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RESEARCH ARTICLE

GLYCATED HEMOGLOBIN AND THE ACTIVITIES OF CARBOHYDRATE METABOLIC ENZYMES IN THE RED BLOOD CELLS OF TYPE 2 DIABETIC PATIENTS

[1]Thangarasu Rajakumar, [1]Sekar Subashini, [2]Mangipudi Ramakrishna Rao, and [1,*]Kodukkur Viswanathan Pugalendi

[1]Department of Biochemistry and Biotechnology, Faculty of Science, Annamalai University, Annamalainagar-608 002, Tamilnadu, India
[2]Faculty of Medicine, Rajah Muthiah Medical College and Hospital, Annamalai University, Annamalainagar-608 002, Tamilnadu, India

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ABSTRACT

Glycated hemoglobin (HbA_{1c}) is one of the parameter to assess the diabetic condition. In this study, the activities of carbohydrate metabolic enzymes were assessed and correlated with HbA_{1c} in the red blood cells (RBCs) of type 2 diabetes mellitus (T2DM) patients. Thirty diabetic patients (taking insulin and different antidiabetic drugs) from Rajah Muthiah Medical College and Hospital, between the age 40 and 70 were chosen and they were not suffering from any other illness and fifteen non-diabetic control subjects of the same age groups were also chosen for comparison. Index of glycemic control (plasma glucose, insulin, blood hemoglobin and glycated hemoglobin) and the activities of carbohydrate metabolic enzymes (hexokinase, phosphogluco isomerase, aldolase, pyruvate kinase and glucose 6-phosphate dehydrogenase) were measured. Diabetic patients have significantly higher plasma glucose, glycated hemoglobin and lower insulin and hemoglobin. Activities of hexokinase, phosphogluco isomerase, aldolase, pyruvate kinase and glucose 6-phosphate dehydrogenase were significantly lower in diabetic patients when compared with non-diabetic control subjects. Statistical analysis indicated a correlation of glycated hemoglobin with hexokinase (r= -0.858, p= 0.000), phosphogluco isomerase (r= -0.546, p= 0.000), aldolase (r= -0.591, p= 0.000), pyruvate kinase (r= -0.360, p= 0.023) and glucose 6-phosphate dehydrogenase (r= -0.449, p= 0.004) in non-diabetic control subjects and diabetic patients. Inspite of regular intake of antidiabetic drugs or insulin, the index of glycemic control showed significant increase implying impaired control of diabetes mellitus. Hexokinase showed a significantly strong negative correlation to glycated hemoglobin. RBCs hexokinase activity has been inversely correlated with glycation which could act as an index in evaluation of glycation in diabetic condition.

INTRODUCTION

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction and failure of various organs. Hypertension and abnormalities of lipoprotein metabolism are often found in people with diabetes (ADA, 2012). Mortality and morbidity associated with diabetes is mainly due to complications arising from it which include neuropathy, nephropathy, vasculopathy and retinopathy (Amos *et al*., 1997). Diabetes mellitus is a life-long disease, which makes many people worry about the quality and longevity of their life after being diagnosed with it. The complications of diabetes are influenced not only by the duration of diabetes but also by the average level of chronic hyperglycemia (DCCT, 1995; Stratton *et al*., 2000) which is measured most reliably with glycated hemoglobin assay. In normoglycemic subjects, a small proportion of adult hemoglobin is attached to a carbohydrate moiety thus creating what is called glycated hemoglobin (Burm *et al*., 1975). Glycated hemoglobin is the total glycohemoglobin present in red blood cells. The N-terminal valine of the 4-polypeptide chains and all the free ε-amino groups of lysine residues are the likely glycation site on the adult hemoglobin molecules. The glycation of the N-terminal of the valine residue of the β-chain predominates and accounts for approximately 60% bound glucose (Krishnamurti and Steffes, 2001). Total glycated hemoglobin includes all glycated fractions, comprising HbA_{1c} as well as hemoglobin glycated at sites other than the N-terminus of the beta chain e.g. epsilon amino groups on lysine residues. The concentration of HbA_{1c} depends on both the concentration of glucose in the blood and the life span of the erythrocytes. Because erythrocytes are in the circulation for approximately 120 days, HbA_{1c} represents the integrated glucose concentration over the preceding 8-12 weeks (Goldstein *et al*., 2004). Erythrocyte metabolism is mostly restricted to two main pathways. The Embden-Meyerhof pathway (EMP) is mainly involved in adenosine triphosphate (ATP) and 2, 3-diphosphoglycerate (2, 3 DPG) generations in the erythrocytes, while the pentose phosphate pathway (PPP) generates NADPH, as a source of reducing equivalents to protect erythrocytes from oxidation. The activities of the enzymes pyruvate kinase and glucose 6-phosphate dehydrogenase are often tested as key-enzyme of EMP and PPP, respectively. In human, glycosylation affects the function of hemoglobin binding to 2, 3 DPG site, thus influencing the regulation of the affinity with oxygen (Jones and Peterson, 1981). In this study, we made an attempt to finding these enzymes activities in correlation with glycation of hemoglobin in T2DM patients.

