The Role of Advanced Glycated End Products and Microalbuminuria on Developing Diabetic Retinopathy in Type 2 Diabetes Mellitus

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Abstract

Diabetic retinopathy is a frequent microvascular complication of diabetes mellitus. We analyzed the association of serum level of major advanced glycated end product mainly Ne-carboxymethyl-lysine with prevalence of developing of diabetic retinopathy in Type 2 diabetic patients, a case control study were confirmed on patients with type 2 diabetes mellitus whom were examined by an ophthalmologist at AL- Hilla Teaching Hospital and patients classified into proliferative and non proliferative diabetic retinopathy and the level of Ne-carboxymethyl-lysine were 2.2±0.45 and2.9±0.24 among NPDRP and PDRP respectively and the level among control diabetic patients without retinopathy 1.2±0.2 and the statistical analysis were revealed that were significant difference between patients and control at p value <0.05. Estimation of microalbuminuria among diabetic patients revealed that prevalence of microalbuminuria among patients with NPDRP is (68%) and among patients with PDRP are (80%) .while prevalence among diabetic patients without retinopathy are (33%). The odd ratio for association between microalbuminuria and diabetic retinopathy is calculated and the results is 2.5 which indicate that patients with positive microalbuminuria is at higher risk for developing retinopathy and microalbuminuria is considered risk marker for developing diabetic retinopathy.

Keywords: advanced glycated end products, carboxymethyl-lysine, diabetic retinopathy, proliferative diabetic retinopathy, risk marker, odd ratio.

Introduction

Diabetic retinopathy (DR), can be defined as damage to microvascular system in the retina due to prolonged hyperglycemia[1]. DR is the most common and specific microvascular complication of diabetes, It remains a
major cause of visual impairment worldwide among the people in working age and is a leading cause of visual loss in older patients[2].

DR is broadly classified as either non proliferative diabetic retinopathy (NPDR) or proliferative diabetic retinopathy (PDRP). These depend on microvascular changes in the retina as studied by ophthalmoscopy [3] . Fundus abnormalities in diabetic patients have a progressive course, from mild retinopathy non proliferative, where microaneurisms are the main feature[4,5], to severe proliferative disease with neovascularization of the disc, retina and iris[6,7].

Advanced glycation end products (AGEs) have been implicated as causal factors in the complications of diabetes mellitus[8]. The term “AGEs” refers to post translationally glycated modifications on end-standing aminogroups on proteins, lipoproteins, lipids and nucleic acids that non-enzymatically have undergone irreversible dehydration and condensation processes via various reactive intermediates. [9]. The modification itself is often referred to as an “adduct”. There are several alternative routes into forming AGEs, and the predominant substrate fuelling the glycation is glucose. Possible pathway for formation AGEP are showed in figure no.1[10].

**Figure 1** pathway for formation AGEP.

One of important AGEs is Nε-carboxymethyllysine (CML) which formed from (glycoxidation of AGEs [11]. AGEs believed to be of pathogenica importance in microvascular complications developing[12].

Microalbuminuria is now defined as a urine albumin excretion between 20 and 200 μg/min (or 15 to 150 μg/min overnighth) [13]. However, these values are currently detectable by semi-quantitative dipstick tests, and can be accurately measured by several widely available sensitive methods such as ELISA, RIA, and nephelometry[14].

Microalbuminuria is often present at the time of diagnosis either due to insidious nature and asymptomatic initial years of type 2 diabetes, or its positive association with insulin resistance[15]. The importance of microalbuminuria as an independent predictor of progressive renal disease and cardiovascular
mortality was thereafter realized in a number of prospective and epidemiological studies particularly in patients with diabetes and hypertension[16,17] The prevalence of microalbuminuria among diabetic patients is (15-20-%).

Microalbuminuria in diabetic patients is a risk marker not only for kidney and cardiac disorders but also for severe Persistence ocular morbidity[18].

**Material and Method**

A case control study was done on 125 patients with NPDRP and PDRP as well as control groups

The subjects participated in this study were classified into following:-  
**Group one:** 75 patients with 2 DM–75 (46 males and 29 females ) with retinopathy ranging from NPDR to proliferative retinopathy. Non proliferative ranging into mild, moderate and severe diabetic retinopathy  
**Group two:** patient with type 2 diabetes mellitus without retinopathy  
**Group three:** apparently healthy subjects were chosen as healthy controls, they were non smoker, non alcohol, and did not have any history of chronic diseases.

