

Original Article

Use of glycosylated hemoglobin in diagnosing pre-diabetes and diabetes in patients with hyperthyroidism

Qian Liu^{1*}, Wei Wang^{1,2*}, Chao Chen¹, Shandong Ye^{1,2}

¹Department of Endocrinology, The First Affiliated Hospital of University of Science and Technology of China (Anhui Provincial Hospital), Division of Life Sciences and Medicine, University of Science and Technology of China, 17 Lujiang Road, Hefei 230001, Anhui, China; ²Laboratory for Diabetes, Department of Endocrinology, The First Affiliated Hospital of University of Science and Technology of China (Anhui Provincial Hospital), Division of Life Sciences and Medicine, University of Science and Technology of China, 17 Lujiang Road, Hefei 230001, Anhui, China. *Equal contributors.

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Abstract: Glycosylated hemoglobin (HbA_{1c}) \geq 6.5% proposed as one of the criteria for the diagnosis of diabetes in the American Diabetes Association (ADA), 2010. For next year, the World Health Organization (WHO) 2011 guidelines recommended HbA_{1c} as a criterion for diagnosing diabetes. But the study of HbA_{1c} as a standard for screening and the diagnosis of diabetes for patients with hyperthyroidism is rare. Abnormal glucose tolerance is common in patients with hyperthyroidism. In the late fasting state and postprandial state, hyperthyroidism leads to increased Cori cycle activity; in the fasting state, hyperthyroidism leads to an enhanced demand for glucose. After the diagnosis of hyperthyroidism, an oral glucose tolerance test was performed. In 508 hyperthyroid patients, were matched for age, fasting blood glucose, 2-hour postglucose load plasma glucose, and hemoglobin A1c. Our study showed that the prevalence of glucose intolerance in hyperthyroid state was 39.1% [impaired pre-diabetes (IGR) 35.6% and diabetes mellitus (DM) 3.5%]. HbA_{1c} testing has the advantages of preanalytic stability, greater clinical convenience, and assay standardization. The diagnostic cut-off point of HbA_{1c} \geq 6.5% misses a substantial number of patients with hyperthyroidism accompany abnormal glucose tolerance. How to reduce the risk of systematic bias inherent in HbA_{1c} testing alone. The results show that the diagnostic cut-off point of HbA_{1c} of IGR in patients with hyperthyroidism was 5.4%, where the sensitivity was 55.9% and the specificity was 64.39%. The diagnostic cut-off point of HbA_{1c} for DM in patients with hyperthyroidism was 5.5%, where the sensitivity was 79.71% and the specificity was 73.16%. HbA_{1c} reflects the different glucose metabolic status in patients with hyperthyroidism, and the diagnostic cut-off points of HbA_{1c} for screening pre-diabetes and DM are 5.4% and 5.5%, respectively.

Keywords: Type 2 diabetes mellitus, hyperthyroidism, glycosylated hemoglobin, impaired glucose regulation, pre-diabetes

Introduction

With the aging of the population, reduced physical exercise, and obesity, diabetes mellitus has become a significant public health problem, early diagnosis and treatment of diabetes are very important [1]. Early lifestyle intervention can effectively reduce the incidence of diabetes and the occurrence of complications [2].

The criterion for the diagnosis of diabetes or pre-diabetes in patients with hyperthyroidism is fasting plasma glucose (FPG) [3] and 2-hour plasma glucose (2 hPG) after 75-g oral glucose load (oral glucose tolerance test; OGTT) [4]. The

HbA_{1c} assay is used as the most reliable means of assessing chronic glycemia, is widely accepted. Its close association with risk for long-term complications, established in epidemiologic studies and clinical trials, has led to the establishment of specific A1c targets for diabetes care with the goal of delaying or preventing the development of long-term complications. Diabetes treatment is expressed as the percentage of hemoglobin that is glycosylated and adjusted based on the A1c results. The vast majority of assays have been standardized worldwide, through the National Glycohemoglobin Standardization Program, the assay used in the Diabetes Control and Complications

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Trial (DCCT), which established the relationship between A1C levels and risk for long term diabetes complications.

