Molecular Vision 2025; 31:276-280 <http://www.molvis.org/molvis/v31/276>
Received 22 March 2025 | Accepted 22 September 2025 | Published 24 September 2025

Serum pro-brain natriuretic peptide is a novel molecular biomarker of proliferative diabetic retinopathy

Prachi Sharma,[1] Sandeep Saxena,[1] Shivani Pandey,[2] Shashi Kumar Bhasker,[1] Apjit Kaur,[1] Abbas Ali Mahdi,[2] Levent Akduman[3]

[1]Department of Ophthalmology, King George's Medical University, Lucknow, India; [2]Department of Biochemistry, King George's Medical University, Lucknow, India; [3]Eye Institute, Saint Louis University, Saint Louis, MO

Purpose: Brain natriuretic peptide (BNP) is a polypeptide hormone that has diuretic, natriuretic, vasorelaxant, cardiac antihypertrophic, and antifibrotic properties. The significance of serum proBNP as a molecular biomarker of the severity of diabetic retinopathy (DR) was studied for the first time.

Methods: This cross-sectional study was conducted at a tertiary care center. The study comprised 66 consecutive cases of type 2 diabetes mellitus (DM-2). The guidelines of the American Diabetes Association were used to identify DM-2. There were 22 participants who had DM without retinopathy (No DR), 22 who had non-proliferative DR (NPDR), 22 who had proliferative DR (PDR), and 22 who served as controls. Complete ophthalmological examinations were performed on all participants. Serum proBNP levels were measured using electrochemiluminescence (ECL). Receiver operating characteristics (ROC) analysis was performed to study the diagnostic accuracy of serum proBNP and to analyze the area under the curve (AUC).

Results: The average levels of serum proBNP (pg/ml) were 14.90 ± 12.04 for the control group, 27.76 ± 12.19 for the No DR group, 278.40 ± 105.39 for the NPDR group, and 118.53 ± 44.62 for the PDR group. Analysis of variance (ANOVA) revealed a significant increase in serum pro-BNP from No DR to NPDR ($F = 97.985$, $p < 0.001$) and a decrease in serum proBNP in the NPDR to PDR groups ($F = 96.897$, $p < 0.001$). Regarding the AUC analysis coefficient, a cutoff value of 125.15 pg/ml for serum proBNP was found to be significantly associated with the occurrence of PDR.

Conclusions: Serum proBNP is a reliable molecular biomarker for the occurrence of PDR.

A modern epidemic, diabetes mellitus (DM) will be the seventh leading cause of death worldwide by 2030 [1]. According to a pooled study of 35 population-based studies, 93 million people globally are expected to have diabetic retinopathy (DR), of which a million (approximately 18%) have proliferative DR (PDR) [2].

Brain natriuretic peptide (BNP) is a hormone made up of 32 amino acids that is primarily generated by ventricular cardiomyocytes and is known to have diuretic, natriuretic, vasorelaxant, cardiac antihypertrophic, and antifibrotic properties [3]. The chromosome 1 region contains the gene that codes for BNP. Pre-proBNP, a precursor of BNP made up of 134 amino acids, is produced, and the 26-amino-acid N-terminal is then cut off to produce the 108-amino-acid BNP (proBNP). The pro-natriuretic peptide convertases further divide proBNP into the inactive NT-proBNP, which has 76 amino acids, and active BNP, which contains 32 amino acids. Both of these compounds can be found in the plasma. BNP has an estimated half-life of 20 min, whereas NT-proBNP has a half-life of 90–120 minutes. It is simpler to measure and monitor NT-proBNP because of its extended half-life [4]. Rollin et al. conducted a study on 10 cadaveric eyes and confirmed the expression of mRNA corresponding to all three NP receptor subtypes in the human retina [3]. For the first time we conducted a cross-sectional study to examine pro-BNP as a molecular biomarker of the severity of DR.

