurotrophic factor level

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Abstract

Diabetes mellitus (DM) is a metabolic disorder of the proteins, lipids, and carbohydrates, results in hyperglycemia. Abnormalities in the function of insulin on target cells, its release from beta cells, or both may contribute to DM. The purpose of this research was to assess the progression of diabetic retinopathy (DR) to the levels of serum brain-derived neurotrophic factor (BDNF), glycated hemoglobin (HbA1c), and biochemical parameters. The study included 44 normal control subjects, 44 diabetic participants, who were separated into four groups based on their diabetes status and the results of fundoscopic examination. A commercial enzyme-linked immunosorbent assay kit was used to measure the levels of BDNF in the serum. The analysis revealed that diabetics had significantly lower serum BDNF levels than non-diabetics ($p< 0.001$). Also, there was a significant reduction in BDNF levels with the development of proliferative diabetic retinopathy in comparison with diabetics without DR ($p < 0.001$). In conclusion, serum BDNF levels decreased significantly in diabetics with and without DR compared to apparently healthy individuals, as well as with the progression of DR.

Keywords: BDNF, DM, Diabetic retinopathy, Best corrected visual acuity.

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Introduction

Diabetes mellitus (DM) is a group of metabolic disorders characterized by hyperglycemia caused by abnormalities in insulin secretion, insulin action, or both. DM causes long-term damage, dysfunction, and failure of various organs, including the eyes, kidneys, nerves, heart, and blood vessels.[1]

A prevalent and distinct micro vascular complication of DM that progresses over time is diabetic retinopathy (DR).[2] A group of professionals developed the International Clinical Disease Severity Scale for DR to simplify the classification of the disease[3]. The stages of retinopathy are no apparent retinopathy (nDR); mild/ moderate non-proliferative retinopathy (mNPDR); severe non-proliferative retinopathy (sNPDR); and proliferative diabetic retinopathy (PDR).[3] Without treatment, severe forms of DR, like diabetic macular edema (DME) and PDR, can lead to blindness.[2]

According to epidemiologic research, 1 in 3 people with DM have DR, and 1 in 10 has PDR or

DME.[4] According to population surveys, half of people with DM are still misdiagnosed and that many are ignorant of their risk of developing DR and other consequences.[5] When DR severity levels are correctly identified for each eye, it is possible to estimate the likelihood that DR will proceed and cause visual loss, which enables the selection of the best referral channels, check-in times, and treatment suggestions.[6]

Neurotrophins are a class of proteins that include brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), and neurotrophins NT-3 and NT-4/5. Neurotrophins promote both the survival and the resilience of neurons against damage. BDNF is a type of neurotrophin that protects neuronal tissue while also improving central nervous system (CNS) performance.[7] BDNF is expressed in peripheral organs, adipose tissues, and activated immune cells in addition to the CNS. It is required for neuronal survival and proliferation in healthy brains. It also affects inflammatory homeostasis in the injured CNS.[8] BDNF is essential for retinal ganglion cells survival and differentiation, encouraging the development of axons and dendrites in rejuvenated retinal ganglion cells.[9] This research study was carried out to determine the correlation of the progression of DR to the levels of BDNF as a DR marker, as well as to HbA1c levels and other biochemical parameters.

Subjects and Methods

The protocol of the study was reviewed and approved by the Ethics Committee of the Faculty of Medicine for Girls, Al-Azhar University, Cairo, Egypt (Approval number: 2022031250, dated March 2022). A written, informed consent was obtained from each participant before included in the study and after explaining any possible complications.

This case-control study included 88 participants of both genders. During the period between April 2022 and June 2022, patients were enrolled from the Ophthalmology Department of Al-Zahraa University Hospital's. The participants were split into two groups: group I of 44 diabetic patients and group II of 44 apparently healthy controls of matched age and gender.

This study enrolled adult patients of both sexes with type 2 diabetes mellitus (T2DM). According to the American Diabetes Association's (ADA) criteria. Patients were deemed to have diabetes if their hemoglobin A1c (HbA1c) level was higher than 6.5%, their two-hour plasma glucose level higher than 200 mg/dl (11.1 mmol/L), their random plasma glucose level higher than 200 mg/dl (11.1 mmol/L), or their fasting blood glucose (FBG) level higher than 126 mg/dl (7.0 mmol/L).[1]

The International Clinical DR Disease Severity Scale[3] was used to grade the severity of retinopathy. One eye was randomly selected. Diabetic patients were divided according to fundus examination into diabetic patients without diabetic retinopathy (nDR, n=11), diabetic patients with proliferative diabetic retinopathy (PDR, n=11), diabetic patients with mild/moderate non- proliferative diabetic retinopathy (mNPDR, n=11), and diabetic patients with severe non- proliferative diabetic retinopathy (sNPDR, n=11).

