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MEASUREMENT OF ADIPONECTIN IN VITREOUS AND PLASMA OF PATIENTS WITH PROLIFERATIVE DIABETIC RETINOPATHY AND ITS CORRELATION WITH VASCULAR ENDOTHELIAL GROWTH FACTOR, PIGMENT EPITHELIAL DERIVED FACTOR AND INSULIN LIKE GROWTH FACTOR-1.

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ABSTRACT

Aim: Purpose of this study was to measure adiponectin (APN) and to compare with already known players of retinopathy namely VEGF, PEDF and IGF-1 in vitreous and plasma of patients with PDR. *Methods:* Plasma and vitreous samples of 26 patients with PDR who underwent vitrectomy were analyzed for APN, PEDF, IGF-1 and VEGF by ELISA. Among these 19 patients were underwent laser treatments prior to vitrectomy. Disease matched control samples were collected from 11 Macular Hole (MH) patients. The study was done with prior approval from Institution Ethics Board. *Results:* The vitreous samples from PDR patients (n=29) showed increased level of APN (91.1 ng/ml, $p<0.0001$) when compared to 0.32 ng/ml in vitreous from MH patients (n=11). PDR patients who underwent LASER treatment showed 3 fold increase in vitreous APN (113.5ng/ml, $p < 0.03$), and a significant decrease in VEGF levels ($p < 0.002$) than those who had not. Vitreous APN, VEGF and IGF -1 levels were significantly elevated in patients with PDR ($p < 0.000$) as compared to those with MH. Plasma PEDF was significantly elevated in patients with PDR ($p < 0.000$) and positively correlated with plasma glucose ($p< 0.009$). Plasma APN in PDR and whole study group positively correlated with HDL ($p < 0.03$). *Conclusions:* Increased vitreous APN and a negative correlation with VEGF after LASER treatment was observed in PDR. Increased PEDF in plasma and its positive correlation with plasma glucose was also seen in PDR group.

KEY WORDS: **Angiogenesis; Adiponectin; PEDF; VEGF; PDR, Photocoagulation.**

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INTRODUCTION

Diabetes mellitus has become a common disease with devastating complications. One of the most serious micro vascular complications of diabetes which leads to blindness is proliferative diabetic retinopathy. Prolonged hyperglycemia, accumulation of advanced glycation end products, secretion of proangiogenic growth factors, inflammatory cytokines and adhesion molecules cause the loss of pericytes, basement membrane thickening and micro-vascular abnormalities leading to retinal neovascularization in PDR [1]. Pan retinal photocoagulation (PRP) is used to treat PDR as PRP is presumed to reduce, hypoxia and to destroy abnormal blood vessels [2]. Adipokines play very important roles in the regulation of local metabolic processes. Several numbers of adipokines are involved in the pathogenesis of PDR. An elevated level of vitreous leptin in PDR was reported by Maberley etal [3]. Presence of leptin receptor in fibro vascular membrane [4] and its angiogenic nature [5] indicate its involvement in the pathogenesis of PDR. In addition, elevated levels of vitreous TNF α [6], MCP-1, IL 6 [7], VEGF [8] and IGF-1, [9] were also reported in PDR. These adipokines help in cell proliferation and neovascularization. Conversely, PEDF is a known antiangiogenic adipokine, which is being reported to have a protective role in the pathogenesis of PDR [10]. It is secreted as 50 kDa protein by retinal pigment epithelial cells. Besides its antiangiogenic role, PEDF also plays roles in protecting neural cells and in the development of photoreceptors and their survival in retina [10]. Recently, Maeda et al, shown that PEDF could block RAGE-induced APN gene suppression in adipocytes when exposed to AGE through its antioxidative properties [11]. One of the important reports by Adamis et al demonstrated elevated levels of the potent angiogenic factor, VEGF in vitreous of PDR patients [8]. Based on this fact, the existing treatment for PDR aims to stop abnormal blood vessel formation by targeting VEGF, which is involved in the disease pathogenesis. Anti-VEGF drugs for treating PDR, namely Bevacizumab, Ranibizumab and Pegatunib sodium, are reported to be useful but have, their own limitations [12]. Besides, anti VEGF treatments, adenoviral vector-delivery of pigment epithelium-derived factor [13] and somatostatin analogues to inhibit IGF-1 were also considered for the control of pathological retinal angiogenesis [14]. IGF 1, another mediator of angiogenesis was also elevated in PDR [9]. Localization of IGF-1 and its receptor in the retina [15] indicates its essential role in the pathogenesis of PDR. IGF-1 increases VEGF secretion through HIF1α dependent and independent manner in cultured ARPE 19 cells [16]. Apart from its proangiogenic activity, it plays a pivotal neuroprotective role in retinal ganglion cells and also improves survival of *in vitro* neuro-retinal cells even under hypoxic condition [17]. APN is a circulating peptide hormone derived from adipocyte tissues. The oligomeric and globular forms of APN are regulators of energy homeostasis. APN is reported to have antidiabetic, antiinflammatory, antiangiogenic, antiatherogenic and antihypertensive properties [18, 19]. ADIPOR1 and ADIPOR2 serve as receptors for globular and full length APN. These receptors mediate AMP activated protein kinases, PPARα pathway, glucose uptake and the oxidation of fats in liver and muscles [20]. It is reported that APN ameliorates retinopathy through the attenuation of inflammation, neovascularization and fibrosis in mice [21]. APN suppresses VEGF-stimulated human coronary artery endothelial cell migration via cAMP/PKA dependent signaling [22]. This is an important effect which implies that APN is a regulator of vascular processes associated with diabetes and atherosclerosis. It is interesting to note that APN is expressed in choroidal tissues and is capable of inhibiting the LASER induced choroidal neovascularization up to 78% through intraperitoneal injections in an experimental mouse model [23]. Zietz et al have observed an elevated APN level in the serum of patients with type 2 diabetes and PDR [24]; The same group showed the presence of

