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ORIGINAL ARTICLE

Statistical Analysis on Threshold of Haemoglobin A1C (HBA1c) for Diabetes Diagnosis and the Relationship Between HBA1c and Plasma Glucose Concentrations in Chinese Diabetic High-Risk Groups

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SUMMARY

Background: **The goal is to evaluate the threshold of hemoglobin A1C (HBA1c) for screening test among Chinese patients with diabetes and high-risk groups in the endocrinological department and identify the relationship between HbA1c and plasma glucose.**
Methods: **Experimental design: This study is based on the data selected from patients without clinical intervention enrolled in the Endocrinology Department and Admission Office in our hospital. It uses the four-point plasma glucose modeling and trapezoidal integration method to analyze the relationship between HbA1c and each plasma glucose threshold in an oral glucose tolerance test (OGTT).**
Setting: Harbin, China, from January 1st of 2010 to December 31st of 2012.
Participants: 2,853 16 - 85 year-old patients who came to our Endocrinology Department to take venous blood measurements and OGTT.
Selection criteria: The OGTT and HbA1c were performed simultaneously, unless acidosis was present, without considering past history of diabetes and oral hypoglycemic drugs or insulin treatment, or other basic combined diseases. Pregnant patients were excluded.
Results: **The area under the receiver operating characteristics curve (ROC) was 0.902 (95% confidence interval 0.890 to 0.914) for HbA1c alone and 0.915 (0.906 to 0.925) for fasting plasma glucose (FPG) alone. The HbA1c threshold of 6.5% showed the highest Youden index of 64.4%, and significantly higher sensitivity (81.1%, 79.3% to 82.7%) than FPG ≥ 7.0 mmol/L (69.8%, 67.8% to 71.8%) ($p < 0.0001$) and higher specificity (83.3%, 80.4% to 85.8%) than HbA1c ≥ 6.3% (76.3%, 73.2% to 79.3%) ($p < 0.0001$) in detecting diabetes, together with a low negative likelihood ratio of 0.2. In addition, the threshold of 1/2 hour postprandial glucose and that of 1 hour postprandial glucose are 10.6 mmol/L and 13.6 mmol/L, respectively. Thus, the relative contribution of FPG increased gradually with increasing levels of HbA1c: 15.9% in the lowest vs. 44.0% in the highest quintile ($p < 0.001$). The relative contribution of 1-hour postprandial glucose decreased progressively from the lowest (25.0%) to the highest quintile of HbA1c (14.2%, $p < 0.001$).**
Conclusions: **These findings suggest that the optimal HbA1c threshold of 6.5% as a screening criterion for diabetes and high-risk groups may be acceptable. This paper is trying to put forward the thresholds of 1/2-hour plasma glucose and 1-hour plasma glucose for diagnosing diabetes. The relative contribution of FPG increased gradually with increasing levels of HbA1c; however, the contribution of postprandial glucose decreased progressively.**
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KEY WORDS

plasma glucose, HbA1c, optimal threshold, relative contribution, diabetes

INTRODUCTION

Oral glucose tolerance test (OGTT) has long been recognized as the golden standard of diabetic diagnosis by ADA/WHO organization [1,2]. However, the prevalence and reproducibility of OGTT is restricted due to the requirement that participants need to take 75 g glucose in the fasting state and OGTT is time consuming. The method of exclusively using blood glucose to screen for diabetes can cause missed diagnoses. Because of lower individual day variability, a reflection of average level of blood glucose within 2 to 3 months, and no need for fasting, the test of HbA1c is recognized as a useful tool [3-6] for screening and diagnosing diabetes. In 2006, it is noted that the American Diabetes Association (ADA) still could not define HbA1c as a criterion for diagnosing diabetes until 2009, when an International Expert Committee recommended that HbA1c $\geq$ 6.5% could be used to diagnose diabetes. Subsequently, in 2011, the ADA summarized and approved this recommendation. Meanwhile, they also expressed that the selection of HbA1c threshold for diagnosing diabetes could be limited by many factors [7,8]. Nowadays, there is no universally accepted criterion [9,10] for the selection of HbA1c threshold in diabetic diagnosis, due to varied testing methods in different locations, racial variation, different study populations, and changed erythrocyte life span, etc. It needs to be further verified what the HbA1c threshold for diabetic diagnosis is and whether it has a direct relationship with the plasma glucose points in OGTT.

