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Serum cortisol is a biomolecular biomarker for severity of diabetic retinopathy

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Purpose: Cortisol and prolactin are multifunctional hormones essential for various metabolic processes in the human body. This study evaluated for the first time the association between serum cortisol and prolactin levels and severity of diabetic retinopathy (DR) and their role as biomolecular biomarkers for disease progression.

Methods: A tertiary care center–based cross-sectional study was conducted. Forty-six consecutive cases of type 2 diabetes mellitus (DM) were included. Retinopathy was graded according to the Early Treatment Diabetic Retinopathy Study (ETDRS) classification: diabetes with no retinopathy (NoDR, n = 15), nonproliferative DR (NPDR, n = 16), and proliferative DR (PDR, n = 15). Healthy controls (n = 15) were also included. All study participants underwent complete ophthalmological evaluations. Serum levels of cortisol and prolactin were analyzed using the chemiluminescence microparticle assay method. Statistical analysis was performed using ANOVA, univariate and multivariate ordinal logistic regression, and receiver operating characteristics (ROC) area under the curve (AUC).

Results: The mean serum cortisol levels (µg/dl) were 10.25±1.380 for the NoDR group, 12.00±2.540 for the NPDR group, 13.19±2.170 for the PDR group, and 8.22±2.97 for the control group. The mean serum prolactin levels (ng/ml) were 13.13±1.97 for the NoDR group, 11.04±2.59 for the NPDR group, 7.84±1.17 for the PDR group, and 7.38±3.34 for the control group. ANOVA showed a statistically significant increase in serum cortisol levels ($F = 12.87$, $p<0.001$) and a decrease in serum prolactin levels ($F = 19.31$, $p<0.001$) with severity of DR. However, the multivariate ordinal logistic regression analysis showed serum cortisol is a statistically significant independent predictor for severity of DR (odds ratio (OR) = 0.49, 95% confidence interval (CI) = 0.36–0.68, $p<0.001$). The AUC analysis of the serum cortisol levels to discriminate between severity of DR showed statistically significant diagnostic accuracy (NoDR group: AUC = 0.787, $p<0.001$; NPDR group: AUC = 0.852, $p<0.001$; PDR group: AUC = 0.887, $p<0.001$). Serum cortisol levels of >9.5 µg/dl and >10.2 µg/dl were found to be statistically significantly associated with occurrence of NPDR and PDR, respectively.

Conclusions: Statistically significantly elevated serum cortisol levels are observed before development of signs of DR. Serum cortisol levels are statistically significantly associated with severity of DR and serve as a sensitive and specific biomolecular biomarker for disease progression.

Diabetes mellitus (DM) affects 463 million people all over the world according to the 2019 International Diabetes Federation Atlas. Diabetic retinopathy (DR) is the most common cause of blindness in adults in developing countries. DR has an intricate process which starts with loss of capillary cells leading to increased vasopermeability, ischemia, and hypoxia. This increases the production of vascular endothelial growth factor (VEGF) which activates the formation of new fragile blood vessels inside the retina [1-3].

Cortisol, also known as the stress hormone, is released as a body response to combat physical and psychological stress. Cortisol is a steroid hormone synthesized in a multistage process from cholesterol in the zona fasciculata of the adrenal gland. The primary function of cortisol is increasing the blood glucose levels through gluconeogenesis [4]. The secretory rates of serum levels of cortisol are high in the morning but low in the late evening. The plasma cortisol level ranges between 5 µg/dl around midnight and 23 µg/dl in the morning [4].

Prolactin is a protein hormone secreted by the pituitary gland. Prolactin stimulates the mammary glands for milk production and plays an important role in lactation [5]. Healthy serum prolactin levels for women range from 5.180 ng/ml to 26.53 ng/ml and for men, range from 3.46 ng/ml to 19.4 ng/ml. Prolactin has extensive effects in reproduction, angiogenesis, osmoregulation, immune system functioning, and regulation of metabolic pathways for energy production [6,7]. Prolactin reduces the threshold for glucose-stimulated insulin secretion and has effects on growth of pancreatic β-cells which indicates that prolactin has some protective effect against type 2 DM [8]. The role of prolactin in angiogenesis and metabolic pathways could have a protective effect against DM and DR. Serum prolactin-derived vasoinhibins,

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which are proteins obtained after proteolytic cleavage of prolactin have been shown to be protective in DR [9]. The present study evaluated the association between serum cortisol and prolactin levels and severity of DR.

