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Vitreous and Serum Levels of Apelin in Type 2 Diabetic Patients with Diabetic Retinopathy

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ABSTRACT

Background: Diabetic retinopathy (DR) is the most common microvascular complication of diabetes, and is the leading cause of blindness and visual impairment in the working-age population. Retinal neovascularization is the most important clinical features of proliferative DR (PDR). Apelin is an endogenous ligand of the G protein-coupled receptor, which has been shown to be involved in retinal angiogenesis.

Objective: To study vitreous and serum levels of apelin in type 2 diabetic patients with diabetic retinopathy.

Patients and methods: Case control study was conducted on total 60 subjects, 40 type 2 diabetic patients and 20 non diabetic subjects as control group. Diabetic patients were divided into 2 groups.

Results: Serum and vitreous levels of apelin were significantly higher in patients with PDR compared to NPDR and control group (P< 0.001). There was non-significant correlation between serum and vitreous apelin. Serum apelin showed lower cut off value (>4.45 ng/ml (P<0.001) with 96.7% sensitivity and 96.7 specificity) for detection of PDR compared to control group than vitreous apelin. Also serum apelin showed lower cut off value >3.55 ng/ml for detection of NPDR compared to control group.

Conclusion: Elevation of serum apelin in patients with PDR compared to NPDR and control group suggesting the role of apelin in development of retinal neovascularization and diabetic retinopathy. So antagonizing effect of apelin can be used as targeted therapy for treatment of diabetic retinopathy. We can rely on serum apelin for early detection of diabetic retinopathy than vitreous apelin.

Keywords: Type 2 diabetes mellitus, proliferative diabetic retinopathy (PDR), non-proliferative diabetic retinopathy (NPDR), Serum and vitreous apelin.

INTRODUCTION

Diabetic retinopathy (DR) is one of the most serious microvascular complication of diabetes mellitus and is one of the leading causes of blindness in working-age population. There are many angiogenic factors including cytokines, inflammatory cells, growth factors, have been identified to play important roles in the pathogenesis of retinal neovascularization including vascular endothelial growth factor (VEGF) and apelin which is a newly discovered adipokines involved in many metabolic disorders [(1)].

Apelin is produced and secreted by both human and mouse white adipose tissue. Studies have shown that apelin was involved in vascular pathophysiology and act as an angiogenic factor stimulating retinal endothelial cells' proliferation, migration and vascular tube formation [(2)].

Furthermore, studies found that inhibition of apelin/apelin receptor (APJ) system prevented rapid abnormal vessel growth. Deficiency of apelin successfully inhibited hypoxia-induced retinal angiogenesis in mice despite upregulation of VEGF. Apelin was shown to affect many biological functions in mammals, such as adjusting the neuroendocrine, cardiovascular, and immune systems by autocrine, paracrine, endocrine and exocrine signaling [(3)].

Animal studies demonstrated that inhibition of apelin-APJ system facilitated retinal vessel maturation in ischemic retinopathy model, and inhibition of apelin expression switched endothelial cells from proliferative to mature state in pathological retinal angiogenesis [(4)]. So in our study we assessed serum and vitreous level of apelin in diabetic retinopathy to detect possible role of apelin in development of diabetic retinopathy and its use as a serum marker in early detection of diabetic retinopathy and as a targeted therapy of retinopathy.

Aim of the work to study serum and vitreous level of apelin in type 2 diabetics with diabetic retinopathy.

PATIENTS AND METHODS

Study design:

Case control study was conducted on total 60 subjects, 40 type 2 diabetic patients and 20 non-diabetic subjects suffering from other conditions necessitating vitrectomy such as retinal detachment as control group. Diabetic patients were divided into 2 groups. Group1: 20 type 2 diabetic patients with proliferative retinopathy (PDR); group 2: 20 type 2 diabetic with non-proliferative retinopathy. They were collected from endocrinology and ophthalmology outpatient clinics of Ain Shams University Hospitals

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during the period from February 2017 to October 2018.

