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A STUDY TO CORRELATE SERUM PROLACTIN LEVELS IN PATIENTS WITH DIABETIC RETINOPATHY

Chandrashekhar K[1], Mohammad Nizamuddin H Attar [2]*, MD Juned[3,]
Sushma S Biradar[4,] Mehak Budhiraja[5] Rajashekhar Dyaberi[6]

[1,6]Associate Professor, [2,3,4]Senior Resident, [5]Junior Resident, Department of General Medicine, Karnataka Institute of Medical Sciences Hubli India

***Corresponding Author and reprint request to**: Dr Mohammad Nizamuddin H Attar

ABSTRACT

Background: Diabetes mellitus is a major medical problem throughout the world. Diabetes causes an array of long-term systemic complications that have considerable impact on the patient as well as society, as the disease typically affects individuals in their most productive years. **Objective:** To correlate Serum Prolactin levels in patients with Diabetic retinopathy. **METHODS:** A total of 189 patients of diabetic retinopathy were enrolled in the study. Details of their medical and personal history, viz. age at diagnosis of diabetes, duration of the diabetes, nature and duration of treatment received, glycaemic control and compliance to the treatment were obtained. The diabetic retinopathy status was classified according to the ETDRS classification system. **RESULTS:** A mean HbA1C levels for each grade of DR: in Mild NPDR the HbA1c was found to be 8.33%, Moderate NPDR was found to be 8.88%, severe NPDR the HbA1c was 9.92% and for PDR the HbA1c was 11.01%. A mean Serum Prolactin levels for each grade of DR: in Mild NPDR the serum prolactin levels was found to be 8.76ng/dl, Moderate NPDR 8.57ng/dl, for Severe NPDR it was 7.28ng/dl, and it was 5.94ng/dl for PDR. A mean duration

of diabetes for each grade of DR: in mild NPDR the mean duration of diabetes was found to be 8.45yrs and in moderate NPDR it was 9.65yrs, whereas in severe NPDR group it was 11.75yrs and for PDR group it was 12.58 years.

A significant correlation ($p < 0.05$) was also found between DR and the duration of diabetes as increasing severity of NPDR and PDR were seen in patients who were diabetic for more than ten years and statistically significant correlation was found between increasing severity of diabetic retinopathy and the decreasing levels of serum prolactin.

CONCLUSION: A statistically significant correlation was found between increasing severity of diabetic retinopathy and the decreasing levels of serum prolactin.

KEYWORDS: Diabetic retinopathy, HbA1C, serum prolactin, vasoinhibins

INTRODUCTION: Some patients with diabetes under good metabolic control develop diabetic retinopathy where as people with poorly controlled diabetes remain free of diabetic retinopathy which cannot be explained easily and requires further research in this horizon. From analysis of the data of DCCT revealed that more than 40% of the patients with poor metabolic control remained free of diabetic retinopathy. [1]

Such under researched areas of pathophysiology of diabetic retinopathy factors including Prolactin and vasoinhibins must be studied in detail given their influence on

the halting of progression of diabetic retinopathy and that they merit further investigation as important targets for therapeutic interventions.

The importance of hormones in the progression of diabetic retinopathy is indicated by their role on glucose metabolism, blood pressure, and blood vessel growth, which are decisive factors in the natural history of the disease

An early event of vascular damage diabetic retinopathy is characterised by the loss of pericytes and endothelial cells causing in acellular and ischemic capillaries. Decreased blood flow increases vasopermeability and causes the accumulation of extracellular fluid and hard exudates that decrease vision when the macula is involved. Further, intraretinal hemorrhages and capillary occlusion produce new areas of ischemia, this hypoxia stimulates the production of proangiogenic factors, such as vascular endothelial growth factor (VEGF). In the later part of disease, the new fragile capillar ies invade and bleed into the vitreous, giving rise a fibrovascular tissue that results in retinal detachment and blindness.

