HbA1c as a Diagnostic Criteria in Diabetes Mellitus A Comparison with Mini-Oggt

Dr Swati Tiwari, Dr Vaishali Jain, Dr S.S.Biswas, Prerna Vyas Gokhale

(Department of Biochemistry, L.N. Medical College, Kolar Road, Bhopal, MP, India)

Abstract: HbA1c is not merely a marker for monitoring diabetes but has been accepted as a marker for its diagnosis too. The diagnostic efficiency of the 6.5% cutoff recommended by ADA was evaluated against the standard mini oral glucose tolerance test (OGTT) in our local population to find an optimum cutoff of HbA1c in Bhopal. Estimation of HbA1c, fasting plasma glucose and 2 hour post 75 gms oral glucose challenge plasma glucose was done in 270 subjects. HbA1c diagnosed comparable but insignificantly fewer number of patients as diabetic (p>0.05) but significantly more as pre-diabetic (p<0.001). ROC analysis of various HbA1c cutoffs against the standard mini-OGTT showed 60% sensitivity and 92% specificity at recommended cutoff of HbA1c >= 6.5. Negative Predictive Value (NPV) and Positive Predictive Value (PPV) were found to be 83% and 77% respectively. The optimum sensitivity of 72% and specificity of 82% was obtained at HbA1c cut off 6.1; for which NPV and PPV were found to be 85% and 57% respectively. In conclusion, HbA1c proved to be slightly inferior but comparable to mini-OGTT in detecting diabetes. The use of the lower optimum cutoff 6.1 is recommended in this population to increase the yield of diabetics and minimize the gap with mini-OGTT. HbA1c detected significantly more pre-diabetics than mini-OGTT, hence it may be used to screen the local population.

Key words: Diabetic, Prediabetic, Glycosylated hemoglobin, Oral Glucose Tolerance Test.

I. Introduction

Asia has been the epicenter of diabetes in the world, with India and China leading the way in the prevalence of diabetes. In 2000, India had more people (31.7 million) with estimated diabetes mellitus than any other country in the world, followed closely by China (20.8 million). (1) China has since overtaken India as the diabetic capital of the world. It has about 92 million diabetics which is far more than the 62 million estimated in India. (2) However, in some urban areas of south India, prevalence of diabetes is as high as nearly 20%. (3) The gold standard in the diagnosis of diabetes has been the oral glucose tolerance test (OGTT). However, the oral load of glucose and the half-hourly sample collection makes it a difficult test to carry out in such large numbers. Using simply the fasting plasma glucose (FPG) for the diagnosis of diabetes has its disadvantages, as it often misses out on cases of diabetes detected by the post challenge plasma glucose (PCPG), besides fasting itself is an inconvenience. (4) Glycosylated hemoglobin (HbA1c) does not require fasting, and hyperglycemia being the cause of complications of diabetes, a long term measure of hyperglycemia (HbA1c) is a better marker of the severity of the disease than a single measurement of glucose. (5)

An International Expert Committee (IEC) with members appointed by the American Diabetes Association (ADA), the European Association for the Study of Diabetes, and the International Diabetes Federation recommended that HbA1c > 6.5% be considered as diagnostic of diabetes. (5) Their recommendation was based on the evidence of ROC (Receiver Operating Characteristic) analysis data that the cut point of HbA1c for diabetes specific retinopathy was 6.5%. Retinopathy has widely been accepted as the best criterion for comparing glycaemic measures because it is a specific, objective, and relatively early clinical complication of diabetes. (6)

In 2010, the ADA accepted this criterion. They recommended that HbA1c > 6.5% be included as a criterion for diagnosis of diabetes, and people with HbA1c between 5.7-6.4% be considered at risk of developing diabetes in the future. (7) In 2011, the WHO gave a conditional acceptance to the use of HbA1c for the diagnosis of diabetes subjecting it to stringent quality assurance tests and standardization criteria, besides mandating the exclusion of conditions which precludes its accurate measurement. (8)

