SERUM PROLACTIN LEVELS IN TYPE-II DIABETES MELLITUS WITH RETINOPATHY PATIENTS IN AND AROUND HYDERABAD

Mohammed Abdullah Saad[1], Mohammed Sabiullah[2], N. Vani N[3].

[1]*Resident specialist, Department of Biochemistry, Niloufer Hospital, Hyderabad, Telangana.*
[2]*Associate professor, Department of Biochemistry, Osmania Medical College, Koti, Hyderabad, Telangana.*
[3]*Professor and Head, Department of Biochemistry, Osmania Medical College, Koti, Hyderabad, Telangana.*

ABSTRACT

Background & Objectives: Diabetes is a disease of metabolic dysregulation. Hyperglycemia and oxidative stress play a role in the development of late diabetic complications. Increased retinal vasopermeability occurs early in diabetes and is crucial for the development of sight-threatening diabetic retinopathy. Serum prolactin is proteolytically processed to vasoinhibins that inhibit the excessive retinal vasopermeability related to diabetic retinopathy. The aim is to study the role of serum prolactin in the pathogenesis of retinopathy in type 2 diabetics and to correlate the changes in serum prolactin levels. **Methods:** A case control study was done with 90 patients divided into 3 groups (healthy controls, Type 2 Diabetes without retinopathy and Type 2 Diabetes with retinopathy) with inclusion and exclusion criteria. Fasting blood samples were collected and fasting plasma glucose, serum Prolactin were measured. Multiple comparisons between different groups were done using ANOVA test. **Results:** In the present study decreased serum prolactin levels were observed in patients of Type 2 Diabetes with retinopathy compared to Type 2 Diabetes without retinopathy and controls. Mean ± S.D of Fasting plasma glucose, was highest in Group 3. Mean ± S.D of serum prolactin (7.737 ± 2.63) was low in Group 3. **Conclusion:** The circulating levels of prolactin were decreased in patients with diabetic retinopathy due to glycosylation and were higher in diabetic patients without retinopathy and healthy controls. Increased prolactin levels influence the progression of retinopathy after its intraocular conversion to vasoinhibins which can reduce the pathological retinal vasopermeability in diabetes and increase in prolactin can be considered to have protective role in pathogenesis of diabetic retinopathy and also may be considered as a treatment option for retinopathy.

Key words: Diabetic retinopathy, Prolactin, Retinal vasopermeability, Vasoinhibins.

INTRODUCTION

Diabetes Mellitus is a chronic disease which has serious metabolic disturbances in carbohydrate, protein and fat metabolism occurring due to insulin insufficiency or its action. Excessive release of free radicals due to increase in oxidative stress in diabetes, leads to diabetic complications such as retinopathy [1].

In diabetic retinopathy, loss of pericytes and endothelial cells causing abnormally permeable retinal capillaries. In early stages, increased retinal vasopermeability leads to intraretinal hemorrhages and exudates that, along with capillary closure, produces nonperfusion areas. Eventually, the resulting hypoxia stimulates the production of proangiogenic factors locally, such as vascular endothelial growth factor (VEGF); the newly formed blood vessels (neovascularization) extend and bleeds into the vitreous gradually, causing detachment of the retina from the accompanying fibrous tissue leading to loss of vision in the patients [2].

A polypeptide hormone prolactin, synthesized in the anterior pituitary cells. Cathepsin-D or matrix metalloproteases cleaves serum prolactin to produces peptide fragments known as Vasoinhibins [3,4] which reduces vasodilation and also prevents angiogenesis and retinal vasopermeability associated with diabetes [5].

Current treatments for diabetic retinopathy such as laser photo-coagulation and vitrectomy, are usually effective but may be damaging as well, and can only treat the later stages of the disease. Hence, working on new strategies to prevent both increased retinal vasopermeability and angiogenic responses has become a major research focus.

MATERIAL AND METHODS

Study design: Case control analytical study

Ethics approval: The study was approved by the institutional ethics committee and informed consent was taken from the all participants.