***Corresponding author:** Kodukkur Viswanathan Pugalendi,
Department of Biochemistry and Biotechnology, Faculty of Science, Annamalai University, Annamalainagar-608 002, Tamilnadu, India

MATERIALS AND METHODS

Patients

Thirty patients of T2DM from Rajah Muthiah Medical College and Hospital, Annamalai University, Chidambaram and fifteen non-diabetic control subjects in the same age group were selected for this study.

Diabetic patients and non-diabetic control subjects were from both sexes and ranged in age from 40 to 70 years. All patients were controlling their blood sugar by taking oral hypoglycemic drugs (biguanides or sulphonylureas) or by insulin injection. Diabetic patients and non-diabetic control subjects were not suffering from any other illness. The study was approved by the Institutional Human Ethics Committee of Rajah Muthiah Medical College and Hospital, Annamalai University, Chidambaram and written consent was obtained from each patient after they had been given a detailed explanation of the study.

Chemicals

Phospho (enol) pyruvic acid, D-fructose 1, 6-bisphosphate and all the biochemicals and chemicals used in this experiment were of analytical grade and purchased from Sigma Chemical Co., St. Louis, MO, USA. Reagent kits for glucose, hemoglobin, glycated hemoglobin were purchased from Agappe Diagnostics, Ernakulam, India.

Blood collection

Fasting blood was collected in heparin and EDTA tubes by venous arm puncture. The blood was centrifuged at 2000 rpm for 10 min and the plasma was separated by aspiration. Heparinised plasma was used for glucose estimation. EDTA blood was used for evaluation of glycated hemoglobin.

Determination of Glucose, Insulin, Hemoglobin and Glycated Hemoglobin

Plasma glucose was estimated by the method of Trinder, (1969) using a reagent kit. Plasma insulin was measured by the method of Burgi *et al*., (1988). Hemoglobin in the blood was estimated by the method of Drabkin and Austin, (1932). HbA_{1c} is determined from blood by immunoturbidimetric latex assay method of ADA, (2001) which is based on the principle of agglutination reaction.

Determination of Carbohydrate Metabolic Enzymes

Hexokinase was assayed by the method of Brandstrup *et al*. (1957). Phosphogluco isomerase was determined by the method of Horrocks *et al*. (1963). Aldolase was assayed by the method of Sibley and Lehninger, (1948). Pyruvate kinase was assayed by the method of Valentine and Tanaka, (1966). Glucose 6-phosphate dehydrogenase was assayed by the method of Bergmeyer, (1984).

Statistical analysis

All values are expressed as means ± standard deviation (SD). The diabetic patient group was compared to the non-diabetic control subject group using the two-tailed Student's t-test for unpaired data. Correlation between groups was tested by the Pearson test. Statistical analyses were performed using Statistical Package for the Social Sciences version 16.0 (SPSS Inc., Chicago, IL, USA). *P* value less than 0.05 was considered to indicate statistical significance.