Among the present therapeutic options for diabetic retinopathy, laser photocoagulation remains the most efficient method for preventing blindness. However, despite of its efficacy, the destructive nature of the laser damages native tissue and has other significant side effects. [2]

 PRL is important hormone for lactation, is also known to exert a wide variety of actions in different horizons like reproduction, osmoregulation, immune response, brain function, behaviour, energy metabolism, and angiogenesis. [3] Among the previous ly mentioned effects, the last two can have safeguarding effect against diabetes and diabetic retinopathy.

PRL acts on pancreatic b-cells to increase proliferation, survival, and synthesis and secretion of insulin66. These effects occur through the classic janus kinase (JAK)/signal transducers and activators of transcription (STAT) pathway that leads to the upregulation of cyclin D267 and the increase in the of glucose transporter 2, glucokinase, and pyruvate dehydrogenase activities. PRL also triggers b-cell proliferation by upregulating the expression of tryptophan hydroxylase-1, which increases serotonin levels. It was observed in experimental studies male and female PRL receptor null mice had decreased islet density, b-cell mass, and insulin synthesis and were glucose intolerant. [4]

 PRL protected against the development of hyperglycaemia in diabetic rats, by increasing islet cell number and reducing mononuclear cell infiltration, hence inflammation and apoptosis. [5]

There is another important mechanism by which prolactin hormone can prevent progression and promote regression of diabetic retinopathy changes, by proteolytic conversion to vasoinhibins.

ROLE OF VASOINHIBINS IN RETINA: Prolactin hormone produced elsewhere is split by Cathepsin D, Matrix Metallo- Proteases (MMP), and Bone Morphogenetic Protein-1 to produce vasoinhibins. [6] Cathepsin D is the prime protease cleaving PRL in the pituitary gland during the secretory process. [7] whereas MMPproduces vasoinhibins in the extracellular space and at the target tissue level like retina. [8]

Vasoinhibins has direct action on endothelial cells to inhibit vasopermeabilit y, vasodilation, and angiogenesis stimulated by several vasoactive substances, includ ing: VEGF, basic fibroblast growth factor (b-FGF), interleukin 1-b, bradykinin, and acetylcholine. Also, vasoinhibins promote the apoptosis-mediated regression of blood vessels.

Vasoinhibins signal through a receptor different from the PRL receptor to inhib it activation of the Ras-Raf-MAPK pathway, the Ras-Tiam1-Rac1-Pak1 pathway, and the Ca21/calmodulin-activation of endothelial nitric oxide synthase (eNOS), which has a protective role in ROS mediated injury.

They also increase protein phosphatase 2A-induced dephosphorylation/ inactivation of eNOS, the activation of proapoptotic proteins of the Bcl-2 family, and the NFjB-mediated activation of caspases.

In many experimental studies Vasoinhibins inhibited the proliferation of blood vessels in the chick embryo chorioallantoic membrane, in coronary vessels, and in several tumour models.

The local injection of vasoinhibins in corneas of rats inhibited corneal angiogenes is stimulated by bFGF and VEGF. [9]

Radioactive PRL injected intracardially in the rats was detected in the retina, the choroids, and the ciliary body. [10]   The ciliary body, which transports the of plasma proteins to ocular fluids. [11] expresses the PRL receptor on its surface, and the genetic deletion of the PRL receptor inhibits the hyperprolactinemia-induced accumulation of retinal vasoinhibins.

When antibodies blocking these vasoinhibins were injected it stimulated angiogenes is in the cornea 78 and in the retina. [12] The intraocular transfection of small interfering RNA blocking the local expression of PRL stimulated retinal vascular proliferation and vasodilation. [12] In one experimental study vasoinhibins were immunologically depleted in neonatal rats that reduced the apoptosis of the hyaloid vasculature, proving that vasoinhibins have role in the physiological regression of intraocular blood vessels after birth. [13]

These findings implicate vasoinhibins as major inhibitors of ocular blood vessels and raise the possibility that altering their levels could influence the progression of diabetic retinopathy

 Since impaired renal function is common in patients with diabetes mellitus, increased renal elimination could explain lower systemic concentrations of PRL-V. According to Ben-Jonathan et al. glycosylation may alter proteolytic cleavage of PRL. [14]

This may result in the decreased proteolytic production of PRL-V, and thus enhanced glycosylation due to hyperglycaemia could explain decreased levels of PRL-V. So present study was conducted to correlate Serum Prolactin levels in patients with Diabetic retinopathy

MATERIALS AND METHODS: This Descriptive correlative study was conducted among 189 diabetic patients referred for evaluation of diabetic retinopathy at the Department of General Medicine KIMS, Hubballifor a period of 18 months.