DOI: 10.5455/ijcbr.2017.34.09

eISSN: 2395-0471
pISSN: 2521-0394

Correspondence: Mohammed Sabiullah, Associate professor, Department of Biochemistry, Osmania Medical College, Koti, Hyderabad, Telangana. Email: mohammadsabiullah@gmail.com

Study location: Investigations were performed at the Department of Biochemistry, Osmania

Medical College/ Osmania General Hospital, Hyderabad.

Study duration: December 2014 to May 2016.

Sample size: case control study was done with 90 patients

Inclusion criteria:

Cases: Case samples were collected from diabetic patients of age group 35-65 years, Department of Retina, Sarojini Devi Eye Hospital, Mehdipatnam, Hyderabad.

Control: 30 healthy male voluntary blood donors of same age were taken as controls.

Exclusion criteria: Females, due to significant variations in hormone levels at different ages, medical history of prolactinoma, hypothyroidism, chronic renal failure, liver cirrhosis, Treatment with Drugs that increase prolactin levels, Recent psychologic stress, recent hospitalization, acromegaly and cushing disease.

Grouping:

Group 1 included healthy controls who were matched for age.

Group 2: Diabetic patients those individuals who were recently diagnosed as diabetic. They were under glycaemic control by receiving either Insulin or oral hypoglycaemic drugs and didn't have any complications. This group of patients had a normal fundus examination.

Group 3: Chronic cases of Diabetic, also under treatment with antidiabetic drugs. They had developed retinopathy and were selected based on fundus examination by an ophthalmoscope. The minimum requirement for a diagnosis of retinopathy was the presence of background retinopathy classified as microaneurysms, haemorrhages, exudates or venous beading. [6]

Methodology:

Patient details including age, sex, medical history, onset, duration and complications of Diabetes were filled in a proforma. 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).[7] The remaining serum was stored at –200C in an aliquot for serum prolactin estimation. Grossly hemolysed and lipemic were excluded. Serum prolactin was measured by ELISA method. [8]

Statistical analysis: Data analysis was done using GraphPad Prism software version 7.0. Descriptive results are expressed as Mean±SD of various parameters in di□erent groups, to assess the significance of the di□erences observed in the mean values of di□erent parameters observed in di□erent groups studied, the data is subjected to ANOVA test. The significance of di□erence of mean values of di□erent groups and within the groups is represented by p value <0.05 is considered as significant. Pearson's correlation was done to assess the correlation of parameters within each group.

Table 1. Study parameters in all groups

Parameter	Group 1	Group 2	Group 3
	Mean ±S.D	Mean ±S.D	Mean ±S.D
F.P.G (md/dl)	84.20± 9.152	127.7± 35.90	163.7± 76.88
S. PRL (ng/ml)	12.84±5.77	17.98±5.631	7.737± 2.63

Table 2. Pearsons Correlation between parameters in control group

		FPG	S.PRL
FPG	Pearsons correlation		0.166
	Sig. (2tailed)N		0.382
S.PRL	Pearsons correlation	0.166	
	Sig. (2tailed)N	0.382	

Table 3. Pearsons Correlation between parameters in group 2

		FPG	S.PRL
FPG	Pearsons correlation		0.006
	Sig. (2tailed)N		0.975
S.PRL	Pearsons correlation	0.006	
	Sig. (2tailed)N	0.975	

Table 4. Pearsons Correlation between parameters in group 3

		FPG	S.PRL
FPG	Pearsons correlation		-0.378
	Sig. (2tailed)N		0.039
S.PRL	Pearsons correlation	-0.378	
	Sig. (2tailed)N	0.039	

RESULTS

The Mean ± SD of all the parameters studied in the total cases were significantly di□erent from those of controls (p<0.05). Mean value of Serum prolactin was lowest in Group 3 followed by Group 1 and Group 2.