The main drawbacks of OGTT are time-consuming, poor reproducibility, and individual difference of glucose absorption. The glycosylated hemoglobin (HbA_{1c}) has the advantages of neither requiring a morning blood draw nor an overnight fast. In addition, it is rarely affected by short-term lifestyle changes. HbA_{1c} shows less intra-individual biologic variability and relatively stable. It has become the gold standard for monitoring glycemic control in patients with diabetes and also been recommended as screening and diagnosis index for pre-diabetic stages and diabetes by World Health Organization (WHO) [5, 6]. However, the study of HbA_{1c} as a standard for screening and the diagnosis of diabetes for patients with hyperthyroidism is rare. We carried out this study to detect the cut-off point of HbA_{1c} in diagnosing pre-diabetic stages and diabetes in patients with hyperthyroidism.

Materials and methods

Participants

Between January 2013 and March 2016, 508 patients with hyperthyroidism were recruited from the ward and clinic in Anhui Provincial Hospital. OGTT criteria for determining diabetes status were identified according to the WHO 2010 criteria. The study protocol was approved by the Institutional Review Board. Informed consents were obtained from patients before inclusion into this study.

Measurements

The diagnosis of hyperthyroidism was identified according to the American Thyroid Association/American Association of Clinical Endocrinologists Guidelines for Hyperthyroidism, established based on the presence of suppressed serum thyrotropin levels (TSH < 4.2 mIU/L) and elevated serum thyroid hormones (FT3>3.8 pmol/L or FT4>22 pmol/L).

The participants were instructed to an overnight fast of 10-12 h before examination and had at least 3 days of unrestricted diet (< 150 g of carbohydrate per day) and OGTT was performed in the morning. The fasting blood glu-

cose, 2 hPG and HbA_{1c} of 508 patients (325 males and 183 females, 18-80 years old, median age 39.8 years old) were detected. They had no history of diabetes. According to the 2010 WHO definition, the participants were divided into 5 groups: NGT was defined as FPG < 6.1 mmol/L and/or 2-hPG < 7.8 mmol/L, impaired fasting glucose (IFG) was defined as 6.1 mmol/L ≤ FPG < 7.0 mmol/L and 2 hPG < 7.8 mmol/L, impaired glucose tolerance (IGT) was defined as FPG < 6.1 mmol/L and 7.8 mmol/L ≤ 2 hPG < 11.1 mmol/L. IFG and IGT: 6.1 mmol/L ≤ FPG < 7.0 mmol/L and 7.8 mmol/L ≤ 2 hPG < 11.1 mmol/L; DM: FPG ≥ 7.0 mmol/L or 2 hPG ≥ 11.1 mmol/L. The impaired glucose regulation (IGR) group included IFG and IGT participants. Blood samples were collected before glucose ingestion and 2 h after a 75-g anhydrous glucose load. FPG and 2 hPG were measured by oxidase method. The HbA_{1c} was measured by high performance liquid chromatography assay.

Statistical analysis

Statistical analyses were performed using SPSS for windows 20.0. Difference between groups was tested using univariate analysis of variance adjusting for body mass index (BMI). Sex and age in frequencies using the chi-square test. Difference in means between groups was tested using univariate analysis of variance (unianova) adjusting for age and sex and in frequencies using the chi-square test. The receiver operating characteristic curve (ROC) was plotted and the optimal cut-off point was the point on the ROC curve closest to the (0, 1) point. The optimal specificity and sensitivity of using HbA_{1c} to predict pre-diabetes or diabetes defined by the OGTT was calculated. The predictive value was calculated to determine the probability of patients with hyperthyroidism who having diabetes (or pre-diabetes) given a positive (or negative) HbA_{1c} result. The likelihood ratio is defined as the probability of an negative or positive HbA_{1c} result given the presence of diabetes (or pre-diabetes) divided by the probability of the result given the absence of diabetes (or pre-diabetes), thus negative likelihood ratio (-LR) = (1-sensitivity)/specificity and positive likelihood ratio (+LR) = sensitivity/(1-specificity) [7, 8]. Statistical significance was considered as $p < 0.05$.