Correspondence to: Sandeep Saxena, Department of Ophthalmology, King George's Medical University, Lucknow, India; email: sandeepsaxena2020@yahoo.com

METHODS

Sixty-six type 2 DM (DM-2) patients aged 40 to 65 participated in the study, which was conducted at a tertiary care center. The study abided by both the Declaration of Helsinki and the Association for Research in Vision and Ophthalmology's policy on including human participants in research. The research was carried out after receiving clearance from the institutional ethics committee. All participants provided voluntary written informed consent. According to the American Diabetes Association's [5] recommendations, DM-2 was identified by a fasting plasma glucose level of ≥ 126 mg/dl, a 2 h oral glucose tolerance test result of ≥ 200 mg/dl, and a glycosylated hemoglobin (HbA1c) level of more than 6.5%. After using the Charan–Biswas formula to determine the appropriate sample size, the Early Treatment Diabetic Retinopathy Study (ETDRS) classification [6] of DR was used to divide 66 consecutive DM-2 patients into the following

groups: cases of DM without retinopathy (No DR, n = 22), non-proliferative DR (NPDR, n = 22), and PDR (n = 22). The classification was done following assessments by two experienced examiners. This study also included 22 healthy controls (n = 22) aged 40–65 years. Patients who refused to grant permission or who had other illnesses, including congestive heart failure, chronic renal disease, pulmonary disorder (lung disease with right heart failure or acute respiratory distress syndrome), and essential hypertension, which potentially elevate serum proBNP, were excluded.

Data collection: The patients' demographic data, including their sex and age, were recorded. The best-corrected visual acuity (BCVA) of each subject based on the logMAR scale was recorded. All individuals had undergone complete ophthalmological examinations with indirect ophthalmoscopy and slit-lamp biomicroscopy with + 90 diopters (D) assessments. A Zeiss Fundus Camera FF 450 Plus with an image size of 2588 × 1958 and a pixel width of 0.0054 (Carl Zeiss Meditec AG 07740, Jena, Germany) was used in fundus fluorescein angiography to examine the retina.

Laboratory analysis: A 5-ml-capacity metal-free plastic syringe and a 24-gauge stainless steel needle were used to obtain participant blood samples by aseptic venipuncture. Fasting and post prandial blood glucose concentration measurement was performed by automatic biochemical analyzer. Mass spectroscopy technique was used for HbA1c level measurement. The kinetic enzymatic approach was used to quantify serum urea [7]. Without deproteinization, the modified Jaffe technique was used to assess serum creatinine [8]. Serum proBNP levels were measured using the Roche Elecsys cobas e 411 (Roche Diagnostics Limited, Basel, Switzerland) based on electrochemiluminescence (ECL). The total duration of the assay was 18 min and the measuring range was 5–35,000 pg/ml.

Statistical analysis: The study's assessed outcomes included demographic data (sex and age), clinical data (BCVA of the poorer eye), and biochemical data (blood glucose fasting, serum proBNP, HbA1c, blood glucose PP, serum creatinine, and urea). Mean and standard deviation (SD) values were used to summarize the data. One-way analysis of variance (ANOVA) was used to evaluate the differences between the groups. After establishing normality with the Shapiro–Wilk test and confirming the homogeneity of variance across groups with the Levene test, the importance of the average variation between the groups was evaluated using the Newman–Keuls post-hoc test. Categorical (or discrete) group comparisons were made using the chi-squared test (χ^2). Pearson correlation analysis was carried out to examine the bond between the factors. To assess the efficacy of serum proBNP as a molecular biomarker of the severity of DR, receiver operating characteristic (ROC) curve analysis was performed. The area under the curve (AUC), specifically the ROC curve, was used to measure the accuracy of the test. The accuracy of the diagnostic test was rated using the conventional academic points system: fair = 0.7–0.8; good = 0.8–0.9; and outstanding = 0.90–1. SPSS software was used for the analyses (Windows version 21.0, Chicago, IL).

RESULTS

Table 1 presents the demographic data, laboratory parameters, and logMAR visual acuity for the control, No DR, NPDR, and PDR groups. ANOVA showed that the groups were in the same age bracket ($p = 0.961$). ANOVA also revealed comparable gender frequencies across the groups ($p = 0.117$). Therefore, all groups were comparable in terms of age and gender ($p > 0.05$).

PP and blood glucose F levels, HbA1c, serum creatinine and urea levels, and BCVA all increased linearly with the severity of DR. ANOVA revealed significant differences in levels of serum proBNP across the groups ($F = 96.897$, $p < 0.001$).

Participants' demographic, clinical, and biochemical data were correlated using the Pearson correlation method, and the results revealed a statistically significant relationship between blood proBNP levels and BCVA ($r = 0.24$, $p < 0.001$), blood glucose PP ($r = 0.36$, $p < 0.05$), blood glucose F ($r = 0.47$, $p < 0.001$), serum creatinine ($r = 0.36$, $p < 0.05$), HbA1c ($r = 0.36$, $p < 0.05$), and serum urea ($r = 0.29$, $p < 0.05$).