Mean value of fasting plasma glucose, was highest in Group 3 followed by Group 2 and Group 1. In Group 1& 2, Serum prolactin was positively correlated with fasting plasma glucose, but was not statistically significant. In Group 3, Serum prolactin was negatively correlated with fasting plasma glucose but it was statistically significant. Results showed that type 2 Diabetes patients with retinopathy had lower levels of serum prolactin when compared to type 2 Diabetes patients without retinopathy and healthy controls.

DISCUSSION

Diabetic retinopathy may be the most common microvascular complication of diabetes. The proposed pathophysiological mechanisms by which diabetes may leads to development of retinopathy are as follows:[24]

- Hyperglycemia increases the flux of sugar molecules through the polyol pathway, causing sorbitol accumulation in cells, eventually producing osmotic stress considered to be an underlying mechanism in the development of diabetic microvascular complications, including diabetic retinopathy.
- Hyperglycemia can promote the nonenzymatic formation of advanced glycosylated end products (AGEs) which are thought to damage the cell.
- High glucose levels can stimulate free radical production and reactive oxygen species formation causing cell injury.
- Growth factors, including vascular endothelial growth factor (VEGF), growth hormone, and transforming growth factor β, have also been postulated to play important roles in the development of diabetic retinopathy.

Vasoinhibins are a family of prolactin (PRL) fragments with antiangiogenic property[9] that inhibit ischemia-induced retinal angiogenesis[10] and prevent increased retinal vasopermeability associated with diabetes.[11] This proteolytic product of prolactin acts as a potent inhibitor of angiogenesis in vivo and in vitro, inhibiting the proliferation of endothelial cells.[12,13] Vasoinhibins are considered as endogenous regulators of angiogenesis and vascular function.[14] In vitro experiments and in vivo studies revealed that vasoinhibins inhibit neovascularization by[15] apoptosis-mediated vascular regression, thus being a potent inhibitor of angiogenesis in the retina[16] and elsewhere. In addition, vasoinhibin can inhibit endothelial cell proliferation and vasopermeability in the retinal vessels of diabetic patients, induced by vascular endothelial growth factor (VEGF)[12]. Serum prolactin levels were significantly lower in group 3 when compared to group 1&2, implying type 2 Diabetes patients with microvascular complications like retinopathy had highest levels of glucose than type 2 Diabetes patients without microvascular complications and controls. It can be explained as diabetic patients with complications had poor glycaemic control, leading to the increased glycation of proteins, forming advanced glycated end products. The protective e□ect of vasoinhibin (PRL-V) against neovascularization (angiogenesis) may be reduced in diabetic patients because of the lower levels of PRL due to increased glycosylation of PRL (G- PRL) which exhibits decreased receptor binding and increased metabolic clearance. [17,18] This puts the diabetics at an increased risk for the developing of retinopathy in future. Study conducted by Arnold E. et al. [19] showed significant decreased in serum prolactin levels in the Type 2 diabetic patients with diabetic retinopathy in later stages, and observed significant di□erence in serum prolactin levels between healthy control and Type 2 diabetic patients with proliferative diabetic retinopathy. And also, the di□erence of serum prolactin levels between individuals with Type 2 Diabetics without retinopathy and Type 2 Diabetes with later stages of diabetic retinopathy was significant. Martini et al. [20] have demonstrated that fragment of human prolactin (hPRL) induces apoptosis of endothelial cells. Low levels of serum prolactin may not prevent the development of new blood vessel in the retina of diabetic patients. Struman et al. [21] have shown that recombinant N-terminal fragments of human prolactin have antiangiogenic properties, which can prevent the neovascularisation in the retina of diabetic patients. Carmen Gonzalez et al. [22] Showed that prolactin fragments inhibit vascular endothelial growth factor (VEGF)- induced eNOS (Nitric oxide synthase) activation and endothelial cell proliferation [12] and concluded that those fragments can block the activation of eNOS which results in inhibition of angiogenesis both in vivo and in vitro. In comparison with the present study, Mooradian A. et al. [23] measured fasting serum prolactin levels in 72 diabetic males and compared the results with those of 63 healthy males. The diabetic group had significantly higher serum prolactin levels than the control group. 18% percent of the diabetics studied had serum prolactin levels above the normal range for males (> 20 ng/ml). But there was no correlation between serum prolactin levels and presence of clinically apparent retinopathy in this study. Celina García et al. [11] observed prolactin to be an important systemic inhibitor of diabetes-induced retinal hypervasopermeability after its intraocular conversion to vasoinhibins, which act directly on endothelial cells to block blood vessel growth, dilation, and permeability and to promote apoptosis-mediated vascular regression. Promoting vasoinhibin activity to counteract vascular endothelial growth factor and reduce excessive retinal vasopermeability in diabetic retinopathy emerges from this study as a potentially powerful therapeutic approach for effective treatment of diabetic macular edema and other vasoproliferative retinopathies.

