Pigment Epithelium-Derived Factor in Proliferative Diabetic Retinopathy
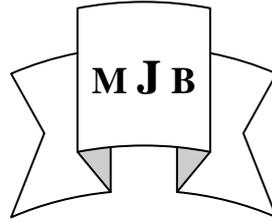
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Abstract

Diabetic retinopathy (DR) is the most common microvascular complication of diabetes mellitus. Pigment epithelium-derived factor (PEDF) is a strong inhibitor of angiogenesis. Our aim was to address the predictive value of anti-angiogenic marker(PEDF) for progression of DR.

A total of 118 subjects (healthy, diabetic without retinopathy and diabetic retinopathy) were studied.Serum angiogenic inhibitor PEDF were determined and the relationship between the DR, levels of PEDF, age, HbA1c, and duration of diabetes were evaluated.

The mean of PEDF in sera of patients with proliferative diabetic retinopathy (6.74 ± 2.20 Pg/ml) was significantly higher (p=0.01) than that of healthy control (4.25 ± 0.81 Pg/ml);So The levels of PEDF increases with progress of diabetic retinopathy and thus, increased levels of PEDF in the blood indicate microvascular damages in diabetic patients and may be predictor of the progression of DR.

Keywords: Diabetic retinopathy, Pigment epithelium-derived factor.

مستوى عامل الصباغ في مصل مرضى اعتلال الشبكية السكري المتقدم

الخلاصة

اعتلال الشبكية السكري هي احدى مضاعفات الاوعية الدموية الدقيقة الأكثر شيوعا في مرض السكري و عامل الصباغ (PEDF)هو مثبط قوي لتولد أوعية دموية غير طبيعية لذلك الهدف من الدراسة هومعرفة وتحديد القيمة التنبؤية للعلامات المضادة للوعائية لتطور اعتلال الشبكية السكري.

شملت الدراسة ١١٨ شخص منهم اصحاء واخرون مصابون بمرض السكري دون اعتلال الشبكية السكري وبوجود اعتلال الشبكية السكري. تم قياس عامل الصباغ في المصل وجرى تقييم العلاقة بين اعتلال الشبكية السكري ،مستويات PEDF، العمر، نسبة HbA1c، ومدة المرض.

اظهرت النتائج ان متوسط مستوى المصل PEDF في المرضى الذين يعانون اعتلال الشبكية المتقدم (٦،٧٤ بيكوغرام / مل) أعلى من بكثير من الاشخاص الاصحاء (٤،٢٥ بيكوغرام / مل).

من ذلك نستنتج ان مستويات PEDF يزيد مع تقدم اعتلال الشبكية السكري وبالتالي فان زيادة مستويات PEDF في الدم تشير إلى أضرار الاوعية الدموية الدقيقة في مرضى السكري، وربما يكون مؤشرا لتقدم اعتلال الشبكية السكري.

الكلمات الدليلية: اعتلال الشبكية السكري، عامل الصباغ.

Introduction

Diabetic retinopathy (DR) is one of the most common complications of diabetes, affecting millions of working adults worldwide, in which the retina progressively damaged leading to blindness [1].

In the year 2000, there were around 171 million people with diabetes globally, and by 2030, it is estimated that this number would increase to 366 million [2]. As the number of persons with diabetes increases, the development of microvascular complications like retinopathy also rises. DR is responsible for 4.8% of the 37 million cases of blindness throughout the world [3]. The mechanisms underlying the development of DR are not fully understood; however, with early detection and treatment, visual loss may be limited. The magnitude of damage caused by these microvascular complications of diabetes stresses the need for sensitive markers of screening for retinopathy.

❖ **The two broad categories of DR are:**

a. Non Proliferative Diabetic Retinopathy (NPDR)

Non proliferative diabetic retinopathy is characterized by retinal micro-aneurysms (Ma), intraretinal hemorrhages (blot, dot, or flame), hard exudates, soft exudates (cotton wool spots), venous looping, and/or venous beading (VB). Moderate to severe hemorrhage or micro-aneurysms (H/Ma) are significant risk factors for progression to PDR [4].

b. Proliferative Diabetic Retinopathy (PDR)

The most severe form of DR is PDR. Most patients with PDR are at significant risk for vision loss. Characteristics of the disease include new vessels on or within one disc diameter (1 DD) of the optic disc (NVD) or new vessels elsewhere in the retina outside the disc and 1 DD from disc [5].

