ALDOSE REDUCTASE ACTIVITY FROM ERYTHROCYTE OF DIABETES MELLITUS PATIENTS

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Abstract

Diabetes often referred to by doctors as diabetes mellitus, describe a group of metabolic diseases in which the person has high blood glucose (blood sugar), either because insulin production is inadequate, or because the body’s cells do not respond properly to insulin or both. The acceleration of the polyol pathway elicits various metabolic imbalances in those tissues that undergo insulin dependent uptake of glucose. The rate-limiting step of this polyol pathway is aldose reductase (ALR2). We investigated the relationship between ALR2 levels with diabetic neuropathy (DN) and diabetic retinopathy (DR) by measuring ALR2 activity in erythrocytes. We examined 59 type 2 diabetic subjects (T2D) with DN and DR and 30 normal subjects in this clinical case-control study. Clinical evaluation of DN in T2D patients was done by nerve conducting velocity (NCV) test and for DR fundus examination was done. ALR2 activity levels along with glucose and glycosylated hemoglobin (HbA1C) levels in erythrocytes were determined. Patients with DN and DR showed higher activity for ALR2 than normal individuals. In patients with DR had longer duration of diabetes than patients with DN. Other parameters like HbA1C, blood sugar level showed no significant relation with the result. Based on our result we may conclude that high value of ALR2 has a role in diabetes. Patients with this high value of ALR2 may be more prone to diabetic complications like neuropathy, retinopathy.

Keywords: Type 2 diabetes (T2D), Diabetic Neuropathy (DN), Diabetic Retinopathy (DR), Aldose Reductase (ALR2).

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INTRODUCTION

Diabetes Mellitus (DM) is emerging as an important public health issue all over the globe including India. Diabetes is characterized by hyperglycaemia resulting in various short-term metabolic changes in lipid and protein metabolism and long-term irreversible vascular changes. These include diabetes-specific complications of the micro-vasculature system (retinopathy, nephropathy and neuropathy) and complications of the macro-vasculature (atherosclerosis leading to heart disease, stroke and peripheral vascular disease) which are present in the non-diabetic population, but have a two to five-fold increase in diabetic subjects. The incidence of diabetes is predicted to double over the next decade. Recent data obtained from the Diabetes Control and Complications Trial clearly indicate in patients with insulin-dependent diabetes mellitus (IDDM) the onset and progression of long-term diabetic complications is effectively delays and slows with intensive insulin treatment (Diabetes Control and Complications Trial Research Group) [1].

A major part of the cellular glucose is phosphorylated into glucose 6-phosphate by hexokinase. A minor portion of nonphosphorylated glucose enters the so-called polyol pathway, an alternate route of glucose metabolism. The rate-limiting step of this polyol pathway is aldose reductase (EC 1.1.1.21). The polyol pathway involves two enzymatic reactions: firstly glucose is reduced to sorbitol by the action of aldose reductase (AR) and then Sorbitol is subsequently oxidised to fructose by sorbitol dehydrogenase. In normal glucose (5.5 mg) condition, AR-catalyzed reduction represents less than 3% of total glucose utilization, whereas in the presence of high glucose (20mg), more than 30% of the glucose is used by AR [2]. It only suggests that the profound increase in the AR-catalyzed reductive pathway may impose a significant strain on NADPH supply. Under hyperglycemia the hexokinase is saturated with ambient glucose. So the increased flux of glucose through the polyol pathway accounts for as much as one-third of the total glucose turnover [2]. This leads to overflow of the products of the polyol pathway along with depletion in reduced nicotinamide adenine dinucleotide phosphate (NADPH) and the oxidized form of nicotinamide adenine dinucleotide (NAD1), the cofactors used in the pathway. This acceleration of the polyol pathway thus elicits various metabolic imbalances in those tissues that undergo insulin independent uptake of glucose. The early tissue damage in the “target” organs of diabetic complications, such as ocular lens, retina, peripheral nerve, and renal glomerulus is provoked by such metabolic perturbation [3]. In this study, we examined the activity of ALR2 in erythrocytes obtained from diabetic patients with neuropathy and retinopathy. These data
demonstrate that erythrocyte ALR2 activity is significantly elevated in diabetic patients with neuropathy and retinopathy as compared with patients without diabetes.

**METHODOLOGY**

**Subjects and Study Design:** A hospital-based prospective case control study was conducted. The study protocols were approved by the Institutional Ethics Committees of the institute involved. Type 2 diabetic patients (T2D) were recruited from the patients who visited the Out Patient Department of Endocrinology, Ramakrishna Mission Seva Pratishthan, Kolkata, India. A total of 59 T2D subjects (48 with Neuropathy, 11 with Retinopathy) and 30 normal subjects were investigated. Consent was obtained from the patients after they were given an explanation of the study details. A complete history of each patient, with respect to age, gender, clinical symptoms, diabetes type and duration, medication, and socioeconomic background, was collected using questionnaire. The Nerve Conducting Velocity (NCV) was evaluated of every patient. For Retinopathy the fundus of each subject was evaluated by both direct and indirect ophthalmoscopy.

**Sample Collection and Processing:** 1 ml of whole blood was drawn from the subjects into anticoagulant tubes and immediately transported to the laboratory on ice. Red blood cells (RBC) were separated by centrifugation (centrifugation done at 10,000 × g for 10 minutes), washed thrice with saline, and stored at −40 °C until further analysis.