CONCLUSION

In this study, we observed decreased serum prolactin levels in patient with diabetic retinopathy when compared to diabetic patients without retinopathy and healthy controls. Thus we concluded that serum prolactin proves to be important in the pathogenesis and progression of diabetic retinopathy and promoting vasoinhibin (fragment of prolactin) activity may reduce excessive retinal vasopermeability and neovasularisation in diabetic retinopathy and is emerging as a potentially powerful therapeutic approach for effective treatment of proliferative diabetic retinopathy and other vasoproliferative retinopathies.

Acknowledgment: We acknowledge the services provided by MDR unit Osmania medical college, Hyderabad to carry out this research.

Conflict of interest: Nil

REFERENCES

1. WHO (1980). Techn. Rep. Ser., No.66.
2. Caldwell RB, Bartoli M, Behzadian MA, El-Remessy AE, Al-Shabrawey M, Platt DH, Caldwell RW. Vascular endothelial growth factor and diabetic retinopathy: pathophysiological mechanisms and treatment perspectives. Diabetes Metab Res Rev 2003;19:442–455.
3. Clapp C, Thebault S, Arnold E, Garcia C, Rivera JC & Martínez de la Escalera G. Vasoinhibins: novel inhibitors of ocular angiogenesis. American Journal of Physiology. Endocrinology and Metabolism 2008 295 pp: 772–778.
4. Clapp C, Thebault S, Jeziorski MC, Martínez De La Escalera G. Peptide hormone regulation of angiogenesis. Physiol Rev 2009;89: pp: 1177–1215.
5. Clapp C, Aranda J, Gonzalez C, Jeziorski MC & Martínez de la Escalera G. Vasoinhibins: endogenous regulators of angiogenesis and vascular function. Trends in Endocrinology and Metabolism 2006;17: 301–307.
6. Lang GE. Characterisation and relevance of di□erent diabetic retinopathy phenotypes Pg 13 - 39 (chapter 2) Laser treatment of diabetic retinopathy (chapter 4) Pg 48 - 69, Diabetic retinopathy Vol. 39.
7. Trinder P. Determination of glucose in blood using glucose oxidase with an alternative oxygen receptor. Ann. Clin. Biochem. 1969;6:24–27.
8. ELISA (CALBIOTECH KIT) PR234F
9. Clapp C, Thebault S, Jeziorski MC, Martínez De La Escalera G. Peptide hormone regulation of angiogenesis. Physiol Rev 2009;89: pp: 1177–1215.
10. Pan H, Nguyen NQ, Yoshida H, Bentzien F, Shaw LC, Rentier-Delrue F, et al. Molecular targeting of anti-angiogenic factor 16K hPRL inhibits oxygen-induced retinopathy in mice. Invest Ophthalmol Vis Sci. 2004;45:2413–2419.
11. Garcia C, Aranda J, Arnold E, The bault S, Macotela Y, Lo pez-Casillas F, et al. Vasoinhibins prevent retinal vasopermeability associated with diabetic retinopathy in rats via protein phosphatase 2A- dependent eNOS inactivation. J Clin Invest. 2008;118:2291–2300.
12. Clapp C, Martial JA, Guzman RC, Rentier-Delure F, Weiner RI. The 16-kilodalton N-terminal fragment of human prolactin is a potent inhibitor of angiogenesis. Endocrinology.1993;133: 1292–1299.