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Table 1. Comparison of clinical and laboratory data among three groups ($\bar{x} \pm s$)

Group	n	Age (years)	BMI (kg/m ²)	FPG (mmol/L)	2 hPG (mmol/L)	HbA _{1c} (%)
NGT	272	37.0±13.2	21.4±2.2	4.74±0.55	5.88±1.19	5.3±0.2
IGR	167	43.4±33.4*	21.4±1.8	5.11±0.69*	8.84±1.08*	5.5±0.3*
DM	69	48.0±12.3*	21.2±2.0	6.29±2.20*	13.0±4.31*	6.0±0.4*

Analysis of variance. FPG = Fasting plasma glucose; 2 hPG = 2-hour plasma glucose; BMI = Body mass index. * $p < 0.001$.

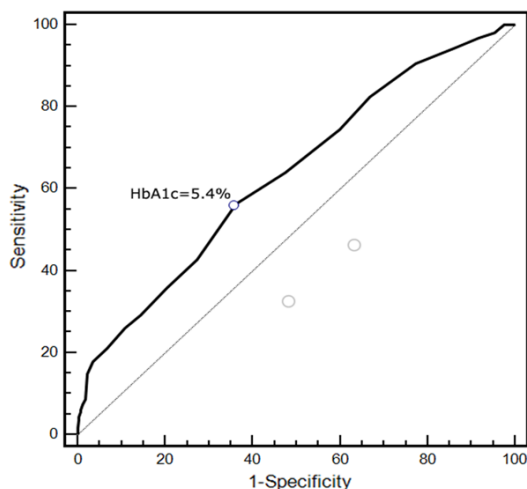


Figure 1. The cut-point of HbA_{1c} ROC curve for diagnosing pre-diabetes.

Results

The status of glucose metabolism in the subjects

Between January 2013 and March 2016, 508 patients were recruited from the ward and clinic. A total of 69 participants had diabetes, 167 had pre-diabetes, and 272 were non diabetic, according to the 2010 WHO diagnostic criteria for diabetes, (**Table 1**). A total of 325 participants were 325 male, and 183 were female. The mean ages were 39.8±13.2 years old. The participants were distributed by baseline A1C in three groups, with 3.5% with HbA_{1c} ≥ 6.5%, 35.6% between 5.6 and 6.4%, and 60.9% between 4 and 5.6%. The percentage of patients with abnormal glucose metabolism as classified by OGTT levels was higher than by HbA_{1c} criteria in patients with hyperthyroidism-32.9% vs. 35.6% for pre-diabetes and 13.6% vs. 3.5% for diabetes, respectively. **Table 1** presents showed that DM group had significantly higher HbA_{1c}, FPG and 2 hPG levels than those with pre-diabetes ($p < 0.001$), and also patients with pre-diabetes had significant-

ly higher HbA_{1c}, FPG and 2 hPG levels than those with NGT ($p < 0.001$).

HbA_{1c} in pre-diabetes diagnosis

The optimal HbA_{1c} cut-off point in patients with hyperthyroidism for detecting pre-diabetes alone was 5.4%. The area under the curve (AUC) for detecting pre-diabetes was 0.636. The sensitivity decreased and specificity increased as the cut-off point increased (**Figure 1** and **Table 2**). And the +LR was 1.57, -LR was 0.68. When lowering the diagnostic cut-off value of HbA_{1c} to 5.4%, 63.4% of those with pre-diabetes were detected.

HbA_{1c} in diabetes diagnosis

The optimal HbA_{1c} cut-off point in patients with hyperthyroidism for detecting DM alone was 5.5%. The AUC for detecting pre-diabetes was 0.832. The sensitivity decreased and specificity increased as the cut-off point increased (**Figure 2** and **Table 3**). And the +LR was 2.97, -LR was 0.28. When lowering the diagnostic cut-off value of HbA_{1c} to 5.5%, 82.6% of those with DM were detected.