Inclusion criteria: Type 2 Diabetic men and Post-menopausal women with diabetic retinopathy.

Exclusion criteria: Patients with medical history of Prolactinoma

Patients on Liver Cirrhosis and Chronic Renal Failure (stage 4 and 5). Premenopausal Women.

Patients who are treatment with drugs that alter prolactin levels.

Patients with Type 1 Diabetes.

Not giving consent. Relevant history regarding the patient's diabetes was taken

- Age of onset of diabetes (first diagnosed)

- Duration of diabetes

- Nature and duration of treatment received

- History regarding patient's glycaemic control and compliance to the treatment.

Patient's examination was then performed as per the proforma.

FIGURE 21: FUNDUS CAMERA USE IN RETINA CLINIC, KIMS, HUBBALLI

A general physical examination was performed followed by a complete ophthalmological examination. All the findings were documented in the proforma and verified by the guide for the study. The fundi were evaluated by Direct ophthalmoscopy.

In our study the cut off values for normal were as follows:

1) HbA1C levels between 4.5 and 5.7%

2) Total serum cholesterol ≤ 200 mg/dl

3) LDL ≤ 130 mg/dl

4) HDL ≤ 55 mg/dl for males and between 45 and 65 mg/dl for females

5) LDL ≤ 160 mg/dl

6) Creatinine: between 0.3 and 1.3 mg/dl

7) Proteinuria (measured in 24-hour urine volume) ≤ 0.15 g

High blood pressure was defined as systolic pressure > 140 mmHg and/or diastolic pressure >110 mmH

REFERANCE RANGE OF SERUM PROLACTIN: The calculated production rate of PRL ranges from 200 to 536 μg/day/m2 and the metabolic clearance rate ranges from 40 to 71 mL/minute/m2.PRL is cleared rapidly, half-life ranging from 26 to 47 minutes. PRL secretion occurs episodically in 4 to 14 secretory pulses over 24 hours, each lasting 67 to 76 minutes. The serum prolactin levels for all ages were 10.7 ± 3 ng/ml in healthy male and 11.6 ± 2.8 ng/ml in healthy female. [15] Fasting blood samples were collected from all the study participants of each group under aseptic conditions in serum vacutainers (Red cap) and estimated fasting plasma glucoses levels by Trinder's method (Glucose oxidase- Peroxidase). The serum was stored at –200C in an aliquot for serum prolactin estimation. Grossly haemolysed and lipemic were excluded. Serum prolactin was measured by ELISA method.

STATISTICAL ANALYSIS: Data was analysed using SPSS 22 version software. Categorical data was represented in the form of Frequencies and proportions. Chi-square test was used as test of significance for qualitative data. Continuous data was represented as mean and standard deviation. **ANOVA (Analysisof Variance)** was the test of significance to identify the mean difference between more than two groups for quantitative data. **p value** (Probability that the result is true) of <0.05 was considered as statistically significant after assuming all the rules of statistical tests.

RESULTS: According to the observations made, the mean age of the patients in mild DR and moderate DR was 58.22 and 58.1 years respectively, almost the same age. Whereas mean age in severe category of DR was 65.33 years. Mean age in PDR Category was 76.58 years. Hence, we observed higher grades of retinopathy was observed in elderly population. Table 1

