

ORIGINAL ARTICLES

Systemic Soluble Tumor Necrosis Factor Receptors 1 and 2 in Diabetic Retinopathy

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ABSTRACT

To investigate the association of serum amyloid A (SAA) protein and soluble tumour necrosis factor receptor1 and 2 (sTNF-R1 and sTNF-R2) with severity of diabetic retinopathy. This study included 45 diabetic patients (15 diabetic patients without retinopathy, 15 with non proliferative diabetic retinopathy (NPDR) and 15 with proliferative diabetic retinopathy (PDR) compared to 15 non-diabetic control subjects. Methods: Levels of SAA, sTNF-R1, and sTNF-R2 were measured by enzyme-linked immunosorbent assay (ELISA). Diabetic retinopathy was assessed by fundus photography. Results: A direct association of sTNF-R1 and sTNF-R2 was found for no DR, nonproliferative DR (NPDR) and proliferative DR, respectively. These associations remained significant after adjusting for age, gender, body mass index, glyco-sylated hemoglobin, diabetes duration, systolic blood pressure, and serum creatinine. Conclusions: Levels of sTNF-R1 and sTNF-R2 are highly correlated with DR suggesting that inflammation and insulin resistance may play a critical role in the development of DR. These may be useful biomarkers for DR, aiding in etiologic studies and possibly identifying at-risk patients for active intervention.

Key words: diabetic retinopathy, sTNF-R1, sTNF-R2, serum amyloid A

Introduction

Diabetic retinopathy (DR), a frequent micro vascular complication of diabetes, is the leading cause of preventable blindness in working-age adults (Mohamed *et al*, 2007). Inflammation, a nontraditional risk factor, has been suggested as being involved in the pathogenesis of DR and other microvascular and macrovascular complications (West *et al*, 2001).

However, the role that inflammatory processes play in DR has not been elaborated extensively, and current evidence remains unclear and inconsistent (Klein *et al*, 2009 & Nguyen *et al*, 2009). In this study, we measured the plasma levels of 3 inflammatory biomarkers, serum amyloid A (SAA) protein and soluble tumor necrosis factor receptors 1 (sTNF-R1) and 2 (sTNF-R2), in patients with type II diabetic subjects with and without DR. We chose to study these particular biomarkers because they have been linked to key etiologic components of inflammation and insulin resistance in diabetes and obesity. Serum amyloid A, an acute-phase protein, is produced by the liver and adipose tissue during an acute inflammatory process. It has been shown that SAA directly mediates obesity-associated inflammation and is a sensitive marker for obesity-associated diseases, such as cardiovascular disease and diabetes (Zhao *et al*, 2010).

Another key inflammatory marker, tumor necrosis factor (TNF)- α , and its receptors may also have an important role in DR. Previous studies have shown that the level of soluble TNF receptors increases in the serum and vitreous of patients with proliferative diabetic retinopathy (PDR) with type I diabetes (Limb *et al*, 2001). This study evaluates the association of SAA, sTNF-R1, and sTNF-R2 with severity of DR in a large group of type II diabetic subjects.

Materials and Methods

The studied subjects were 45 diabetic patients with type 2 diabetes, their age ranged from 56-65 years. They were classified into 3 groups:

- Group1: 15 diabetic patients without retinopathy.
- Group2: 15 diabetic patients with non proliferative diabetic retinopathy (NPDR).
- Group3: 15 diabetic patients with proliferative diabetic retinopathy (PDR). They were compared to 15 normal non diabetic control subjects.

- Full ophthalmological examination and medical history was taken for each subject including:
- Intraocular pressure measurement by applanation tonometry.