A complete ophthalmologic examination, fundus photography, optical coherence tomography, and/or fluorescein angiography were performed at the Ophthalmology Department, Al-Zahraa University Hospital's.

Patients with type 1 diabetes mellitus, a history of diabetic nephropathy, neoplastic disease, infectious disease, auto-immune disease, or an eye condition that hides retinal abnormalities were all excluded from the research.

All participants underwent the following investigations: complete clinical information, including age, gender, marital status, smoking history, disease duration and medications taken. Comprehensive medical and ophthalmological history, including any instances of intra-ocular surgery, trauma, or ocular disease.

Best corrected visual acuity (BCVA), Bio microscopic investigation with a slit-lamp, slit-lamp bio microscopy with a + 90 D non-contact lens and indirect ophthalmoscopy was used to

examine the fundus. An examination of the fundus was used to confirm whether DR was present or not.

A venous blood sample (6 ml) was drawn from each study subject and split as follows: an aliquot of 2 ml of blood was placed in a tube with EDTA as anticoagulant for complete blood count (CBC) and assessment of plasma HbA1c. A fully automated hematology analyzer was used to perform CBC (Sysmex XP, Kobe, Japan), according to the manufacturer's instructions. A fully automated chemistry analyzer (Cobas C 311, Germany) based on turbidimetric inhibition immunoassay with a reference range between 4 % and 5.6 % was used to measure HbA1c. For testing kidney and liver function, serum was isolated from 2 ml of blood. A fully automated chemical analyzer was used to perform kidney and liver function testing (Cobas C 311, Germany), according to the manufacturer's instructions. Finally, sera were separated from 2 ml blood and used to test for BDNF. Assessment of BDNF was performed using a commercial ELISA Kit (Cat. No. E1302Hu, Bioassay Technology Laboratory, China), according to the manufacturer's instructions.

Statistical analysis

Data were coded and entered using the statistical package for the Social Sciences (SPSS) version 26 (IBM Corp., Armonk, NY, USA). Data was summarized using mean and standard deviation for normally distributed quantitative variables or median and interquartile range for non-normally distributed quantitative variables and frequencies (number of cases) and relative frequencies (percentages) for categorical variables. Comparisons between groups were done using unpaired t test or analysis of variance (ANOVA) with multiple comparisons post hoc test in normally distributed quantitative variables while non-parametric Kruskal-Wallis test and Mann-Whitney test were used for non-normally distributed quantitative variables (Chan, 2003a). For comparing categorical data, Chi square ($\chi 2$) test was performed. Exact test was used instead when the expected frequency is less than 5 (Chan, 2003b). The receiver operating characteristic (ROC) curve analysis was performed to determine the diagnostic ability of BDNF to distinguish between DM patients and controls. A *p*-value less than 0.05 was considered statistically significant.

Results

There was no difference in gender ratios or ages between the studied groups (p = 0.224 and p = 0.235, respectively). The average ages of the diabetic and control groups were 58.07 ± 9.45 and 55.93 ± 7.18 years, respectively. The duration of DM in the nDR, PDR, mNPDR and sNPDR subgroups, were 10.8± 5.31, 21.18 ±9.14, 12.59± 6.98, and 16.50 ±9.95 years, respectively and the difference between the subgroups was statistically significant (p = 0.029). Descriptive data for DM patients (group I) are displayed in Table 1

Table 1. Descriptive data of the 44 Diabetic patients (Group I).

		Group I Diabetic patients	
		no.	%
DM treatment	Oral treatment	17	38.6%
	Insulin	21	47.7%
	Oral and Insulin treatment	6	13.6%
Associated systemic disease	YES	24	54.5%
	NO	20	45.5%
	Hypertension	22	50.0%
	Thyroid	1	2.3%
	Ischemic heart disease	1	2.3%
Diabetic Duration (years) mean±SD		15.27±8.75	

DM : diabetes mellitus; SD=Standard deviation.