Our study was based on patient data selected from the first ones who came to the Endocrinology Department of the second affiliated Hospital of Harbin Medical University and analyzed the relationship between HbA1c and each blood glucose point in an oral glucose tolerance test (OGTT) in order to identify the HbA1c threshold in diabetic diagnosis for people with diabetic high-risk groups. Meanwhile, we evaluated the thresholds for diagnosing diabetes with 1/2-hours plasma glucose (1/2-hPG) and 1-hour plasma glucose (1-hPG). We adopted four-point blood glucose modeling (not used previously) to assess the relative contribution of each blood glucose threshold in an OGTT to HbA1c.

MATERIALS AND METHODS

First, we logged into the computer laboratory information system of the Second Affiliated Hospital of Harbin Medical University, and collected HbA1c and OGTT data from January 1, 2010 to December 31, 2012 within the limited scope of all data from patients enrolled through the Endocrinology Department and admission office without clinical intervention and had blood drawn on the same day for FPG of HbA1c and OGTT (data not conform to the requirement of blood-drawing on the same day was automatically excluded). At the same time, we also collected the information related to gender and age. We collected 2,887 cases, not including those involved with ketosis or aged less than 16. We then had a total of 2,853 subjects aged 16 to 85. Case collections did not consider past history of diabetes, diabetes type identification, and oral hypoglycemic drugs or insulin treatment, or other basic combined diseases and complications related to diabetes. Pregnant patients were ruled out.

The test method [11], operating criteria, and testing apparatus of OGTT: (blood was drawn between 5:00 and 9:00 am after an 8-hour overnight fast) OGTT was performed in accordance with the criteria defined by the World Health Organization [11]. After a 10 to 12 hours overnight fast, subjects ingested a solution containing 75 g glucose over a 5-minute period, and venous blood samples were collected at 0, 30, 60, and 120 minutes for determination of plasma glucose by an automated glucose oxidase method (D360plus, SINO-INNOVA Medical Science & Technology Co., Ltd. China). The test method, operating criteria, and testing apparatus of HbA1c [12]: (blood was drawn between 5:00 and 9:00 am after an 8-hours overnight fast) Tests for HbA1c were performed using a high performance liquid chromatographic method on an analyzer (VARIANT II TURBO, Bio-Rad Laboratories, Hercules, CA, USA). Normal glucose tolerance (NGT): fasting plasma glucose (FPG) $<$ 5.6 mmol/L and 2-hour plasma glucose (2-hPG) $<$ 7.8 mmol/L. The defining criterion of IGR [13] was: IFG 5.6 mmol/L $\leq$ FPG $<$ 7.0 mmol/L; IGT 7.8 mmol/L $\leq$ 2-hPG $<$ 11.1 mmol/L. The defining criterion of DM [14] was: FPG $\geq$ 7.0 mmol/L and/or 2-hPG $\geq$ 11.1 mmol/L.

Statistical analysis

We used SPSS version 13.0 for all statistical analyses. We presented continuous variables as means (SD), except for skewed variables, which we presented as medians (interquartile range). We expressed categorical variables as percentages. We used Pearson's correlation

analysis to investigate the association of two variables. We used the method described by Hanley and McNeil [15] to compare the two area under the receiver operating characteristics curves (ROC). We examined the sensitivity and specificity of HbA1c with the ROC to identify participants as having undiagnosed diabetes. The Youden index [16] maximum was used as a basis to determine the best cutoff value. The relative contributions of fasting and post-load glucose were compared over quintiles of HbA1c by using one-way ANOVA, followed by a Student-Newman-Keuls test. Linear trends of the relative contributions of fasting and post-load glucose were tested. We considered p-values less than 0.05 to be statistically significant for a two-sided test.

Validation of the model using a four-point glucose profile and calculation using the trapezoidal integration method.