METHODS

A tertiary care centre based cross sectional study comprising of 46 consecutive patients of type-2 DM, between age group of 40-65 years was undertaken. Authors of this study confirm adherence to the doctrines of the Declaration of Helsinki and to Association for Research in Vision and Ophthalmology (ARVO) statement of human subjects. This study was performed after approval was received from the Institutional Ethics Committee of King George's Medical University, Lucknow, India. Voluntary written informed consent was obtained from all study participants. The study included patients with type 2 DM. Type 2 DM was diagnosed according to American Diabetes Association (ADA) guidelines which include fasting plasma glucose level ≥126 mg/dl, 2 h plasma glucose level ≥200 mg/dl during an oral glucose tolerance test, and glycosylated hemoglobin (HbA1c) >6.5% [10]. A total of 46 consecutive patients with type 2 DM, age 40–65 years, were screened for DR and divided into three groups according to Early Treatment Diabetic Retinopathy Study (ETDRS) classification and guidelines by two experienced observers [11]: cases of DM without retinopathy (NoPDR, n = 15), nonproliferative diabetic retinopathy (NPDR, n = 16), and proliferative diabetic retinopathy (PDR, n = 15). Fifteen nondiabetic healthy controls were also included. Patients who did not give consent or had any history of medical conditions that affect serum prolactin and cortisol levels, such as medical history of prolactinoma, hypothyroidism, chronic renal failure, liver cirrhosis, treatment with drugs that increase or decrease serum levels of cortisol or prolactin, pregnancy and breastfeeding, hypertension, recent physical or psychological stress, recent hospitalization, Addison's disease, acromegaly, or Cushing's disease, were excluded from the study. In asymmetric involvement, the eye with the more severe form of the disease was included.

Data collection: Patients' demographic details such as age and sex were documented. The best-corrected visual acuity (BVCA) of all participants was documented on the logMAR scale. All study participants underwent a detailed ophthalmological examination, including slit-lamp biomicroscopy with +90 diopters (D) evaluation and indirect ophthalmoscopy. All cases also underwent fundus fluorescein angiography using a Zeiss fundus camera FF 450 Plus with a pixel width of 0.0054 and an image size of 2588 × 1958 (Carl Zeiss Meditec AG 07,740, Jena, Germany) for evaluation of the fundus.

Laboratory analysis: Fasting blood samples from study participants were collected with aseptic venipuncture between 12:00 p.m. and 12:30 p.m. Blood samples were drawn through aseptic venipuncture using 5 ml metal-free plastic syringe fitted with a 24 Gauge stainless steel needle (Nirlife, one use, manufactured by NIRMA LIMITED, Sachana Gujarat India) under contamination controlled conditions and blood samples were collected in appropriate 4 ml VACUTAINERs (VAKU-8, Hindustan Syringes and Medical Devices Limited manufactured in Faridabad INDIA). For separation of serum blood was transferred to glass tubes. The tubes containing blood were set on to the stand and left for 30 min to allow the blood to clot. Soon after, the samples were centrifuged at 1000 x g for 10 min and serum was carefully removed in to another tube. Postprandial blood samples for blood glucose were collected 2 h after lunch. Fasting blood glucose (blood glucose F), postprandial blood glucose (blood glucose PP), and glycosylated hemoglobin (HbA1c) were measured on an autoanalyzer using the standard protocol [12,13]. Serum urea was measured with the kinetic enzymatic method with urease and glutamate dehydrogenase [14]. Serum creatinine was measured with the modified Jaffe method without deproteinization [15]. Serum cortisol and prolactin levels were measured with the chemiluminescence microparticle assay method using the Abbott ARCHITECT immunoassay analyzer i-1000 (Abbott Diagnostics, Wiesbaden, Germany) [16,17].

Statistical analysis: The outcome measures of the study were demographic characteristics (age and sex), clinical characteristics in the form of the BCVA of the eye with the more severe form of disease (BCVA worse eye), and the biochemical parameters serum prolactin, serum cortisol, blood glucose F, blood glucose PP, HbA1c, hemoglobin, serum urea, and serum creatinine. Data were summarized as mean ± standard deviation (SD). Groups were compared with one-way ANOVA (ANOVA), and the significance of the mean difference between the groups was found with the Newman-Keuls post hoc test after normality was ascertained with the Shapiro-Wilk test and homogeneity of variance between groups with Levene's test. Categorical (discrete) groups were compared with the chi-square (χ^2) test. Pearson correlation analysis was performed to assess associations between the variables. Independent predictors for severity of DR were assessed by using univariate and multivariate ordinal logistic regression analysis. Receiver operating characteristics (ROC) curve analysis was performed to assess serum cortisol as a biomolecular biomarker for severity of DR. A two-tailed (α = 2) p value of less than 0.05 was considered statistically significant. Analyses were performed on SPSS software (Chicago, IL; Windows version 17.0).

Table 1. Demographic, clinical and biochemical parameter levels.