TABLE 1: MEAN AGE OF THE PATIENTS IN DIFFERENT CATEGORIES OF DIABETIC RETINOPATHY

| | CATEGORY | | | | | | | | p value |
| | Mild | | Moderate | | Severe | | PDR | | |
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Age	58.22	11.27	58.1	13.31	65.33	13.98	76.58	8.18	< 0.001*
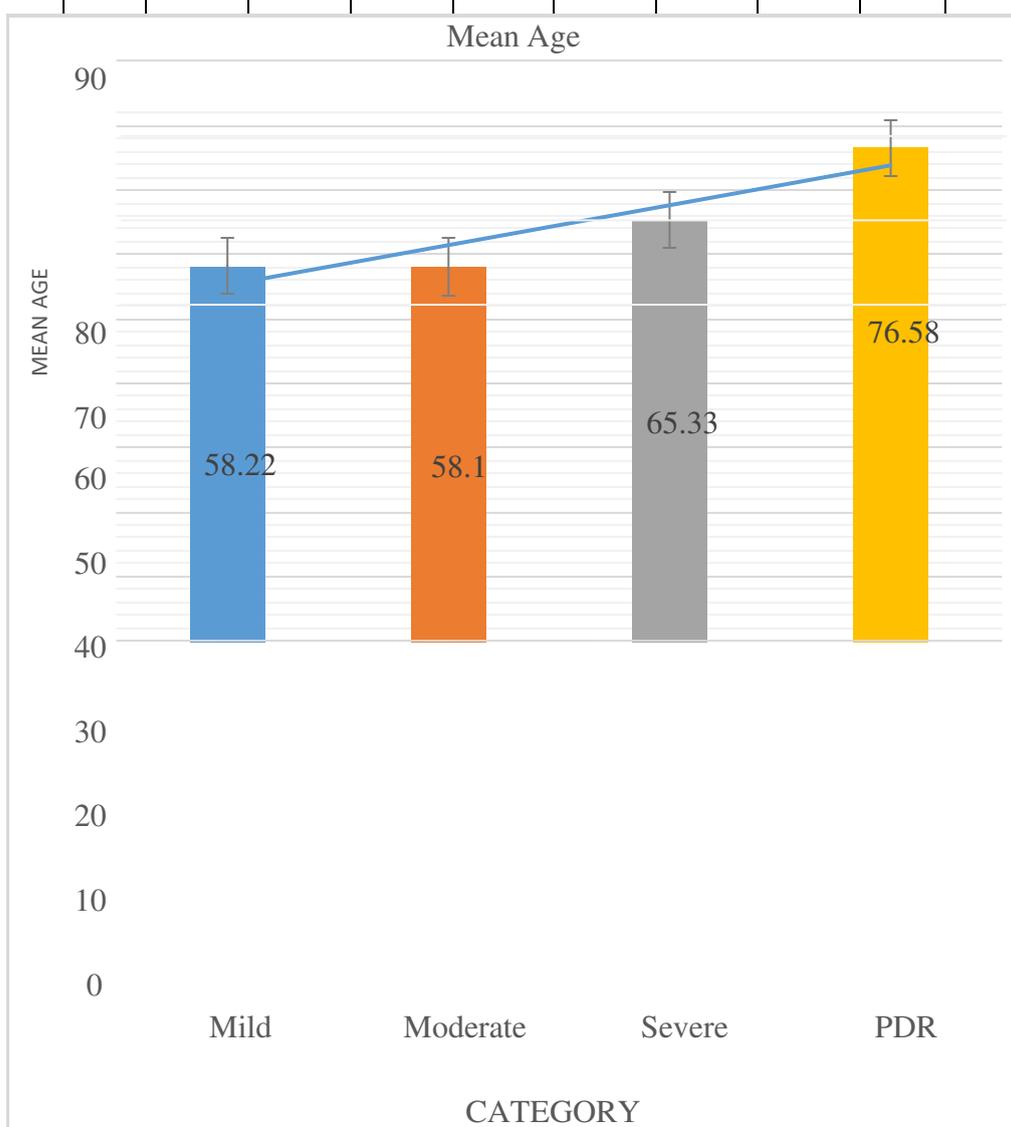
Figure1: **Mean Age of the Patients in Different Categories of Diabetic Retinopathy**

TABLE 2: COMPARISON OF MEAN DURATION OF DIABETES BETWEEN DIFFERENT CATEGORIESOF DIABETIC RETINOPATHY.

