Assessment of pentosidine and TNF-α levels in Iraqi diabetic patients with retinopathy

Jassim Mohammed Jabur
Assistant Prof., Biochemistry Department, College of Medicine, University of Babylon, Hillah, Iraq
*Corresponding author email: jassim.jmjcc@gmail.com

Qassem Farhoud Al-Rubaie
Assistant Prof., Biochemistry Department, College of Medicine, University of Babylon, Hillah, Iraq

Ban Mahmood Shaker Al-Joda
Assistant Prof., Biochemistry Department, College of Medicine, University of Babylon, Hillah, Iraq

Abstract---Diabetic retinopathy (DR), the most common retinal vascular consequence of diabetes mellitus (DM), is a leading cause of vision problems in people of working age. DR is usually asymptomatic within the early stages. When left untreated, DR can cause significant vision loss and potentially lead to blindness. Classically, DR was thought to be a retinal microvascular disease. The treatment of DR remains challenging. This study designed as a case-control study. Nineteen patients were involved in this study, divided into two groups (DR group that contain 45 patients, and control groups that contain 45 subject). This study was conducted over a period 6 months' form October 2021 till April 2022. Sample collected form clinic of ophthalmology in Imam Sadiq Hospital and Ophthalmology center in Hilla city. The particular side of the study was performed at the laboratory of the biochemistry department in college of medicine / Babylon university. This study including 100 subjects. This subjects were divided into two group, the first group includes 50 patients with diabetic retinopathy and the second group includes 50 apparently healthy peoples., the age ware ranged between (43-77) years. The results of current study revealed a significant increase in the serum of pentosidine, leptin, TNF-α and C-RP among DR patient than control and significant decrease of serum adiponectin in psoriasis patient than control. The body mass index (BMI) was significantly increase in patients than healthy control.

Keywords---pentosidine, TNF-α levels, diabetic patients, retinopathy.
**Introduction**

Diabetic retinopathy (DR), the most common retinal vascular consequence of diabetes mellitus (DM), is a leading cause of vision problems in people of working age. DR is usually asymptomatic within the early stages. When left untreated, DR can cause significant vision loss and potentially lead to blindness[1]. Classically, DR was thought to be a retinal microvascular disease. However, mounting data shows that retinal neurodegeneration occurs early in the pathophysiology of DR, perhaps contributing to microvascular anomalies[2]. There are numerous clinical studies proving that chronically imbalanced sugar/glucose in the blood damages retina microvasculature, causes the blood-retinal barrier (BRB) to break down, fluid leakage, and intra-retinal hemorrhage in the early non-proliferative phase of DR (NPDR), and causes retinal neovascularization in the proliferative phase of DR (PDR)[3] [4]. Proliferative diabetic retinopathy (PDR) is a stage of DR in which new blood vessels develop on the retina. The majority of severe vision loss is caused by PDR. Consequently, retinal vessels might become permeable, resulting in retinal swelling known as diabetic macular edema (DME). In diabetes, DME is a primary cause of moderate vision loss (MVL)[5].

These vessels, however, are fragile and prone to bleeding. The blood that collects in the vitreous cavity as a result of these hemorrhaging veins adversely hinders vision. Further complications, such as traction retinal detachment leading to documented blindness, may make this permanent. Without therapy for PDR, it is expected that half of all patients will go blind within five years of diagnosis[6], [7]. Diabetic retinopathy progresses from mild Nonproliferative abnormalities, which are characterized by increased vascular permeability, to moderate and severe Nonproliferative diabetic retinopathy (NPDR), which is characterized by vascular closure, to proliferative diabetic retinopathy (PDR), which is characterized by the formation of new blood vessels on the retina and the posterior surface of the vitreous. Macular edema, which is defined as retinal swelling caused by leaky blood vessels, can occur at any stage of retinopathy. These changes can be accelerated by pregnancy, puberty, blood sugar management, hypertension, and cataract surgery[8]. During the first 20 years after the development of diabetes, nearly all people with type 1 diabetes and 60% of people with type 2 diabetes will develop retinopathy[9]. Diabetic eye disease manifests itself in a variety of ways, the most common of which are cataracts and diabetic retinopathy (DR). Diabetics are 25 times more likely than the general population to become blind. Diabetic eye disease is the primary cause of blindness in adults under 75 years old in developed countries[10].