Variables	Control (n=15; %)	NoDR (n=15; %)	NPDR (n=16; %)	PDR (n=15; %)	F/χ^2 value	p value
Age (years)	53.80±6.29	54.33±5.83	56.19±8.77	55.93±5.54	0.46	0.710
Female Male	6 (40.0) 9 (60.0)	11 (73.3) 4 (26.7)	6 (37.5) 10 (62.5)	9 (60.0) 6 (40.0)	5.33	0.149
BCVA (worse eye)	0.37±0.46	0.35±0.27	0.97±0.69	1.63±0.63	18.97	<0.001
Serum prolactin (ng/ml)	7.38±3.34	13.13±1.97	11.04±2.59	7.84±1.17	19.31	<0.001
Serum cortisol (µg/dl)	8.22±2.97	10.25±1.38	12.00±2.54	13.19±2.17	12.87	<0.001
Hemoglobin (g/dl)	12.09±1.39	11.77±0.80	10.71±1.85	11.57±1.25	2.84	0.046
Blood glucose F (mg/dl)	85.07±7.09	139.88±34.91	161.38±65.72	181.11±64.45	10.48	<0.001
Blood glucose PP (mg/dl)	102.93±4.52	218.50±63.46	254.29±86.13	259.12±75.33	18.48	<0.001
HbA1c (%)	4.97±0.34	7.32±1.44	7.63±2.07	9.31±3.44	10.50	<0.001
Serum urea (mg/dl)	31.58±4.27	33.14±11.42	40.83±12.13	43.28±14.35	3.93	0.013
Serum creatinine (mg/dl)	0.91±0.10	0.94±0.26	1.13±0.36	1.31±0.71	2.92	0.042

Abbreviations: SD: standard deviation, NoDR: no retinopathy, NPDR: non-proliferative diabetic retinopathy, PDR: proliferative diabetic retinopathy, BCVA: best corrected visual acuity, ng/ml: nanogram per milliliter, µg/dl: microgram per deciliter, g/dl: gram per deciliter, mg/dl: milligram per deciliter, Blood glucose F: fasting blood glucose, Blood glucose PP: post prandial blood glucose, HbA1c: glycosylated hemoglobin; (Mean ± SD) of four groups (n=61).

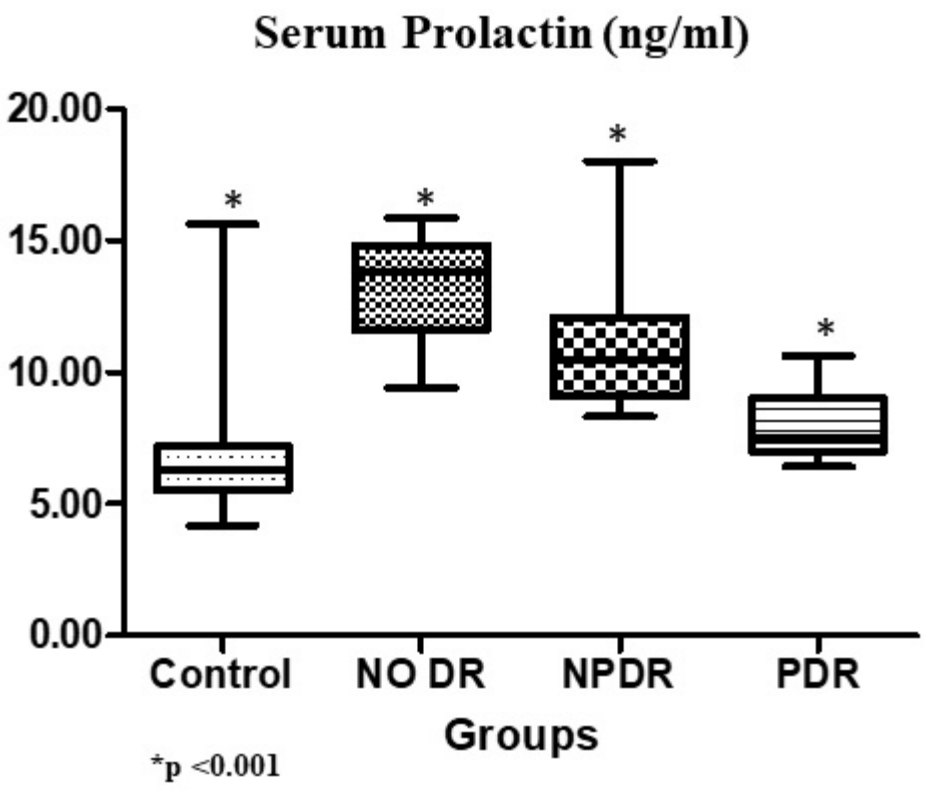

Figure 1. Box and whisker plot showing median and interquartile range for distribution of serum prolactin levels [nanogram per milliliter (ng/ml)] in the four groups [NoDR: no retinopathy (n=15), NPDR: non-proliferative diabetic retinopathy (n=16), PDR: proliferative diabetic retinopathy (n=15) and controls (n=15)] showing decrease in serum prolactin levels with severity of diabetic retinopathy.