For the 2,853 patients, we used four-point plasma glucose (FPG, 1/2-hPG, 1-hPG, and 2-hPG) mean ± standard deviation, which were drawn as shown in the graph (attached figure in supplementary material). Four areas were calculated geometrically from the four-point curve, the area below the baseline value (< 5.6 mmol/L) was ignored, leaving 2,330 cases of FPG > 5.6 mmol/L to analyze the relatively contribution. First, the area under the curve (AUC) above FPG concentrations (AUC1) was calculated above the fasting plasma value and therefore considered as a reflection of the postprandial glycemic responses to 75 g glucose in the fasting state. Second, the AUC > 5.6 mmol/L (AUC2) was calculated above a baseline level equal to 5.6 mmol/L, reflecting the increases in both fasting and postprandial PG. Then we divided AUC1 into three areas, which contained a triangle formed by three points of "abg", a trapezoidal area formed by four points of "bcfg", and a trapezoid region of "cdef". The baseline value of 5.6 mmol/L was chosen because this threshold has been defined as the upper limit of normal PG during fasting or pre-prandial times by the American Diabetes Association (ADA). Therefore, the difference (AUC2 - AUC1) can be considered as an assessment of the increment in FPG values. As a result, the relative contributions of postprandial (1/2-hPG, 1-hPG, 2-hPG) and FPG to the total PG increment were calculated by using the following equations: (AUC_{abg}/AUC2) x 100, (AUC_{bcfg}/AUC2) x 100, and (AUC_{cdef}/AUC2) x 100 for the postprandial (1/2-hPG, 1-hPG, 2-hPG) contribution and [(AUC2 - AUC1)/AUC2] x 100 for the fasting contribution.

RESULTS

In total there were 2,853 samples including 1,569 men and 1,284 women with a median age of 49.0 (interquartile range 40.0 - 57.0 years). We re-evaluated the results of OGTT in the dataset (Table 1): 267 people with an average age of 41.7 (16 - 75 years) with normal glucose tolerance, the 16 - 30 year old group accounted for 25.5%, the proportion of men is 40.5%, hospitalized cases accounted for 13.9%; 498 with impaired glucose regulation with an average age of 48.0 (16 - 83 years), the 16 - 30 year old group accounted for 7.6%, the proportion of men is 54.6%, hospitalized cases accounted for 11.2%; and 2,088 diabetics with an average age of 49.3 (16 - 85 years) and the proportion of men is 56.9%, the 16 - 30 year old group accounted for 3.7%, hospitalized cases accounted for 20.5%. Of the 498 participants with impaired glucose regulation, 116 (24.9%) had impaired fasting glucose, 124 (23.3%) had impaired glucose tolerance, and 258 (51.8%) had impaired fasting glucose with impaired glucose tolerance.

Of the 2,088 participants with diabetes, OGTT was used to assess that 62 subjects (3.0%) had isolated high fasting plasma glucose concentrations, 624 (29.9%) had isolated high two-hour post-load plasma glucose concentrations, and 1,402 (61.7%) had high fasting plasma glucose concentrations with high two hour post-load plasma glucose concentrations.

HbA1c and either fasting plasma glucose or two hour post-load plasma glucose were significantly correlated ($p < 0.001$), with Pearson's correlation coefficients of 0.72 and 0.73; 30-minute post-load plasma glucose and either fasting plasma glucose or two-hour post-load plasma glucose were significantly correlated ($p < 0.001$), with correlation coefficients of 0.88 and 0.76; 60-minute post-load plasma glucose and either FPG or two-hour post-load plasma glucose were significantly correlated ($p < 0.001$), with correlation coefficients of 0.81 and 0.86 on the basis of Pearson's correlation analysis.

The threshold of HbA1c for diagnosing diabetes

The receiver operating characteristics curve (ROC) [17] shown in Figure 1A represented the diagnostic accuracy of HbA1c for undiagnosed diabetes. The area under the curve was 0.902 (95% confidence interval 0.890 to 0.914) for HbA1c alone and 0.915 (0.906 to 0.925) for FPG alone. The two areas were significantly different from each other ($p = 0.04$). These findings coincided with the threshold selected by the closest distance to the left upper corner of the receiver operating characteristics curve, which indicated the best trade-off between sensitivity and specificity.

Table 2 showed the sensitivity, specificity, positive likelihood ratio, negative l-likelihood ratio, positive predictive value, and negative predictive value and Youden index for identifying diabetes at HbA1c thresholds of 6.0 - 7.0%. HbA1c, as the diagnosis of diabetes, boundary value increased with 0.1%, the sensitivity decreased and the specificity increased. An HbA1c threshold of 6.5% showed the highest Youden index of 64.4% among the HbA1c thresholds, and a higher sensitivity of 81.1% ($p < 0.001$) and a highly specificity of 83.3% ($p < 0.001$) for detecting diabetes compared with FPG 7.0 mmol/L. For an HbA1c threshold of 6.5%, the positive likelihood ratio was 4.9, the negative likelihood ratio 0.2, the positive predictive value 93.0% and negative predictive value 61.7%. With the HbA1c level in-

Table 1. Clinical characteristic of participants.