Exclusion criteria:

1. Diabetic patients complicated with infection
2. Systemic lupus erythematosus or rheumatoid arthritis.
3. Diabetic nephropathy, any co-morbid end–organ failure, systematic or ocular historical treatment with anti-VEGF therapy.
4. Previous ocular surgery, and ocular tumors.

Ethical approval and written informed consent:

An approval of the study was obtained from Ain Shams University Academic and Ethical Committee. Every patient signed an informed written consent to participate in this study after full explanation of the study.

Clinical assessment:

All participants were subjected to full detailed medical history including age, sex, body weight, height, BMI, duration of diabetes, and presence of diabetic complications. Ocular examination including a slit-lamp biomicroscopy and dilated ophthalmoscopy. Fundus fluorescein angiography was done in patients with retinal hemorrhage, exudation or microaneurysm. Patients were divided into PDR (PDR) group and NPDR group according to international clinical classification for DR [(5)].

Laboratory assessment:

Biochemical assays included fasting blood glucose (FBG), 2h postprandial glucose (PPG) and glycated hemoglobin (HbA1c), lipid profile, albumin to creatinine ratio (ACR) in urine and serum level of apelin by ELISA were measured in all patients.

Vitrectomy Procedure:

Vitreous body sample collection and determination of apelin concentration through pars plana vitrectomy that was done in a standardized technique involving three pars plana sclerotomy incisions as standard surgical and therapeutic procedure for these cases. The infusion was first shifted to air and any fluid was expressed out of the infusion cannula by air presser, then 23 trocars were inserted 3.5 mm into paras plana posterior to the limbus in pseudophakic eyes and 4 mm in phakic eyes, the infusion was then connected and turned on. Active cutting at 2500 cpm under venturi effect was activated and vitreous was removed from the eye and replaced by air, no fluid was introduced to the eye prior to this step, so the undiluted vitreous was now filling the vitrectomy tube line. The vitrectomy line was disconnected from the vitrectomy machine and reconnected to 10 cc syringe and all vitreous material present in the tube was aspirated in to the syringe which was labeled and sent in ice box to the lab. The concentrations of apelin (ng/ml) in the vitreous was measured by using enzyme-linked immunosorbent assay (ELISA) [(6)].

Statistical analysis

Analysis of data was done using SPSS (Statistical Package for the Social Science) program version 24. Quantitative data were presented as mean and standard deviation. Qualitative data were presented as count and percentage. Student t-test was used to compare quantitative data between two groups and one-way ANOVA was used when more than two groups were to be compared then post hoc test was used to detect the difference between individual groups. Chi-square test was used to compare qualitative data between different groups, Pearson correlation test was used to compare correlation between different continuous variables. ROC curve was used to measure diagnostic validity and determine the best cut off value for some variables. Multiple linear regression analysis was used to measure independent effect of different variables on some outcomes. P value < 0.05 was considered statistically significant. The confidence interval was set to 95% and the margin of error accepted was set to 5%.

RESULTS

There was highly significant difference as regard BMI on comparing 3 groups together; being higher in PDR compared to the other 2 groups. The duration of diabetes mellitus showed highly significant increase in PDR compared to NPDR. The groups didn't vary significantly regarding age and sex (Table 1).

Table (1): Demographic data of the studied groups.

		PDR (n=20)		NPDR (n=20)		Control (n=20)		P value
		Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	
Age (years)		50.27	7.01	51.27	7.05	50.60	7.33	>0.05
BMI (kg/m$^{2)}$		30.80[a]	4.07	27.17[b]	3.46	24.60[c]	3.06	**<0.001**
Duration (years)		11.80	2.58	8.90	1.69			**<0.001**
		N	**%**	**N**	**%**	**N**	**%**	**P value**
Sex	**Male**	17	56.7%	18	60.0%	15	50.0%	>0.05
	Female	13	43.3%	12	40.0%	15	50.0%	
	Total	30	100%	30	100%	30	100%	

Our study showed highly significant elevation of serum and vitreous apelin on comparing the 3 groups together being the highest in PDR. There were highly significant difference as regard FBG, 2HPP, HBA1C and serum triglycerides and LDL cholesterol on comparing different groups together (Table 2).

Table (2): Laboratory data of the studied groups.