RESULTS

Table 1 shows the levels of glucose, insulin, hemoglobin and glycated hemoglobin in non-diabetic control subjects and diabetic patients. The levels of glucose and glycated hemoglobin were significantly higher, insulin and hemoglobin levels were significantly lower in diabetic patients when compared with non-diabetic control subjects. Table 2 shows the activities of carbohydrate metabolic enzymes (hexokinase, phosphogluco isomerase, aldolase, pyruvate kinase and glucose 6-phosphate dehydrogenase) in non-diabetic control subjects and diabetic patients. The activities of all these enzymes were significantly lower in diabetic patients when compared with non-diabetic control subjects. Figures 1-5 show the Pearson correlation between the level of HbA_{1c} and the activities of hexokinase (r= -0.858, p= 0.000), phosphogluco isomerase (r= -0.546, p= 0.000), aldolase (r= -0.591, p= 0.000), pyruvate kinase (r= -0.360, p= 0.023) and glucose 6-phosphate dehydrogenase (r= -0.449, p= 0.004), respectively, in non-diabetic control subjects and diabetic patients. Hexokinase showed a significantly strong negative correlation to glycated hemoglobin.

DISCUSSION

Absence of rapid insulin secretion in response to a rise in plasma glucose is the hallmark of type 2 diabetes (Weyer *et al*., 1999; Pfeifer *et al*., 1981) and the decline in beta cell function determines the progression towards a need for insulin therapy (Prospective Diabetes Study Group UK, 1995). Despite much higher blood glucose levels Type 2 diabetic patients demonstrated lower total insulin output. This could be explained by the failure of pancreatic beta cells to respond appropriately to the prevailing blood glucose levels. The hypoinsulinaemia observed among type 2 diabetic patients in this study, is in agreement with earlier studies in African (Omar and Asmal, 1983; Asmal and Leary, 1975; Wicks and Jones, 1973) and African-American (Osei *et al*., 1993) type 2 diabetic

Table 1. Plasma (fasting) glucose, insulin and blood hemoglobin, glycated hemoglobin in non-diabetic control subjects and diabetic patients

Parameters	Non-diabetic control subjects (n=15)	Diabetic patients (n=30)	*p*- Value
Glucose (mg/dL)	87.97 ± 2.43	146.04 ± 22.76	0.001
Insulin (μU/mL)	8.50 ± 1.50	5.72± 0.95	0.001
Hemoglobin (g/dL)	12.87 ± 1.31	11.10 ± 1.18	0.001
Glycated hemoglobin (%)	5.51 ± 0.24	8.75 ± 1.57	0.001

Values are expressed as means ± standard deviation. Two-tailed Student's t-test.

Table 2. Carbohydrate metabolic enzyme activities in the RBCs of non-diabetic control subjects and diabetic patients

Parameters	Non-diabetic control subjects (n=15)	Diabetic patients (n=30)	[a]Normal range (μmol/g Hb)	[b]Lysine content (No.)	*p*- Value
Hexokinase (Unit*/min/g Hb)	1.56 ± 0.18	0.64 ± 0.21	1.0-2.5	59	0.001
Phosphogluco isomerase (Unit#/ min/g Hb)	42.46 ± 2.59	37.11 ± 2.49	38.8-82.8	35	0.001
Aldolase (Unit@/min/g Hb)	2.64 ± 0.37	1.60 ± 0.29	1.5-4.9	26	0.001
Pyruvate kinase (Unit§/min/g Hb)	12.66 ± 1.15	10.30 ± 1.64	11.1-18.9	37	0.001
Glucose 6- phosphate dehydrogenase (Unit¶/min/g Hb)	9.89 ± 0.66	8.05 ± 0.97	7.9-16.3	27	0.001

Values are expressed as means ± standard deviation. Two-tailed Student's t-test.
U* - μmol of glucose phosphorylated per min. U† - μmol of fructose liberated per min. U‡ - μmol of glyceraldehyde formed per min.
U§ - μmol of pyruvate formed per min. U¶- μmol of NADPH formed per min.