Characteristics	Total (n = 2,853)	Men (n = 1,569)	Women (n = 1,284)
Age (years)	49.0 (40.0 - 57.0)	47.0 (40.0 - 55.0)	52.0 (43.0 - 58.0) *
FPG (mmol/L)	7.1 (5.9 - 8.9)	7.2 (6.1 - 8.9)	6.9 (5.7 - 9.0) *
1/2-hPG (mmol/L)	11.8 (9.8 - 14.2)	11.9 (10.1 - 14.1)	11.6 (9.6 - 14.2) *
1-hPG (mmol/L)	14.9 (12.2 - 17.7)	15.3 (12.7 - 17.7)	14.5 (11.5 - 17.7) *
2-hPG (mmol/L)	14.5 (10.3 - 17.9)	14.5 (10.7 - 17.6)	14.5 (9.6 - 18.3)
HbA1c (%)	7.0 (6.2 - 8.4)	7.1 (6.3 - 8.3)	7.0 (6.1 - 8.4) *
Outpatient-No. (%)	2,332 (81.7)	1,228 (78.3)	1,104 (86.0) *
NGT-No. (%) (5.6)	267 (9.4)	108 (6.9)	159 (12.4) *
IGR-No. (%) (5.6)	498 (17.5)	272 (17.3)	226 (17.6)
Diabetes-No. (%)	2,088 (73.2)	1,189 (75.8)	899 (70.0) *
FPG ≥ 7.0 mmol/L and 2-hPG ≥ 11.1 mmol/L-No. (%)	1,402 (49.1)	814 (51.9)	588 (45.8) *
FPG < 7.0 mmol/L and 2-hPG ≥ 11.1 mmol/L-No. (%)	624 (21.9)	334 (21.3)	290 (22.6)
FPG ≥ 7.0 mmol/L and 2-hPG < 11.1 mmol/L-No. (%)	62 (2.2)	41 (2.6)	21 (1.6)

Values are median (interquartile range) unless stated otherwise.
FPG - fasting plasma glucose, 2-hPG - 2-hour post-load plasma glucose, 1/2-hPG - 1/2-hour post-load plasma glucose, 1-hPG - 1-hour post-load plasma glucose, NGT - normal glucose tolerance, IGR - impaired glucose regulation. * - $p < 0.05$ compared with men. Analyzed by Wilcoxon Two-Sample Test and χ^2 test.

creased, the positive hierarchical likelihood ratio, the chance of HbA1c diagnosis at high risk of diabetes was correspondingly increased. When HbA1c < 5.9%, the hierarchical likelihood ratio was 0.1, with the increase of HbA1c, 6.2 - 6.4% the ratio was 0.5; 6.5 - 6.7% was 1.0; after a significant increase, 7.1 - 7.3% was 2.5, when 7.4 - 7.5% was 7.8, > 7.6% that was 28.4 (shown in the table in the supplementary material specification). Subsequently, we compared the sensitivity and specificity of HbA1c thresholds of 6.5% (as recommended by the international expert committee) and 6.3% (recommended by a study from a community population of Shanghai, China [18]) with a FPG threshold of 7.0 mmol/L. The sensitivities of an HbA1c threshold of 6.5% and this FPG concentration in diagnosing diabetes were 81% and 69.8%, respectively ($p < 0.0001$), specificities were 83.3% and 100.0% ($p < 0.0001$), respectively. However, the sensitivity and specificity of an HbA1c threshold of 6.3% were 86.1% and 76.3%, which also differed significantly from that of FPG ($p < 0.0001$). At an HbA1c threshold of 6.3% (Table 2), the positive and negative predictive values were 90.8% and 66.7% and the positive and negative likelihood ratios were 3.6 and 0.2, Youden index was 62.4%. Interestingly, the specificity of an HbA1c threshold of 6.5% was higher than that of an HbA1c threshold of 6.3% ($p = 0.0007$).

