

Role of Endothelin -1 in Plasma and Vitreous Humour in Type 1 and Type 2 Diabetic Retinopathy

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Purpose: The aim of our study was to compare the level of Endothelin 1 in plasma and vitreous between type 1 and type 2 diabetes mellitus and control group and understand its role in diabetic retinopathy.

Design: Prospective randomized case control study.

Methods: This study was conducted on three groups. Group 1 were type I diabetic patients, Group 2 were type II diabetics and group 3 were controls (complete healthy non-diabetic individuals). Full ophthalmological examination was done to all our cases. Endothelin level in vitreous and plasma was estimated together with glycated hemoglobin HbA1c and other parameters were estimated in all patients and controls.

Results: Plasma Endothelin 1 level was significantly elevated in diabetics compared to controls (normal subjects). Vitreous endothelin 1 was significantly lower in diabetics compared to controls. There was a positive correlation between glycated hemoglobin HbA1C and serum Endothelin-1 ET-1 level in both type 1 and 2 diabetes mellitus. Endothelin 1 level is elevated in poorly controlled diabetic patients.

Conclusion: Endothelin-1 plays an important role in mediating retinal changes in diabetics.

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Introduction

Diabetic retinopathy "a micro vascular disease" is a leading cause of blindness in the world. Vasoactive factors like endothelins, by the virtue of the micro-vascular regulation as well as by other effects, possibly play on important roles in the pathogenesis of diabetic retinal microangiopathy [1].

Diabetic Retinopathy is a significant complication of both type 1 and type 2 diabetes mellitus. The prevalence of diabetic retinopathy is 23.8% in Egyptian diabetic patients. The prevalence of retinopathy is dependant on the duration of diabetes, being rare before five years but present in 95% of patients 15 years after diagnosis. Other factors reported to be associated with diabetic retinopathy include poor glycemic control, hypertension and genetic factors [2].

Endothelin-1 (ET-1) a novel 21 amino acids Vasoconstrictive peptide secreted by endothelial cells, has been thought to play a role in various forms of diabetic vascular disease [3].

Ocular tissue is a rich source of ET-1 peptide expression that has been shown to maintain ocular blood flow in vivo [4]. Accordingly alteration in ET-1 production and action has been hypothesized to contribute in diabetic retinopathy and other ocular vascular disease [5].

The aim of this work was to compare the levels of Endothelin-1 in plasma and vitreous fluid of type 1 and type 2 diabetic patients with retinopathy in comparison to non-diabetic individuals as controls and this could help to know the significance of ET-1 as a new marker for diabetic retinopathy or no.

Patients and Methods

This study was conducted on 300 patients divided into three groups.

Group (1) = included 120 patients with type 1 diabetes mellitus.

Group (2) = included 120 patients with type 2 diabetes mellitus.

The duration of diabetic retinopathy in both groups was between 10 and 20 years to fix the duration in both groups for reliable comparison and all cases were manifested with diabetic retinopathy (different grades).

Group (3) = 120 individuals of the same age group, non-diabetic and without any neither systemic nor ocular disease.

All patients were subjected to full ophthalmological examination. Fundus examination had been done by indirect ophthalmoscope and biomicroscopy. Blood and vitreous samples were taken from all fundus. Under complete aseptic condition, the vitreous samples were taken using the vitrectomy probe. Both blood and vitreous samples were sent directly for laboratory examination.

The blood samples were subjected to radioimmunoassay of Endothelin-1, glycated Hemoglobin Assay (HbA1C) by Colometric method, serum cholesterol and triglycerides, finally renal and liver functions. Urine analysis

was also done for detection of microalbuminuria by dipstick method. Conjunctival swab for culture and sensitivity was also done. Also ECG (Electro Cardio Gram) and blood pressure measurement had been assessed in all our cases.

The following patients were excluded from our study to avoid the other causes that change endothelin-1 level, as patients with cardiac ischemia, hypertensive > 140/90 mmHg, patients with microalbuminuria to exclude nephropathy and patients with abnormal lipid profile.

All data were collected, analyzed and reported.

Results

Three hundred cases were included in this study, divided into three equal groups (group 1 = Type 1 DM), (Group 1 = Type II DM) and (Group 3 = Non diabetic subjects as controls).

Patient's characteristics are shown in table 1. All were selected from Ain-Shams university hospitals, out-patient clinic after written informed consent and acceptance of hospital medical and ethical committee. Baseline data was not statistically significant differ ($P > 0.05$). Fundus examination details are shown in table 2.

Table 1: Patient's data

Patient's characteristics	Group 1 (Type 1 DM)	Group 2 (Type 2 DM)	Group 3 (Controls)	P value
Age in years	56.9 $\pm$ 3.3	58.4 $\pm$ 7.1	59.1 $\pm$ 11.2	P > 0.05
Sex (M/F)	92/28	89/31	74/46	P > 0.05
Number	120	120	120	P > 0.05
Duration of Diabetes in years	26 $\pm$ 11.7	22 $\pm$ 6.7	-	P > 0.05
Duration of diabetic retinopathy in years	18 $\pm$ 5.4	16 $\pm$ 8.2	-	P > 0.05
Medication taken	Insulin	Oral hypoglycemic in 22 Insulin in 98	-	

Table 2: Fundus Examination

Fundus examination	Group 1 (Type 1 DM)	Group 2 (Type 2 DM)	Group 3 (Controls)
PDR	72	48	-
Severe NPDR	36	36	-

