USE OF GLYCATED HEMOGLOBIN (HbA_{1c}) AND IMPAIRED GLUCOSE TOLERANCE IN THE SCREENING OF UNDIAGNOSED DIABETES IN THE MALAYSIAN POPULATION

Rajes Qvist, Ikram Shah Ismail, Karuthan Chinna** and Sekaran Muniandy*

Departments of Medicine and *Molecular Medicine, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia

**Department of Quantitative Methods, Faculty of Information Technology and Quantitative Sciences, University Technology Mara, Shah Alam, Selangor, Malaysia

ABSTRACT

Although HbA_{1c} is widely accepted as a useful index of mean blood glucose in type 2 diabetic patients its usefulness as screening test for diabetes has been controversial. The present study was undertaken to determine whether the level of HbA_{1c} predicted diabetes in a prediabetic group of subjects. Plasma lipids, oral glucose tolerance, HbA_{1c} was determined in 90 normal control subjects, 57 offspring of one type 2 diabetes mellitus parent and 11 diagnosed type 2 diabetes mellitus individuals. The mean age of participants was 44.5 yrs (not significantly different amongst the three groups) and the mean body mass index was 26.8 (not significantly different amongst the three groups). Two hours after a 75 g glucose challenge, the offspring had a significantly higher plasma glucose level (mean = 7.1 mmol/L, p value = 0.002) than the normals. Similarly the HbA_{1c} values were higher in the offspring than in the normals (mean = 5.78%, p value = 0.016). Besides the significantly higher values for oral glucose tolerance test and HbA_{1c}, the diabetics also were significantly higher for triglycerides (mean = 2.25mmol/L), total cholesterol (mean = 6.24mmol/L) and systolic blood pressure (mean = 138.45mm Hg) than the offspring (P value = 0.031, 0.006, 0.010) and the normals (P value = 0.026, 0.018, 0.002) respectively. The mean values of diastolic blood pressure, LDL cholesterol and HDL cholesterol were not significantly different amongst the three groups.

KEY WORDS

Diabetes mellitus, HbA_{1c}, Prediabetic, Fasting blood glucose, Insulin resistance.

INTRODUCTION

Type 2 diabetes mellitus (DM) which has devastating consequences on life expectancy and quality of life, imposes large economic costs, and currently affects 8.2% of the Malaysian population (1). Despite major advances in the treatment of hyperglycemia, risk factors for cardiovascular disease (CVD) and other diabetes complications, type 2 DM remains a chronic degenerative condition that is difficult to treat (2). Type 2 DM results from the interaction between genetic predisposition, behavioral and environmental risk

factors (3). Although the genetic basis of type 2 DM has yet to be identified there is strong evidence that such modifiable risk factors as obesity and physical inactivity are the main non genetic determinants of the disease (4,5,6). Prevention of diabetes is a worthy cause especially as diabetes is linked to contemporary lifestyle (7). Recently the Diabetes Prevention Programme has shown that it was possible to prevent or delay diabetes among individuals at high risk for diabetes, regardless of sex, age and race/ethnicity (8). The diagnosis of diabetes mellitus is based on an elevated plasma glucose concentration in the fasting state and after an oral glucose load. The complications of diabetes are influenced not only by the duration of diabetes but also by the average level of chronic hyperglycemia (9) which is measured most reliably with the HbA_{1c} assay. Impaired glucose tolerance is an intermediate category between normal glucose tolerance and overt diabetes (10,11) and it can be identified by the response to a oral glucose tolerance test. Subjects with impaired glucose tolerance have an increased risk of type 2 DM (12). The aim of this study is to

Address for Correspondence :

Dr. Sekaran Muniandy

Department of Molecular Medicine

Faculty of Medicine, University of Malaya

Kuala Lumpur, Malaysia, Tel: 603-79674953

E-mail: sekaran@um.edu.my

evaluate the usefulness of HbA_{1c} as an early marker in nondiabetic high risk group. In our study the group consisted of adults whose parents were diabetic and who form an important target group for early intervention aimed at preventing diabetes.

MATERIALS AND METHODS

Subjects: One hundred and forty seven adult individual men and women were recruited from Klang Valley and from the diabetic clinic of University of Malaya Medical centre. They were classified into one of three groups; diabetic (n=11), offspring (first degree relatives) of one type 2 DM parent (n=57) or normal (n = 90). The exclusion criteria for first degree relatives as determined by questionnaire responses were a history of cardiovascular disease, diabetes, hypertension, dyslipidaemia, pregnancy, any permanent illness or incapability of fasting for eight hours. Informed consent was obtained from all subjects and the study was approved by University of Malaya, Faculty of Medicine's Medical ethics committee and carried out in accordance with the Helsinki declaration.