Figure 1 shows the distribution of serum proBNP levels (picogram per milliliter [pg/ml]) among the four study groups. Table 2 presents the results of ROC curve analysis, which revealed that serum proBNP is a good marker for discriminating between No DR and NPDR (AUC = 0.99, $p < 0.001$), No DR and PDR (AUC = 0.99, $p < 0.001$), and NPDR and PDR (AUC = 0.99, $p < 0.001$). A cutoff value of < 125.15 pg/ml discriminated the cases of NPDR and PDR with 97% sensitivity and 68.2% specificity.

DISCUSSION

The present study assessed serum proBNP's role as a novel molecular biomarker and its relationship with the severity of DR. Serum proBNP is an established biomarker used to determine a prognosis during heart failure after myocardial infarction [9]. Heart failure is strongly affected by natriuretic peptides (NPs). The atrium and ventricles primarily express and produce atrial natriuretic peptide (ANP) and BNP. The vasculature, bones, and the central nervous system produce

Table 1. The demographic data, biochemical and clinical indicators of cases (n=66) and controls (n=22).

Variables	Control	NoDR	NPDR	PDR	F/χ^2 value	p-value
Age (years)	55.18±5.18	54.82±5.47	54.23±6.78	54.77±6.11	0.097	0.961
Male	12 (54.5%)	11 (50.0%)	15 (68.2%)	18 (81.8%)	6.93	0.117
Female	10 (45.5%)	11 (50.0%)	7 (31.8%)	4 (18.2%)		
Hemoglobin (gm%)	12.68±0.71	12.31±1.00	11.04±0.61	11.43±0.57	22.590	<0.001
Fasting plasma glucose (mg/dl)	88.41±20.40	112.77±14.98	125.76±36.08	150.32±46.04	14.445	<0.001
2-h plasma glucose (mg/dl)	121.95±17.99	166.91±19.84	201.37±51.09	211.59±66.80	18.496	<0.001
HbA1c (%)	4.76±0.49	7.72±0.44	8.48±1.50	9.09±1.13	80.925	<0.001
Serum urea (mg/dl)	19.06±31.97	18.41±4.33	29.41±4.29	36.56±12.87	46.640	0.002
Serum creatinine(mg/dl)	0.83±0.17	0.94±0.22	1.13±0.22	1.66±0.39	27.422	<0.001
Serum Pro BNP (pg/ml)	14.90±12.04	27.76±12.19	278.40±105.39	118.53±44.62	96.897	<0.001
BCVA (logMAR; RE)	0.30±0.18	0.38±0.13	0.66±0.49	1.15±0.44	26.834	<0.001
BCVA (logMAR; LE)	0.25±0.15	0.43±0.14	0.57±0.39	1.18±0.54	28.717	<0.001

NoDR: no diabetic retinopathy ; NPDR: non proliferative diabetic retinopathy ; PDR: proliferative diabetic retinopathy ; gm: gram ; mg/dl: milligram/deciliter ; HbA1c: glycated hemoglobin ; pg/ml: picogram/milliliter ; RE: right eye and LE: left eye.

C-type natriuretic peptide (CNP) most often [10]. Cyclic guanosine monophosphate (cGMP) is increased by both natriuretic peptide receptor-A (NPR-A) and NPR-B, activating cGMP-dependent protein kinase (PKG). PKG also activates downstream effectors that are involved in cell proliferation, growth, inflammation, and apoptosis [11]. Studies testing the expression of NPs in rat retina confirmed the presence of receptors for NP in ganglion and muller cells [12]. Immunohistochemistry confirmed the presence of BNP in rat amacrine cells. The presence of NPR-B was observed by immunohistochemistry in cultured rat GABAergic amacrine cells. These findings established the neuromodulatory role of