RESULTS

The association of different outcome variables (demographic, clinical, and biochemical) with severity of DR (the control group to the NoDR group to the NPDR group to the PDR group) is summarized in Table 1. In the comparison of the mean age of the four groups, ANOVA showed similar ages among the group (F = 0.46, p = 0.710). Similarly, in the comparison of the gender (male/female) frequency (%) of the four groups, the chi-square test showed similar gender frequency among the groups (χ^2 = 5.33, p = 0.149).

The mean serum cortisol, blood glucose F and PP, HbA1c, serum urea, serum creatinine, and BCVA showed a linear increase with severity of DR. The Newman-Keuls test showed statistically significantly (p<0.05 or p<0.01 or p<0.001) different and higher serum prolactin, serum cortisol, blood glucose F and PP, and HbA1c levels in the NoDR, NPDR, and PDR groups compared to the control group except serum prolactin between the control group and the PDR group. Furthermore, the mean serum levels of prolactin decreased statistically significantly (p<0.05 or p<0.001) in the NPDR and PDR groups compared to the NoDR group and were statistically significantly lower in the PDR group compared to the NPDR group (Figure 1). In contrast, the mean serum levels of cortisol were statistically significantly (p<0.05 or p<0.01) different and higher in the NPDR and PDR groups compared to the NoDR group (Figure 2).

The Pearson correlation analysis of the serum levels of cortisol and prolactin with the demographic, clinical, and biochemical parameters of all participants (control + NoDR + NPDR + PDR, n = 61) showed a statistically significant association between the serum cortisol levels with BCVA (r = 0.36, p<0.01), blood glucose F (r = 0.50, p<0.001), blood glucose PP (r = 0.53, p<0.001), HbA1c (r = 0.33, p<0.01), serum urea (r = 0.30, p<0.05), and creatinine (r = 0.32, p<0.05). However, the serum level of prolactin did not correlate statistically significantly.

The univariate and multivariate ordinal logistic regression analyses showed the serum cortisol levels and HbA1c (serum cortisol: multivariate odds ratio (OR) (95% confidence interval (95% CI) = 0.49 (0.36–0.68), p<0.001; HbA1c: multivariate OR (95% CI) = 0.58 (0.40–0.84), p = 0.004) are statistically significant and independent predictors for severity of DR. However, the serum prolactin levels did not show statistically significant results.

The ROC curve analysis showed statistically significant diagnostic accuracy of serum cortisol in discriminating

controls and cases (NoDR: area under the curve (AUC) = 0.787, p<0.001, NPDR: AUC = 0.852, p<0.001, PDR: AUC = 0.887, p<0.001) summarized in Table 2. Further, a cutoff value of >9.3 µg/dl discriminated between the controls and the NoDR cases with 80.00% sensitivity (95% CI = 51.9–95.4) and 73.33% specificity (95% CI = 44.9–92.0; Figure 3A). Similarly, a cutoff value of >9.5 µg/dl discriminated between the controls and the NPDR cases with 81.25% sensitivity (95% CI = 54.3–95.7) and 73.33% specificity (95% CI = 44.9–92.0; Figure 3B). Further, a cutoff value of >10.2 µg/dl discriminated between the controls and the PDR cases with 86.67% sensitivity (95% CI = 59.5–98.0) and 80.00% specificity (95% CI = 51.9–95.4; Figure 3C).

DISCUSSION

Elevated serum cortisol levels have been found to be associated with DM [18]. High serum prolactin levels have been found to be associated with lower prevalence of diabetes and impaired glucose regulation [19].

The present study included cases of DR. We found a significant increase in serum cortisol levels with severity of DR. Significantly elevated serum cortisol levels were observed even before the development of signs of DR. A statistically significant positive correlation was observed between serum cortisol levels and logMAR BCVA, HbA1c, blood glucose F and PP, serum urea, and creatinine levels.

According to the univariate analysis, serum levels of cortisol, BCVA, blood glucose F and PP, urea, and HbA1c were found to be statistically significant predictors for severity of DR. However, in the multivariate analysis, serum cortisol levels and HbA1c showed a statistically significant association with the severity of DR.

In the ROC curve analysis, the AUC showed serum cortisol is a sensitive and specific biomarker for severity of DR. Serum cortisol levels of >9.5 µg/dl and >10.2 µg/dl were found to be statistically significantly associated with the occurrence of NPDR and PDR, respectively.