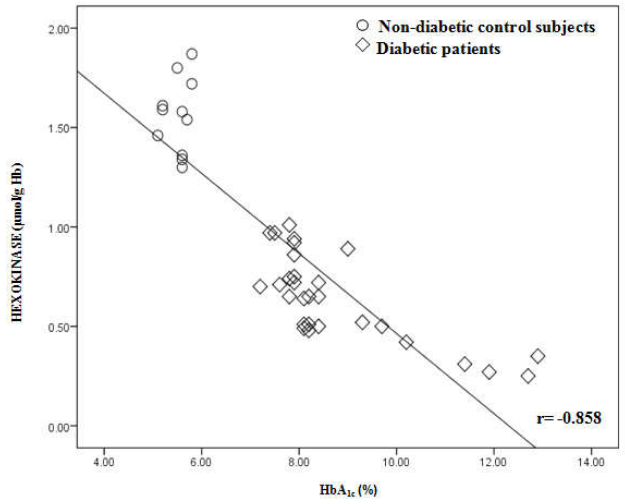

Fig. 1. The correlation between the levels of HbA_{1c} and hexokinase enzyme activities in non-diabetic control subjects and diabetic patients

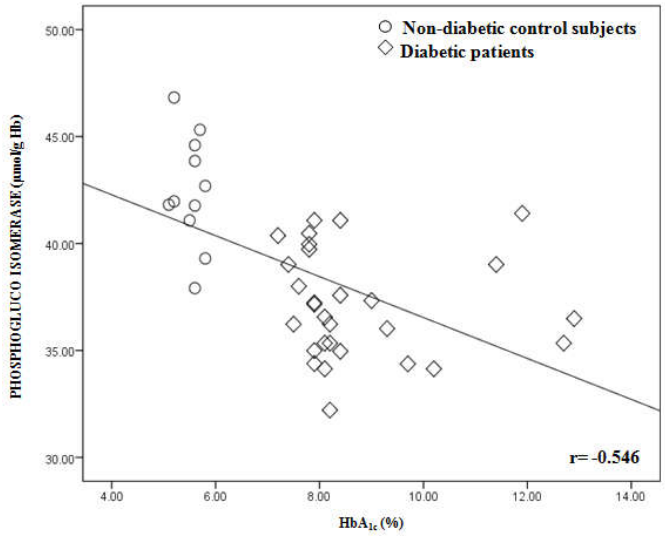

Fig. 2. The correlation between the levels of HbA_{1c} and phosphogluco isomerase enzyme activities in non-diabetic control subjects and diabetic patients

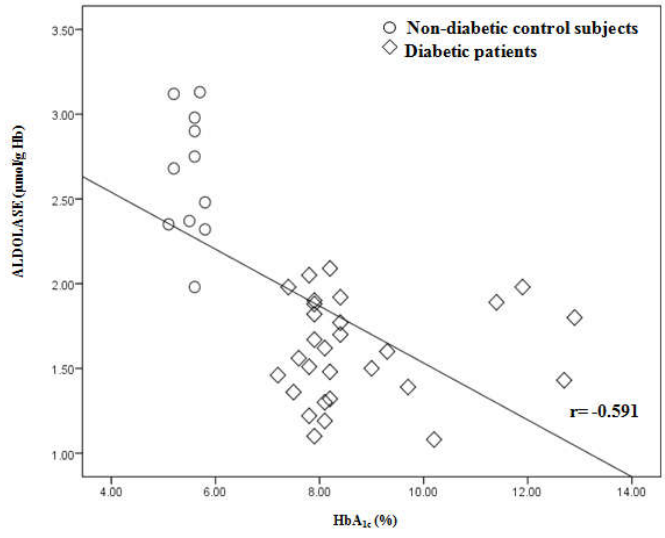

Fig. 3. The correlation between the levels of HbA_{1c} and aldolase enzyme activities in non-diabetic control subjects and diabetic patients

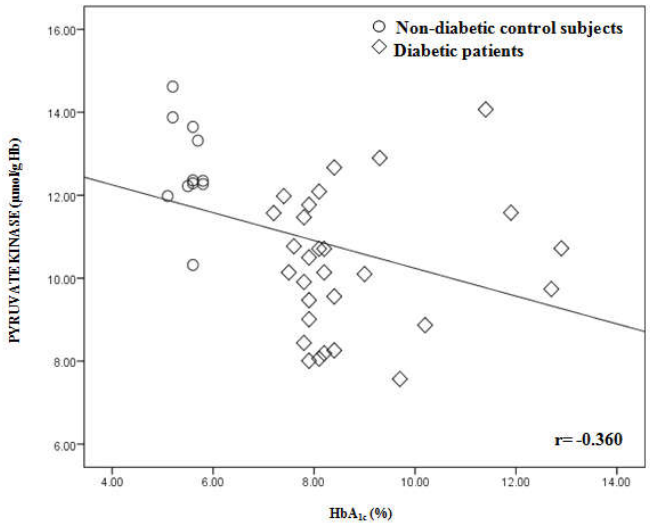

Fig. 4. The correlation between the levels of HbA_{1c} and pyruvate kinase enzyme activities in non-diabetic control subjects and diabeticpatients