	PDR (n=20)		NPDR (n=20)		Control (n=20)		P value
	Mean	SD	Mean	SD	Mean	SD	
S. apelin (ng/ml)	6.37[a]	1.03	3.75[b]	0.53	3.37[c]	0.59	**<0.001**
V. apelin (ng/ml)	9.88[a]	1.81	7.23[b]	0.77	4.06[c]	0.87	**<0.001**
FBG (mg/dl)	171.00[a]	32.37	163.10[a]	37.13	86.07[b]	8.73	**<0.001**
2 Hpp (mg/dl)	261.90[a]	7.32	204.90[b]	5.42	121.10[c]	14.91	**<0.001**
HbA1C %	10.78[a]	1.77	8.91[b]	1.10	4.45[c]	0.47	**<0.001**
LDL cholesterol (mg/dl)	241.20[a]	47.36	189.83[b]	21.65	184.00[b]	11.68	**<0.001**
S. TG (mg/dl)	179.73[a]	38.59	165.70[b]	16.97	143.33[c]	14.42	**<0.001**

There was highly significant positive correlation between serum apelin and BMI, duration of diabetes mellitus, HbA1C and serum LDL cholesterol and with 2HPP, while there was non-significant correlation with age and serum triglycerides and FBG (Table 3).

Table (3): Correlation between serum apelin, clinical and laboratory data in diabetic patients.

	Serum apelin	
	r*	P value
Age (years)	-0.15	>0.05
BMI(kg/m^2	0.47	**<0.001**
Duration(years)	0.48	**<0.001**
FBS(mg/dl)	0.07	>0.05
2Hpp(mg/dl)	0.37	**0.004**
HbA1C%	0.48	**<0.001**
LDL cholesterol (mg/dl)	0.55	**<0.001**
Triglycerides(mg/dl)	0.20	>0.05

*Pearson correlation coefficient.

There was highly significant positive correlation between vitreous apelin and BMI, duration of diabetes mellitus, HBA1C and LDL cholesterol, and with 2HPP and serum triglycerides, while there was non- significant correlation with age and FBG (Table 4).

Table (4): Correlation between vitreous apelin, clinical and laboratory data in diabetic patients:

	Vitreous apelin	
	r*	P value
Age (years)	0.04	>0.05
BMI (kg/m^2	0.46	**<0.001**
Duration (years)	0.39	**0.002**
FBS (mg/dl)	0.08	>0.05
2Hpp (mg/dl)	0.33	**0.01**
HbA1C %	0.40	**0.002**
LDL cholesterol (mg/dl)	0.39	**0.002**
Triglycerides (mg/dl)	0.35	**0.01**

*Pearson correlation coefficient.

There was no significant correlation between serum and vitreous apelin in PDR and NPDR. (Table 5)

Table (5): Correlation between serum and vitreous apelin in diabetic patients.

Vitreous apelin	Serum apelin	
	r*	P value
PDR group	-0.14	>0.05
NPDR group	0.03	>0.05

*Pearson correlation coefficient

In the multiple regression analysis with all the significant variables from the Spearman's correlation analyses confirmed that only BMI remained significantly associated with vitreous apelin, while there were non-significant associations of other variables with serum and vitreous apelin (Tables 6 and 7).

Table (6): Regression analysis of factors affecting serum apelin in diabetic patients

	Unstandardized Coefficients		Standardized Coefficients	Sig.	95.0% Confidence Interval for B	
	B	Std. Error	Beta		Lower Bound	Upper Bound
2Hpp mg/dl	-0.004	0.004	-0.204	>0.05	-0.012	0.003
HbA1C%	0.023	0.142	0.044	>0.05	-0.262	0.309
LDL cholesterol mg/dl	0.009	0.005	0.384	>0.05	-0.001	0.019
BMI kg/m^2	0.016	0.042	0.091	>0.05	-0.068	0.101
Duration (years)	0.131	0.069	0.264	>0.05	-0.007	0.269

Table (7): Regression analysis of factors affecting vitreous apelin in diabetic patients