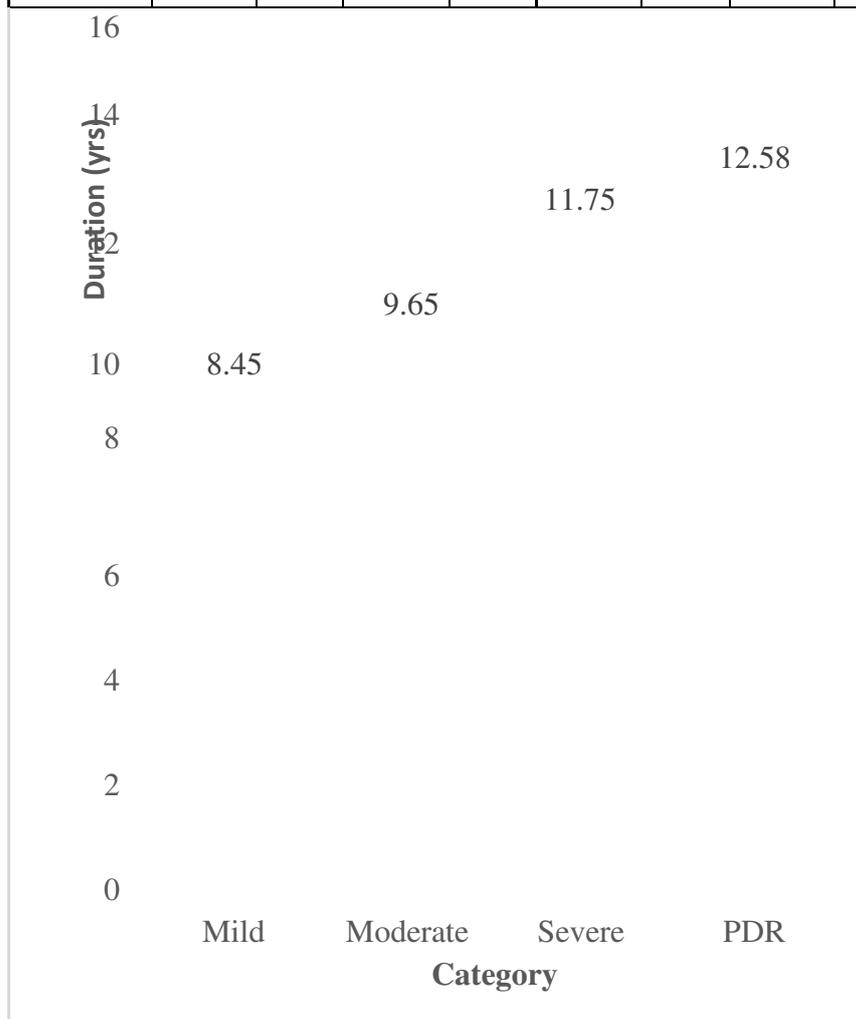| | CATEGORY | | | | | | | | p value |
| | Mild | | Moderate | | Severe | | PDR | | |
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Duration (years)	8.45	3.92	9.65	3.44	11.75	2.85	12.58	1.98	< 0.001*

Figure 2: Comparison of Mean Duration of Diabetes between different categories of Diabetic Retinopathy.

- In the Mild NPDR group the mean duration of diabetes was 8.45yrs with SD of3.92yrs
- The Moderate NPDR group the mean duration of diabetes was 9.65yrs with SDof 3.44yrs
- The Severe NPDR group the mean duration of diabetes was 11.75yrs with SDof 2.85yrs
- The PDR group the mean duration of diabetes was 12.58yrs with SD of 1.98yrs

- These numbers were analysed statistically, yielding a p value of <0.05, whichwas statistically significant.