Figure 2A showed the distribution of HbA1c in those with NGT, pre-diabetes (including IFG and/or IGT), and DM. It could be seen that there was considerable overlap among three categories with respect to the HbA1c levels. Using the cutoff of 6.5%, 82.8% of subjects with DM would be correctly identified; however, 23.7% of subjects with pre-diabetes and 3.7% of subjects with NGT would be included in this category. In Figure 2B, we further divided pre-diabetes and diabetes into different categories for analysis. It could be seen further that using the cutoff of 6.5% as the optimal threshold to diagnose diabetes, 61.1% of subjects with only 2-hPG rising diabetes, 59.7% of subjects with only FPG rising diabetes, and 90.9% with 2-hPG and FPG all rising diabetes would be correctly identified. Nevertheless, 19.4% of subjects with IGT, 12.1% with IFG, and 31.0% with IFG and IGT would be included in this category.

1/2-hPG and 1-hPG cutoff points for the diagnosis of diabetes

We also attempted to provide an idea to adopt 1/2-hPG and 1-hPG in OGTT for diabetic diagnosis and tested different thresholds of sensitivity, specificity, positive predictive value, negative predictive value, positive likelihood ratio, negative likelihood ratio and Youden index to compare with FPG threshold of 7.0 mmol/L. As a result, we listed the positive results in Table 2 showing that the thresholds of 1/2-hPG and 1-hPG were 10.6 mmol/L and 13.6 mmol/L, respectively. The sensitivities were 81.4% and 82.3%, the specificities were 79.1% and 91.6%, their Youden indices were the highest among the different thresholds, 60.5% and 74.0%.

Table 2. Sensitivity, specificity, positive predictive value, negative predictive value, positive likelihood ratio, negative likelihood ratio and Youden index of the curve of HbA1c, 1/2-hour, 1-hour plasma glucose, FPG and 2-hour plasma glucose (n = 2,853).

Criterion	Thresh-old	Sensi-tivity	Speci-ficity	Positive likelihood ratio	Negative likelihood ratio	Positive predictive value	Negative predictive value	Youden index	Accu-racy	Area under curve
HbA1c	> 6.0	92.4	60.4	2.3	0.1	86.4	74.5	52.8		
(%)	> 6.1	90.6	66.9	2.7	0.1	88.2	72.3	57.5		
	> 6.2	88.4	72.2	3.2	0.2	89.7	69.4	60.5		
	> 6.3	86.1	76.3	3.6	0.2	90.8	66.7	62.4		
	> 6.4	83.5	80.7	4.3	0.2	92.2	64.2	64.2		
	> 6.5 *	81.1	83.3	4.9	0.2	93.0	61.7	64.4	82.8	0.902 (0.890 - 0.914)
	> 6.6	77.9	86.3	5.7	0.3	93.9	58.9	64.2		
	> 6.7	74.8	89.0	6.8	0.3	94.9	56.4	63.8		
	> 6.8	71.8	90.9	7.9	0.3	95.5	54.2	62.7		
	> 6.9	68.7	92.4	9.1	0.3	96.1	51.9	61.1		
	> 7.0	65.2	94.3	11.3	0.4	96.9	49.8	59.5		
1/2-hPG (mmol/L)	> 10.6 *	81.4	79.1	3.9	0.2	91.4	60.9	60.5	81.0	0.888 (0.876 - 0.900)
1-hPG (mmol/L)	> 13.6 *	82.3	91.6	9.8	0.2	96.4	65.5	74.0	84.9	0.945 (0.937 - 0.953)
FPG (mmol/L)	> 7.0	69.8	100.0	-	0.3	100.0	54.8	69.8	78.1	0.915 (0.906 - 0.925)
2-hPG (mmol/L)	> 11.1	97.0	100.0	-	0.0	100.0	92.4	97.0	97.8	0.993 (0.990 - 0.995)

Values in parentheses are 95% confidence intervals.