Cortisol has the function of increasing blood glucose levels through gluconeogenesis. Cortisol also plays a functional role in suppressing the immune system, aiding metabolism of fat, sugar, and carbohydrates, and increasing the body's response in physically and psychologically stressful conditions. Increases in serum levels of cortisol lead to increases in blood glucose levels causing worsening of diabetic control. This leads to an increase in all diabetes-related vasculopathy, including retinopathy [20-22].

The molecular mechanisms of action of cortisol in the pathogenesis of DR are not fully understood. The plausible

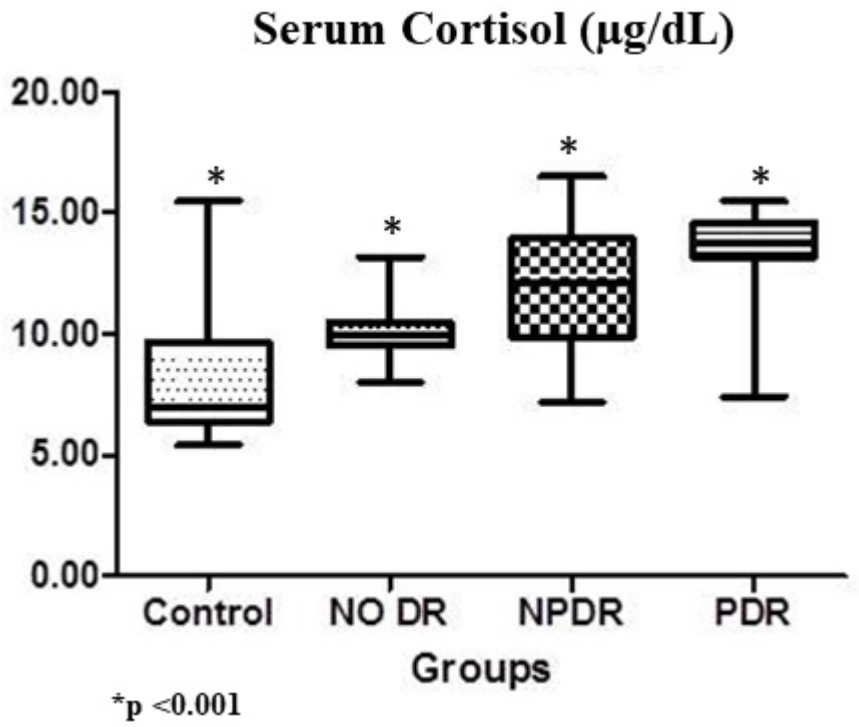

Figure 2. Box and whisker plot showing median and interquartile range for distribution of serum cortisol levels [microgram per deciliter (µg/dl)] in the four groups [NoDR: no retinopathy (n=15), NPDR: non-proliferative diabetic retinopathy (n=16), PDR: proliferative diabetic retinopathy (n=15) and controls (n=15)] showing increase in serum cortisol levels with severity of diabetic retinopathy.

Table 2. Diagnostic accuracy of serum cortisol (mg/dl) to discriminate control and cases using ROC curve analysis.

Group	Criterion (cut off) value	Sensitivity (95% CI)	Specificity (95% CI)	+PV	-PV	AUC	Z value	p value
NoDR	>9.3	80.00 (51.9–95.4)	73.33 (44.9–92.0)	75.0	78.6	0.787	3.38	<0.001
NPDR	>9.5	81.25 (54.3–95.7)	73.33 (44.9–92.0)	76.5	78.6	0.852	5.02	<0.001
PDR	>10.2	86.67 (59.5–98.0)	80.00 (51.9–95.4)	81.2	85.7	0.887	6.10	<0.001

Abbreviations: +PV: positive predictive value, -PV: negative predictive value, AUC: area under the curve, CI: confidence interval, NoDR: no retinopathy, NPDR: non-proliferative diabetic retinopathy, PDR: proliferative diabetic retinopathy; (n=61)