HbA1c is known to be affected by ethnicity, irrespective of the state of glycaemia. (9) (10)Blacks have widely been reported to have higher levels of HbA1c than whites. (11) Asians and American Indians have also been reported to have higher levels of HbA1c than local whites. (12) This study was therefore carried out in our local population in Bhopal to find out the diagnostic efficiency of the recommended cutoff of HbA1c >= 6.5% against the standard mini OGTT and to find an optimum cutoff of HbA1c in our population.
II. Material And Methods

This study was a hospital based cross-sectional study conducted on 270 men and women visiting IPD/OPD of Medicine department of the J.K. Hospital and LN Medical College, Kolar, Bhopal. The study group included 162 males and 108 females who were between 18 to 80 years of age. They included 59 Young (<40 years), 107 Middle aged (40-59 years) and 104 Old people (>= 60 years). A structured questionnaire was used to seek general details of the subject’s economic status, diet, smoking, alcohol consumption, medical and drug history. Those excluded from the study were already diagnosed cases of diabetes, pregnant women, patients taking medication causing rapid rise in plasma glucose, patients of hemolytic anaemia, iron deficiency anaemia and hemoglobinopathies, patients with hemoglobin variants, history of chronic blood loss or recent blood transfusion. Study was conducted after clearance by Institutional Ethics Committee and after obtaining informed consent of the subjects. Sample size was calculated on the basis of a previous study. (13) Modified mini OGTT was performed taking only two venous blood samples, one after overnight fasting (10-12 hours) and the other two hours after a 75 Grams of oral anhydrous glucose load (in 300 ml water). The fasting samples were used for the estimation of FPG and HbA1c. Fluoride vials were used to collect samples for glucose estimation and EDTA vials for HbA1c. All parameters were analyzed on fully automated analyzer. Glycosylated hemoglobin was estimated using turbidimetric inhibition immunoassay and glucose was estimated using glucose oxidase/ peroxidase method.

The data obtained was analyzed using software IBM SPSS version 16, Chicago, USA. The statistical tests used were chi-square test, odds ratio and Pearson’s correlation. The difference between groups for the above tests was considered significant if the P value was less than 0.05. ROC (Receiver Operating Characteristic) analysis was done to assess sensitivity and specificity of the recommended cutoff of HbA1c against the standard mini-OGTT and also to decide on an optimum cutoff value of HbA1c in our population for the diagnosis of diabetes. Negative and positive predictive values (NPV and PPV) and likelihood ratios (LR +ve and LR -ve) were calculated for each cutoff.

III. Results

Subjects diagnosed diabetic by mini-OGTT were slightly more than by HbA1c, but it was not statistically significant (p=0.122). In contrast, the number of patients diagnosed pre-diabetic were significantly more by HbA1c (p<0.001), while the number found to be normal were significantly more by mini-OGTT (p<0.001). [Table 1] HbA1c showed significant association with mini-OGTT in diagnosing diabetics ($\chi^2 = 112$, p value <0.001)

The odds of diagnosing diabetic to not diagnosing diabetic were 44% more when diabetes was diagnosed by mini-OGTT than by HbA1c. However, this difference was not statistically significant (p = 0.08). The odds of diagnosing pre-diabetes to normal were 90% less by mini-OGTT than by HbA1c. This difference was statistically significant (p<0.001). [Table 2]

Significant (p<0.001) correlation of HbA1c was found with both FPG (r=0.744) and PCPG (r=0.766). [Table 3] Scatter plot of FPG with HbA1c [Fig 1] was better than that of PCPG with HbA1c [Fig 2] although both confirmed the linear relationship.

