The Role of Hba1c as a Diagnostic Test for Type 2 Diabetes Mellitus in Bangladesh.

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Abstract:

Background: Type 2 diabetes mellitus is a serious chronic disease with micro vascular complications such as retinopathy, nephropathy and neuropathy and macro vascular complications such as cardiac, peripheral arterial and cerebrovascular disease. **Objective:** The aim of the study was to investigate the value of HbA1c as a diagnostic test for type 2 diabetes mellitus in Bangladeshi individuals. **Methods:** This cross sectional study was conducted in the Department of Biochemistry, Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh. A total 657 patients, who were attended in the one point sample collection centre of Bangabandhu Sheikh Mujib Medical University for oral glucose tolerance test (OGTT) from 1st April 2014 to 30th June 2014, were purposively enrolled in this study. According to WHO criteria and based on OGTT findings study subjects were categorized into Normoglycemic (257), IFG (82), IGT (174), and DM (347). Fasting plasma glucose, HbA1c and plasma glucose at 2 hour after glucose load on OGTT was done from all the study subjects. **Results:** With a cut-off value of 6.1%, HbA1c had a maximal sensitivity and specificity of 97.0% and 49.0% respectively with a positive predictive value 65.5% and a negative predictive value 94.0%. HbA1c had a sensitivity of 93.0% and a specificity of 63.0% was calculated with a cut-off value of 6.5% with positive predictive value 77.5% and negative predictive value 90.0%. Both fasting plasma glucose levels and 2 hour plasma glucose levels were showed significant positive correlation with HbA1c ($r = 0.788$, $P = 0.000$ and $r = 0.800$, $P = 0.000$ respectively). **Conclusion:** The study suggests that measurement of HbA1c could be used to make diagnosis of T2DM in the Bangladeshi population.

Key words: HbA1c, Type 2 diabetes mellitus, diagnosis, oral glucose tolerance test.

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Introduction:

The term diabetes mellitus (DM) describes a metabolic disorder with heterogeneous aetiologies which is characterized by chronic hyperglycemia and disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both[1]. The long term relatively specific effects of diabetes include development of retinopathy, nephropathy and neuropathy[2]. People with diabetes are also at increased risk of cardiac, peripheral arterial and cerebrovascular disease[3]. Rates of T2DM have increased markedly since 1960 in parallel with obesity. As of 2010 there were approximately 285 million people diagnosed with the disease compared to around 30 million in 1985[4,5]. By 2030, the worldwide prevalence of adult DM is expected to rise to 7.7%, which roughly translates to 439 million affected individual[6]. In 2010, the International Diabetes Federation estimated that 5.7 million (6.1%) and 6.7 million (7.1%) of people living in Bangladesh is suffering from diabetes and impaired glucose tolerance (IGT) respectively. By 2030, that number of diabetic population is expected to rise to 11.1 million. This explosion on diabetes prevalence will place Bangladesh among the top seven countries in terms of the number of people living with diabetes in 2030[7].

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Based on current recommendations, diagnosis of DM requires the presence of a fasting plasma glucose concentration of ≥7.1mmol/L, or a 2 hour plasma glucose level of ≥11.1 mmol/L on an oral glucose tolerance test (OGTT). On the other hand, international committee members selected by the American Diabetes Association (ADA) and the Alliance for European Diabetes Research (EURADIA) recently suggested that glycosylated hemoglobin (HbA1c) could be used as an alternative for making diagnosis of DM[8]. The committee concluded that an HbA1c level of ≥6.5% was diagnostic for DM, without requiring a determination of plasma glucose levels. However, the use of standard glucose measurements is still recommended for individuals when HbA1c assays are deemed unreliable[9].

HbA1c is formed as a result of the addition of a stable glucose molecule to the N-terminal group of an HbA0 molecule via a non enzymatic glycation process[10], and is considered a reliable indicator of the glycemic status of the previous 3 months[11].

Despite the cloud of controversy regarding the limitations of HbA1c for making diagnosis of DM, many experts believe that HbA1c may be superior to the OGTT in daily clinical practice as there was no need of preparation of patient, no fasting is necessary, sample collection and test procedure is simple, there was no variation of test result like plasma glucose[12].

The aim of this study was to investigate the value of HbA1c as a diagnostic test for T2DM in Bangladeshi individuals.

Methods :

This cross sectional study was undertaken in the Department of Biochemistry, Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh. Patients, who were attended in the one point sample collection centre of Bangabandhu Sheikh Mujib Medical University for oral glucose tolerance test (OGTT) from 1st April 2014 to 30th June 2014, were purposively enrolled in this study.