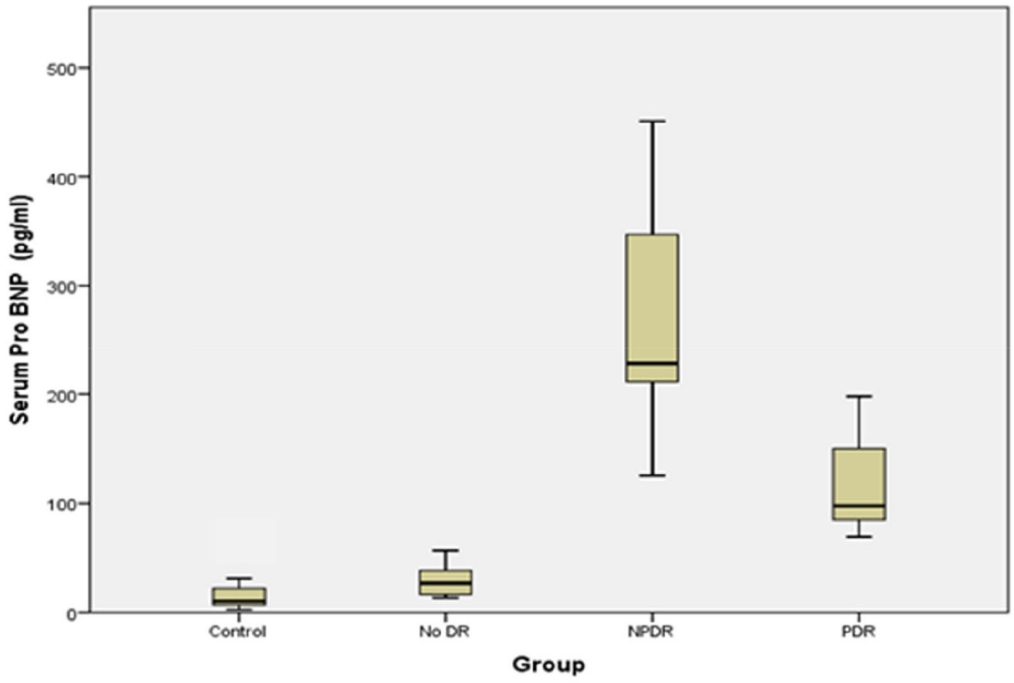

Figure 1. Box and whisker plot showing the median and interquartile range for the distribution of serum proBNP levels (picogram per milliliter [pg/ml]) in the four groups (control: n = 22; No DR: n = 22; NPDR: n = 22; PDR: n = 22).

Table 2. ROC curve analysis for serum pro BNP.

Test result variable(s)		Area	Standard error	Asymptotic 95% CI	
				Upper-bound	Lower-bound
Serum Pro BNP (pg/ml)	No DR to NPDR	0.99	0.01	1.000	1.000
	No DR to PDR	0.99	0.01	1.000	1.000
	NPDR to PDR	0.97	0.03	1.000	0.927

NPs [13]. However, the role of NPs in the severity of DR has not been studied.

The role of vascular endothelial growth factor (VEGF) in angiogenesis has been established, having been shown to lead to neovascularization in the retina [14]. NPs are unique in their actions as they regulate both the action and production of VEGF. NPs inhibit the activation of numerous key signaling molecules that are crucial for VEGF angiogenesis. These include the p38 MAP kinase family members extracellular signal-related kinase (ERK) and c-Jun N-terminal kinase (c-JNK) [15]. NP signaling also impedes transforming growth factor-β1 (TGF-β1)-induced pericyte loss [16]. The discussion above suggests that enhancing endogenous NP/NPR-A/cGMP signaling may open up new therapeutic avenues by revealing a new therapeutic target for treating retinopathies linked to neovascularization [15,16].

In this study, mean levels of serum BNP were discovered to increase from No DR to NPDR. On the contrary, the levels were found to significantly decrease from NPDR to PDR. This can be explained by the fact that BNP protects pericytes from apoptosis and decreases retinal vascularization by diminishing VEGF secretion via TGF-β1 and cyclic guanosine monophosphate signaling in pericytes and astrocytes [15,16]. With the occurrence of the proliferative stage, BNP levels go down, hence lowering the protective guard. In a study done on the murine retina, NPR-A/cGMP signaling was seen to exert local preventive properties through the proposed mechanism of the inhibition of stress-induced pericyte apoptosis and a decrease in the hypoxic induction of VEGF in astrocytes [13].

Serum proBNP was found to be an excellent molecular biomarker of the severity of DR. The cutoff value of serum proBNP for distinguishing between NPDR and PDR, according to our study, was 125.15 pg/ml. Hence, the evaluation of serum proBNP levels in cases of NPDR can predict the occurrence of PDR. Limitations of the present study include the small sample size and cross-sectional design, as causality could not be determined.

Conclusion: Serum proBNP is a novel molecular biomarker of the severity of DR.