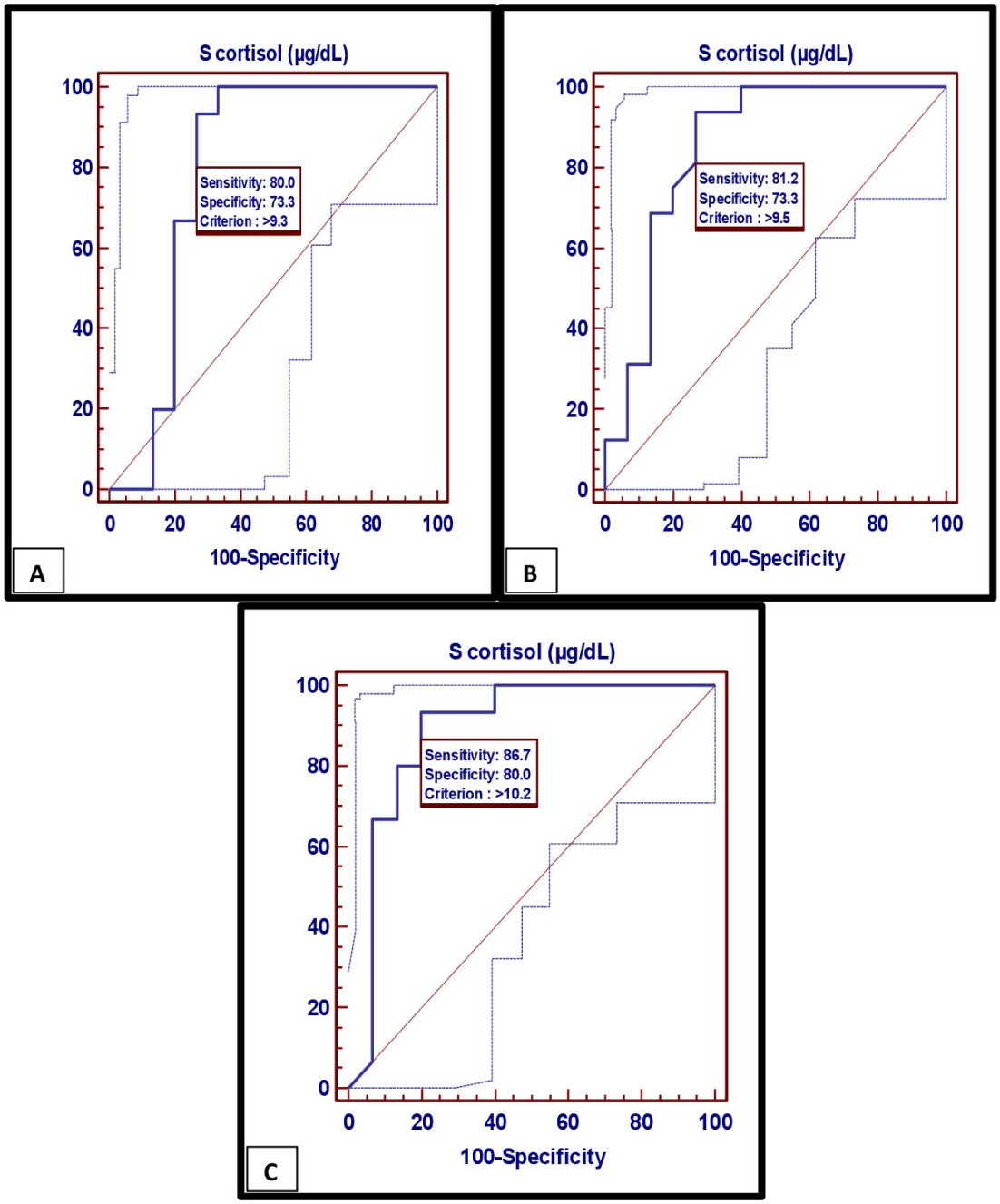

Figure 3. ROC curve analysis. **A**: Sensitivity and specificity of serum cortisol (S cortisol) to discriminate controls and no retinopathy (NoDR) cases using receiver operating characteristics (ROC) curve analysis. Discrimination between controls and NoDR cases with 80.00% sensitivity (95% confidence interval (CI)=51.9-95.4) and 73.33% specificity (95% CI=44.9-92.0) at cut off value of >9.3 microgram per deciliter (µg/dl). **B**: Sensitivity and specificity of serum cortisol to discriminate controls and non-proliferative diabetic retinopathy (NPDR) cases using ROC curve analysis. Discrimination between controls and NPDR cases with 81.25% sensitivity (95% CI=54.3-95.7) and 73.33% specificity (95% CI=44.9-92.0) at cut off value of >9.5µg/dl. **C**: Sensitivity and specificity of serum cortisol to discriminate controls and proliferative diabetic retinopathy (PDR) cases using ROC curve analysis. Discrimination between controls and PDR cases with 86.67% sensitivity (95% CI=59.5-98.0) and 80.00% specificity (95% CI=51.9-95.4) at cut off value of >10.2 µg/dl.

mechanisms are linked to hyperglycemia and insulin resistance which are affected by glucocorticoid, growth hormones, and growth factors in DM. As a result of hyperglycemia, increased flux of glucose through the polyol and hexosamine pathways, activation of protein kinase C (PKC), and increased formation of advanced glycation end products occur which lead to oxidative stress [23]. This results in initiation of inflammation-mediated release of inflammatory markers (intercellular adhesion molecule (ICAM), tumor necrosis factor alpha (TNF-α), matrix metalloproteases (MMP), and interleukin 6 (IL-6)) and growth factor imbalances [23]. Moreover, PKC activation can lead to activation of mitogen-activated protein kinase (MAPK) and phosphorylation of some principal transcription factors that also increase the gene expression of various stress-related components [24]. Activation of MAPK has a role in the form of a signaling component for VEGF and as a regulator of endothelial cell permeability [25,26]. In previous studies, we highlighted an association between the increase in serum VEGF and ICAM-1 levels and changes in the retinal structure and severity of DR [27,28].