ROC curve with mini-OGTT as the standard test for diagnosing diabetes and HbA1c as the screening test showed Area Under the Curve 0.822 and p value <0.001, showing that HbA1c was a significant predictor of diabetes as diagnosed by OGTT. [Fig 3] ROC analysis of various HbA1c cutoffs against the standard mini-OGTT showed 60% sensitivity and 92% specificity at the ADA recommended cutoff of 6.5. LR+ was 3.4 and LR- was 0.20 at that level. The optimum specificity and sensitivity of 72% and 82% respectively was obtained at HbA1c cutoff 6.1. [Table 4]

<table>
<thead>
<tr>
<th>Diabetic status</th>
<th>Mini-OGTT</th>
<th>HbA1c</th>
<th>$\chi^2$</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>159</td>
<td>95</td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Pre-diabetic</td>
<td>26</td>
<td>109</td>
<td>51</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diabetic</td>
<td>85</td>
<td>66</td>
<td>2.4</td>
<td>0.122</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Category</th>
<th>Odds by Mini-OGTT</th>
<th>Odds by HbA1c</th>
<th>Odds Ratio</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetic/Non-diabetic</td>
<td>0.46</td>
<td>0.32</td>
<td>1.44</td>
<td>0.61-2.11</td>
<td>0.08</td>
</tr>
<tr>
<td>Pre-diabetic/Normal</td>
<td>0.16</td>
<td>1.65</td>
<td>0.10</td>
<td>0.05-0.15</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Test parameter1</th>
<th>Test Parameter 2</th>
<th>r</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HbA1c</td>
<td>FPG</td>
<td>0.744</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HbA1c</td>
<td>PCPG</td>
<td>0.766</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
HbA1c as a Diagnostic Criteria in Diabetes Mellitus A Comparison with Mini-Oggt

Table 4 Performance of different HbA1c cutoffs against mini-OGTT

<table>
<thead>
<tr>
<th>HbA1c Cutoff (gm%)</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Accuracy</th>
<th>NPV</th>
<th>PPV</th>
<th>LR+</th>
<th>LR-</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.7</td>
<td>82%</td>
<td>34%</td>
<td>58%</td>
<td>88%</td>
<td>42%</td>
<td>0.73</td>
<td>0.13</td>
</tr>
<tr>
<td>5.8</td>
<td>80%</td>
<td>62%</td>
<td>62%</td>
<td>86%</td>
<td>44%</td>
<td>0.81</td>
<td>0.15</td>
</tr>
<tr>
<td>5.9</td>
<td>74%</td>
<td>67%</td>
<td>67%</td>
<td>87%</td>
<td>49%</td>
<td>0.97</td>
<td>0.15</td>
</tr>
<tr>
<td>6.0</td>
<td>71%</td>
<td>76%</td>
<td>69%</td>
<td>85%</td>
<td>50%</td>
<td>1.03</td>
<td>0.17</td>
</tr>
<tr>
<td>6.1 (ADA)</td>
<td>72%</td>
<td>82%</td>
<td>74%</td>
<td>85%</td>
<td>57%</td>
<td>1.35</td>
<td>0.17</td>
</tr>
<tr>
<td>6.2</td>
<td>65%</td>
<td>87%</td>
<td>78%</td>
<td>86%</td>
<td>64%</td>
<td>1.79</td>
<td>0.16</td>
</tr>
<tr>
<td>6.3</td>
<td>63%</td>
<td>90%</td>
<td>79%</td>
<td>84%</td>
<td>68%</td>
<td>2.2</td>
<td>0.18</td>
</tr>
<tr>
<td>6.4</td>
<td>60%</td>
<td>92%</td>
<td>81%</td>
<td>83%</td>
<td>74%</td>
<td>2.9</td>
<td>0.19</td>
</tr>
<tr>
<td>6.5 (ADA)</td>
<td>60%</td>
<td>92%</td>
<td>82%</td>
<td>83%</td>
<td>77%</td>
<td>3.4</td>
<td>0.20</td>
</tr>
<tr>
<td>6.6</td>
<td>56%</td>
<td>95%</td>
<td>82%</td>
<td>83%</td>
<td>78%</td>
<td>3.6</td>
<td>0.19</td>
</tr>
<tr>
<td>6.7</td>
<td>54%</td>
<td>96%</td>
<td>82%</td>
<td>82%</td>
<td>84%</td>
<td>5.3</td>
<td>0.21</td>
</tr>
</tbody>
</table>