**Pathogenesis of Diabetic Retinopathy DR**

The cause of DR is unknown. It is a multifactorial illness with a complicated etiology. The precise mechanisms by which elevated blood glucose levels cause diabetic complications are unknown. Regardless, hyperglycemia is known to have metabolic consequences on the retina, causing microvascular damage[11]. Diabetic retinopathy is a retinal microangiopathy. It involves changes in the vascular wall as well as the blood’s rheological characteristics. Capillary occlusion is caused by a combination of these conditions, resulting in retinal ischemia and angiographically visible leaking. The loss of pericytes and endothelial cells, as well
as the thickening of the basilar membrane, are characteristic histological abnormalities. Microaneurysms, or areas of capillary wall inflating outward, are pathognomonic[12]. Hyperglycemia-induced changes in biochemical pathways, such as increased flux of advanced glycation end products/receptors (AGE/RAGE), polyol pathway, protein kinase C (PKC) activation, and hexosamine pathway, produce oxidative stress, which causes the rupture of the BRB, pericytes' demise, and increased vascular permeability, which leads to progression to advanced DR stages and the development of diabetes[13], [14].

**Pentosidine**

A biomarker for advanced glycation endproducts, or AGEs, is pentosidine. This broad family of chemicals has a well-characterized and easily detectable member. Pentosidine, which is derived from the pentose ribose, creates fluorescent cross-links between arginine and lysine residues in collagen. It is made when amino acids react with the Maillard reaction products of ribose. It is valuable for detecting accumulated damage to proteins (advanced glycation endproducts) caused by non-enzymatic browning interactions with carbs, despite the fact that it is only present in trace amounts among tissue proteins. Advanced glycation end products (AGEs) are nonenzymatically glycated and oxidized proteins or lipids that come into contact with aldose sugars. Schiff bases and Amadori products are formed as a result of early glycation and oxidation reactions. AGEs are formed when proteins and lipids are further glycated, causing molecular rearrangements. Advanced protein glycosylation, a nonenzymatic modification of tissue proteins in vivo by physiologic sugars, appears to play a key role in the etiology of diabetes complications. Advanced glycation end products (AGEs) are sugar-derived changes that may have a role in the pathophysiology of age-related illnesses affecting connective tissue, lens, blood vessels, and neurons. Because tissue damage occurs in diabetes, nearly all in vivo investigations of AGEs have focused on diabetes rather than age-related illnesses[15].

**TNF-α**

TNF-α also known as cachectin, is a powerful pro-inflammatory cytokine that is expressed as a 26 kDa membrane-bound protein that is then cleaved by TNF-converting enzyme (TACE) to release a soluble 17 kDa monomer that forms homotrimers in circulation. It was first discovered in the serum of endotoxin-treated mice as a mediator of transplantable tumor necrosis. TNF-α is cytocidal or cytostatic for some tumor cells in tissue culture, according to subsequent research. Furthermore, new research suggests that TNF-α may have a role in either the pathogenesis of infection, tissue injury, and inflammation or as a helpful mediator in host defense, immunology, and tissue homeostasis[16]. TNF is not normally detectable in healthy people, but it is discovered at higher serum and tissue levels in inflammatory and infectious diseases, and serum levels are linked to infection severity[17]. Although monocyte/macrophage cells are the primary producers of TNF in inflammatory diseases, TNF can also be produced by mast cells, T and B lymphocytes, natural killer (NK) cells, neutrophils, endothelial cells, smooth and cardiac muscle cells, fibroblasts, and osteoclasts.[18].
Materials and Methods

This study was conducted over a period 6 months' from October 2021 till April 2022. Sample collected from clinic of ophthalmology in Imam Sadiq Hospital and Ophthalmology center in Hilla city. The particular side of the study was performed at the laboratory of the biochemistry department in college of medicine / Babylon university. This study including 90 subjects. This subjects were divided into two group, the first group includes 45 patients with diabetic retinopathy and the second group includes 45 apparently healthy peoples., the age ware ranged between (43-77) years. Venous blood samples were drawn from diabetic retinopathy and control subjects by using disposable syringe (5 ml) in the sitting position. For mL of blood was obtained from each subject by vein puncture and pushed slowly into two tube (1.5 ml blood in EDTA tube for ESR study and 2.5 ml blood in gel tube for ELISA and RBS study). blood in gel tube allowed to clot at room temperature for 10 -15 minutes and then centrifuged at 3000 RPM for approximately 10minutes then serum was obtained and then the blood serum was isolated, split into aliquots an Eppendorf tube stored at (-20°C) until analysis (measure pentosidine, leptin, adeponectine TNF-α and CRP).

Results

The means and standard deviation of age in patients and control as shown in Table (3-1). Total patients with DR were (45) included in this study whose age ranged (43-75) years; with mean ± SD (60.3 ± 8.0) year. Control group (45) apparently healthy subjects with an age range (48-76) years; with mean ± SD (62.2 ± 7.8) year. as showed in table (1). Amongst forty-five patients with DRP who contributed to this study, there were 29(68%) males and 16(23%) females, as shown in Figure (1). the mean ± SD of pentosidine, TNF-α, HbA1c and glucose levels for DRP patients and control respectively (2.5±1.5, 1.7±0.4), (140.8±26.4, 123.0±18.8mg/dl) (5.41±0.46, 11.9±2.2 %), (102.9±20.7, 247.5±95.8g/dl) P-value 0.001, as showed in table (2).