Prolactin is cleaved by cathepsin D, MMP, and bone morphogenetic protein 1 to antiangiogenic 16 kg-Dalton prolactin by retinal tissue [29]. These molecules have been reported to play a vital role in preventing angiogenesis in healthy retinal tissue. Serum prolactin-derived vasoinhibins, which are obtained after proteolytic cleavage of prolactin, show a protective role in DR by inhibiting the migration of endothelial cells [29]. This inhibition of proliferation of endothelial cell seems to be mediated by blocking of the extracellular signal regulated kinase (ERK) and MAPK signaling pathway (also known as the Ras-Raf-MEK-ERK pathway). The blockade of Ras activation is followed by downstream inhibition of VEGF-induced translocation of Raf-1 to the plasma membrane and then followed by blockade of the activation of MAPK and ERK [30]. This inhibits capillary endothelial cell migration by activation of apoptosis in capillary endothelial cells [31,32]. Therefore, patients with higher serum prolactin levels are at a lower risk for development and progression of DR, owing to the protective effects of vasoinhibins. In the present study, it was observed that all patients with DM had higher serum prolactin levels (within the normal physiologic range) than the healthy controls. A decrease in mean serum prolactin levels was observed with severity of DR. However, no statistically significant association was found in the multivariate analysis.

The present study had several limitations: It was a cross-sectional study and had a small sample size. This study has highlighted for the first time that serum cortisol levels are significantly increased before the development of signs of DR. Elevated serum cortisol levels are associated with severity of DR and serve as a biomolecular biomarker for disease progression.

REFERENCES

1. Frank RN. Diabetic retinopathy: current concepts of evaluation and treatment. Clin Endocrinol Metab 1986; 15:933-69. [PMID: 3536206].
2. Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. Diabetes Care 2004; 27:1047-53. [PMID: 15111519].
3. Bandello F, Lattanzio R, Zucchiatti I, Del Turco C. Pathophysiology and treatment of diabetic retinopathy. Acta Diabetol 2013; 50:1-20. [PMID: 23277338].
4. Barrett KE, Barman SM, Brooks HL, Yuan JX-J. The Adrenal Medulla & Adrenal Cortex. In: Ganong's Review of Medical Physiology, 26e. New York, NY: McGraw-Hill Education; 2019.
5. Barrett KE, Barman SM, Brooks HL, Yuan JX-J. The Pituitary Gland. In: Ganong's Review of Medical Physiology, 26e. New York, NY: McGraw-Hill Education; 2019.
6. Clapp C, Thebault S, Jeziorski MC, Martinez De La Escalera G. Peptide hormone regulation of angiogenesis. Physiol Rev 2009; 89:1177-215. [PMID: 19789380].
7. Freeman ME, Kanyicska B, Lerant A, Nagy G. Prolactin: structure, function, and regulation of secretion. Physiol Rev 2000; 80:1523-631. [PMID: 11015620].
8. Brelje TC, Scharp DW, Lacy PE, Ogren L, Talamantes F, Robertson M, Friesen HG, Sorenson RL. Effect of homologous placental lactogens, prolactins, and growth hormones on islet B-cell division and insulin secretion in rat, mouse, and human islets: implication for placental lactogen regulation of islet function during pregnancy. Endocrinology 1993; 132:879-87. [PMID: 8425500].
9. Triebel J, MacOtela Y, De La Escalera GM, Clapp C. Prolactin and vasoinhibins: Endogenous players in diabetic retinopathy. IUBMB Life 2011; 63:806-10. [PMID: 21913303].
10. . American Diabetes Association. Standards of medical care in diabetes: classification and diagnosis of diabetes. Diabetes Care 2015; 38:Suppl 1S8-16. .
11. 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].
12. Pocino K, Molinario R, Manieri R, Bianucci L, Capoluongo E. The Hemo One Autoanalyzer for Glycated Hemoglobin Assay. Lab Med 2016; 47:119-23. [PMID: 27069029].
13. Ilanchezhian SP, Rajini T. Samuel BR. A Comparative Study of Blood Glucose Level Measurement between Glucometer,

Semi-Auto Analyzer and Auto-analyzer. Int J Pharm Sci Rev Res 2017; 44:36-9. .