The mean HbA1c levels in the nDR, PDR, mNPDR and sNPDR subgroups were 8.15±2, 8.30 ± 1.39, 8.65±2.09 and 8.85 ± 2.26, respectively. Nevertheless, there was no difference between the subgroups (p = 0.832). Table 2 displays a comparison of CBC and the studied biochemical parameters in DM patients (group I) and controls (group II).

Table 2. Comparison of blood cell counts and studied biochemical parameters among the study groups.

	Group I (Diabetic patients) (n=44)	Group II (Apparently healthy control) (n=44)	p value
WBCs $\times 10^9$/L (mean±SD)	7.66±2.55	7.44±2.50	NS
Hb (gm/dl) (mean±SD)	12.76±1.43	12.37±2.04	NS
Platelet ($\times 10^9$/L) (mean±SD)	288.75±84.64	266.52±73.68	NS
Serum ALT U/L (mean±SD)	12.66±8.47	20.30±12.45	< 0.001
Serum AST U/L (mean±SD)	22.82±12.99	22.11±9.21	NS
Serum Urea mg/dl (mean±SD)	32.61±12.81	28.84±13.71	NS
Serum Creatinine mg/dl (mean±SD)	0.83±0.30	0.75±0.31	NS
Glycated Hemoglobin A1c % (HbA1c) (mean±SD)	8.48±1.91	------	------

P > 0.05 is not significant (NS). SD=Standard deviation ; CBC; complete blood count: WBCs=White blood cells ; Hb=Hemoglobin ; ALT= Alanine Aminotransferase ; AST= Aspartate Aminotransferase.

The mean blood BDNF level in the diabetic group was 19.04±4.47 ng/ml, significantly reduced than in the control group, 32.24±6.36 ng/ml (p < 0.001). Additionally, the mean serum BDNF level in the nDR, PDR, mNPDR, and sNPDR subgroups was significantly lower in comparison to the control group (p<0.001 for all). Among the DR subgroups, the level of BDNF in the PDR subgroup was significantly lower than in the nDR subgroup (p = 0.004) (Figure 1).

The mean BCVA was significantly lower in the diabetic group than the control group (0.37±0.25 and 0.85±0.17, respectively, p< 0.001). In addition, the mean of BCVA level in the nDR, PDR, mNPDR and sNPDR subgroups, was considerably significantly reduced than in the control group (p=0.002, p<0.001, p<0.001 and p<0.001, respectively). The BCVA level was significantly reduced in the PDR subgroup compared to nDR and mNPDR subgroups (p=0.007, p=0.033, respectively) (Figure 2).

The BDNF and BCVA levels were significantly positively correlated in the diabetic patient group (r = 0.453, P = 0.002) (Figure 3). The BDNF level and duration of DM were significantly negatively correlated in the diabetic patient group (r = -0.332, P = 0.028) (Figure 4).

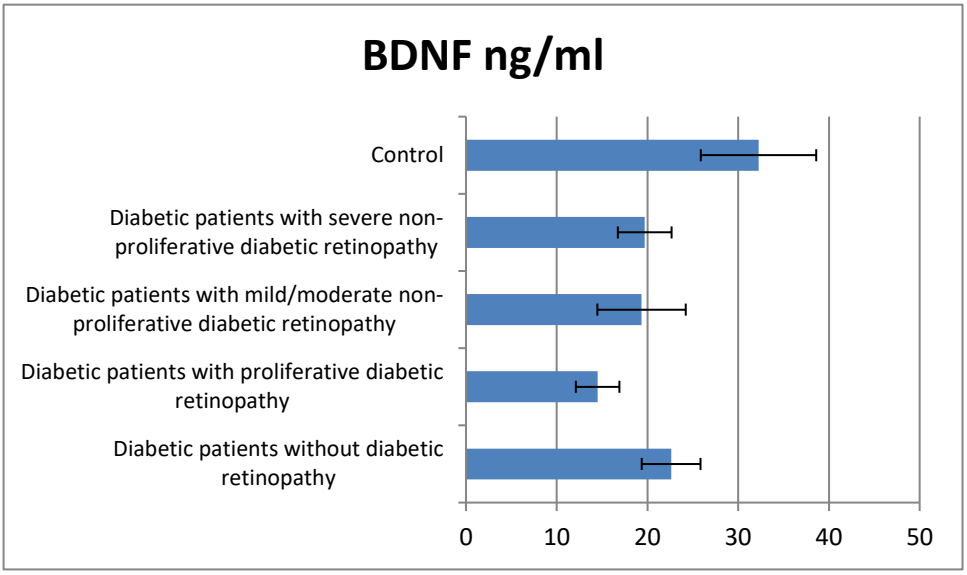

Figure 1. Comparative diagrams illustrating serum BDNF levels of diabetic patients' subgroups with apparently healthy control subjects.