**Exclusion criteria**

1-Congestive heart failure  
2-Urinary tract infection  
3-Fever  
4-Peripheral neuropathy  
5-Renal disease  
7 - Patients with type 1 diabetes mellitus  
8- Gestational diabetes

**Methods**

1. OxiSelect™ Advanced Glycation End Product (AGE) Kit for determination CML in serum by ELISA [19] . The standard curve for estimation CML are shown in figure 2

![Figure2](image-url): Standard curve of CML

2. **Microalbuminurea** determined Strips for the, semi- quantitative in vitro determination of urinary albumin up to a concentration of 100 mg/L.

**Principle**

Immunological detection of human albumin by means of soluble antibody gold-conjugate. Excess conjugate is retained in a separation zone containing immobilised human albumin immunological[20].

Statistical analysis were performed using SPSS17:0(SPSS Inc, Chicago ,11,USA ) The odds ratio
(Ors) and 95% confidence intervals (CIs) for possible risk factors in diabetic retinopathy were calculated. Statistical significance was defined as P < 0.05

**Results**

1. **Descriptive study for DRP patients groups**

The demographic and medical characteristics of patients are shown in table 1.

**Table 1** Patients clinical characteristics

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>DRP</th>
<th>No DRP</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (Years)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NPR</td>
<td>56±8</td>
<td>55.5± 4.5</td>
<td>0.8</td>
</tr>
<tr>
<td>PR</td>
<td>51± 9.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>46</td>
<td>M 16</td>
<td>0.3</td>
</tr>
<tr>
<td>F</td>
<td>29</td>
<td>F 9</td>
<td></td>
</tr>
<tr>
<td><strong>BMI(KG/M²)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NPR</td>
<td>M 27±4.2</td>
<td>M 30±2.1</td>
<td>0.5</td>
</tr>
<tr>
<td>PR</td>
<td>F 27±4.2</td>
<td>F 24±2.5</td>
<td></td>
</tr>
<tr>
<td><strong>HbA1c%</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NPR</td>
<td>8.2±0.8</td>
<td>7.6± 0.6</td>
<td>0.005*</td>
</tr>
<tr>
<td>PR</td>
<td>10.2±2.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Duration in years</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NPR</td>
<td>17±6</td>
<td>5.5</td>
<td>0.001*</td>
</tr>
<tr>
<td>PR</td>
<td>12±6.2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

2. **Microalbuminuria and DRP**

Microalbuminuria was estimated in diabetic retinopathy and control group and the relationship between positive microalbuminuria and developing of DRP are represented in figure 3

**Figure 3** Relationship between microalbuminuria and DRP
3. The relationship between gender and microalbuminuria: The relationship between gender and developing of diabetic retinopathy were represented in figure 4.

Figure 4 The relationship between gender and microalbuminuria.

3. The relationship between duration of DRP and microalbuminuria: The association of microalbuminuria with duration are representing in figure 5.

Figure 5: The relationship between duration of DRP and microalbuminuria.

4. DRP and CML

Nε-propionyl CML is belonged to one of advanced glycated end products was measured by ELISA technique and mean± SD are summarized in table 1.

Table 2 Mean and standard deviation of CML in diabetic retinopathy and control.

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>CML Mean ± SD (Ug/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild DRP</td>
<td>1.8±0.3</td>
</tr>
<tr>
<td>Moderate DRP</td>
<td>2.2±0.45</td>
</tr>
<tr>
<td>Severe DRP</td>
<td>2.6±0.2</td>
</tr>
<tr>
<td>Proliferative DRP</td>
<td>2.9±0.24</td>
</tr>
<tr>
<td>Control DNR</td>
<td>1.2±0.2</td>
</tr>
<tr>
<td>Control HC</td>
<td>0.6±0.05</td>
</tr>
</tbody>
</table>
The comparism of CML level between diabetic retinopathy (proliferative and non proliferative) with control group are represented in figure 6.

**Figure 6** The comparism of Nε-carboxy(methyl)lysine (CML) in sera of type 2 diabetic patients with and without retinopathy.