	Unstandardized Coefficients		Standardized Coefficients	Sig.	95.0% Confidence Interval for B	
	B	Std. Error	Beta		Lower Bound	Upper Bound
2Hpp mg/dl	-0.006	0.005	-0.170	>0.05	-0.017	0.004
HbA1C %	0.124	0.198	0.142	>0.05	-0.273	0.521
LDL cholesterol mg/dl	0.002	0.007	0.057	>0.05	-0.011	0.016
BMI kg/m^2	0.160	0.058	0.535	0.008	0.043	0.277
Duration years	0.136	0.096	0.165	>0.05	-0.057	0.328

Also our study showed that serum apelin showed a highly significant cut off value >4.50 ng/ml (P<0.001) with 96.7% sensitivity and 93.3% specificity to differentiate between PDR and NPDR. While serum apelin showed a highly significant cut off value >4.45 ng/ml (P<0.001) with 96.7% sensitivity and 96.7 specificity to differentiate between PDR and control. Also vitreous apelin showed a highly significant cut of value >8.30 ng/ml (P<0.001) with 73.3% sensitivity and 100% specificity to differentiate between PDR and NPDR. Vitreous apelin showed a highly significant cut off value >5.80 ng/ml (P<0.001) with 100%sensitivity and 96.7% specificity to differentiate between PDR and control group. While serum apelin showed a highly significant cut off value >3.55 ng/ml (P=0.008) with 70% sensitivity and 60% specificity to differentiate between NPDR and control. While vitreous apelin showed a highly significant cut off value >5.20 ng/ml (P<0.001) with 100% sensitivity and 96.7%spcificity to differentiate between NPDR and control group.

DISCUSSION

Apelin plays a role in stimulating retinal endothelial cells' proliferation, migration and vascular tube formation. Otherwise, the underlying mechanisms which apelin was involved in retinal angiogenesis was unclear. Some studies found that apelin was related with oxidative and inflammation markers [(1)].

Our study showed that there was highly significant elevation of serum apelin in PDR compared to NPDR and control group. This agreed with **Du *et al.*** [(1)] while no statistically significant difference was found between PDR group and NPDR group; this comes in agreement with **Yonem *et al.*** [(7)].

Furthermore we found that vitreous apelin was highly significantly elevated in PDR compared to NPDR and control group. This coincides with **Tao *et al.*** [(8)], who also found that serum and vitreous apelin was significantly higher in PDR compared to control group.

Our study showed that there was highly significant positive correlation between serum apelin and BMI, HbA1C and with 2HPP. This agreed with **Yu *et al.*** [(9)] and **Cavallo *et al.*** [(10)] who found that apelin levels in serum were increased and associated with glucose homeostasis, and there was highly significant positive correlation between serum apelin and BMI in T2DM and this was consistent with **Du *et al.*** [(1)] suggesting that apelin levels maybe associated with obesity.

While **Cavallo *et al.*** [(10)] found that obese patients with T2DM had significantly higher apelin levels than non-diabetic obese subject, confirming that increased apelin levels are directly associated with the presence of diabetes rather than obesity itself.

Our results showed that there was highly significant positive correlation of serum apelin and LDL cholesterol. This agreed with **Li *et al.*** [(11)].

Also we found highly significant positive correlation of vitreous apelin with Hba1c, low density lipoprotein, BMI and duration of diabetes. Unfortunately there are limited data about vitreous apelin and different clinical and laboratory parameters.

Our study showed that there was no correlation between serum and vitreous apelin. There are limited data about vitreous apelin and its correlation with serum apelin.

CONCLUSION

Elevation of serum apelin in patients with PDR compared to NPDR and control group suggesting the role of apelin in retinal neovascularization. Also serum apelin (4.5 ng/ml) showed higher sensitivity and lower cut off value in detection of diabetic retinopathy compared to invasive vitreous apelin, so serum apelin is good marker for diabetic retinopathy. Understanding its role in development of diabetic retinopathy makes it hopeful target for treatment of diabetic retinopathy.

DECLARATIONS

1. Ethics approval ----this study was approved by the ethics committee of Ain Shams University with approval number FWA 000017585
2. The authors declare no conflict of interests
3. There was no financial funding from any institution
4. We declare receiving no grants for this study.

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