Discussion

Hyperthyroidism refers to a form of thyrotoxicosis in excessive thyroid hormone. The symptoms and signs of hyperthyroidism are manifested as appetite stimulation, polyuria and so on. Glucose metabolic disorders often got in patients with hyperthyroidism [9]. In China, the prevalence of glucose intolerance in patients with thyrotoxicosis has been shown to be as high as 2%-57% [10]. The abnormal rate of glucose metabolism in patients with hyperthyroidism is higher than patients without hyperthyroidism. If HbA_{1c} test result is equivocal, for example, between 6% and 6.5%, it should be confirmed with a plasma glucose test (fasting plasma glucose, 2 hour post glucose load plasma glucose, or oral glucose toler-

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Table 2. Sensitivity, specificity, positive likelihood ratio and negative ratio of different HbA_{1c} levels to diagnose pre-diabetes

HbA _{1c} (%)	Sensitivity (%)	Specificity (%)	+LR	-LR
>5.3	63.98	52.52	1.35	0.69
>5.4*	55.9	64.39	1.57	0.68
>5.49	55.28	64.39	1.55	0.69
>5.5	42.86	72.66	1.57	0.79
>5.6	36.02	79.5	1.76	0.8
>5.7	29.19	85.61	2.03	0.83
>5.8	26.09	89.21	2.42	0.83
>5.9	21.12	93.17	3.09	0.85
>6	18.01	96.4	5.01	0.85
>6.1	14.91	97.84	6.91	0.87
>6.2	8.7	98.2	4.83	0.93
>6.3	7.45	98.92	6.91	0.94
>6.4	6.21	99.28	8.63	0.94
>6.48	5.59	99.28	7.77	0.95
>6.5	4.35	99.64	12.09	0.96

*The optimal cut-off point of HbA_{1c} for diagnosing IGR in patients with hyperthyroidism was 5.4% according to the ROC curve, with the sensitivity of 55.9% and the specificity of 64.39%.

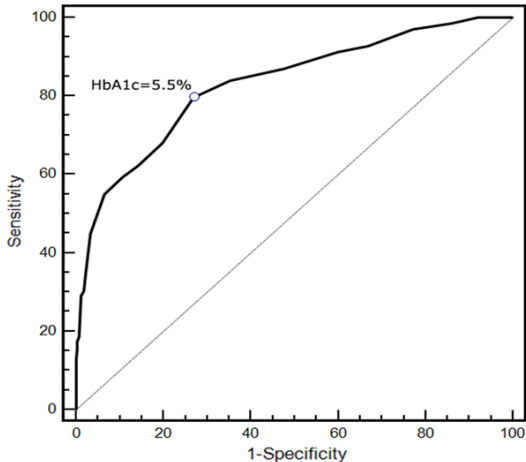


Figure 2. The cut-point of HbA_{1c} ROC curve for diagnosing diabetes.

ance test). Indeed, our work demonstrates that a diagnostic cut-off point for HbA_{1c} of $\geq 6.5\%$ misses a substantial number of patients with type 2 diabetes, including some with fasting hyperglycemia, and misses most patients with impaired glucose tolerance. Although the specific mechanism remains unknown, these patients have been found to have hyperinsulinemia, impaired glucose tolerance and insulin

resistance. The impairment in insulin secretion, appears to contribute to both impaired glucose intolerance and fasting glucose to carbohydrate loads in the hyperthyroid state. In addition, decreased glucose utilization [11, 12], changes in gastric emptying, blunted insulin receptor binding, hepatic glucose production via influence of glucose and catecholamines on enhanced lipid oxidation have been demonstrated [13, 14]. Excessive thyroid hormone stimulates increased metabolism in many tissues, reductions in weight, and leading to an increased demand for glucose, increased uptake of glucose, result in improvements in markers of glycemic control.