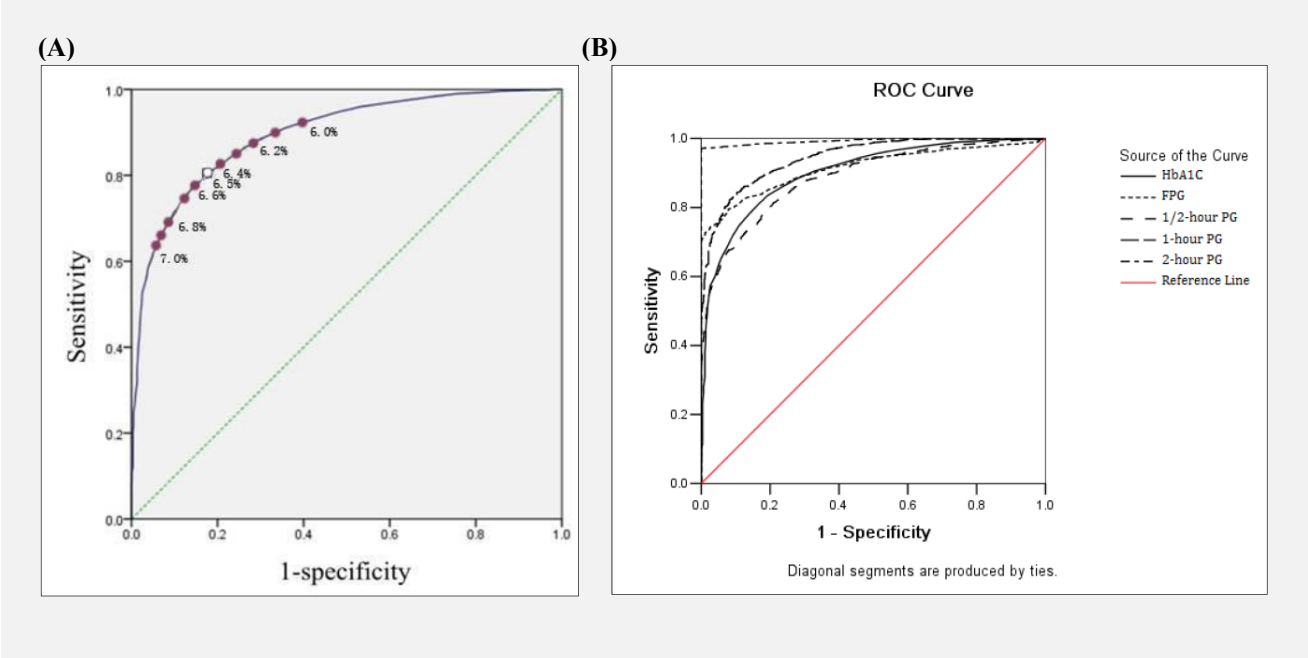

Figure 1. (A) Receiver operating characteristics curve of HbA1c for detecting diabetes at each possible HbA1c threshold. (B) Receiver characteristics curves of HbA1c, fasting glucose, 1/2-hPG, 1-hPG, and 2-hPG. (——HbA1c, - - - -FPG, — —1/2hPG, — — —1-hPG, — — —2-hPG, ——Reference Line).