Figure 1: Scatter plot of HbA1c with FPG

Figure 2: Scatter plot of HbA1c with PPBS

Figure 3: ROC curve with mini-OGTT as standard test for diagnosing diabetes and HbA1c as screening test
IV. Discussion

HbA1c fared well against mini-OGTT in diagnosing diabetes as it diagnosed an almost similar number i.e. 66 as diabetic (24%) to 85 (31%) by mini-OGTT. Of these, 55 were diagnosed diabetic by either criterion. The odds of diagnosing diabetic/non-diabetic were 44% higher by mini-OGTT, but again not statistically significant. Hjellestad et al reported in Norwegian patients that HbA1c diagnosed a similar percentage (14.6%) as diabetic as OGTT (12%). (14) Incani et al reported in Italian participants that prevalence of diabetes by the two criteria was similar i.e. 28% by OGTT and 24% by HbA1c in an obese cohort and 11% by both OGTT and HbA1c in a diabetes screening cohort. (15) This trend is largely followed for HbA1c cutoff 6.5% but gets reversed on taking the HbA1c cutoff higher. Raman et al took HbA1c cutoff 7% as a treatment limit in an undiagnosed group at high risk of developing diabetes and reported significantly more diabetics (49%) by HbA1c compared to OGTT (17%). (16) HbA1c proved comparable to mini-OGTT by other measures too. It showed significant association with mini-OGTT in diagnosing diabetics and was a significant predictor of diabetic status by mini-OGTT (AUC was 0.82) in ROC analysis. Hjellestad et al reported similar but slightly lower AUC (0.73) for ROC analysis of HbA1c against OGTT. (14) In this study, HbA1c had high LR+ i.e. 3.4 and low LR- i.e. 0.20, so it showed high probability of being positive and low probability of being negative in diabetics. HbA1c expectedly showed significant correlation with fasting and post-challenge 2 hour plasma glucose (r=0.744 for fasting and r=0.766 for post challenge plasma glucose). Studies by Riet et al and Ghazanfari et al reported similar correlation of HbA1c with FPG and PCPG, the correlation being stronger in cases of known diabetes than otherwise. (17) (18) HbA1c diagnosed 109 (40%) prediabetics as compared to 26 (10%) by mini-OGTT, which was significantly higher (p-value<0.001). Hjellestad et al also reported twice the amount of pre-diabetic by HbA1c than by OGTT (70% vs 33%) in his study on Italian patients. (14) Rivers et al also reported in a study on Bahamian adolescent students a much higher yield of pre-diabetics by HbA1c (16%) compared to OGTT (1%), attributing fluctuations to hormonal changes. (19) Incani et al reported conversely a higher yield of prediabetics by OGTT rather than by HbA1c i.e. 65% to 43% in the diabetic screening cohort and a similar yield i.e. 29% to 27% respectively in the obese cohort. (15)

In this study, the ADA recommended cutoff of 6.5 HbA1c showed very good specificity (92%) but relatively modest sensitivity (60%). NPV was slightly higher than PPV i.e. 83% and 77% respectively. In a large cross sectional study among adults in Chandigarh, Kumar et al reported similar results with HbA1c >= 6.5% against OGTT having specificity 88%, sensitivity 65%, NPV 96% and PPV 75%. (20) Studies by Hjellestad et al and Riet et al have confirmed this trend of lower sensitivity and higher specificity at HbA1c >= 6.5%. (17) Incani et al reported results similar to our study in an obesity clinic cohort, but lower sensitivity and PPV in a diabetes screening cohort. (15) Engelgau et al used diabetic retinopathy to define diabetes instead of OGTT in a cross section of Egyptians. At HbA1c >= 6.5%, they reported sensitivity 51% and specificity 86% in a subpopulation not receiving antihyperglycaemic medication, while sensitivity was 79% and specificity was 75% in the total population. (21) Their findings in the untreated subpopulation are close to the findings in this study. Overall, most studies reported this trend of sensitivity lower than specificity and PPV lower than NPV for HbA1c >= 6.5%, although the figures varied from one study to another depending on the population studied and the methodology.