APN in vitreous from 5 patients who underwent vitreoretinal surgery, as a proof of concept. However, there is no report on APN levels in vitreous from PDR and MH patients to date. This study has estimated, for the first time, the levels of APN, in surgically removed vitreous samples and compared with that of VEGF, IGF- 1 and PEDF. The likely role of these adipokines in disease pathogenesis is discussed based on the results.

MATERIALS AND METHODS

Research design and subjects

The study was conducted in strict adherence to the guidelines of the Helsinki Declarations and with the approval of the Institution Ethics Board. The study was conducted for a period of 2 years, and patients who underwent vitrectomy as part of their treatment for PDR and MH were included. Patients aged between 35 and 70 years with PDR, suffering from diabetes for more than 10 years were the test subjects. Patients in the same age group with MH were recruited as controls. Patients who were under any anti-VEGF, statins, piogioglitozone treatment and those having coronary heart diseases, renal failure, sepsis and malignancy were excluded. Almost all of the patients were on insulin and/or oral anti diabetic drugs. In a total, 40 vitreous samples (from 37 subjects), 29 from PDR and 11 from MH surgery were included during this study period. Out of 29 PDR cases, 21 patients had undergone vitrectomy within 3 to 12 months after LASER treatment. In addition, 14 control subjects without diabetes were included for the measurement of biochemical risk factors of PDR, as well as the factors APN, PEDF, IGF 1 and VEGF. Blood and vitreous samples were collected with proper consent from all test subjects. All patients recruited in the study had undergone a complete slit lamp and fundus examination and their clinical ocular findings were graded for the presence of vitreous hemorrhage, retinal detachment, presence and absence of neovascularization in the retina or optic disc prior to vitrectomy. Patients with MH were with an idiopathic full thickness retinal defect of more than 400 microns with posterior vitreous detachment were included. Similar patients with diabetes or with macular edema were also excluded. Undiluted vitreous samples were aspirated during vitrectomy and transported to laboratory immediately. Vitreous samples were centrifuged, aliquoted and stored at – 80^{o}C till the assays were carried out. Assays were performed with in one week from the time of collection. Routine biochemical assays comprising of plasma glucose, total cholesterol, triglycerides, high density lipoprotein (HDL) and total protein using a fully automated Dade Behring RxL Max (Siemens, USA) were conducted. Glycosylated hemoglobin was estimated by boronate affinity assay and Microalbumin in urine by immunometric assay (Nycocard reader II, Axis Shied, Norway). All the assays were done on the day of collection. Both vitreous and plasma levels of APN, IGF 1 and VEGF, were measured using quantikine ELISA kit (R&D, USA). PEDF was measured using chemikine PEDF ELISA kit (Chemicon International, USA). EDTA plasma was separated and stored at – 80^{o}C till the assays were carried out.

Statistics

Mann Whitney test was used to evaluate the significance of measured parameters between patients with PDR and disease matched MH controls, in both vitreous and plasma. Pearson Correlation was used to calculate the *r* and *p* values. Statistical analysis was done by SPSS software version 16.

RESULTS

Details of study subjects such as duration of diabetes, treatment done and medication used are given in supplement table 1. Table 1 shows the levels of physical and biochemical risk factors for PDR in the pool of study participants. The control group was age, and BMI matched to the PDR group. Biochemical analysis of the known risk factors showed increased plasma glucose, triglycerides, and glycosylated hemoglobin in the PDR group with statistical significance. 35% of patients with PDR had > 200 mg/L while 65 % of them had > 20 mg/L of urine microalbumin while, in all control subjects, it was < 20 mg/L. APN,

VEGF and PEDF levels, were measured using ELISA and appropriate standards were run every time along with the samples while performing the immunoassay. Sensitivity of the ELISA kits used is APN: 0.24 ng/mL, PEDF: 0.09 ng/mL, VEGF: 9 pg/mL, IGF- 1: 0.026 ng/mL

Table 1
Physical and Biochemical parameters of patients with and without PDR.