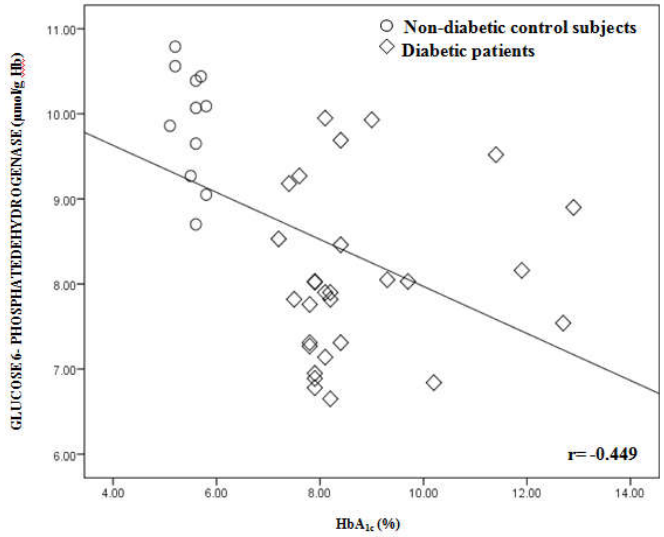

Fig. 5. The correlation between the levels of HbA_{1c} and glucose 6-phosphate dehydrogenase enzyme activities in non-diabetic control subjects and diabetic patients

populations. In our study, glucose level significantly higher and hemoglobin level significantly lower in diabetic patients when compared with non-diabetic control subjects. Glycated hemoglobin was found to increase in diabetic patients and the amount of increase is directly proportional to the fasting blood glucose level (Goodarzi *et al*., 2006). In our study, glycated hemoglobin level significantly higher in diabetic patients when compared with non-diabetic control subjects. Various studies prove that the amount of carbohydrate attached to the HbA_{1c} increases with increasing duration of the diabetes (Sampson *et al*., 2002). Hexokinase and glucose 6-phosphate dehydrogenase (G6PD) activities have been decreased, which may be due to loss of insulin receptors (Garvey *et al*., 1985). The decreased activity of G6PD enzyme in diabetic condition may result in the diminished functioning of hexose monophosphate shunt and thereby decreasing the production of reducing equivalents such as NADH and NADPH (Weber and Convery, 1996). Impairment of hexokinase activity suggests that the impaired oxidation of glucose via glycolysis, which leads to its accumulation, resulting in hyperglycemia. A partial or total deficiency of insulin causes a derangement in carbohydrate metabolism that decreases the activities of several key enzymes, including hexokinase, phosphofructokinase and pyruvate kinase (Hikino *et al*., 1989), resulting in impaired peripheral glucose utilization and augmented hepatic glucose production.

Phosphogluco isomerase is a widely distributed glycolytic enzyme present in liver, muscle, bone, brain, lung, erythrocytes and leucocytes in decreasing order of activity (Siegel and Bing, 1956; White, 1956; Bing *et al*., 1957). In our study, hexokinase, phosphogluco isomerase, aldolase, pyruvate kinase and glucose 6-phosphate dehydrogenase activities were significantly lower in the RBC of diabetic patients when compared with non-diabetic control subjects. Hexokinase, the first enzyme of glycolysis is having maximum number of lysine. The enzyme showed only 40% of its activity in diabetic patients. Since all other enzymes depend on this for their substrates, the activities of those enzymes were also reduced. In conclusion, inspite of regular intake of antidiabetic drugs or insulin, the index of glycemic control showed significant increase implying impaired control of diabetes mellitus. Hexokinase showed a significantly strong negative correlation to glycated hemoglobin. RBCs hexokinase activity has been inversely correlated with glycation which could act as index in evalution of glycation in diabetic condition.

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