Role of Cytokines in Diabetic Retinopathy

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Abstract

Background: Diabetic retinopathy is a progressive vision threatening complication of diabetes. Raised plasma level of tumor necrosis factor and other cytokines are accepted as the key in early events in the development of vascular disease.

Objective: The aim of this study was to assess the level of some cytokines in diabetic retinopathy and its effect on the severity of diabetic retinopathy.

Patients and Methods: This study was conducted on 60 diabetic patients and 15 healthy subjects as a control group. Diabetic patients were subdivided into 4 groups, group 1 included 15 cases without retinopathy, group 2 included 15 cases with non proliferative DR, group 3 included 15 cases with preproliferative DR, group 4 included 15 cases with proliferative DR. Full ophthalmological examination was done and any posterior segment pathologies was excluded. Blood samples were taken and quantitative determination of glycohemoglobin (HbA1C), ELIZA assay was used to determine the levels of different cytokines. Urine samples were collected to determine the level of ketone bodies and to test for microalbuminuria.

Results: There was a significant correlation between the level of tumor necrosis factor (TNF) and the degree of retinopathy, the mean level in group 1 (40.7 ± 5.3) in group 2 (98.5 ± 6.8), in group 3 (116.1 ± 6.2), in group 4 was (313.7 ± 71). Its level was also elevated in vitreous samples in group 3 and 4, group 3 (216 ± 17), group 4 (260.13 ± 7). Interlukin 6 showed marked elevation in blood and vitreous in diabetic patients with advanced retinopathy, group 3 and 4, group 3 (107 ± 120) blood, vitreous (120±31), group 4 (253.7 ± 130) blood, vitreous (200± 11). There was a strong correlation between ketosis and elevated TNF and interlukin 6.

Conclusion: In diabetic retinopathy the increased level of some cytokines as tissue necrosis factor and interlukin 6 may stimulate endothelial cells for neovascularization process, there was a significant correlation between clinical grades of diabetic retinopathy and the expression levels of cytokines.

Key Words: Diabetic retinopathy – Cytokines – TNF – interlukin.

Introduction

DIABETIC retinopathy is a progressive vision threatening complication of diabetes, characterized by capillary occlusion, formation of microvascular lesions and retinal neovascularization [1].

The exact cause of diabetic microvascular disease is unknown. It is believed that the exposure to hyperglycemia over an extended period results in a number of biochemical and physiological changes that ultimately cause vascular endothelial damage.

Raised plasma level of tumor necrosis factor (TNF) can cause induction of pro-inflammatory cytokines and adhesion molecules, which are accepted as a key in early event in the development of vascular disease and arteriosclerosis [2].

Diabetic retinopathy is one of the most common complications in diabetics and affects nearly all patients with type I diabetes mellitus and more than 60% of type II diabetes mellitus within 15 years after diagnosis. Hiperglycemia is known to be the primary pathogenic factor. Diabetic retinopathy is a progressive disease affecting the structure and cellular composition of the microvasculature [3].

Elevated blood level of TNF is validated as a marker of vascular inflammation which can result in the development of vascular disease and other sclerosis [4].

Tumor necrosis factor and other cytokines or adhesion molecules such as platelet derived molecule 1 and vascular adhesion molecule (VCSM), have been reported to be increased in human vitreous in proliferative diabetic retinopathy. High glucose level has a direct effect on interlukin-6 and this induce activation of signals and vascular endothelial growth factor release [8].

Aim of work:

The aim of this study was to assess the level of some cytokines (tumor necrosis factor and
interlukin 6) in diabetic retinopathy and its effect on the severity of diabetic retinopathy.

**Patients and Methods**

The study was performed in Ain-Shams University Hospital, from June 2008 to October 2009. It included 60 diabetic patients (45 with diabetic retinopathy and 15 cases without retinopathy) (32 men and 28 women), their age ranged between 43 to 67 years with a mean of (49.6±7.1ys) and 15 normal healthy subjects as a control group (7 men and 8 women) with mean age (42.3±10.4ys).