PARAMETERS	PDR (n = 26)	MH (n = 11)	CONTROLS (n=14)	*P* Value
BP Systolic (mm Hg) [Mean ± SD]	150.96 ± 21.36	132.09 ± 19.1	115.7 ± 11.6	
BP Diastolic (mm Hg) [Mean ± SD]	85.0 ± 8.6	82.6 ± 6.2	73.0 ± 9.72	
Random blood Glucose (mg/dL) [Mean ± SEM]	196.5 ± 14.03	117.6 ± 6.61	94.43 ± 1.98	^ *P < 0.000* * *P < 0.000*
HbA1c (%) [Mean ± SEM]	7.34 ± 0.22	6.07 ± 0.30	5.26 ± 0.12	^*P < 0.001* * *P < 0.001*
Serum Total Cholesterol (mg/dL) [Mean ± SEM]	175.7 ± 11.4	178.9 ± 11.8	168.4 ± 8.06	
Serum Triglycerides (mg/dL) [Mean ± SEM]	146.5 ± 16.2	137.2 ± 22	99.8 ± 9.45	* *P < 0.014*
Serum HDL Cholesterol (mg/dL) [Mean ± SEM]	43.2 ± 2.11	45.2 ± 3.88	47. 4 ± 1.6	
Serum protein (gm/ dL) [Mean ± SEM]	7.3 ± 0.1	7.67 ± 0.17	7.54 ± 0.10	
Microalbumin in urine (mg/ L) [Mean ± SEM]	35% > 200 and 65 % > 20	100% cases < 20	100% cases < 20	

Significance comparison between PDR and MH) * Significance comparison between PDR and Control

Elevated Plasma PEDF correlated positively with glucose

There was no significant difference in the levels of plasma APN (Fig 1A); VEGF (Fig 1B); IGF -1(Fig 1D), between the PDR group and MH or with the control group; however, plasma PEDF (Fig 1C), levels were significantly increased in the PDR group compared with control (Plasma PEDF: 4.35 ± 0.95 versus 0.54 ± 0.06 µg/mL $p < 0.000$). Plasma PEDF was positively correlated with plasma glucose in PDR group (fig 2B) ($p< 0.009$) and also in the whole study group (fig 2D) ($p< 0.001$).

Plasma APN and its correlation with HDL

Plasma APN was positively correlated with HDL (fig 2A) ($p < 0.03$) in patients with PDR and also in the whole study group (fig 2C) ($p < 0.03$) whereas plasma VEGF, and IGF-1 did not correlate with any other parameters.

Elevated vitreous adipokines in PDR

Vitreous APN (fig 3A), VEGF (fig 3B), and IGF 1 (fig 3D) were significantly higher in PDR than in MH, Whereas, PEDF (fig 3C) does not show any significant difference in comparison to MH The levels of adipokines are as follows, APN: 91.1 ± 17.7 versus 0.32 ± 0.31ng/mL, ($p < 0.0001$); VEGF: 1.23 ± 0.29 ng/mL versus, not detectable levels, ($p < 0.0001$), IGF - 1: 1.95 ± 0.13 versus 0.82 ± 0.16 ng/mL, ($p < 0.0001$) and PEDF: 5.89 ± 0.89 versus 3.65 ± 0.67 ng/mL. Further within the PDR group, patients who underwent LASER treatment prior to surgery showed a significant increase in vitreous APN (ng/mL), decreased VEGF (ng/mL) than those who had not undergone any LASER treatment (APN: 113.5 ± 22.6 versus 37.4 ± 5.7, $p < 0.03$, VEGF: 0.99 ± 0.24 versus 1.87 ± 0.83, $p < 0.002$, fig 3 A, C). In addition, PEDF levels are increased, and IGF -1 levels are not altered much in the PDR patients who had undergone LASER treatment, but there is no statistical significance between their levels.

Vitreous APN positively correlated with IGF-1 and negatively correlated with VEGF

Vitreous growth factors were correlated with each other. Total of six combinations (APN/VEGF, APN/PEDF. APN/IGF-1, VEGF/IGF-1, PEDF/IGF-1 & VEGF/PEDF) were created, and Pearson's correlation was

done in PDR group and same was repeated in the PDR patients who had undergone laser prior to vitrectomy. We observed a positive correlation between vitreous APN and IGF-1 (r = 0.38, p < 0.04; Fig 4: C) and negative correlation between VEGF and IGF -1(r = -0.55, p < 0.002; Fig 4: D) in vitreous of PDR group whereas, in patients who underwent LASER treatment prior to surgery, APN negatively correlated with VEGF(r = - 0.55, p < 0.002; Fig 4: G).

Figure 1

Plasma levels of adipokines (Mean ± SEM). Levels of APN,VEGF,PEDF,IGF-1 in plasma of control (n = 14), patients with MH (n =11), patients with PDR (n = 26).