The ANOVAs analysis for comparism control group are represented in mean among different groups for following table patients with diabetic retinopathy and

**Table 3** A nova analysis for comparism CML between control and DRP patients.

<table>
<thead>
<tr>
<th>Comparism group</th>
<th>Diabetic retinopathy</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control DM NDRP</td>
<td>MildNDRP</td>
<td>0.001*</td>
</tr>
<tr>
<td></td>
<td>Mod.NDRP</td>
<td>0.001*</td>
</tr>
<tr>
<td></td>
<td>Severe NDRP</td>
<td>0.001*</td>
</tr>
<tr>
<td></td>
<td>PDRP</td>
<td>0.001*</td>
</tr>
</tbody>
</table>

(P-value of < 0.05* was considered to be statistically significant)

Advanced glycated end product as CML are considered risk factor for developing diabetic microvascular complication as diabetic retinopathy and The associated between CML with chronic hyperglycemia among diabetic retinopathy patients were represented by Linear regression in figure 7.
Figure 7 linear regression of CML and blood Glucose.

Table 4 The odd ratio for independent variables as possible risk factor for DRP.

<table>
<thead>
<tr>
<th>Independent variable</th>
<th>Odd ratio</th>
<th>95% CI</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>DURATION &gt;5</td>
<td>5.3</td>
<td>1.9-13.4</td>
<td>0.001*</td>
</tr>
<tr>
<td>INSULIN R AND DRP</td>
<td>4.5</td>
<td>1.6-9.4</td>
<td>0.001*</td>
</tr>
<tr>
<td>FAMILY HISTORY</td>
<td>3.2</td>
<td>1.3-6.4</td>
<td>&lt;0.01*</td>
</tr>
<tr>
<td>Hyperglycemia and DRP</td>
<td>2.6</td>
<td>1.44.3</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Microalbuminuria and DRP</td>
<td>2.5</td>
<td>1.4</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Obesity and DRP</td>
<td>1</td>
<td>0.7-0.9</td>
<td>0.3</td>
</tr>
</tbody>
</table>

* significant DRP diabetic retinopathy ,CI confidence interval

Discussion
The age of patients varied from 43 to 68 years old (average 56±8±51±9.4years for NPDRP and PDRP). Patients 'profile data such as sex, family history and type of treatments are shown in Tables 1.

In figure 3 were revealed that microalbuminuria is associated with developing and progression of diabetic retinopathy ,the prevalence of microalbuminuria among patients with non proliferative DRP is 68% ,and among patients with proliferative DRP are 80% ,while prevalence among diabetic patients without retinopathy are 33%.

The data in figure 4 were shown the developing of microalbuminuria among males is more prevalent than females, the microalbuminuria is 60% among males and (40%)among female in patients with diabetic retinopathy.

The microalbuminuria in patients with diabetic retinopathy were increased with increasing in duration of diabetes mellitus ,meaning that microalbuminuria and duration represented risk factors for developing diabetic retinopathy.
Microalbuminuria is considered as marker and predictor for developing diabetic retinopathy, by calculation of odd ratio for microalbuninuria and developing diabetic retinopathy the odd ratio is 2.5 and the odd ratio for increase duration of DM and developing diabetic retinopathy is 5.3 which mean that microalbuminuria and duration are considered risk factors for developing diabetic retinopathy. Microalbuminuria considered as biomarker for detection of microvascular complication in diabetes mellitus as diabetic retinopathy most resent studies agree with this result[21,22] . and some other studies may opposite this result [23,24].

We performed this a case-control study and had been analyzed the association of CML serum levels with advanced stages of diabetic retinopathy. The higher the serum of CML level the higher the likelihood for advanced stages diabetic retinopathy. Therefore CML levels provided a novel progressive risk marker for developing and progression of diabetic retinopathy.

A studies were indicated that there were a direct link of advanced glycation end products, including the late oxidative product Ne-carboxymethyl-lysine with diabetic microvascular complications so CML has a role in developing microvascular complication in diabetic patients that lead to difference Vascular dysfunction, including basement membrane thickening, increased vascular permeability and prothrombotic state, and decreased blood flow and it is a ubiquitous trait of microvascular disease of the retina, nephron, and peripheral nerve [25,26]. Since CML can engage receptors of signal transduction for AGE (RAGE), therefore CML can directly activate key cell signalling pathways and modulate gene expression. Most importantly RAGE expression has been found in the retina, mesangial compartment concomitant with AGE/CML accumulation and may therefore provide a direct link of CML levels and diabetic complications[.27]. Our study suggests that factors influencing levels of protein and lipid glycation and oxidation leading to increased level of late oxidative product of CML and this are of considerable importance in microvascular complications. Factors determining differential CML levels could have a major clinical impact and may lead to a wide range of pathologies including vascular complications[28]. The relation ship between CML and developing and progression of diabetic retinopathy were confirmed by other studies [29,30].

References
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