**Diabetic patients were divided into four groups:**

- **Group (1) (15 cases):** Included patients without retinopathy.
- **Group (2) (15 cases):** Included patients with NPDR (Non proliferative diabetic retinopathy).
- **Group (3) (15 cases):** Included patients in the pre proliferative stage (pre-PDR).
- **Group (4) (15 cases):** Included patients with PDR (Proliferative diabetic retinopathy).

All cases were subjected to full ophthalmological history and examination, including assessment of the external eye, anterior segment and fundus examination. Refraction and measurement of intraocular pressure were mandatory in all our cases. Cases with any other ocular diseases especially posterior segment pathologies were excluded from our study.

Fluorecine angiography was done to all cases. Analysis of eye fundus pictures was based on the International Diabetic Retinopathy division as non proliferative DR, pre PDR, proliferative DR.

Blood and urine samples were taken from all our cases for laboratory examination by lab. doctors. 10mL of whole blood were collected and divided into 3 types of tubes, one with EDTA and two plane tubes for collection of serum. Immunoassay for direct photometric determination of glyco hemoglobin (HbA1C) quantitative determination of HbA1C% is preformed for long term control of diabetes mellitus. HbA1C values provide an indication of the average glucose level over the proceeding 4-8 weeks. ELISA assay was used to determine the levels of different cytokines.

Urine samples were collected in a clean container and subjected to laboratory examination for microalbuminuria and ketone bodies as soon as possible. The samples were taken after complete insurance of healthy urinary tract as it should not be collected in presence of acute urinary tract infection. Ketone bodies values were reported in um/L. To test for micro albuminuria urine specimen should be frozen, turbid specimen centrifuged at 300 RPM for 10 minutes prior to analysis, urine may be stored at 4°C for at least one week.

Finally a vitreous sample through intravitreal aspiration under complete sterile condition was taken and sent to the laboratory for quantitative assessment of cytokines in the vitreous of diabetic patients.

All data were collected, analyzed and recorded.

**Results**

This study was conducted on 60 diabetic patients (45 with diabetic retinopathy of differently grades and 15 without retinopathy) and 15 normal subjects as a control group. The diabetic group was divided into four subgroups according to the degree of retinal affection. The mean age of all cases was (49.1±6.3 years) in group 1, (49.6±9.3 years) in group 2, (50.1±7.3 years) in group 3, and (50.2±8.4 years) in group 4. Sex distribution difference was statistically insignificant among all groups.

HBA1C% was statistically significantly elevated in group (4) more than other groups being 10.5% in group (4), 8.7% group (3), 6.9% in group (2) and 5.5% in group (1).

The mean Glucose level was 85.5±6.1mg/mL in group (1), in group (2) it was 192.3±40.1 mg/mL, 225.1±61.1mg/mL in group (3) and finally it was 375.6±60.9 in group (4).

Microalbumurin was statistically significantly high in group (4) than in other study groups (222.5±22.4), being 1.6±1.1 in group (1), 22.1±16.4 in group (2), and 100±16.4 in group (3).

There was a significant correlation between tumor necrosis factor (TNF) level in blood and the degree of retinopathy. The range was 100-400 with a mean of (313.7±71) in group (4), 90-320 with a mean of (116.1±6.2) in group (3), in group (2) 20-300 with a mean of (98.5±6.8) while in group (1) the range was 21-48 with a mean of (40.7±5.3). It's level was also elevated in vitreous sample in group (4) and (3), its mean level in vitreous is shown in Table (2) below.

Thus TNF (one of cytokines) was markedly elevated in blood and vitreous of patients with
advanced grades of diabetic retinopathy returning to a lesser values after control of diabetes and laser treatment of retinopathies. Thus, TNF may be regarded as a diagnostic and prognostic factor in diabetic retinopathy, being highly significantly elevated in blood and vitreous of diabetic patient in pre proliferative and proliferative stages.