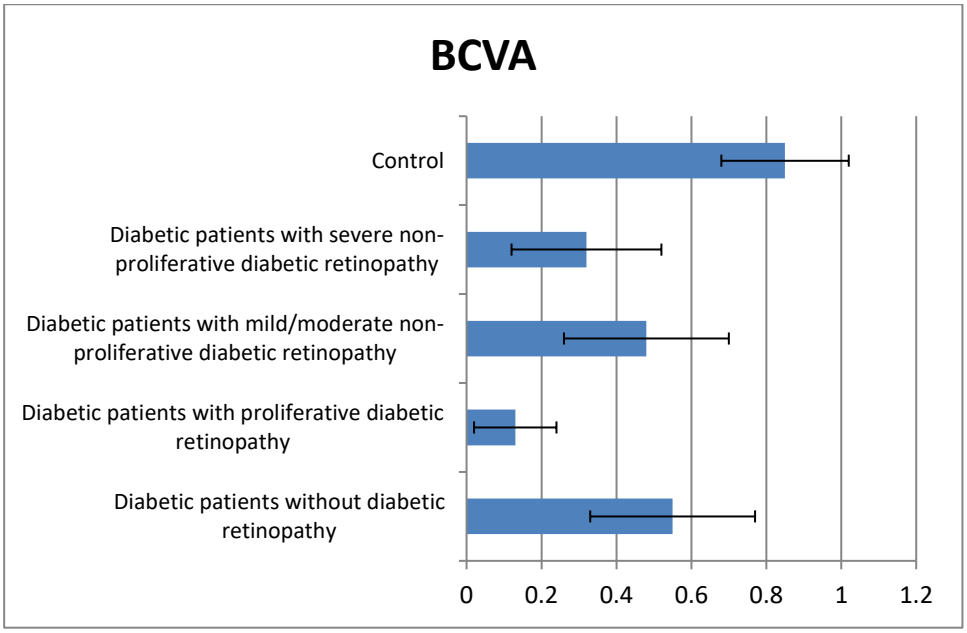

Figure 2. Comparative diagrams illustrating BCVA levels of diabetic patients' subgroups and apparently healthy control subjects.

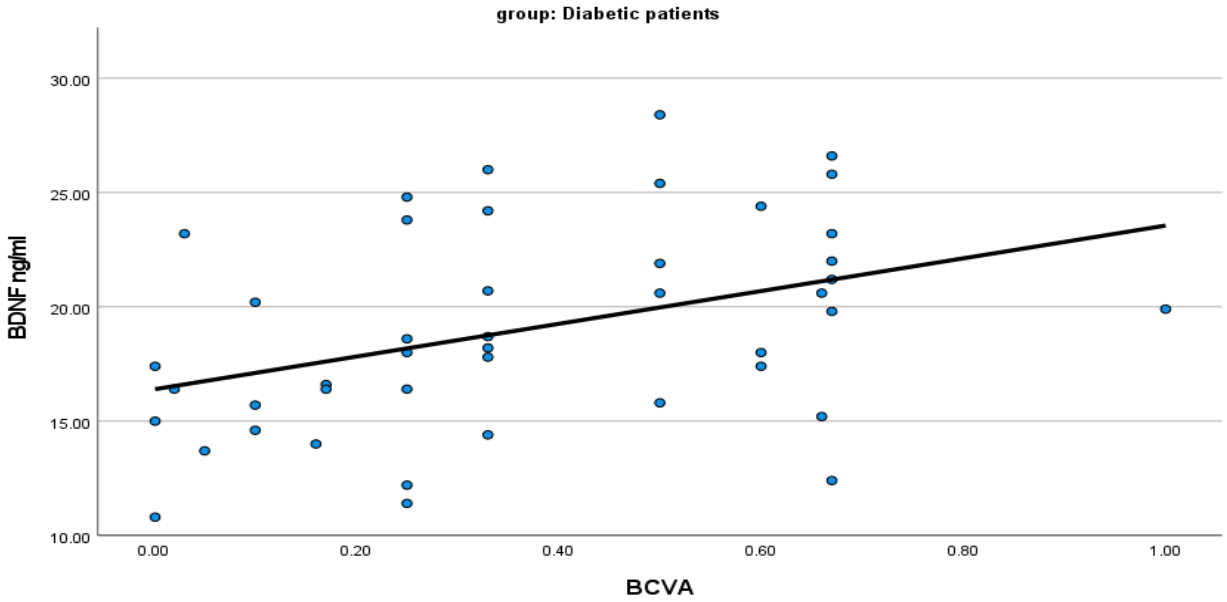

Figure 3. The correlation coefficient between BDNF and BCVA in the diabetic patient group (r = 0.453, *p* = 0.002).