HbA_{1c} is formed by glycation of N-terminal valine residue of the β -chain of globin, which indicates a patient's glycemic status over previous 12 weeks [15]. HbA_{1c} has been recommended to diagnose and screen DM by WHO and the endorsement of influential DM societies [16]. The optimal cut-off points for screening undiagnosed DM have been proposed to be 5.3-6.2% [8, 17-19] based on following reasons: 1) HbA_{1c} does not require patients to be fasting; 2) HbA_{1c} level is not affected by short-term lifestyle change [20]; 3) HbA_{1c} reflects long-term glycemia compared with plasma glucose; 4) Microvascular complications such as retinopathy and nephropathy and the severity of the neuropathy is tied to the change in the HbA_{1c} [21]; 5) HbA_{1c} assessing methods are standardized and reliable. Nevertheless, there were differences in the cut-off points of HbA_{1c} for diagnosing DM between patients with hyperthyroidism and the general population, due to their different abnormal glucose metabolism. 6) HbA_{1c} has the advantages of not requiring an overnight fast or a morning blood draw. In addition, it is familiar and generally available to clinicians in developed countries. It is less likely than glucose to be affected by short-term lifestyle changes. HbA_{1c} is relatively stable at room temperature and shows less intra-individual biologic variability (4% coefficient of variation) than fasting or postglucose load glucose levels. In addition, the precision and accuracy of the HbA_{1c} assay have benefited from an international effort to improve assay standardization.

The low sensitivity of the HbA_{1c} diagnostic criteria underestimated the prevalence of pre-diabetes and diabetes in patients with hyper-

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Table 3. Sensitivity, Specificity, positive likelihood ratio and negative ratio of different HbA_{1c} levels to diagnose diabetes

HbA _{1c} (%)	Sensitivity (%)	Specificity (%)	+LR	-LR
>5.4	84.06	64.71	2.38	0.25
>5.5*	79.71	73.16	2.97	0.28
>5.6	68.12	80.15	3.43	0.4
>5.7	62.32	85.66	4.35	0.44
>5.8	59.42	89.34	5.57	0.45
>5.9	55.07	93.38	8.32	0.48
>6	44.93	96.69	13.58	0.57
>6.1	34.78	97.79	15.77	0.67
>6.2	30.43	98.16	16.56	0.71
>6.3	28.99	98.9	26.28	0.72
>6.4	18.84	99.26	25.62	0.82
>6.5	17.39	99.63	47.3	0.83

*The optimal cut-off point of HbA_{1c} for diagnosing DM in patients with hyperthyroidism was 5.5% with the sensitivity of 79.71% and the specificity of 73.16%.

thyroidism. In this study, 508 patients with hyperthyroidism who carried out OGTT were recruited into the study. Total 69 patients, 13.6% of all patients with hyperthyroidism, were diagnosed with DM, and 167 patients, 32.9% of all patients, were diagnosed with IGR. The HbA_{1c} values of 5.6%-6.4% and \geq 6.5% were used as the cut-off points for diagnosing IGR and DM in general population, respectively, but the optimal cut-off point of HbA_{1c} for diagnosing IGR was 5.4% according to the ROC curve, with the sensitivity of 55.9% and the specificity of 64.39%. The optimal cut-off point of HbA_{1c} for diagnosing DM in patients with hyperthyroidism was 5.5% with the sensitivity of 79.71% and the specificity of 73.16%. Compared with the results of OGTT, the optimal cut-off point of HbA_{1c} that had coincidence rate of 82.6% for diagnosing DM in patients with hyperthyroidism was 5.5%, which is lower than that in general population. The difference can be explained by following reasons: 1) The established value of HbA_{1c} in ADA is correlated with the risk for retinopathy, but this cut-off point is established in hyperthyroidism; 2) The patients with hyperthyroidism have different metabolic characteristics. In patients with hyperthyroidism, abnormal glucose metabolism is mainly featured with higher postprandial blood glucose.

In summary, we found 46.5% of patients with hyperthyroidism were affected by IGR or DM

in this study. HbA_{1c} at the cut-off points of 5.4% and 5.5% could be used to diagnose and screen pre-diabetic state and DM in patients with hyperthyroidism. Considering its good compliance, we recommend HbA_{1c} as a novel diagnostic tool for screening abnormal glucose metabolism in patients with hyperthyroidism. Further study is needed to address this issue in a larger sample size.

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Disclosure of conflict of interest

None.

Address correspondence to: Shandong Ye and Wei Wang, Department of Endocrinology, The First Affiliated Hospital of University of Science and Technology of China (Anhui Provincial Hospital), Division of Life Sciences and Medicine, University of Science and Technology of China, 17 Lujiang Road, Hefei 230001, Anhui, China. Tel: +86-551-62283472; E-mail: ysd196406@163.com (SDY); hfww2001@163.com (WW)

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