From each patient, blood samples were obtained at 0800 hours and onward following 10 hours fast, from the antecubital vein in a sitting position, for the determination of HbA1c as well as fasting plasma glucose level. All patients were then subjected to a 75-gm OGTT on the same day, and second blood samples were obtained 2 hour after glucose loading for determination of plasma glucose level. For determination of HbA1c whole blood samples were collected in vacuum tube contains EDTA and for plasma glucose measurement blood samples were collected in fluoride tube.

An NGSP-approved for the percent determination of HbA1c in human whole blood using ion-exchange high-performance liquid chromatography (HPLC) method on a D-10 Hemoglobin A1c program (Bio-Rad, Japan) analyzer. Blood samples were automatically diluted on the D-10 and injected in the analytical cartridge. The D-10 delivers a programmed buffer gradient of increasing ionic strength into the analytical cartridge, where the hemoglobin is separated base on their ionic interactions with the cartridge material. The separated hemoglobin then passes through the flow cell of the photometer, where changes in absorbance at 415 nm were measured. Glucose measurements were made on the same day as HbA1c measurements, using the glucose oxidation method on Dead Behring (Bio-Rad, Japan) auto analyzer. Subjects were categorized into 4 groups based on their OGTT results, according to the criteria put forth by the WHO: normoglycemic (NG), impaired fasting glucose (IFG), impaired glucose tolerance (IGT), and diabetes mellitus (DM).

Statistical analyses were performed using SPSS windows version 20. Values for HbA1c and plasma glucose levels were provided as mean ± standard deviation. The sensitivity, specificity, positive predictive values, and negative predictive values for both tests were calculated by plotting a receiver operating characteristic (ROC) curve. Correla-

tion analyses between fasting plasma glucose, 2 hour plasma glucose, and HbA1c levels were performed using Spearman's correlation test. A p-value of less than 0.05 was considered indicative of statistical significance.

Results:

A total of 657 consenting participants (360 male and 297 female) were included in this study. Based on OGTT results and according to criteria put forth by the WHO, 257 individuals were categorized as normoglycemic, 82 were IFG, 174 were IGT and 347 were DM. The demographic characteristics and laboratory findings of the study population have been summarized in the Table.

Table-I

Demographic and Laboratory findings of study population

Parameters	Total (N = 657)	N G	IFG	IGT	D M
A ge (Y ears)	47.88 ±11.33				
Sex (M , F)	360, 297				
FPG (m m o/L)	7.87±3.42	5.08 ± 0.57	6.43± 0.29		10.50 ±3.18
		(N = 257)	(N = 82)		(N = 218)
2 h P G (m m o/L)	12.56 ±5.33	6.64±0.75		9.30±0.98	16.52±4.28
		(N = 136)		(N = 174)	(N = 347)
HbA 1c (%)	8.08±2.36				

NG = normoglycemic IFG= Impaired fasting gluease IGT =Impained glucose to leramee DM=Diabe tesmellitng

Values provided as mean ± standard deviation; HbA1c-glycosylated hemoglobin; NG- normoglycemic group; IFG- impaired fasting glucose group; IGT- impaired glucose tolerance group; and DM- diabetes mellitus group.

The OGTT was considered the gold-standard test for the diagnosis of DM. The area under the ROC curve for the diagnosis of DM by HbA1c was 0.90 ($P < 0.001$) (Figure 1). With a cut-off value of 6.1%, HbA1c had a maximal sensitivity and specificity of 97.0% and 49.0% respectively with a positive predictive value 65.5% and negative predictive value 94.0%. With a cut-off value of 6.5%, HbA1c had a sensitivity of 93.0% and a specificity of 63.0% respectively with positive predictive value 77.5% and negative predictive value 90.0%.

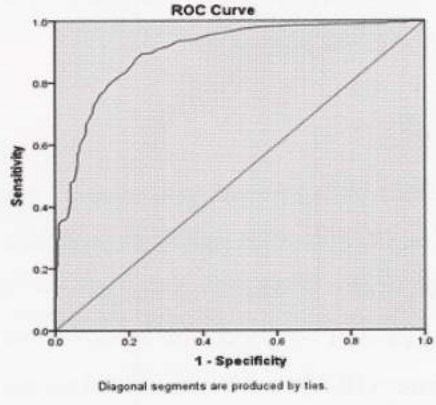

Fig-1: *Receiver operating characteristic curve for HbA1c in patient with diabetes mellitus.*

A significant positive correlation was observed between HbA1c, fasting plasma glucose ($r = 0.788$, $P = 0.000$), and 2 h plasma glucose ($r = 0.800$, $P = 0.000$) levels in patients with DM (Figures 2 & 3).