REFERENCES

1. Mathers CD, Loncar D. Projections of global mortality and burden of disease from 2002 to 2030. PLoS Med 2006; 3:e442[PMID: 17132052].
2. Yau JW, Rogers SL, Kawasaki R, Lamoureux EL, Kowalski JW, Bek T, Chen SJ, Dekker JM, Fletcher A, Grauslund J, Haffner S, Hamman RF, Ikram MK, Kayama T, Klein BE, Klein R, Krishnaiah S, Mayurasakorn K, O'Hare JP, Orchard TJ, Porta M, Rema M, Roy MS, Sharma T, Shaw J, Taylor H, Tielsch JM, Varma R, Wang JJ, Wang N, West S, Xu L, Yasuda M, Zhang X, Mitchell P, Wong TY. Meta-Analysis for Eye Disease (META-EYE) Study Group. Global prevalence and major risk factors of diabetic retinopathy. Diabetes Care 2012; 35:556-64. [PMID: 22301125].
3. Rollín R, Mediero A, Roldán-Pallarés M, Fernández-Cruz A, Fernández-Durango R. Natriuretic peptide system in the human retina. Mol Vis 2004; 10:15-22. [PMID: 14737067].
4. Cao Z, Jia Y, Zhu B. BNP and NT-proBNP as Diagnostic Biomarkers for Cardiac Dysfunction in Both Clinical and Forensic Medicine. Int J Mol Sci 2019; 20:1820-[PMID: 31013779].
5. . American Diabetes Association. Standards of medical care in diabetes: classification and diagnosis of diabetes. Clin Diabetes 2015; 33:97-111. .
6. ETDRS Research Group Grading diabetic retinopathy from stereoscopic color fundus photographs—an extension of the modified Airlie House classification. ETDRS report number 10. Early Treatment Diabetic Retinopathy Study Research Group. Ophthalmology 1991; 98:786-806. [PMID: 2062513].
7. Tabata M, Murachi T. A chemiluminometric method for the determination of urea in serum using a three-enzyme bioreactor. J Biolumin Chemilumin 1988; 2:63-7. [PMID: 3213592].
8. Vaishya R, Arora S, Singh B, Mallika V, Arora S. Modification of Jaffe's kinetic method decreases bilirubin interference: A preliminary report. Indian J Clin Biochem 2010; 25:64-6. [PMID: 23105886].
9. Palladini G, Campana C, Klersy C, Balduini A, Vadacca G, Perfetti V, Perlini S, Obici L, Ascari E, d'Eril GM, Moratti R, Merlini G. Serum N-terminal pro-brain natriuretic peptide is a sensitive marker of myocardial dysfunction in AL amyloidosis. Circulation 2003; 107:2440-5. [PMID: 12719281].
10. Kuwahara K. The natriuretic peptide system in heart failure: Diagnostic and therapeutic implications. Pharmacol Ther 2021; 227:107863[PMID: 33894277].

11. Burtenshaw D, Cahill PA. Natriuretic peptides and the regulation of retinal neovascularization. Arterioscler Thromb Vasc Biol 2020; 40:7-10. [PMID: 31869266].

12. Cao LH, Yu YC, Zhao JW, Yang XL. Expression of natriuretic peptides in rat Müller cells. Neurosci Lett 2004; 365:176-9. [PMID: 15246543].

13. Jin Y, Zhong YM, Yang XL. Natriuretic peptides are localized to rat retinal amacrine cells. Neurosci Lett 2007; 421:106-9. Epub2007May26[PMID: 17566658].

14. Bubb KJ, Aubdool AA, Moyes AJ, Lewis S, Drayton JP, Tang O, Mehta V, Zachary IC, Abraham DJ, Tsui J, Hobbs AJ. Endothelial C-type natriuretic peptide is a critical regulator of angiogenesis and vascular remodeling. Circulation 2019; 139:1612-28. [PMID: 30586761].

15. Pedram A, Razandi M, Levin ER. Natriuretic peptides suppress vascular endothelial cell growth factor signaling to angiogenesis. Endocrinology 2001; 142:1578-86. [PMID: 11250939].

16. Špiranec Spes K, Hupp S, Werner F, Koch F, Völker K, Krebes L, Kämmerer U, Heinze KG, Braunger BM, Kuhn M. Natriuretic peptides attenuate retinal pathological neovascularization via cyclic guanosine monophosphate signaling in pericytes and astrocytes. Arterioscler Thromb Vasc Biol 2020; 40:159-74. [PMID: 31619060].

Articles are provided courtesy of Emory University and The Abraham J. & Phyllis Katz Foundation. The print version of this article was created on 24 September 2025. This reflects all typographical corrections and errata to the article through that date. Details of any changes may be found in the online version of the article.