There are many methods to diagnose DR, such as ophthalmoscopy, fluorescent angiography, and fundus photography but all of these ophthalmic diagnostic approaches must be conducted by efficient ophthalmologists and require invasive and expensive procedures. The identification of peripheral blood biochemical parameters including angiogenic profile for DR could be helpful for early detection and management of patients with DR before vision loss.

PEDF is a 50-kDa glycoprotein initially isolated from fetal human retinal pigment epithelial cells [6] and was later found to be expressed in various tissues and cells [7,8], including endothelial cells, osteoblasts [9,10], plasma [11], and liver[12]. PEDF is a member of the serpin super-family of serine protease inhibitors[6]. However, unlike many serpins, PEDF does not inhibit serine proteases[13].it is a multifunctional secreted protein [6] that has anti-angiogenic, antivasoper-meability [14], antiinflammatory [15], antifibrosis [16], antitumorigenic [17] and neurotrophic [18] functions.

PEDF inhibit the migration of endothelial cells in vitro in a dose-dependent manner and was more effective than angiostatin, thrombospondin-1, and endostatin [19]. These results placed PEDF among the most potent natural inhibitors of angiogenesis.

PEDF expression is upregulated by angiostatin [20, 21]. Hypoxia leads to the downregulation of PEDF [21]. This effect is due to the fact that hypoxic conditions cause matrix metalloproteinases (MMPs) to proteolytically degrade PEDF [22]. Secreted PEDF binds a receptor on the cell surface termed PEDF-R [23]. PEDF enhances gamma-secretase activity, leading to the cleavage of the VEGF receptor 1 (VEGFR-1) transmembrane domain [24]. This action interferes with VEGF signaling thereby inhibiting angiogenesis [25].

Aims of the Study

1- Determination the level of PEDF in sera of patients with diabetic retinopathy.

2- Study the relevance between the level of PEDF and DR.

3- Assessment the relation between PEDF, duration of diabetes, age, and HbA1c.

Subjects and Methods

The study was conducted in the city of Hilla, from December 2011 to February 2013, this case-control study enrolled 118 subjects which attended different medical centers including Al-Hilla teaching general hospital, and Marjan medical city. Informed consent was obtained from all participants; the practical side of the study was performed at general health laboratory in Hilla and lab of clinic in Al-Hilla teaching general hospital

Sixty four Patients with DR were divided into 2 groups, group (1): 42 NPDR patients with age mean 53.8 ± 8 years and group (2): 22 PDR patients with age mean 51.8 ±10 years

those were recruited from the Ophthalmological Clinic, and had underwent complete ophthalmological examination, including best corrected visual acuity, and slit-lamp examination with high power condensing lens (78,90diopter) was done after pupillary dilation by tropicamide 1% ophthalmic drops. The examination was performed by senior ophthalmologist.

Control include fifty four subjects: 29 diabetic non retinopathy(DNR) with age mean 49.3 ±13 years and 25 healthy volunteers (HC) with no history of diabetes, or any major clinical disorders with age mean 47.15±13 years.

> **Exclusion criteria**

I. Any acute illness and chronic disease that may interfere with the result of measured parameters
II. Evidence of nephropathy
III. Neuropathy
IV. Coronary heart diseases
V. Hypertension.
VI. Smoking and alcohol.

Serum PEDF was measured using ELISA Kit (BioProducts MD-U.S.A), blood sugar and glycated Hb (quantitative colorimetric method in whole blood by Stanbio Glycohemoglobin (Pre-Fil®) kit-Texas) also measured.

Statistical analysis

Statistical analysis were performed using SPSS 17.0 (SPSS Inc, Chicago, IL, USA), clinical data were compared by one-way analysis of variance (ANOVA) and statistical significance was defined as $P< 0.05$

Results

> **Characters of patients**

The baselines characteristics of studied groups are summarizes in table (1).