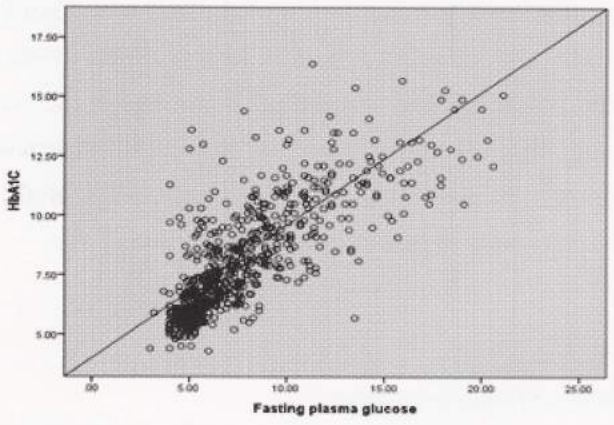

Fig-2: *Correlation curve for fasting plasma glucose and HbA1c (r = 0.788, p = 0.000).*

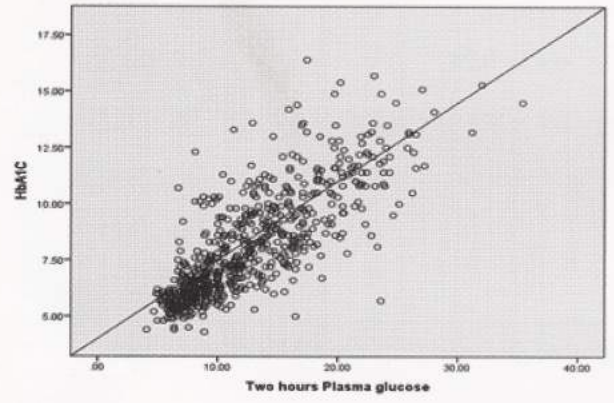

Fig-3: *Correlation curve for 2 h plasma glucose and HbA1c (r = 0.800, p = 0.000).*

Discussion:

In this study fasting plasma glucose and 2 hour plasma glucose on OGTT showed significant positive correlation with HbA1c (r = 0.788, p = 0.000 and r = 0.800, p = 0.000). Riet et al.[13] reported that weak positive correlations between HbA1c and fasting plasma glucose levels (r = 0.46), as well as 2 hour plasma glucose (r = 0.33) levels, were observed in individuals from the general population. Ginis et al.[14] reported that both fasting plasma glucose and 2 h plasma glucose levels were found to correlate moderately with HbA1c levels (r = 0.47, P = 0.001 and r = 0.52, P = 0.000, respectively) and were consisted with our observations.

Several studies have investigated the value of HbA1c for the diagnosis of DM with a cut-off level of 6.1%. In such a study by Tavintharan et al.[15] a sensitivity of 81% was reported with a specificity of 84%. Similarly, Ko et al.[16] reported on a sensitivity and specificity of 77.5% and 78.8%, respectively. With a cut-off value for the diagnosis of DM of 6.1%, HbA1c had a sensitivity of 81.8% and a specificity of 80%, with positive and negative predictive values of 80.2% and 81.05%, respectively showed by Ginis et al.[14]. Comparable results were observed in our study for diagnosis of DM by HbA1c with a cut-off value of 6.1% with sensitivity 97%, specificity 49%, with positive predictive value 65.5% and negative predictive value 94.0%.

Kumar et al.[17] reported on a sensitivity of 65% and a specificity of 88% with positive and negative predictive values of 75.2% and 96.5% respectively for diagnosis of DM by HbA1c with a cut-off value of 6.5%. Similar study done by Ginis el al.[14] also reported that a sensitivity of 56.8% and a specificity of 89.2% for diagnosis DM with a cut-off value of 6.5%. In our study with a cut-off value of 6.5%, HbA1c had a sensitivity of 93.0% and a specificity of 63.0% with positive predictive value 77.5% and negative predictive value 90.0%.

HbA1c reflects average plasma glucose over the previous eight to 12 weeks[18]. It can be performed at any time of the day and does not require any special preparation such as fasting. These properties have made it the preferred test for assessing glycemic control in people with diabetes. More recently, there has been substantial interest in using it as a diagnostic test for diabetes and as a screening test for persons at high risk of diabetes[19]. Owing in large part to levels or performing an OGTT, and day-to-day variability in glucose, an alternative to glucose measurements for the diagnosis of diabetes has long been sought. HbA1c has now been recommended by an International

Committee and by the ADA as a means to diagnose diabetes[19].

For diagnosis of DM by measurement of HbA1c alternate to the OGTT as a reliable test would require the determination of an optimal cut-off value and further studies on a larger scale are required in order to validate HbA1c assays as a reliable diagnostic test for DM and as a screening test for persons at high risk of diabetes in Bangladeshi individuals.

Conclusion:

It was concluded from this study that measurement of HbA1c could be used to make diagnosis of T2DM in the Bangladeshi population.

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