- Slit lamp examination to determine anterior chamber depth and presence of iris neovascularization . Indirect ophthalmoscopy and biomicroscopy to evaluate the grade of vitreous proliferation and determine the presence and nature of macular oedema.
- The pre-operative findings were recorded and the clinical disease severity was classified, according to the presence and extent of active fibrovascular tissue, vitreous hemorrhage, tractional retinal detachment (with or without retinal tears). Recent vitreous hemorrhage was excluded to avoid affecting the vitreous samples.
- Fundus fluorescein angiography was done using a 50 field fundus camera, 5 ml of 10% sodium fluorescein was injected in the anti cubital vein and photography was carried out.
- Angiography was performed in patients with diabetic retinopathy to differentiate between non proliferative and proliferative retinopathies.
- Electroretinogram ERG, Flash ERG used RETI system, program ISCEV-ERG GF parameter of background and stimuli of the steps correspond with minimum standard ISCEV.
- Flash ERG was subnormal in amplitude and oscillatory potentials was found in all PDR patients.
- Oscillatory potentials (OPs) are very sensitive to ischemia in localized retinal areas. Therefore, in situations where the a- and b-waves remain normal in waveform and amplitude, OP recordings can indicate mild retinal ischemia in the inner retina. ⁽¹⁰⁾.

Exclusion criteria:

only those patients who did not have any hepatic or GIT and renal diseases were selected. Any patient with serum creatinine > 1.2 mg/dl or urinary albumin excretion > 150 mg/24hrs was not included in this study. Also any patient with local eye disease such as, cataract, glaucoma or uveitis was excluded from the study.

Samples of venous blood were collected, part on EDTA to estimate HbA_{1c}, and the other part centrifuged and serum was separated and stored at – 80°C until assayed.

Statistical analysis:

Data was expressed as mean \pm SD. The four groups were compared using the Anova; single factor test. The degree of association between the variables was assessed using Pearson's correlation coefficient (r), where values of $p < 0.05$ were considered significant.

Results:

Clinical characteristics of the patients are presented in table 1.

The results of this study are illustrated in table 2 and figures 1-4.

The mean serum levels of sTNF-R1&2 were significantly elevated in all diabetic groups compared to controls. Also serum levels of sTNF-R1&2 in PDR patients (group3) were significantly elevated compared to NPDR (group2) and diabetics without retinopathy ($p < 0.001$).

The mean serum levels of SAA were significantly decreased in all diabetic groups compared to controls.

Also serum levels of SAA in PDR patients (group3) were significantly decreased compared to diabetics without retinopathy ($p < 0.001$). While no significant difference between PDR (group3) and NPDR (group2) in SAA serum levels ($p > 0.05$).

Table1: Demographics of diabetic patients and controls included in this study

	Controls	Group 1	Group 2	Group 3
Number (n)	15	15	15	15
Sex (M/F)	5/10	8/7	7/8	9/6
Mean age \pm SD	55.7 \pm 7.6	63.6 \pm 9.2	63 \pm 8.8	64.1 \pm 8.3
Duration of diabetes	-	12.6 \pm 8.4	12.6 \pm 8.4	12.6 \pm 8.4
Serum Glucose (mg/dl)	99.9 \pm 3.4	180 \pm 5.5	190 \pm 6.1	227 \pm 10.2

Table 2: Comparison of the different studied parameters among all diabetic groups.

	Controls	Group 1	Group 2	Group 3	p value
Number (n)	15	15	15	15	
HbA _{1c} %	6.9 \pm 0.8 a	9.4 \pm 1.2 b	9.7 \pm 1.1 b	10.7 \pm 1.5 b	$p < 0.001$
sTNF-R1 (ng/ml)	1.37 \pm 14.9 a	3.82 \pm 90.8 b	5.27 \pm 116 c	7.10 \pm 130 d	$p < 0.001$
sTNF-R2 (ng/ml)	6.6 \pm 1.8 a	9.9 \pm 0.9 b	11.8 \pm 0.58 c	12.68 \pm 0.6 c	$p < 0.001$
SAA (ug/ml)	6.6 \pm 1.8 a	2.9 \pm 0.9 b	1.8 \pm 0.58 c	1.68 \pm 0.6 c	$p < 0.001$

$p < 0.05$ is statistically significant. Groups with different letters have a statistically significant difference.