Moderate NPDR	12	3	-
Normal	-	-	96 cases
Myopic Fundus	-	-	24 cases

Plasma endothelin-1 level was significantly increased in group II mean $(18.7) \pm 2.1$ P value < 0.0001 in comparison with controls, while in group I its mean level was 9.5 ± 3.5 , $P < 0.0001$ compared also to control (3.9 ± 1.2). This indicate marked increase in plasma level of endothelin 1 in group 1 (Type 2 DM) in correlation with group 1 & controls. Table 3

Table 3: Mean Endothelin -1 level

	Group 1	Group 2	Group 3
Mean plasma endothelin- 1	9.5 ± 3.1	18.7 ± 2.1	3.9 ± 1.2
Mean vitreous endothelin -1	8.4 ± 1.8	10.6 ± 2	21 ± 2

The mean value of endothelin -1 in vitreous of group I was 8.4 ± 1.8 , being 10.6 ± 2 in group II which were significantly lower than control (21 ± 2) (P value < 0.001). There is significant lowering of vitreal endothelin 1 in diabetic patients in comparison with control.

The endothelin -1 level in vitreous was markedly decreased in proliferative diabetic retinopathy its mean was 6.9 ± 3.2 in comparison with severe non-proliferative diabetic retinopathy being 9.5 ± 1.2 while in moderate diabetic non-proliferative retinopathy being 12.9 ± 2.2 . Its mean level in group 3 with normal fundus was 21 ± 2 . Endothelin -1 level in vitreous normally high and lowered with the progression of retinopathy being at lower levels in cases with PDR, this results may correlate the vitreous level of endothelin 1 with the pathogenesis of diabetic retinopathy.

There was positive correlation between glycated hemoglobin HbA1C and serum Endothelin level (ET-1) in both diabetic groups.

Discussion

Vasoactive factors like endothelin possibly play important roles in the pathogenesis of diabetic microangiopathy

[6]. This study was novel in comparison of the levels of Endothelin-1 in plasma and vitreous fluid of type 1 and type 2 diabetic patients with retinopathy in comparison to non-diabetic individuals as controls.

This study showed a significantly increased plasma level of endothelin-1 in type II diabetes mellitus with diabetic retinopathy especially severe grades. These results are consistent with Moris et al. study (1995) [7], who showed that patients with type II DM had increased plasma level of ET-1 in contrast to others who showed that endothelin 1 level was unchanged [8].

Endothelin-1 level was significantly increased in type I DM compared to non-diabetic group, however it is less than its level in type II DM. These results were supported by previous works done by (Haak et al. 1992) [9] who showed increased ET-1 level in type I DM, although others reported decreased level [10].

Our results showed positive correlation between glycated hemoglobin HbA1C and serum endothelin-1 level in both diabetic groups and this may be a possible explanation for elevated ET-1 level in diabetics due to hyperglycemia and Advanced Glycation End products (AGEs) [11].

Also we proved that the level of ET-1 in type II DM is more than in type I. Some authors explain this finding by that type II DM is often considered a hyperinsulinemic state and the patients with type I DM on insulin, therapy exhibiting fluctuation between insulinemia and glycemia, increased insulin concentrations are known to increase ET-1 from human endothelial cells. Recent Studies showed that insulin increases the ET-1 receptors in vascular tissue [12].

From this point of view elevation of ET-1 in the proliferative stage of diabetic retinopathy suggested the role of ET-1 in the pathogenesis of diabetic retinopathy as elevated ET-1 level leads to retinal vasoconstriction that could lead to

reduction of blood flow in the retinal capillary bed leading to ischemia which is the main trigger factor in process of proliferation [13].

Our study showed a significant decrease in endothelin-1 level in vitreous of diabetic patients compared with non-diabetic individuals. Also ET-1 level intravitreal was significantly decreased in proliferative stage of diabetic retinopathy compared with non-proliferative stages of diabetic retinopathy, the more the advanced the stage of diabetic retinopathy the more the decrease in vitreous ET-1. These results were supported by other studies [14]. The decreased rather than increased immuno-reactive endothelin-1 level may simply reflect severe endothelial injury of retinal vessels caused by diabetic microangiopathy as a result of high serum endothelin-1 level especially in patients with advanced proliferative diabetic retinopathy.

Chakrabasti et al. (1996) [13] study, showed over expression of endothelin-1 receptors in retina responsible for mediating retinal capillary vasoconstriction with increased sensitivity leading to retinopathy.

It is however, not clear what impact altered expression of endothelins and their receptors possibly have a role in the development of diabetic retinal lesions in the long-term. It has been postulated that loss of contractile function of pericytes in diabetes might be related to changes in endothelin 1 [15].

Endothelins have been shown to regulate vascular endothelial growth factor production. The long-term consequences of an augmented endothelin system possibly further contribute to long-term cellular changes [16]. Increased Extracellular matrix protein synthesis and subsequent structural changes such as basement membrane thickening are hallmarks of diabetic retinal micro-angiopathy [17].

The results of this study indicated that endothelin-1 plays an important role in mediating retinal changes in diabetics.

It is elevated in poorly controlled diabetes so tight glycemic control and prophylactic treatment of diabetic patients may be beneficial.

A farther longer study with more number of patients is recommended for more accurate confirmation of a significance of ET-1 as a sensitive and specific marker for diabetic retinopathy.

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