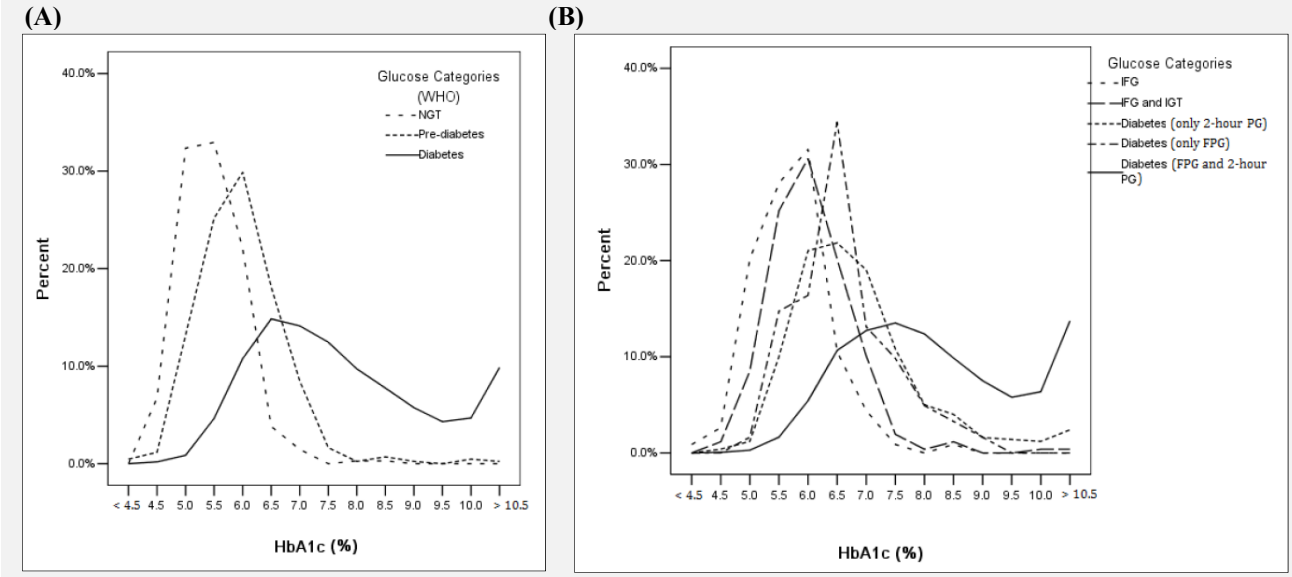

Figure 2. (A) HbA1c distribution among subjects with NG, pre-DM, and DM. (- - - NGT, ----- Pre-diabetes, ——— Diabetes) (B) HbA1c distribution among subjects with IFG, IFG and IGT, Diabetes (only 2-hPG), Diabetes (only FPG), Diabetes (FPG and 2-hPG). (- - IFG, — — IFG and IGT, ----Diabetes < only 2-hPG >, - - - Diabetes < only FPG >, ——— Diabetes < FPG and 2-hPG >).

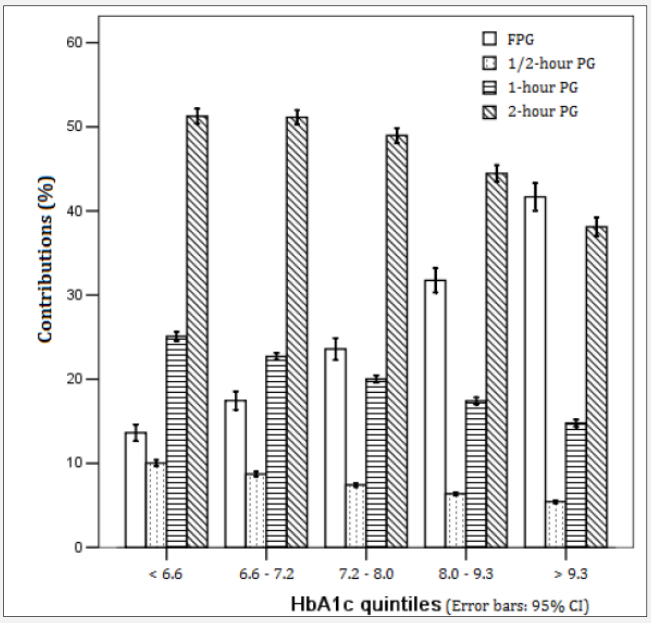

Figure 3. Relative contributions of postprandial (1/2-hPG, 1-hPG, 2-hPG) and fasting hyperglycemia (%) to the overall diurnal hyperglycemia over quintiles of HbA1c. (FPG, 1/2-hPG, 1-hPG, 2-hPG).

Accordingly, the sensitivity of FPG threshold of 7.0 mmol/L was 69.8% and Youden index was 69.8%. It was also interesting that the sensitivities of the threshold of 1/2-hPG 10.6 mmol/L and that of 1-hPG 13.6 mmol/L were higher than that of FPG threshold of 7.0 mmol/L ($p < 0.0001$, $p < 0.0001$).

Moreover, all plasma glucose concentrations during OGTT and HbA1c were significant predictors of the future risk of diabetes ($p < 0.001$). The area under the receiver operating characteristics curves (AUC_{ROC}) of 2-hPG was 0.993 (0.990 - 0.995), accordingly, 1 hour-AUC_{ROC} and FPG-AUC_{ROC} were 0.945 (0.937 - 0.953) and 0.915 (0.906 - 0.925), respectively, HbA1c-AUC_{ROC} was 0.902 (0.890 - 0.914), and the smallest was 1/2 hour-AUC_{ROC}, 0.888 (0.876 - 0.900). Nevertheless, the area under the curves for HbA1c was significantly smaller than that of FPG ($p = 0.04$), 1-hPG ($p < 0.001$), 2-hPG ($p < 0.001$), but not significantly different from that of 1/2-hPG ($p = 0.06$). The area under the receiver operating characteristics curve for 1/2-hPG was smaller than that of FPG, 1-hPG, 2-hPG, while 1-hPG was 0.945 (0.937 - 0.953) which was above FPG ($p < 0.0001$) and under 2-hPG ($p < 0.0001$) (Figure 1B, Table 2).