Blood sample collection: Ten milliliters of fasting blood from each recruit was collected in Vacutainer™ tubes containing disodium ethylene diamine tetraacetate (EDTA, 1mg/mL) or containing glycolysis inhibitor (fluoride) or serum separator (SST) for the various tests after an overnight fast of eight hours. For oral glucose tolerance test (OGTT), glucose (75g) was given orally to all participants and another venous blood sample 5mL was collected again after two hours. Blood samples were then subjected to estimation of fasting plasma glucose (FPG), plasma glucose after two hours of glucose challenge (postprandial, PPBG), fasting lipid (total cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides) and HbA_{1c}.

Classification of Subjects : The subjects are classified as having impaired glucose tolerance (IGT) if the two hours plasma glucose (postprandial) level is between 7.8 and 11.0 mmol/L. The subjects are classified as having normal glucose tolerance if the FBG was less than 5.6 mmol/L and the two hours plasma glucose level was less than 7.8 mmol/L as recommended by the American Diabetes Association 2003 (13). They were classified as diabetic if FPG was more than 7.0 mmol/L or if the two hours plasma glucose was more than 11.1 mmol/L. None of the subjects received hypolipidemic drug therapy, or had any renal, hepatic or thyroid disease affecting glucose or lipid metabolism.

Estimation of lipids, FBG, PPBG, and HbA_{1c} : HbA_{1c} was determined by immunoassay using Cobas Integra 800 (Roche

Diagnostics, USA). Plasma was separated immediately by centrifugation at 3000 rpm for 15 minutes at 4°C. Total cholesterol, triglycerides and high density lipoprotein (HDL) were determined using the Dimension clinical chemistry system (Dade Behring Systems, France), and low density lipoprotein (LDL) was determined by using Friedewald's equation (14). Glucose was determined using the Dimension clinical chemistry system based on the hexokinase–glucose-6-phosphate dehydrogenase system.

Statistical Analysis : Data were expressed as mean $\pm$ standard deviation. Continuous variables were analyzed using one way ANOVA. Two tailed *p* value of less than 0.05 was considered significant. For most of the variables, the Levene's test of equality of variances across groups gave *p* values which were less than 0.05. Thus equality of variances could not be assumed in most cases. Pairwise multiple comparisons were done using Dunnett's T3 test.

RESULTS

The mean age for all participants was 44.5 (not significantly different by the Dunnett's test) and the mean BMI was 26.8 (not significantly different). The anthropometric values and the blood pressure determinations of the diabetics, the first degree relatives and the nondiabetic normals are shown in Table 1. There were no significant differences in the anthropometric measures between the three groups. The systolic blood pressure was significantly higher in the diabetics compared to the normal and the first degree relatives but there was no difference between the normals and the first degree relatives. The levels of blood glucose after two hours of oral glucose administration (PPBG), HbA_{1c}, total cholesterol, triglyceride, high density lipoprotein, and low density lipoprotein are shown in Table 2. The fasting glucose was significantly higher in the diabetics as compared to the normals and the first degree relatives, but there was no significant differences between the normals and the first degree relatives. The two hour glucose determination was significantly higher in the diabetics as compared to the normals and the first degree relatives. The first degree relative demonstrated higher post prandial glucose levels compared to the normals. The HbA_{1c} was significantly higher in the diabetics as compared to the normals and the first degree relatives. The interesting finding in this study is that the HbA_{1c} was significantly higher in the first degree relatives as compared to the normals.

DISCUSSION

Type 2 diabetes mellitus and the complications associated

Table 1: Comparison of "Body Measures"