TABLE 3: COMPARISON OF MEAN SERUM PROLACTIN LEVELS BETWEEN DIFFERENT CATEGORIESOF DIABETIC RETINOPATHY

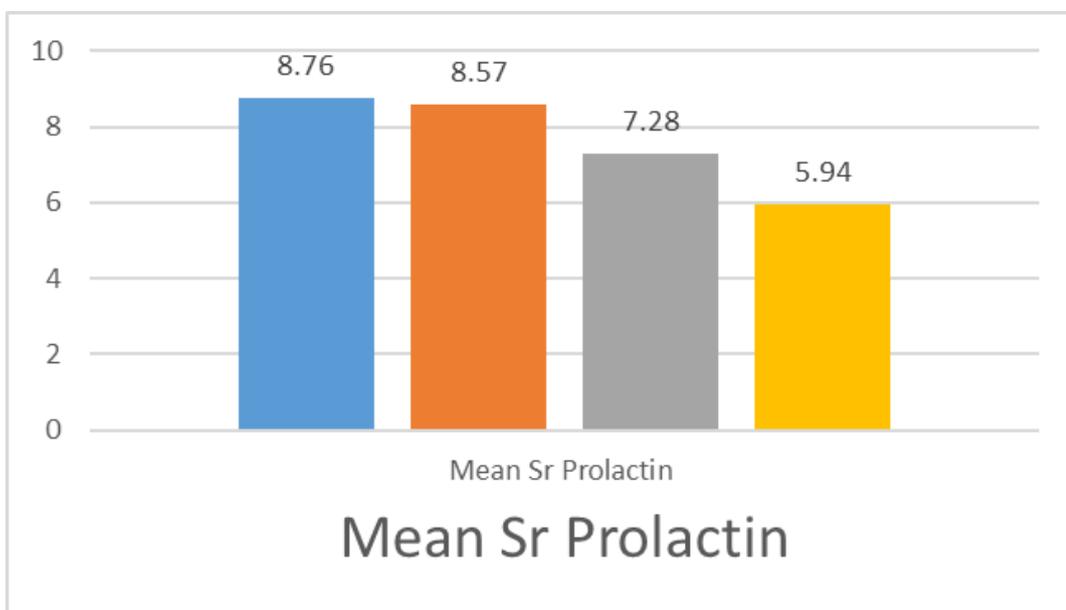| | CATEGORY | | | | | | | | p value |
| | Mild | | Moderate | | Severe | | PDR | | |
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
SR Prolactin	8.76	4.39	8.57	3.36	7.28	1.95	5.94	2.41	< 0.05*

Figure 3: Comparison   of Mean Serum Prolactin levels between differentcategories of Diabetic Retinopathy

From the above table a correlation is inferred for mild NPDR the mean Serum Prolactin levels were **8.76** with SD of **4.39**, moderate NPDR the mean Serum Prolactin levels were **8.57** with SD of **3.36**.

In severe NPDR group the mean Serum Prolactin levels was **7.28** with SD of **1.95** and for PDR the mean Serum Prolactin levels was **5.94** with SD of **2.41**.

There was a statistically significant result observed with the values of mean Serum Prolactin levels which were observed in different grades of diabetic retinopathy.