Table 1 Clinical parameters of the Study Subjects

Parameters	HC	DNR	NPDR	PDR	P-value
No.	25	29	42	22	
Age (years)*	47.15 ±13	49.35 ±13	53.81 ±8	51.80 ±10	0.276
Duration (years)*		5.7 ±4	9.8 ±7	12.2 ±6	0.003
HbA1c (%)		9.2 ±4	7.3 ±2	7.1 ±1	0.167
Patients on insulin therapy (%)		27%	57%	71%	0.082

*Values are given as mean ± S.D.,HC: healthy control, DNR: diabetic non retinopathy, NPDR: non proliferative diabetic retinopathy, PDR: proliferative diabetic retinopathy.

The results of this study revealed no significant difference in age between all diabetic groups (DNR, DNPR, PDR) P value > 0.05 as demonstrated in fig. (1), and there is significant difference in duration of diabetes between diabetic and retinopathy groups (P= 0.003) as shown in fig. (2).
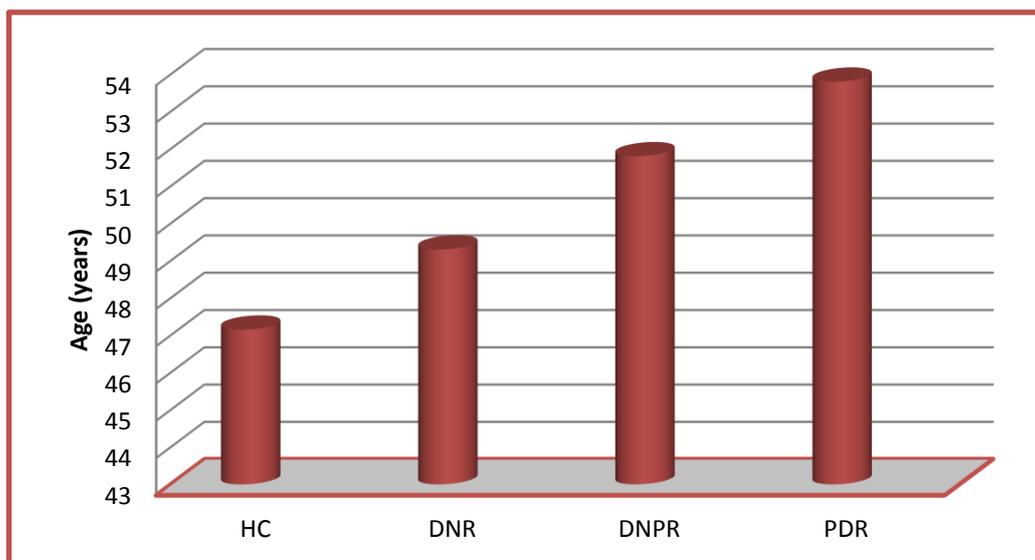

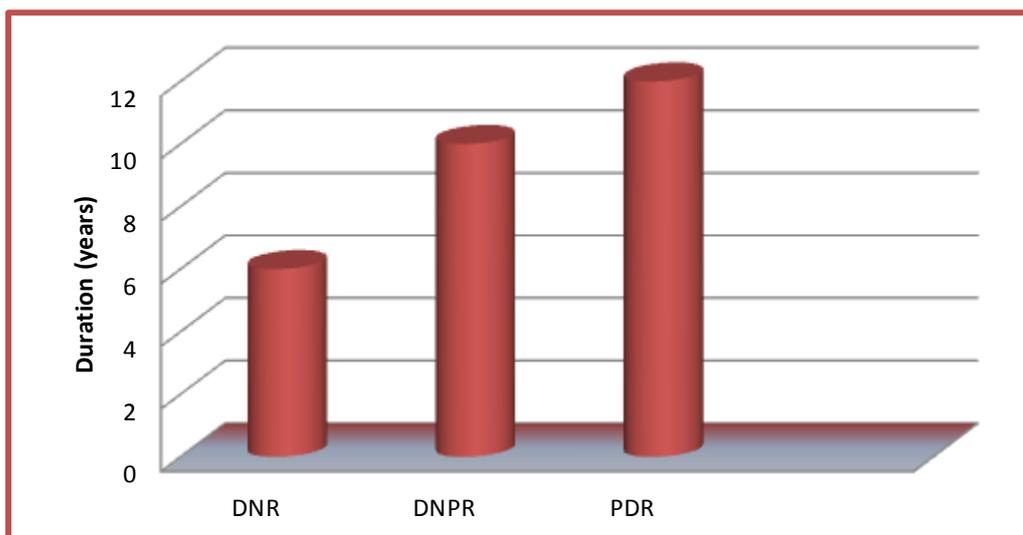**Figure 1** age in years express as mean of all studied groups