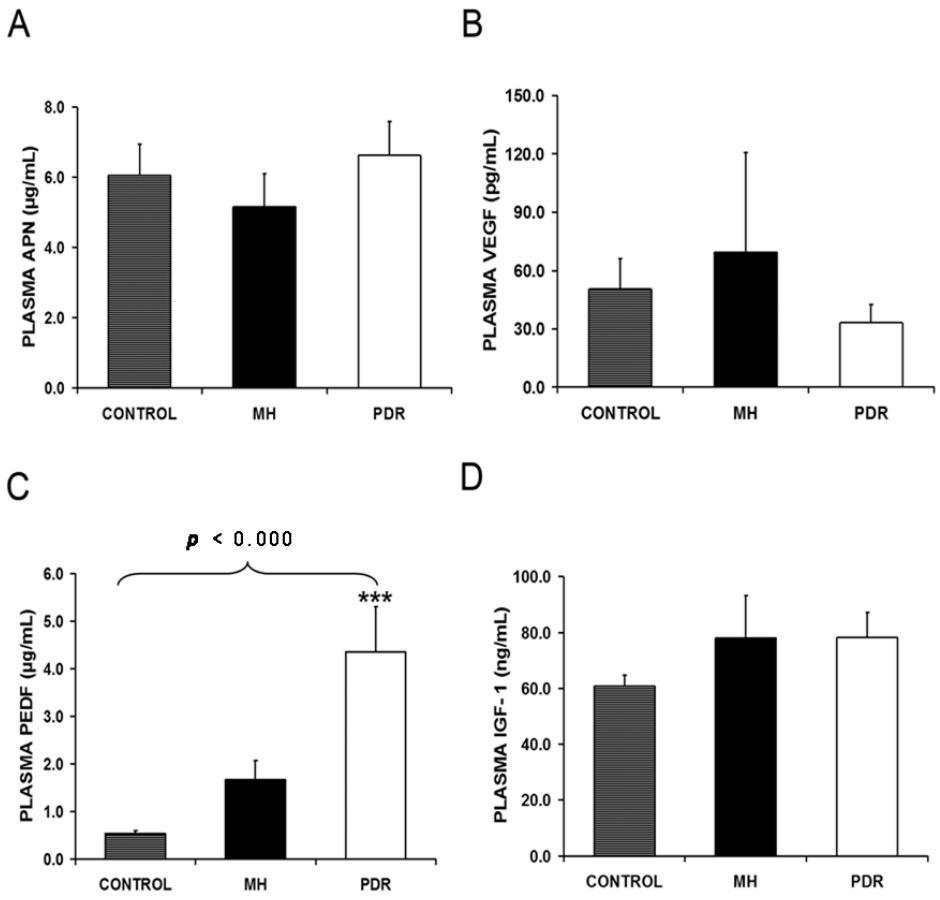

Figure 1: Plasma levels of adipokines (Mean ± SEM)

A) Levels of APN in plasma of control (n = 14), patients with MH (n =11), patients with PDR (n = 26). Plasma APN Levels were not significant among the groups.

B) Levels of VEGF in plasma. Plasma VEGF Levels were not significant among the groups.

C) Levels of PEDF in plasma. Plasma PEDF Levels were significant elevated in the patients with PDR (p<0.000) compared to control***

D) Levels of IGF -1 in plasma. Plasma IGF -1 Levels were not significant among the groups