TABLE 4: MEAN HBA1C COMPARISON BETWEEN DIFFERENT CATEGORIES OF DIABETICRETINOPATHY

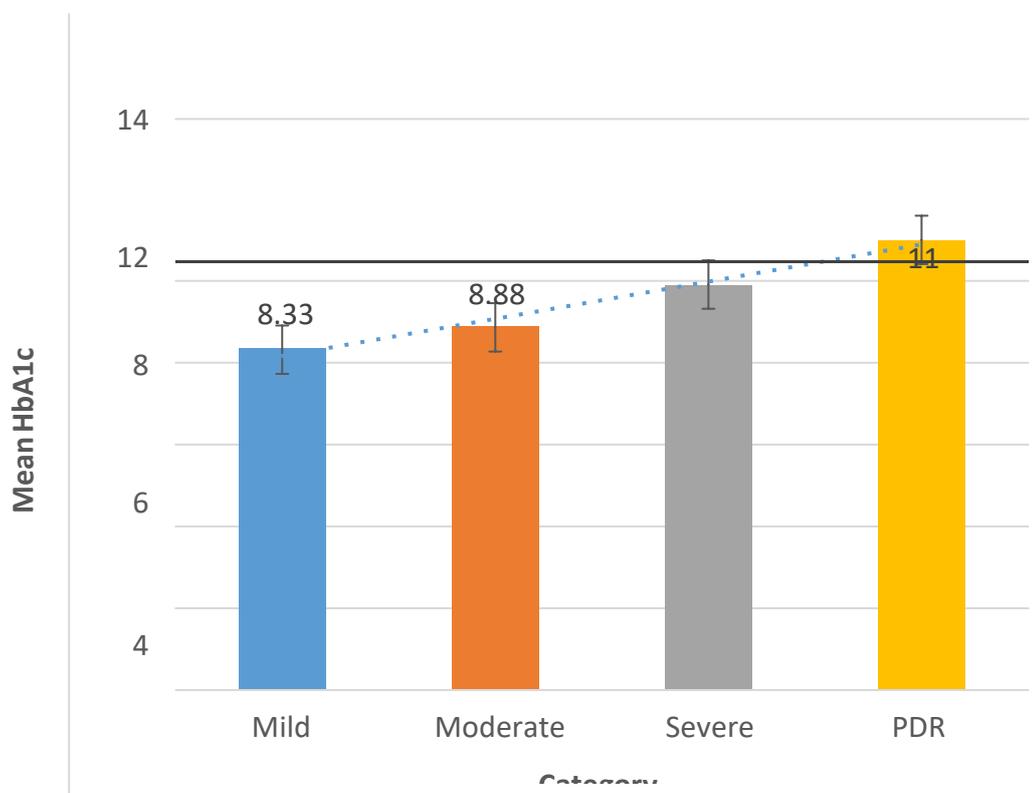|	CATEGORY								p value
	Mild		Moderate		Severe		PDR		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
HbA1c	8.33	2.72	8.88	1.97	9.92	3.07	11	2.41	< 0.001*

Figure 4: Mean HbA1c Comparison between different categories of Diabetic Retinopathy

In mild NPDR group the mean HbA1c was found to be 8.33% with SD of 2.72%.

- In moderate NPDR group the mean HbA1c was 8.88 % with SD of 1.97%.

- In severe NPDR group the mean HbA1c was 9.92 % with SD of 3.07%.

- In PDR group the mean HbA1c 11% with SD of 2.41 %.

- There was a statistically significant result observed with the values of HbA1C,which were observed in different grades of diabetic retinopathy.

Table 5: Mean FBS Comparison between different categories of Diabetic Retinopathy