Figure 2 Duration of diabetes (mean) in all diabetic groups

Meanwhile there is no significant difference in HbA1c mean between DNR, DNPR and PDR groups (P=0.167) but at the same time the incidence of retinopathy is higher in uncontrolled diabetes (HbA1c >7%) than controlled (HbA1c <7%) as revealed in fig. (3).
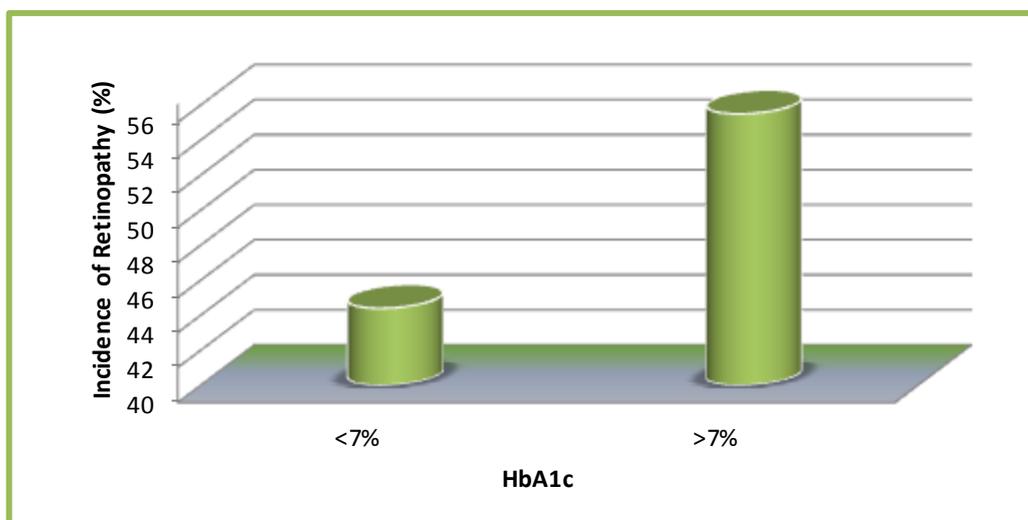

Figure 3 Incidence of retinopathy (%) by glycated hemoglobin value

Also those patients on insulin treatment were found to be 4.5 times more likely to develop retinopathy than those on dietary treatment alone (95% C.I. 0.83-24.18).

> **PEDF**

This study shows that the plasma level of PEDF was significantly different between groups (HC, DNR, NPDR, PDR) (p=0.001) as shown in fig. (4) and significantly higher in PDR group compare to HC group(p=0.002), DNR group (p=0.002) and NPDR group (P=0.001)

An insignificant relation ship between PEDF , HbA1c, age, duration, and insulin treatment (p>0.05) is detected which indicate that PEDF is an independent factor in pathogenesis of diabetes.
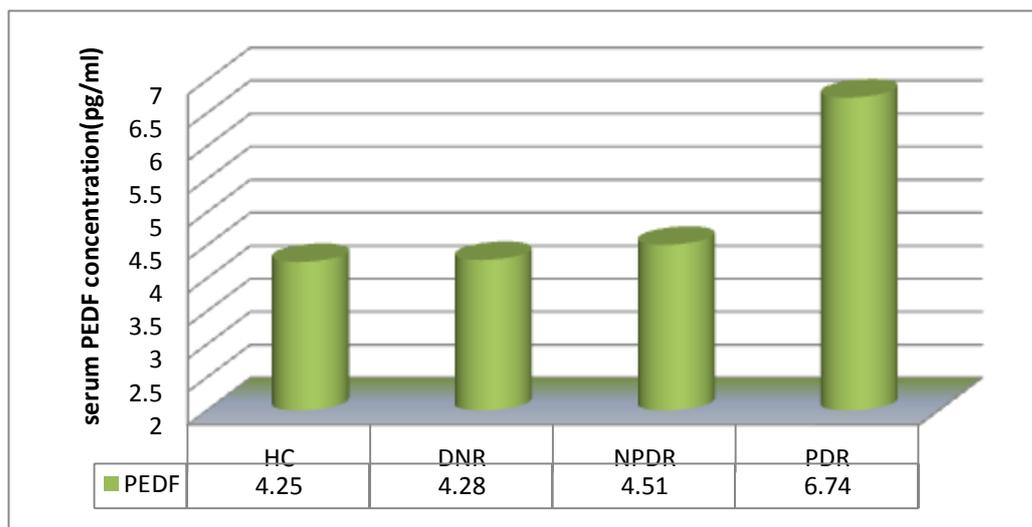
Figure 4 Concentration of serum PEDF (mean) in studied groups