Same number of control, MH, PDR were analyzed for B,C,D

Figure 2
Plasma APN, PEDF correlate with HDL and Glucose respectively

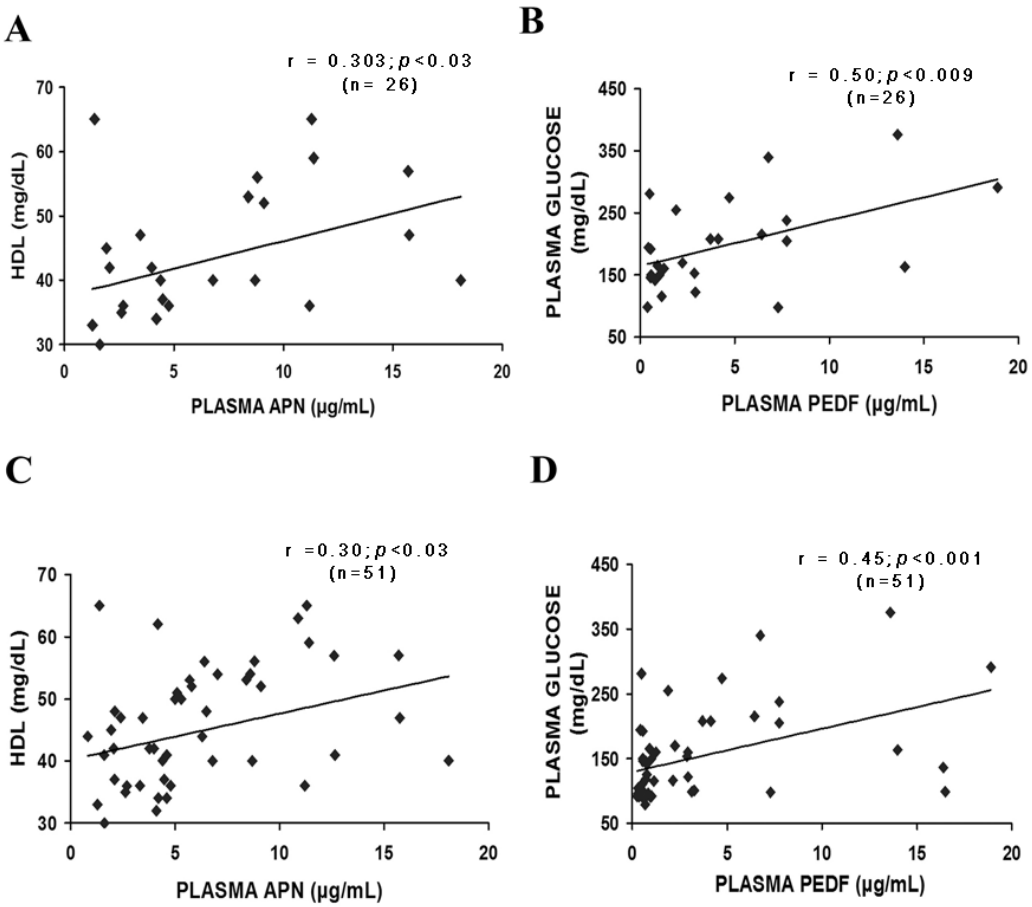

Figure 2: Plasma APN, PEDF correlates with HDL and Glucose respectively
A) Correlation between plasma APN and HDL cholesterol among PDR group
B) Correlation between plasma PEDF and plasma Glucose among PDR group
C) Correlation between plasma APN and HDL cholesterol in total study group
D) Correlation between plasma PEDF and plasma Glucose in total study group

Figure 3

Vitreous levels of adipokines (Mean ± SEM). Levels of APN, VEGF, PEDF,IGF-1 in vitreous of control (n = 14), patients with MH (n =11), patients with PDR (n = 26).

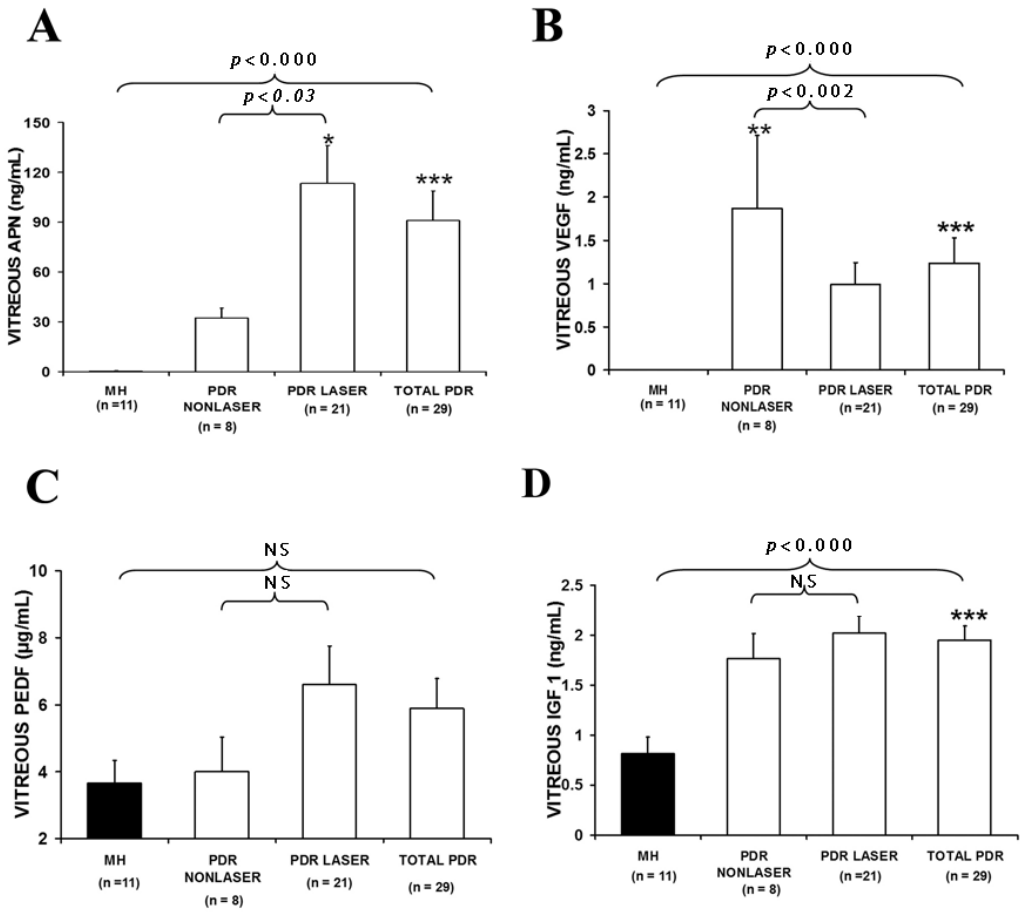

Figure 3: Levels of adipokines in Vitreous fluid. (Mean ± SEM)