Another cytokines that showed marked elevation in blood and vitreous of diabetic patients with advanced retinopathies (group 4 and 3) was interleukin 6 (IL-6) as shown in Table (2).

Thus there was a high significant statistical difference in IL-6 level in blood and vitreous in group (4) and other three groups (Table 2). There was a strong correlation between Ketosis and the elevated levels of TNF and IL6. Ketone bodies was positive in 4 cases in group (4) and 2 cases in group (3) and 1 case in group (2) and no cases in group (1).

Table (1): Clinical parameters of the diabetic group and control group. Values are presented as means ± SD.

<table>
<thead>
<tr>
<th>Clinical parameters</th>
<th>Control (normal)</th>
<th>Group 1 (diabetic no retinopathy)</th>
<th>Group 2 (NPDR)</th>
<th>Group 3 (Pre-PDR)</th>
<th>Group 4 (PDR)</th>
<th>Statist. sign. (p value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of cases</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>N.S</td>
</tr>
<tr>
<td>Age (years)</td>
<td>48±10.2</td>
<td>49±1.6 3</td>
<td>49.6±9.3</td>
<td>50.1±7.3</td>
<td>50±28.4</td>
<td>N.S</td>
</tr>
<tr>
<td>Duration of DM (years)</td>
<td>6.5±2.8</td>
<td>8.4±3.6</td>
<td>9.8±6.3</td>
<td>15.3±4.2</td>
<td></td>
<td>p&lt;0.001 ** p&lt;0.001 **</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>4.2±0.3%</td>
<td>5.5±0.7%</td>
<td>6.9±1.7%</td>
<td>8.7±1.2%</td>
<td>10.5±1.8%</td>
<td>p&lt;0.001 * p&lt;0.001 **</td>
</tr>
<tr>
<td>Mean glucose level (mg/ml)</td>
<td>75.8±5.6</td>
<td>85.5±6.1</td>
<td>192±40.1</td>
<td>225±61.1</td>
<td>375±60.9</td>
<td>p&lt;0.001 * p&lt;0.001 **</td>
</tr>
<tr>
<td>Albumin excretion rate (mg/24)</td>
<td>0.0</td>
<td>1.6±1.1</td>
<td>22.1±16.4</td>
<td>100±16.4</td>
<td>222.5±22.4</td>
<td>p&lt;0.001 * p&lt;0.001 **</td>
</tr>
<tr>
<td>Ketone bodies in blood</td>
<td>0.0</td>
<td>0.0</td>
<td>1 case</td>
<td>2 cases</td>
<td>4 cases</td>
<td>p&lt;0.001 * p&lt;0.001 **</td>
</tr>
</tbody>
</table>

* Difference between control and diabetic groups. ** Between four diabetic subgroups.

Table (2): Circulating levels of cytokines in serum of diabetic and healthy group. Values are presented as means ± SD.

<table>
<thead>
<tr>
<th>Clinical parameters</th>
<th>Control (normal)</th>
<th>Group 1 (diabetic no retinopathy)</th>
<th>Group 2 (NPDR)</th>
<th>Group 3 (Pre-PDR)</th>
<th>Group 4 (PDR)</th>
<th>Statist. sign. (p value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF-α in blood (pg/mL)</td>
<td>0.0</td>
<td>40.7±5.3</td>
<td>98.5±6.8</td>
<td>116.1±6.2</td>
<td>313.7±71</td>
<td>p&lt;0.001 * p&lt;0.001 **</td>
</tr>
<tr>
<td>TNF-α in vitreous (pg/mL)</td>
<td>0.0</td>
<td>12.3±1.1</td>
<td>60.1±17.2</td>
<td>216±17</td>
<td>260±13.7</td>
<td>p&lt;0.001 * p&lt;0.001 **</td>
</tr>
<tr>
<td>IL-6 (pg/mL) in blood</td>
<td>0.5±0</td>
<td>4.7±2.6</td>
<td>107±120</td>
<td>197±150</td>
<td>235±130</td>
<td>p&lt;0.001 * p&lt;0.001 **</td>
</tr>
<tr>
<td>IL-6 (pg/mL) in vitreous</td>
<td>0.0</td>
<td>0.0</td>
<td>106±13</td>
<td>200±1.1</td>
<td></td>
<td>p&lt;0.001 * p&lt;0.001 **</td>
</tr>
</tbody>
</table>