Discussion:

After controlling for confounding risk factors, including age, gender, BMI, HbA_{1c}, diabetes duration, systolic blood pressure, and serum creatinine or urinary albumin: creatinine ratio, both sTNF-R1 and sTNF-R2 were cross-sectionally associated with increasing severity of DR in type II diabetic subjects. These findings reveal novel systemic biomarkers, namely, soluble TNF receptors, which may serve as a biochemical link between 2 important pathophysiologic processes involved: inflammation and insulin resistance (Varma *et al*, 2010). Previous studies have examined a number of systemic biomarkers of inflammation, endothelial dysfunction (Goldberg *et al*, 2009), hemostatic disturbances, angiogenesis (Meleth *et al*, 2005), and homocysteinemia with DR in both type I and II diabetes, but reports for these results have been inconclusive. It has been suggested that the lack of association between DR and systemic biomarkers of inflammation, endothelial dysfunction, and hemostatic disturbances may be because the eye is an immune-privileged area, and therefore inflammatory makers do not cross the blood-retinal barrier (Cheung *et al*, 2010).

The previously examined inflammatory biomarkers include C-reactive protein (Klein *et al*, 2009), TNF- α , cytokine interleukin-6, and were reported to be elevated in patients with PDR (Meleth *et al*, 2005). The latter study combined both type I and II diabetic subjects in the analysis, which may be problematic if the baseline levels of these markers varied between type I and type II diabetes. In the Wisconsin Epidemiologic Study of Diabetic Retinopathy, the authors found that the inflammatory marker TNF- α was associated with the severity of DR in type I diabetic participants with kidney disease (Klein *et al*, 2009).

Endothelial dysfunction, characterized by increased level of soluble vascular cell adhesion molecule and soluble intercellular adhesion molecule-1, has been reported to increase vascular permeability, alter blood flow, increase oxidative stress, and affect angiogenesis (Fukumura *et al*, 2001). Only soluble vascular cell adhesion molecule-1 has been associated with the prevalence and severity of DR in type I diabetic persons with kidney disease, although in an earlier study, a positive association was not seen with either adhesion molecule (Nguyen *et al*, 2009).

Our findings support 2 earlier studies, one examining the level of SAA with severity of DR in Japanese with type II diabetes and another examining the serum levels of TNF- α receptors in type I diabetes in white subjects with active; PDR, quiescent PDR, or no DR and in healthy controls (Kumon *et al* 1994 & Limb *et al*, 2001). The authors from the first study demonstrated no association of SAA with severity of DR, but instead suggested an association with diabetic nephropathy. This observation indicates the mechanism by which SAA acts may be due to impaired metabolism in the liver and kidney, and thus a different mechanism from that which may affect DR in the eyes. In the second study, the authors found that patients with type I diabetes complicated by PDR exhibited significantly higher serum levels of sTNF-R1 and sTNF-R2 than those without retinopathy or healthy individuals (Medvedev *et al*, 1998). It is possible that marked elevation of sTNF-R1 and sTNF-R2 may constitute important clinical and pathophysiologic significance in that soluble TNF receptors may play a role in modulating the biological activity of TNF- α and interfere with the immunologic detection of TNF- α (Moreau *et al*, 1998). Soluble TNF receptors may be a more sensitive marker than TNF- α in determining inflammatory and insulin resistance status in patients with DR.

In recent years, the molecular mechanisms of TNF function have been intensively investigated. One of the earliest studies linking inflammation to insulin resistance in type II diabetes involved animal models of obesity and diabetes (Hotamisligil *et al*, 1993). Increased expression of TNF- α mRNA and protein was observed, and these elevated levels were found both locally within the adipose tissue and systemically in the plasma of these animals. Neutralization of TNF- α using a recombinant TNF- α receptor-immunoglobulin G chimeric protein showed improvement in insulin sensitivity, implicating a direct role of TNF- α in the development of insulin resistance. Subsequent studies have suggested that in addition to TNF- α , increased circulating levels of TNF receptors are also associated with diabetes, obesity, and insulin resistance (Fernandez-Real *et al*, 1998). Our study demonstrates that soluble TNF receptors are also correlated with DR in type II diabetic patients and supports the idea that insulin resistance/ impaired insulin action may have a critical role in the development of DR.

In conclusion, data from this study of type II diabetic subjects indicate that soluble TNF receptors are cross-sectionally associated with DR after adjusting for potential confounders. It is possible that the biological activity of soluble TNF receptors may correlate with clinical disease severity and that this biomarker may provide a link between inflammation and insulin resistance in DR. This finding needs to be replicated in prospective studies.

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