Body Measure	Group			p-value	*Comparison of means using Dunnett's T3 test
	Normal N=90	Offspring N=57	Diabetic N=11		
Age	44.46 ± 11.19	44.49 ± 9.245	45.82 ± 8.460	0.917	No difference
Height	160.50 ± 7.68	162.48 ± 8.04	165.36 ± 8.56	0.086	No difference
Weight	69.14 ± 13.45	70.19 ± 12.64	78.91 ± 6.60	0.061	No difference
BMI	26.74 ± 4.21	26.55 ± 4.45	28.99 ± 3.12	0.98	No difference
Waist	82.21 ± 11.36	82.57 ± 10.37	90.60 ± 7.47	0.052	No difference
Hip	100.91 ± 11.20	102.00 ± 13.16	103.10 ± 4.45	0.766	No difference
Waist/Hip ratio	0.83 ± 0.224	0.82 ± 0.126	0.88 ± 0.072	0.606	No difference
Systolic Blood Pressure	124.58 ± 17.55	126.51 ± 16.63	138.45 ± 4.76	0.038	Diabetic > Normal Diabetic > Offspring
Diastolic Blood Pressure	77.66 ± 11.43	78.76 ± 10.95	81.68 ± 10.05	0.497	No difference

*Pairwise Multiple Comparison using Dunnett's T3 test, $\alpha = 0.05$. Only groups that differ pairwise are indicated in the last column.

with it are one of the major causes of death in Malaysia. Its causes and progress are commonly traced to interacting genetic and behavioral factors. Glucose toxicity is a well established entity that has been shown in type 2 diabetes to cause progressive impairment in insulin secretion and finally leads to insulin resistance (15). In theory treating diabetes mellitus should be simple - just prevent hyperglycemia from causing damage to organs, and not allow hypoglycemia to cause coma as energy supply to brain fails. It is also known that poor glycemic control is an important factor in the generation of increased protein oxidation in type 2 diabetic patients clinically free of complications (16). In practice it is

not so simple to monitor the glucose levels as they fluctuates all the time. The only effective way is to monitor HbA_{1C} levels which gives the average blood glucose level of the preceding two or three months (17).

HbA_{1C} has been widely used as a measure of glycemic control in patients with diabetes mellitus. It has been shown that a HbA_{1C} value of 6.5% is an adequate and useful supportive marker to diagnose diabetes because of its high specificity (18). A borderline (5.6 to 6.4%) or high ($\geq 6.5\%$) level of HbA_{1C} was found to strongly predict future drug treatment for diabetes (19). It has also been shown that the HbA_{1C} levels showed a

Table 2 : Comparison of "Blood Serum Measures"

Body Measure	Group			p-value (power)	*Comparison of means using Dunnett's T3 test
	Normal N=90	Offspring N=57	Diabetic N=11		
Glucose fasting (Prandial)	4.83±0.42	4.95±0.66	8.11±3.31	< 0.001 (>99.9%)	Diabetic > Normal Diabetic > Offspring
Glucose 2 hours (Postprandial)	5.82±0.98	7.12±2.64	16.94±4.28	< 0.001 (>99.9%)	Offspring > Normal Diabetic > Normal Diabetic > Offspring
Triglyceride	1.30±0.79	1.33±0.67	2.25±0.98	0.001 (94.8%)	Diabetic > Normal Diabetic > Offspring
Total Cholesterol	5.53±1.04	5.39±0.97	6.24±0.66	0.037 (62.7%)	Diabetic > Normal Diabetic > Offspring
HDL	1.30±0.35	1.28±0.35	1.14±0.24	0.367 (22.4%)	No difference
LDL	3.64±0.89	3.50±0.85	4.07±0.59	0.133 (41.6%)	No difference
HbA _{1C} (%)	5.54±0.45	5.78±0.52	8.06±2.07	< 0.001 (>99.9%)	Offspring > Normal Diabetic > Normal Diabetic > Offspring

*Pairwise Multiple Comparison using Dunnett's T3 test, $\alpha = 0.05$. Only groups that differ pairwise are indicated in the last column.

significant increase with the duration of diabetes (20). However the role of HbA_{1c} in the prediabetic stage has received little attention although the effectiveness of early intensive intervention for the prediabetic patients defined by plasma glucose is well documented. Although HbA_{1c} is widely accepted as a useful index of mean blood glucose in the treatment of patients with diabetes its use as a screening test for diabetes has been controversial (21). Our results demonstrate that HbA_{1c} can serve as early marker for the identification of people at high risk group. HbA_{1c} estimations can be performed at any time of day and without special patient preparation and is sensitive enough to use as a single diagnostic marker for DM (22). It has also been shown that HbA_{1c} determination in high risk patient to be a convenient alternative to fasting plasma glucose or OGTT to diagnose DM (23). Since poor glycaemic control and age related pathology with duration of diabetes are thought to accelerate degenerative changes in a cooperative manner (24), it is better to identify the people at high risk group during the prediabetic stage.

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