- ***A) Levels of APN in the vitreous fluid of the patients with MH (n = 11), patients with PDR who had not undergone any laser treatment (n=8), patients with PDR who had undergone laser prior to vitrectomy (n=21), patients with PDR (n = 29). APN is significantly elevated in patients with PDR (*** p < 0.000) compared to MH. Among the PDR group, APN is significantly elevated in the PDR Laser (* p < 0.030) [patients who had undergone laser prior to vitrectomy] compared to the non lasered group.***
- ***B) Levels of VEGF in the vitreous fluid. VEGF is significantly elevated in patients with PDR (*** p < 0.000) compared to MH. Among the PDR group, VEGF is significantly decreased in the PDR Laser (** p < 0.002) [patients who had undergone laser prior to vitrectomy] compared to the non lasered group.***
- ***C) Levels of PEDF in the vitreous fluid. PEDF levels doesn't show any significance between PDR (NS - not significant) and MH or among the PDR group.***
- ***D) Levels of IGF- 1 in the vitreous fluid. IGF-1 is significantly elevated in patients with PDR (*** p < 0.000) compared to MH. Among the PDR group, there is no siginificant difference in IGF -1 levels***

Same number of control, MH, PDR were analyzed for B,C,D

Figure 4
Results of correlation studies between adipokines in Vitreous

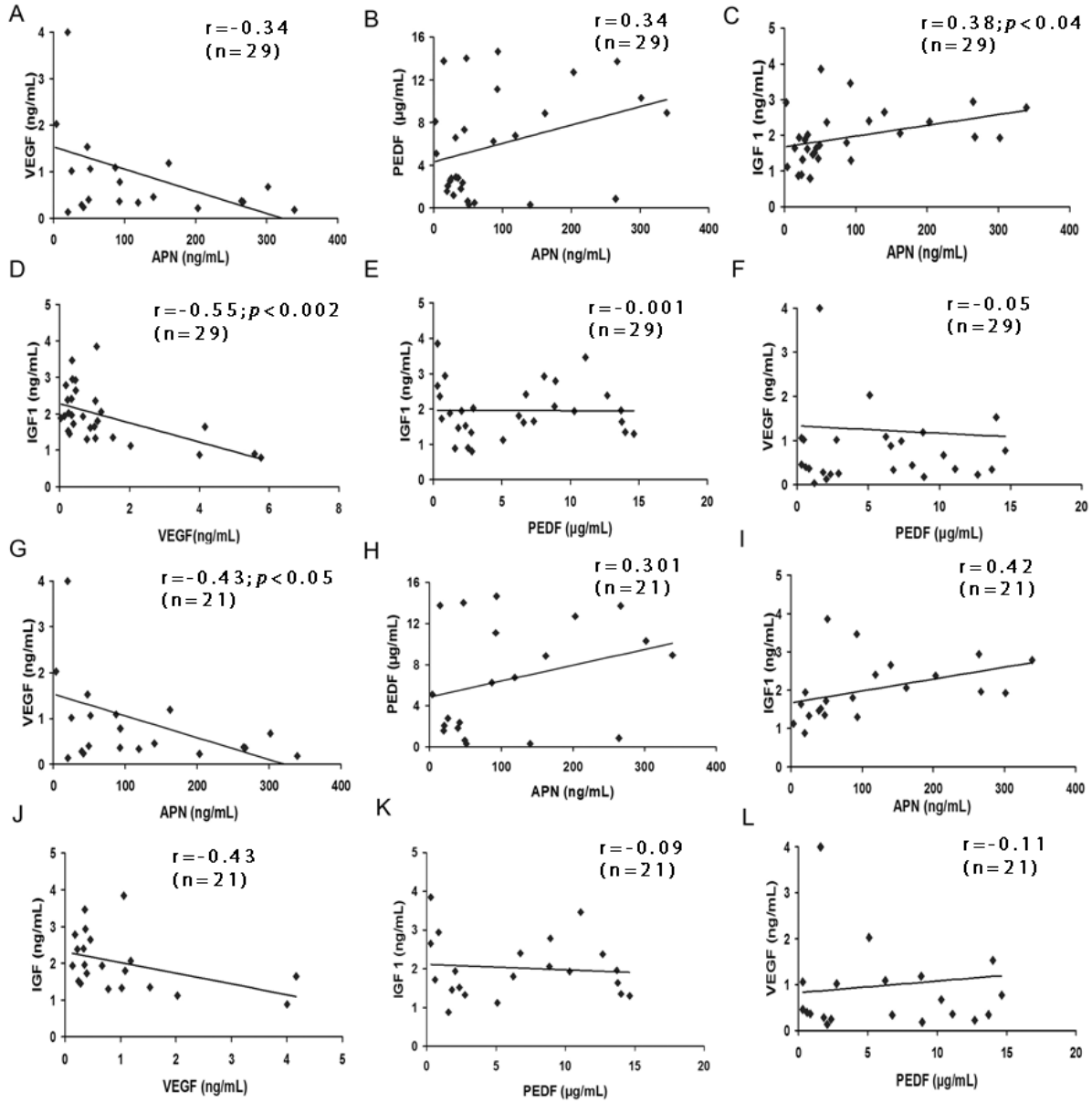

Figure 4: Results of correlation studies between adipokines in Vitreous

- **A) Correlation between vitreous APN and VEGF in PDR group (n = 29)**
- **B) Correlation between vitreous APN and PEDF in PDR group (n = 29)**
- **C) Correlation between vitreous APN and IGF -1 in PDR group (n = 29)**
- **D) Correlation between vitreous IGF -1 and VEGF in PDR group (n = 29)**
- **E) Correlation between vitreous IGF -1 and PEDF in PDR group (n = 29)**
- **F) Correlation between vitreous PEDF and VEGF in PDR group (n = 29)**
- **G) Correlation between vitreous APN and VEGF in PDR group who had undergone laser treatment prior to vitrectomy (n = 21)**
- **H) Correlation between vitreous APN and PEDF in PDR group who had undergone laser treatment prior to vitrectomy (n = 21)**
- **I) Correlation between vitreous APN and IGF -1 in PDR group who had undergone laser treatment prior to vitrectomy (n = 21)**
- **J) Correlation between vitreous IGF -1 and VEGF in PDR group who had undergone laser treatment prior to vitrectomy (n = 21)**
- **K) Correlation between vitreous IGF -1 and PEDF in PDR group who had undergone laser treatment prior to vitrectomy (n = 21)**