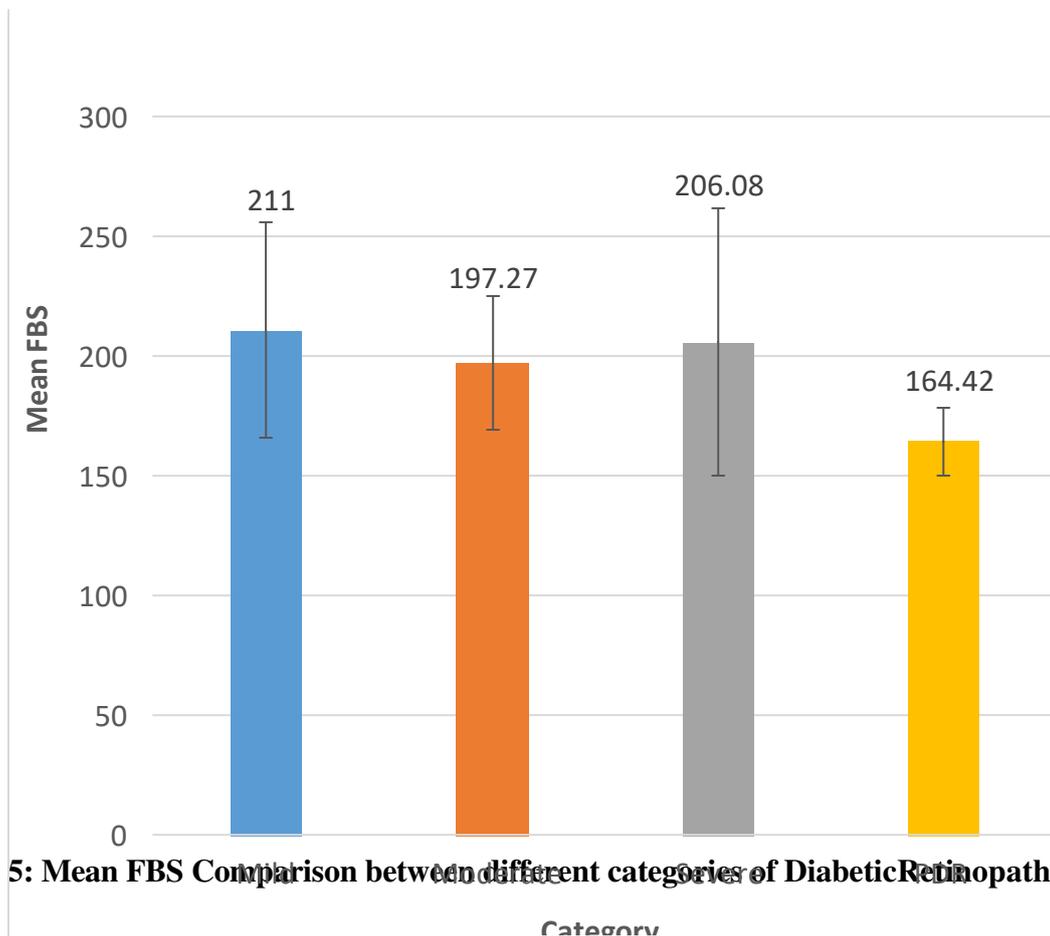|	CATEGORY								p value
	Mild		Moderate		Severe		PDR		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
FBS	211	45.05	197.27	28.06	206.08	55.64	164.42	13.88	0.002*

Figure 5: Mean FBS Comparison between different categories of DiabeticRetinopathy

DISCUSSION: In present study the mean age group of the study population was 57.05 years which was similar to that in studies done by Shokoofeh B [16], whereas Malaz Salah et al [17] and Abdel Moneim et al [18] had 61.57 years and 67.34 years respectively. The difference may be due to population distribution or variation due to geography or environment.

It is evident from these findings that there was a statistically significant worsening of retinopathy and progress of the severity of retinopathy with increasing duration of diabetes in these individuals. (p =<0.05).

In the CURES Eye study, 41.8 per cent had DR after 15yrs of diabetes and severity of DR proportionally increased with longer duration of diabetes. In addition, it has been demonstrated that for every five year increase in duration of diabetes, the risk for DR increased by 1.89 times.

In the Wisconsin Epidemiologic Study of Diabetic Retinopathy (WESDR), the widest and most prolonged population based ophthalmologic survey, reported that higher prevalence of DR was associated with longer duration of diabetes. [19]

In present study the mean Sr PRL level was 7.63 ng/dl, which was comparable to the study population of Mohammed Abdullah Saad et al which was 7.737 ng/dl, whereas Shokoofeh B, et al.and Abdel Moneim et al Sr PRL level was6.29ng/dl and 6.92ng/dl. [2]

In present study the mean HbA1C was 9.53± 2.67, which was comparable to the study population of Shokoofeh B, et al. [16] which was 9.40±1.72 ,whereas , Mohammed Abdullah Saad et al and Abdel Moneim et al [18] HbA1C level was 10.16 ± 2.10 and 9.07 ± 0.22 respectively.

In present study the mean FBS was 190.5mg/dl, which was comparable to the study population of shokoofeh et al which was 187.28, whereas Abdel Moneim et al and AbdelMoneim et al [18] mean FBS was 212.36 mg/dl and 163.7 mg/dl.

CONCLUSION: A statistically significant correlation was found between serum prolactin levels and the severity of diabetic retinopathy with more severe grades of diabetic retinopathy manifesting with lower levels of serum Prolactin

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