13. Duen ãs Z, Torner L, Corbacho AM, Ochoa A, Gutierrez-Ospina G, Lopez- Barrera F, Barrios FA, Berger P, Martinez de la Escalera G, Clapp C 1999 Inhibition of rat corneal angiogenesis by 16-kDa prolactin and by endogenous prolactin-like molecules. Invest Ophthalmol Vis Sci.1999;40: 2498–2505.
14. Clapp C, Aranda J, Gonzalez C, Jeziorski MC & Martínez de la Escalera G. Vasoinhibins: endogenous regulators of angiogenesis and vascular function. Trends in Endocrinology and Metabolism 2006;17:301–307.
15. Corbacho AM, Martínez de la Escalera G, Clapp C. Roles of prolactin and related members of the prolactin/growth hormone/placental lactogen family in angiogenesis. J Endocrino. 2002;173: 219–238.
16. Aranda J, Rivera JC, Jeziorsky MC, Riesgo-Escovar J, Nava G, Lo´ pez- Barrera F, Quiro´ etal. Prolactins are natural inhibitors of angiogenesis in the retina. Investigative Ophthalmology and Visual Science 2005; 46:2947–2953.
17. Pellegrini I, Gunz G, Ronin C, Fenouillet E, Peyrat JP, Delori P, Jaquet P. Polymorphism of prolactin secreted by human prolactinoma cells: immunological, receptor binding, and biological properties of the glycosylated and nonglycosylated forms. Endocrinology.1988;122: 2667- 2674.
18. Ho□mann T, Penel C, Ronin C. Glycosylation of human prolactin regulates hormone bioactivity and metabolic clearance. J Endocrinol Invest.1993;16:807-816.
19. Arnold E, Rivera JC, Thebault S, Moreno-Paramo D, Quiroz-Mercado H, et al. High Levels Of Serum Prolactin Protect Against Diabetic Retinopathy By Increasing Ocular Vasoinhibins. Diabetes.2010;59:3192- 3197.
20. Martini JF, Piot C, Humeau LM, Struman I, Martial

JA, Weiner RI. The antiangiogenic factor 16K PRL induces programmed cell death in endothelial cells by caspase activation. Mol Endocrinol.2000;14: 1536–1549.

21. Struman I, Bentzien F, Lee H, Mainfroid V, D'Angelo G, Go□n V, Weiner RI & Martial JA. Opposing actions of intact and N-terminal fragments of the human prolactin/growth hormone family members on angiogenesis: an e□cient mechanism for the regulation of angiogenesis. PNAS. 1999;96:1246–1251.

22. Gonz´alez C, Corbacho AM, Eiserich JP. 16K-prolactin inhibits activation of endothelial nitric oxide synthase, intracellular calcium mobilization, and endothelium-dependent vasorelaxation. Endocrinology. 2004;145:5714–5722.

23. Mooradian A, Morley J, Billington C, Slag M, Elson M, Shafer R. Hyperprolactinaemia in male diabetics. Postgraduate Medical Journal. 1985;61 (711):11-14.

24. Fong DS, Aiello LP, Ferris FL, Klein R: Diabetic retinopathy. Diabetes Care. 2004; 27:pp: 2540-2553.

How to Cite this article: Mohammed Abdullah Saad1, Mohammed Sabiullah, N. Vani N. Serum Serum Prolactin Levels in Type-II Diabetes Mellitus With Retinopathy Patients in and Around Hyderabad. Int j. clin. biomed. Res. 2017;3(4) : 39-43