*Difference between control and diabetic groups. ** Difference between four diabetic subgroups.

Discussion
Tumor necrosis factor (TNF-α) is thought to be involved in the pathogenesis of diabetic retinopathy and ocular inflammation. TNF-α is a monocyte/macrophage derived pro inflammatory cytokine in response to various stimuli. TNF-α exerts a variety of biological effects, including regulation of adhesion molecules, proliferation, differentiation and cell death [6].

The effect of a high glucose level on interleukin-6 and soluble interleukin-6 receptor induced activation and vascular endothelial growth factor expression in human fibroblasts [6].

The results of our study revealed that increased tumor necrosis factor (TNF) level was highest in group (4) (PDR) and lesser values was found in group (3) (pre PDR), while in group (2) (NPDR) and group (1) (with no retinopathy) it was in a much lower levels in both blood and vitreous.

There was a significant association between TNF level and the clinical grades of diabetic retinopathy (p<0.001). This results was similar to Sushil, et al. [4] study, who reported that TNF level was higher in diabetic patients TNF-α than in normal subjects of matched age groups and they suggested that the elevated TNF γ in diabetic patients is one of the causes of complications associated with diabetics.

Funastu, et al. [7], reported similar results with strong correlation between the level of TNF-α and the grade of diabetic retinopathy.

As regards the interleukin 6 level (another important cytokines in diabetic retinopathy, it was the highest in group (4) (PDR) followed by group (3) (pre PDR) and recorded in lower levels in group 2, and 1. Also there was a significant association between the IL-6 level and the clinical grades of diabetic retinopathy (p<0.001). Funastu, et al. [7]
reported similar results to our study, but they studied the IL-6 level in the aqueous and found a significant correlation between its level in aqueous and the severity of diabetic retinopathy. Their study reported similar results to our study as they found elevated level of IL-6 in human vitreous of diabetic patients with PDR.

Our study revealed that there was correlation between ketosis and TNF level. Ketosis was associated with a high level of TNF in group (4) in four cases and 2 cases in group (3) and 1 case in group (2) (p<0.05). This was in agreement with Sushil, et al. [4], who found that ketosis increase TNF secretion in cell culture model and in type 1 diabetic patients in vivo, as hyper ketonemia in diabetic patients have significantly higher level of TNF-α than normoketonemia diabetic patient (p<0.01).

As regards blood glucose level in our patients, it was the highest in group (4), lesser in group (3), less in group (2) and fairly controlled in group (1) and this was documented by the level of HBAIC which indicate the long-term control of blood glucose level (p<0.001). In agreement with our results Funastu, et al. [7], reported that intensive therapy of subcutaneous insulin reduce the risk of progression of retinopathy by 54%.

The current study revealed that microalbuminuria was higher in group (4) followed by group (3) than in group (2) (p<0.001) and normal value in group (1). Bahedin, et al. [1], reported similar results to our study with higher incidence of microalbuminuria in PDR.

**Conclusion:** From our study we concluded that in diabetic retinopathy the increased concentrations of some cytokines as tissue necrosis factor α and interleukine 6 may stimulate endothelial cells for neovascularization process, there was a significant association between clinical grades of diabetic retinopathy and the expression levels of these cytokines.

**References**

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