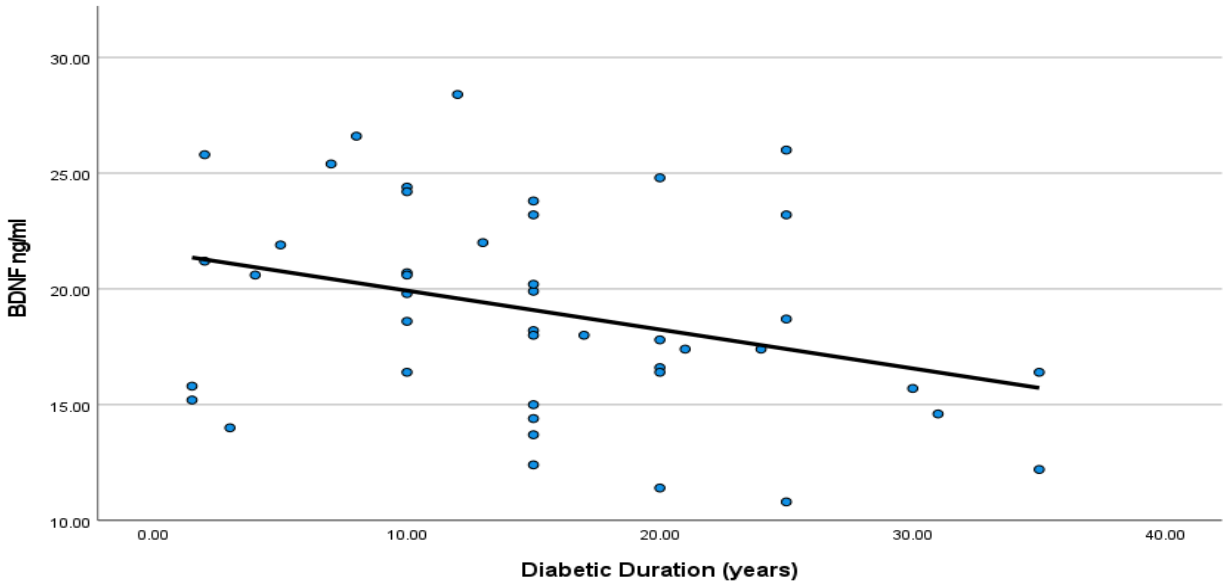

Figure 4. The correlation coefficient between BDNF and duration of DM in the diabetic patient group (r =-0.332, p = 0.028).

Table 3 displays the results of the ROC curve analysis to determine the ability of BDNF to distinguish between DM patients and controls. At a cutoff value of 26.2, the specificity and sensitivity of BDNF were 81.8% and 95.5%, respectively, and that of subgroups (nDR and PDR), (PDR and mNPDR), and (PDR and sNPDR) were (90.9% and 100%), (81.8% and 63.6%), (100% and 72.7%), respectively.

Table 3. The BDNF receiver operating characteristic curve's output data.

	Area Under the Curve	*p* value	95% Confidence Interval		Cut off	Sensitivity %	Specificity %
			Lower Bound	Upper Bound			
A	0.950	< 0.001	0.909	0.991	26.2	95.5	81.8
B	0.992	< 0.001	0.965	1.018	19	100	90.9
C	0.793	0.002	0.605	0.981	15.1	63.6	81.8
D	0.930	< 0.001	0.828	1.031	15.75	72.7	100

The receiver operating characteristic curve output data for: A) diabetic patients and controls; B) subgroups (nDR and PDR); C) subgroups (PDR and mNPDR); D) and subgroups (PDR and sNPDR). *$P \leq 0.05$ is significant.