Discussion

If PEDF concentration predicts adverse outcomes, its measurement may facilitate risk estimation, and PEDF-based interventions might be considered.

PEDF is synthesized in a wide range of human tissues including the lung, brain, kidney, and especially the liver [12], which may contribute to the high levels of PEDF in the blood. PEDF is most likely associated with the metabolism in patients with diabetes mellitus and may be associated with vascular damage. Vascular endothelial growth factor (VEGF) is a strong angiogenic factor, and many studies have demonstrated that VEGF induces the progression of diabetic retinopathy.

Advanced glycation end products (AGEs) in diabetic patients are also involved in the leukostasis and microthrombosis that result in PDR; it has been suggested that PEDF counteracts the effects of VEGF [26], and it also been suggested that PEDF significantly inhibits AGE activity[27] thus, increased levels of PEDF in the blood of patients with the PDR may be a response to counteract the activity of VEGF and AGEs. Previous studies demonstrated that the level of PEDF

was lower in eyes with diabetic retinopathy, especially in eyes with PDR [28-31]. These findings indicated that the decrease of PEDF in the eyes might be involved in the progression of diabetic retinopathy and the degree of retinal neovascularization because, PEDF is a potent anti-angiogenic and antiinflammatory cytokine [32-33], PEDF may be consumed in the eye with diabetic retinopathy to counteract the angiogenic and inflammatory responses of the endothelial cell. Our study is consistence with study done by Nahoko Ogata et al.[34] which found The plasma level of PEDF in the PDR group was significantly higher than that of controls.

The first line in treatment of diabetic patients is diet regime but most patients not obey the rules and doctors will in turn describe drugs (oral glycemic therapy) and when there is no or little response or complication occur insulin treatment will added .

We found that patients on insulin treatment were 4.5 times more likely to develop retinopathy than those on dietary treatment alone. It is understandable that those on insulin were more likely to develop diabetic retinopathy. For type 2 diabetics,

insulin therapy is typically an indication that an individual has had poor blood sugar control on oral hypoglycemics and, as such, it is also an indication of more advanced disease. Diabetic retinopathy would be expected to be more prevalent under these circumstances and this result similar to the results of Xiufen Yang et al and Hanan Fouadi et al [35,36].

American Diabetes Association considers HbA1c one of depending criteria for diagnosis of diabetes and its measuring became important for monitoring glycemic control, in this study we found no significant difference in HbA1c mean between diabetic and retinopathy groups (P=0 .167) which is similar to the findings of many studies :Mojca Globočnik Petrovič, Ying and Takuya Awata [37,38,39] And contrast to finding of other studies like Mostafa Feghhi, and Manaviat MR[40-43] but the incidence of retinopathy is higher in uncontrolled diabetes (HbA1c >7%) than controlled as demonstrated documented that good glycemic control remains crucial in prevention of late diabetic complications . Lastly the study revealed that with increment of the duration the risk of retinopathy increase (p=0 .001) as most of studies revealed such as those done by Jacek P. Szaflik, Shinko Nakamura and Sotoodeh Abhary [37,39,41,43,44]so duration considered as risk factor for diabetic retinopathy[45] which may belong to that when duration of disease increase, exposure to pathological factors increase and development of complication become more likely, in spite that other studies reveal no significant difference in duration between DNR and DR [40,36].

Conclusion

The PEDF level in the blood is elevated in diabetic patients, especially in those with PDR compared to healthy control. However, the results of our present study show that the plasma PEDF levels were significantly higher in patients with PDR. Furthermore, studies with paired sets of plasma and ocular PEDF levels in diabetic patients may reveal the correlation more clearly.

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