14. 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].

15. 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].

16. Grimminger P, Osswald A, Reincke M, Cm R, Bidlingmaier M. A fast and fully automated chemiluminescence immunoassay for the determination of cortisol in serum, plasma, urine and saliva. Congress of Clinical Chemistry and Laboratory Medicine / 10th Annual Meeting of the German Society for Clinical Chemistry and Laboratory Medicine (DGKL), Dresden, Germany, 23rd–26th October, 2013*). Clin Chem Lab Med 2013; 51:1-2. [PMID: 23495396].

17. Comuzio FL, Bolelli S, Mimmi G, Messeri PG, Orlandini A. Configuration and optimization of a common two site immunoassay for human prolactin using a chemiluminescent tracer and an enzymatic tracer. J Biolumin Chemilumin 1989; 4:587-93. [PMID: 2678928].

18. Chiodini I, Adda G, Scillitani A, Coletti F, Morelli V, Di Lembo S, Epaminonda P, Masserini B, Paccoz PB, Orsi E, Ambrosi B, Arosio M. Cortisol secretion in patients with type 2 diabetes: Relationship with chronic complications. Diabetes Care 2007; 30:83-8. [PMID: 17192338].

19. Wang T, Lu J, Xu Y, Li M, Sun J, Zhang J, Xu B, Xu M, Chen Y, Bi Y, Wang W, Ning G. Circulating prolactin associates with diabetes and impaired glucose regulation: a population-based study. Diabetes Care 2013; 367:1974-80. [PMID: 23340889].

20. Sapolsky R, Plotsky P. Hypercortisolism and its possible neural bases. Biol Psychiatry 1990; 27:937-52. [PMID: 2185849].

21. Sapolsky R, Krey L, McEwen B. Stress downregulates corticosterone receptors in a site-specific manner. Endocrinology 1984; 114:287-98. [PMID: 6690273].

22. Sapolsky R, Krey L, McEwen B. Prolonged glucocorticoid exposure reduces hippocampal neuron number: Implications for aging. J Neurosci 1985; 5:1221-6. [PMID: 3998818].

23. Brownlee M. Biochemistry and molecular cell biology of diabetic complications. Nature 2001; 414:813-20. [PMID: 11742414].

24. Geraldes P, King GL. Activation of protein kinase C isoforms and its impact on diabetic complications. Circ Res 2010; 106:1319-31. [PMID: 20431074].

25. Xia P, Inoguchi T, Kern TS, Engerman RL, Oates PJ, King GL. Characterization of the mechanism for the chronic activation of diacylglycerol-protein kinase C pathway in diabetes and hypergalactosemia. Diabetes 1994; 43:1122-9. [PMID: 8070612].

26. Koya D, King GL. Protein kinase C activation and the development of diabetic complications. Diabetes 1998; 47:859-66. .

27. Jain A, Saxena S, Khanna VK, Shukla RK, Meyer CH. Status of serum VEGF and ICAM-1 and its association with external limiting membrane and inner segment-outer segment junction disruption in type 2 diabetes mellitus. Mol Vis 2013; 19:1760-7. [PMID: 23922493].

28. Ahuja S, Saxena S, Akduman L, Meyer CH, Kruzliak P, Khanna VK. Serum vascular endothelial growth factor is a biomolecular biomarker of severity of diabetic retinopathy. Int J Retin Vitr. 2019; 5:1-6. [PMID: 31583119].

29. Pan H, Nguyen NQN, Yoshida H, Bentzien F, Shaw LC, Rentier-Delrue F, Martial JA, Weiner R, Struman I, Grant MB. Molecular targeting of antiangiogenic factor 16K hPRL inhibits oxygen-induced retinopathy in mice. Invest Ophthalmol Vis Sci 2004; 45:2413-9. [PMID: 15223825].

30. D'Angelo G, Martini JF, Iiri T, Fantl WJ, Martial J, Weiner RI. 16K human prolactin inhibits vascular endothelial growth factor-induced activation of Ras in capillary endothelial cells. Mol Endocrinol 1999; 13:692-704. [PMID: 10319320].

31. Clapp C, Thebault S, Jeziorski MC, Martinez De La Escalera G. Peptide hormone regulation of angiogenesis. Physiol Rev 2009; 89:1177-215. [PMID: 19789380].

32. Takahashi T, Ueno H, Shibuya M. VEGF activates protein kinase C-dependent, but Ras-independent Raf-MEK-MAP kinase pathway for DNA synthesis in primary endothelial cells. Oncogene 1999; 18:2221-30. [PMID: 10327068].

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