- **L) Correlation between vitreous PEDF and VEGF in PDR group who had undergone laser treatment prior to vitrectomy (n = 21)**

Supplement table. Patients particulars on age sex, diabetic status , medication and treatment

AGE/ SEX	DURATION OFDIABETES	Vitreous Haemorrage	Retinal Detachment	NVD/NVE	TREATMEN SYSTEMIC	OCULAR
Proliferative Diabetic retinopathy						
40/M	15	+	+	+	Insulin	Post laser vitrectomy
47/F	20	+	+	+	Insulin, T ramcor,T gloeye, E/D Acular	Post laser vitrectomy
48/M	15	+	+	+	insulin	Post laser vitrectomy
54/M	15	+	+	+	Insulin and vitamin tablets	Post laser vitrectomy
55/M	10	+	+	+	Insulin,T glucophage	Post laser vitrectomy
45/M	10	+	+	+	Insulin,T.Glynase	Post laser vitrectomy
55/M	10	+	+	+	ANTI DIABETIC DRUGSTablet name not known to patient	Post laser vitrectomy
47/M	10	+	+	+	T diapod, T atorva ,T livorir,T pionorm,T Clevepod,T Mxgalin	Post laser vitrectomy
45/F	10	+	+	No view	t.Metformin,T.Angiolock,T.Gliclazide	Post laser vitrectomy
63/M	15	+	+	+	Insulin Inj.,Htn medicines	Post laser vitrectomy
54/M	10	+	+	+	PiopodGM2, Remitorva, Voglitor, Diavit forte	Post laser vitrectomy
68/M	20	+	+	+	ANTIHYPERTENSIVE AND ANTI DIABETIC DRUGS	Post laser vitrectomy
55/M	10	+	+	+	T.Gliciride	Post laser vitrectomy
55/M	10	+	+	+	T.Actrapid,T.Alphapress,T.Diltizem, T.Dicaltrol,T.Atorva,T.Hypophos,T.Esomix	Post laser vitrectomy
51/F	10	+	+	+	inj.insulin,T.glucoret forte,T.stamlo	Post laser vitrectomy
53/M	10	+	+	+	T.Dianorm	Post laser vitrectomy
57/F	15	+	+	+	ANTI DIABETIC DRUGS	Post laser vitrectomy
54/F	15	+	+	+	nil	Post laser vitrectomy
60/M	15	+	+	+	T.ozomet,T.Amlopres	Post laser vitrectomy
41/F	17	+	+	+	inj.Insulin	Post laser vitrectomy
58/F	15	-	+	+	T. merthotexate,T. atenova,inj.insulin	Post laser vitrectomy
54/M	12	+	-	No view	Oral hypoglycemics,anti hypertensive and lipid lowering agents	Vitrectomy
56/M	10	+	pvd	No view	Insulin	Vitrectomy
56/M	10	-	+	+	T.Glucomet,C.Diataal,T.Tazlac	Vitrectomy
62/F	10	+	pvd	No view	T.metformin, t.dicynone,T.Losar,T.Revelotoxil	Vitrectomy
57/M	16	+	pvd	No view	Inj.Mixtard,T.Lasix,T.Envas,T.Roseday,T.Ecosprin	Vitrectomy
45/M	10	+			Oral hypoglycemics	Vitrectomy
60/F	10	+	+	No view	Oral hypoglycemics	Vitrectomy
Macular Hole						
61/F	Nil	-	-	-	Nil	Vitrectomy
53/M	Nil	-	-	-	Nil	Vitrectomy
44/M	Nil	-	-	-	Nil	Vitrectomy
60/F	Nil	-	-	-	Nil	Vitrectomy
68/F	Nil	-	-	-	Nil	Vitrectomy
65/F	Nil	-	-	-	Nil	Vitrectomy
62/F	Nil	-	-	-	Nil	Vitrectomy
65/F	Nil	-	-	-	Nil	Vitrectomy
70/F	Nil	-	-	-	Nil	Vitrectomy
61/M	Nil	-	-	-	Nil	Vitrectomy
66/M	Nil	-	-	-	Nil	Vitrectomy