In this study, the optimum sensitivity and specificity, i.e. 72% & 82% were obtained at HbA1c >= 6.1%, which is below the ADA and WHO recommended cutoff of 6.5%. Reports on the optimum cutoff for HbA1c for diagnosing diabetes have varied worldwide, 5.5% in the Gomyo study (22), 5.8% in the Dutch general population, (17) 6.0% in The Diabetes Control and Complications Trial (DCCT), (23) 6.2% in The United Kingdom Prospective Diabetes Study (24). The Australian Diabetes Society Expert Committee agreed with the recommended HbA1c cutoff >= 6.5%, but recommended that lower than 6.5% HbA1c did not rule out a diagnosis of diabetes. The New Zealand Society for the Study of Diabetes favoured the use of HbA1c at >= 6.7% for the diagnosis of diabetes ahead of OGTT in 2011, even though this higher level cutoff of HbA1c further compromises its sensitivity, (25) The Australia AusDiab study recommended that HbA1c < 5.5% be chosen to rule out diabetes and HbA1c >= 7.0% be chosen to rule in diabetes as they found HbA1c < 5.5% provided 99% negative predictive value and HbA1c >= 7.0% provided 100% positive predictive value. (25) However, Raman et al reported only 24% PPV for HbA1c >= 7.0%. (16) The lower PPV shows that there are genuine concerns about the variations in HbA1c assay which need to be standardized. However, some of the variation could be due to the population and methodology being different.

A cross sectional population based Iranian study compared HbA1c against FPG >=126mg/dl rather than full OGTT. They used the cutoff point of HbA1c >= 6.0% recommended by the DCCT and found 86% sensitivity, 78% specificity, 36% PPV and 97% NPV. (18)

In India, Kumar et al reported an optimal cutoff similar to our study i.e. 6.1%, which gave 81% sensitivity and 81% specificity. (20) This lower cutoff increases the sensitivity of the test and avoids missing a
diagnosis of diabetes in a large number of cases. Further, use of a lower than optimal level to rule out diabetes and higher than optimal level to rule in diabetes can also be followed. Although HbA1c with a lower cutoff would have many advantages in screening a population without known diabetes, it has some disadvantages that limit its use. It is costly, has lower sensitivity, 2 hour PCPG is stronger than it in predicting CVD, standardization is often poor leading to unreliability of results, does not unveil within day disturbances in glucose metabolism and it is affected by haemolysis and ethnicity of the population. (26) As a consequence, in situations where performing the OGTT is not feasible, instead of using only FPG or only HbA1c, a combination of both is more predictive than either parameter alone. (13)

The findings and implications of this study were limited by the fact that it was a hospital based study with a limited sample size and levels of HbA1c were not correlated with clinical complications. Future community based study on newly diagnosed diabetics and obese prediabetics could test the sensitivity of a combination of fasting plasma glucose and HbA1c against OGTT and correlate these with microvascular and macrovascular clinical complications.

V. Conclusion

In conclusion, the agreement between the two criteria- HbA1c and mini-OGTT was good for diabetes but poor for pre-diabetes. HbA1c proved to be slightly inferior but comparable to mini-OGTT in detecting diabetes. The use of the optimum cutoff of 6.1 found in this population is recommended to narrow the gap and improve the sensitivity of HbA1c. HbA1c also detected significantly more pre-diabetics than mini-OGTT; hence it may be used to screen the local undiagnosed population.

References


DOI: 10.9790/0853-150401100104 www.iosrjournals.org 104 | Page