Discussion

The fifth most common cause of preventable blindness and the main reason for blindness in people of working age is DR. Effective hyperglycemia management is critical for preventing DR, with long-term goals of maintaining target HbA1c levels and achieving near-normal glycaemia as soon as possible after diagnosis.[10]

In the current investigation, diabetics with varying levels of retinopathy were compared to an apparently healthy control group in terms of their serum levels of BDNF, CBC parameters, and biochemical tests. Since it was shown that serum BDNF levels decrease with age, a control group of apparently healthy age-matched adults was added.[11] DR progression is also influenced by hemodynamic changes such as anemia, as well as diabetes control,[12] therefore, in addition to measuring serum BDNF, CBC parameters and biochemical tests were also performed. According to our research, participants' histories of hypertension were significantly correlated with DR ($p< 0.001$). Prior research has repeatedly shown a substantial correlation between retinal microangiopathies and cardiovascular illnesses.[13] According to findings of a study by Ciccacci et al., 2013 polymorphism of the transcription factor 7-like 2 gene (TCF7L2), contributes to the development of both cardiac autonomic neuropathy and diabetic retinopathy.[14]

According to our study's findings, diabetes patients' serum BDNF levels were lower than those of the control group. Since DM is a neurodegenerative condition, low serum BDNF levels may suggest decreased CNS BDNF production.[15] Our results from BDNF tests in diabetic individuals agreed with earlier research which showed that diabetic individuals had lower levels than non-diabetic controls.[16] Contrary to our study founding, Suwa et al., 2006, reported that newly diagnosed T2DM subjects had higher levels of BDNF compared to non-diabetics. These conflicting findings could be the result of different sampling methods or resistance established via BDNF receptors, which could coexist with insulin.[17]

Additionally, in the present study, diabetics' serum BDNF levels were compared according to whether retinopathy was present or not. The subgroups of PDR versus nDR showed a statistically significant decrease in BDNF. The study by Liu et al., 2010, showed that DR patients' serum levels of BDNF were greater than those of non-diabetic controls, and they hypothesized a correlation between the elevated BDNF levels and the proportion of bone marrow-derived endothelial progenitor cells (EPCs) in patients with PDR.[18] In line with our results, Ola et al., 2013 reported lower levels of BDNF with the development of DR.[19] Their findings showed that BDNF may not be involved in bone marrow EPC recruitment as indicated by Liu et al., 2010.[18] More studies are required to determine the precise mechanism of action that would allow a decreased BDNF level to influence pathological retinal neovascularization.[19]

Our study revealed a significant positive connection between serum BDNF levels and BCVA. To the best of our knowledge, similar information regarding BDNF, however, has not

been previously published. In mice with chronic ocular hypertension, experimental findings suggested that BDNF overexpression can shield the retinal ganglion cells and lessen vision loss.[20] The duration of DM and serum BDNF levels were found to be negatively correlated. According to Liu et al., 2016 research, a prolonged DM duration is associated with decreased blood levels of BDNF.[21]

A study by Ola et al., 2013, evaluated the amount of BDNF in the serum and retina of non-diabetic and streptozotocin-induced diabetic rats. Based on the duration of diabetes, they separated diabetic rats into two groups (3 and 10 weeks). After 3 weeks of diabetes, the BDNF level in the serum was modestly but not significantly lower than in the age-matched control group. However, when compared to age-matched controls, the serum level of BDNF declined considerably in the 10-week group. They proposed that low BDNF levels in diabetes may result in early neurodegeneration and apoptosis in the diabetic retina that could subsequently result in DR's neuro-vascular damage.[19]

In conclusion, T2DM had significantly lower serum BDNF levels than apparently healthy control subjects. Long-term diabetic retinopathy patients with either non-proliferative or proliferative retinopathy had lower serum levels of BDNF than diabetics without retinopathy or apparently healthy controls. Additionally, we observed a positive connection between BDNF and BCVA. A decrease in serum BDNF can be investigated as a potential biomarker for visual acuity affection in diabetic patients.

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Author Contributions

SM is the corresponding author and has a major role in conception and design of study, acquisition of data, Analysis and interpretation of data, drafting and revising the manuscript. EA has a major role in conception and design of study, acquisition of data, Analysis and interpretation of data,drafting the manuscript. AM has a major role in collecting the data doing the ophthalmologic examination and fundus photography of the patients in the study and a major contributor in conception and design of study, acquisition of data, analysis and interpretation of data,drafting and revising the manuscript.The authors have read and approved the manuscript.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Ethical approval

The study protocol was reviewed and approved by the Research Ethics Committee of the Faculty of Medicine for Girls, Al-Azhar University, Cairo, Egypt. (Approval No. 2022031250, dated March 2022).

Informed consent

Informed written consent was obtained from all participants before included in the study.

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