NVD- Neovascularisation on disc
NVE- Neovascularisation elsewhere in retina
PVD – posterior vitreous detachment

DISCUSSION

In the present study, an attempt has been made to analyze the role of adipokines in PDR using the patient's plasma and vitreous in understanding the role of these adipokines in disease pathogenesis. Interestingly, we found that elevated levels of plasma PEDF in PDR than controls and also significantly higher levels of vitreous APN, VEGF, IGF-1 in PDR group when compared to those with MH. High levels of PEDF in the plasma of patients with PDR is not surprising since, it has already been reported that PEDF is one of the most abundant proteins secreted by adipocytes and induces insulin resistance and inflammatory signals in muscles and fat cells [25]. Increased plasma PEDF correlated with increased glucose in this study which also supports the implication of PEDF playing a role in insulin resistance. Blood levels of VEGF and IGF-1 were not different between the groups. Blood levels of APN were increased in PDR group compared to that of controls though the present study did not observe any statistically significant increase. It has been reported by Kato et al that blood levels of APN is elevated in patients with diabetic retinopathy, in addition to its positive correlation, with the severity of the disease [26]. Nevertheless, APN levels positively correlated with HDL cholesterol in this study, which is considered beneficial for vascular diseases [27]. Earlier Zietz et al generated a proof of concept for the presence of APN in vitreous by measuring APN level in just 5 vitreous samples, obtained after vitreoretinal surgery. The reported APN levels ranged from 2.0 to 70.2 ng/ml, which is in line, with our finding (2.28 to 301.8 ng/ml) where a larger sample size (n=29) was used [24]. Presence of APN in the subretinal fluid was reported by Ricker et al. APN levels were significant in PVR, positively correlated with cathepsin. APN is also considered as a predictor of redetachment in post-operative PVR [28]. Recently elevated levels of Aqueous humor APN in PDR patients were reported. These elevated levels may be a protective mechanism in PDR [29]. Ogata and Tombran have recently reported that low and elevated levels of PEDF in the vitreous and plasma respectively in patients with PDR [30]. Adamis reported the elevated levels of vitreous VEGF in PDR.[8] Similarly elevated levels of another angiogenic molecule IGF-1 in vitreous of PDR was also reported.[9] In our study vitreous levels of APN in PDR patients along with other angiogenic players VEGF, PEDF, and IGF-1 was estimated in 29 PDR samples. Further these adipokines levels were subjected to statistical correlation tests to understand their relationship between each other and with the disease pathogenesis. Even though, both VEGF and IGF -1, are proangiogenic we observed, a negative correlation between VEGF and IGF-1 in our study. Possible reason could be among 29 PDR samples 21 samples come under PDR laser group. During laser surgery, the hypoxic environment is reduced so as the VEGF levels [2], but the IGF -1 level doesn't get altered since it is a hypoxic independent adipokine [16]. On the other hand, we observed a positive correlation for APN with IGF-1. Besides its proangiogenic property IGF-1 is a known neuroprotective agent in retinal cells [14]. APN has been shown to have neuroprotective role in brain cells and might have a similar function in ocular tissues as well, where IGF-1 could be a positive regulator in that function. Moreover, there was a negative correlation between APN and VEGF in the vitreous of patients who underwent LASER treatment prior to surgery. It has been reported that APN is expressed in choroidal blood vessels, and this expression is increased upon LASER treatment in mice. Intravitreal and intraperitonial injection of recombinant APN decreased choroidal neovascularization by up to 68% and 78% respectively [23]. We found increased APN in the vitreous of patients with PDR who underwent LASER treatment prior to surgery, and this increase in APN correlates positively with PEDF, indicating that LASER treatment improves APN levels. At this point, we are yet to determine whether APN is pro or antiangiogenic in retina. However, our findings indicate it could inhibit VEGF

expression as reported earlier by Bora et al in APN treated mice with choroidal neovascularization [23]. Thus, its role as an antiangiogenic molecule is convincing.

CONCLUSION

In summary, Adipokines viz APN, VEGF, IGF-1 and PEDF are elevated in vitreous of PDR patients compared to MH. There is a 3 fold increase in APN level in patients, who had undergone LASER treatment, before vitrectomy and negatively correlated with VEGF. In addition, APN is localized in retinal tissues along with its two receptors indicating its strong association with PDR (unpublished observation). Plasma PEDF was elevated in patients with PDR, and positive correlation with plasma glucose was observed. Similarly, Plasma APN positively correlated with HDL cholesterol. However, further studies on APN are needed to elucidate therapeutic application for PDR.

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ABBREVIATIONS

APN – Adiponectin, ADIPOR1- Adiponectin receptor1, ADIPOR2 – Adiponectin receptor2, PDR - Proliferative diabetic retinopathy, MH – Macular hole, VEGF – Vascular endothelial growth factor, PEDF- pigment epithelium derived growth factor, PPARα - peroxisome proliferator–activated receptor, AGE- Advanced glycation end products, RAGE- Receptor for advanced glycation end products, PRP- Panretinal photocoagulation

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