**Aldose Reductase Activity:** A 10% erythrocyte suspension was made by adding 50 mM sodium phosphate buffer, pH 7.4, containing 150 mM NaCl. The suspension was lysed by repeated freezing and thawing (three cycles) and centrifuged to remove insoluble material, if any. ALR2 activity was measured spectrophotometrically using an appropriately diluted hemolysate. AR activity was assayed according to the method described by Hayman and Kinoshita [4] using a SpectraMax spectrophotometer (Molecular Devices, Sunnyvale, CA). One unit was defined as micromoles NADPH oxidized/g Hb/ min. The assay mixture of 1 ml contained 50 μmol potassium phosphate buffer pH 6.2, 0.4 mmol lithium sulfate, 5 μmol 2-mercapto ethanol, 10 μmol DL-glyceraldehyde, 0.1 μmol NADPH and enzyme preparation (hemolysate). The assay mixture was incubated at 37 °C and initiated by the addition of NADPH at 37 °C. The change in the absorbance at 340 nm due to NADPH oxidation was followed.

**Statistical Analysis:** All data were statistically analyzed. Data were expressed as mean±standard deviation.
RESULTS

RESULTS: All data on mean age, duration of diabetes, blood sugar level, glycosylated hemoglobin (HbA1C), aldose reductase activity (ALR2) with respect to gender distribution for nondiabetic as control, diabetes with neuropathy and diabetes with retinopathy are summarized in Table 1.

Table 1: Clinical and Biochemical Features of the Studied Cases

<table>
<thead>
<tr>
<th></th>
<th>No. of Cases</th>
<th>Age Mean±SD (Years)</th>
<th>Duration Mean±SD (Years)</th>
<th>Glucose Mean±SD (mg/dL)</th>
<th>HbA1C Mean±SD (%)</th>
<th>ALR2 Mean±SD (units/g Hb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non Diabetic</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>20</td>
<td>53.8±11.47</td>
<td>0</td>
<td>129.38±21.03</td>
<td>5.9±0.7</td>
<td>1.79±1.2</td>
</tr>
<tr>
<td>Female</td>
<td>10</td>
<td>47.34±6.09</td>
<td>0</td>
<td>115.09±86</td>
<td>5.75±1.2</td>
<td>1.13±0.8</td>
</tr>
<tr>
<td>Neuropathy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>33</td>
<td>54.36±10.08</td>
<td>8.70±7.52</td>
<td>298.83±171.39</td>
<td>8.58±1.14</td>
<td>3.87±1.85</td>
</tr>
<tr>
<td>Female</td>
<td>15</td>
<td>49.8±10.47</td>
<td>12.2±5.80</td>
<td>326±0</td>
<td>8.03±0.33</td>
<td>4.14±5.28</td>
</tr>
<tr>
<td>Retinopathy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>6</td>
<td>58.67±3.06</td>
<td>14.33±1.18</td>
<td>242.83±97.70</td>
<td>8.72±0.06</td>
<td>3.75±0</td>
</tr>
<tr>
<td>Female</td>
<td>5</td>
<td>52.6±3.82</td>
<td>20.4±8.20</td>
<td>209±50.20</td>
<td>7.76±0.52</td>
<td>3.5±0.18</td>
</tr>
</tbody>
</table>

The distribution of age, random glucose, duration of diabetes, glycosylated hemoglobin (HbA1C) and ALR2 activity levels between male and females in nondiabetic control, diabetes neuropathy (DN) and diabetes retinopathy (DR) groups. All the data are represented as mean ± standard deviation (SD).

Table 1 showed that in respect to control cases ALR2 activity in both the neuropathy and retinopathy cases is significantly different but erythrocyte ALR2 activity in male and female cases was not significantly different. In both cases the value of ALR2 activity is higher than the normal individuals. However, the ALR2 activity ranged from 0.25 units/g Hb in the control group to 8.75 units/g Hb in the diabetic neuropathy group. There is no significant correlation between the activity of ALR2 with age, glucose level, diabetes duration, and HbA1C levels in all both the neuropathy and retinopathy groups. Though all the values are significantly low in control cases.

DISCUSSION

It has been hypothesized that uncontrolled hyperglycemia is the major factor to various secondary complications. When intracellular glucose levels are high polyol pathway for glucose metabolism is activated [5] and this activation is immediately linked to hyperglycemia and as a result it develops complications [5,6]. Increased oxidative stress also induces diabetic complications like retinopathy [7]. As ALR2 was present in cells of retina, it became evident for the occurrence of
diabetic retinopathy [8-11]. So ALR2 becomes an intriguing target for the treatment of secondary complications. The present study investigated the activity of erythrocyte ALR2 in DR patients as well as patients with DN. The results showed that the activity of ALR2 is significantly higher in diabetic patients with complications like neuropathy and retinopathy as compared to nondiabetic patients. Though the difference between diabetic neuropathy and diabetic retinopathy is significantly low. Certain increase in oxidative stress had been associated with diabetic complications [7]. In some study it has been found that this increased oxidative stress help in oxidation of certain molecule like cystein which in turn modulate ALR2 activity[12]. So it can be concluded that specific activity of certain protein may also contribute to the development of diabetic complications. In this study we have also found that ALR2 activity is higher in neuropathy and retinopathy cases than normal, though there is no significant differences between diseased state. So from this result we may also conclude that high value of ALR2 may initiate the disease but not involve in its progression. Diabetic complications generate with times. Diabetic neuropathy and retinopathy are such complications which are linked to diabetes duration of patients [13]. In this study we observed that diabetic retinopathy happens with patients who have diabetes for more than 10-15 years on average. In contrast neuropathy happens with patients with a history of diabetes for more than 5 years on average. So this study also supports that diabetes complications have an association with duration of this disease. Other parameters like HbA1C, blood sugar level have no significant difference. Based on our study, we can hypothesize that ALR2 activity may act as an predisposing factor in diabetes. Diabetes patient with certain high ALR2 level is prone to these secondary complications like diabetic neuropathy and diabetic retinopathy.

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REFERENCES