Predictor of pentosidine DRP patients form controls Groups Figure (2) shows the ROC curve between well DRP patients and controls. The test revealed that the area under the curve (AUC) was 0.66 (standard error) 0.18, 95 % CI = 0.92 – 0.99, p=0.0003. The sensitivity and specificity of the test at the cut-off value of ≥ 3.05 ng/mL were 77% and 65%, respectively, positive predictive value(PPV) was 71%, negative predictive value(NPV) was 63% indicating a fair discriminative value.

Table 1
The means and standard deviation of age in patients and control

<table>
<thead>
<tr>
<th>Variable</th>
<th>Study Group</th>
<th>N</th>
<th>Mean ± SD</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>control</td>
<td>45</td>
<td>62.2 ± 7.8</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>case</td>
<td>45</td>
<td>60.3 ± 8.0</td>
<td>0.279</td>
</tr>
</tbody>
</table>
Figure 3.1. Rate of male to female among patients

Table 2
Comparison of pentosidine, TNF-α, HbA1c and glucose level in patients and control groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean ± SE</th>
<th>Pentosidine (ng/mL)</th>
<th>TNF-α (ng/mL)</th>
<th>HbA1c %</th>
<th>glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>2.5±1.5</td>
<td>140.8±26.4</td>
<td>11.9±2.2</td>
<td>247.5±95.8</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1.7±0.4</td>
<td>123.0±18.8</td>
<td>5.41±0.46</td>
<td>102.9±20.7</td>
<td></td>
</tr>
<tr>
<td>T-test</td>
<td>111.93</td>
<td>93.081</td>
<td>96.375</td>
<td>91.345</td>
<td></td>
</tr>
<tr>
<td>P-value</td>
<td>0.0003</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.0001</td>
<td></td>
</tr>
</tbody>
</table>

(P≤0.01)

Figure 2. Criterion values and coordinates of the ROC curve analysis for pentosidine as differentiating patients from control subject.
Discussion

In the current study, pentosidine levels increase in DRP Patients with highly significant differences (P≤0.01). pentosidine is also increase in diabetic nephropathy disease and may have a role in DRP prognosis (19). OPG levels are higher in patients with diabetes mellitus and DRP(20). this result was agreed with some study in which serum pentosidine levels are raised in individuals with DRP, with the goal of predicting complication of DM(21). the present study revealed that Parathyroid hormone (TNF-α) highly increased in DRP patient’s significant differences (P≤0.01) when compared with control group, when interpreting these results, the following issues should be taken into consideration. First, TNF-α can simulate the release and synergistic proliferation of IL-6, IL-8, VEGF and platelet derived growth factor (PDGF).

Second, TNF-α can inhibit the formation and development of retinal vascular endothelial cells, promote apoptosis of endothelial cells, destroy normal function of the vascular wall and influence vascular permeability of the retina. This study showed HbA1c, glucose were higher significantly increased in patient group compare with healthy control where (P≤0.01). Severe retinopathy (NPDR/PDR), however, was more frequent in type 2 than in type 1 diabetic patients in our study, Patients with type 2 diabetes may have been diabetic before diagnosis. Therefore, the duration of diabetes may have exceeded 10 years and may explain why they had the most severe form of retinopathy. Diabetes duration and prolonged poor glycemic control are the main predictors of the prevalence and progression of retinopathy. The natural history of the microvascular complications is closely associated with metabolic control as expressed by the HbA1c levels, a high glycemic burden over time leads to an increased frequency and severity of retinopathy. Other previous study done by [22] reported The concentration of VEGF has been found to be higher in the vitreous of eyes with PDR. Likewise in the serum of obese individuals, elevated antigenic factors including VEGF have been observed partially owing to the presence of oxidative stress providing additional confirmation of the possible link between obesity and PDR [23].

Conclusions

The biochemical parameters included in this study such as pentosidine, leptin and TNF-α are significantly higher in DR patients than healthy control and its influenced by the severity and duration of DR, but adiponectin are significantly higher in healthy control than DR patients and C-RP show non-significant difference between patients and controls group. the body mass index was significantly higher in patients than control, so obesity considered as a comorbid for DR patients. There was a positive correlation between BMI and glucose, also between pentosidine and TNF-α and between leptin and TNF-α and between adiponectin and TNF-α. Pentosidine could be considered as a biomarker of inflammation in DR and is more sensitive and specific than adiponectin so that it’s a good marker for diagnosis of DR patients TNF-α can be used as a new marker for evaluation of disease severity and progression in DR.
References