The relationship between HbA1c and all plasma glucose concentrations in OGTT

In 2,330 cases of FPG > 5.6 mmol/L, HbA1c correlated with FPG, 1/2-hPG, 1-hPG, and 2-hPG, and correlation coefficients were 0.685, 0.638, 0.666, and 0.684, respectively. HbA1c was associated with the area under the curve of the four points' glucose profile and above 5.6 mmol/L (AUC2) with a correlation coefficient of 0.718.

As shown in Figure 3, the relative contribution of fasting glucose increased gradually with increasing levels of HbA1c: 15.9% in the lowest vs. 44.0% in the highest quintile ($P_{trend} < 0.01$). In contrast, the relative contribution of 1/2-hPG decreased progressively from the lowest 10.3% to the highest quintile of HbA1c 5.3% ($P_{trend} < 0.01$), as well as the contribution of 1-hPG from 25.0% to 14.2% ($P_{trend} < 0.01$), and the contribution of 2-hPG in the lowest 48.9% vs. 36.6% in the highest quintile ($P_{trend} < 0.01$) [19]. Moreover, one-hour postprandial glucose showed a higher relative contribution compared with FPG when HbA1c < 6.6%. Then, when HbA1c exceeded 7.2%, the contribution of fasting glucose was significantly higher than that of 1-hPG. While HbA1c > 9.3%, the relative contribution of fasting glucose even went beyond the contribution of 2-hPG, and became the main role to affect blood glucose levels over a period of time. In conclusion, the relative contribution of fasting hyperglycemia increased gradually with increasing levels of HbA1c, whereas the contribution of postprandial glucose decreased progressively. The correlation analysis of 2,330 cases shows the same conclusion: HbA1c and the relative contributions of FPG, 1/2-hPG, 1-h PG, and 2-hPG were also associated, and correlation coefficients were 0.605, -0.526, -0.622, -0.411.

DISCUSSION

We collected the data from 2,853 cases from the Endocrinology Department of the Second Affiliated Hospital of Harbin Medical University. We found that an HbA1c threshold of 6.5% had higher sensitivity and specificity in terms of detecting undiagnosed diabetes compared to that of a FPG threshold of 7.0 mmol/L. Its sensitivity (81.1%) was higher than that of FPG (69.8%), so we can say that HbA1c 6.5% increased the sensitivity of FPG in the diagnosis of diabetes. Namely it can reduce missed diagnosis and is a good complement to FPG. HbA1c 6.5% had the highest Youden index of 64.4% among HbA1c thresholds, which was an evaluation screening testing authenticity. The Youden index, equal to sensitivity plus specificity minus one, suggests the overall ability of detecting diabetes and non-diabetes by the screening test method. The bigger an index is, the better effect and more authenticity the screening test will present. This threshold may be more efficient in the people at high risk of diabetes.

As the testing of HbA1c can be performed at any time without the fasting state or other preparations for patients and does not need fasting for at least 8 hours makes the diagnosis in one day possible. HbA1c is not affected by the short-term changes of lifestyle such as eating and exercise before an examination and can reflect the average level of blood glucose in the past 2 to 3 months before the diagnosis which thus will reflect the long term blood glucose accurately. Therefore, regarding HbA1c as an indicator of diabetic diagnosis presents better reproducibility. Some people put forward the idea that HbA1c can be seen as the gold standard of blood sugar monitoring. HbA1c increases the degree of hypoxia, and diabetic nephropathy, retinopathy, microangiopathy all relate to the modification of other proteins (such as glycosylation and lipid peroxidation). So HbA1c can be regarded as the evaluation method of dangerous elements other than the use of diabetic diagnosis. Type 1 diabetes mellitus control and DCCT of America or Type 2 diabetes mellitus control and UKPDS all regard HbA1c as an important indicator of diabetes mellitus. While HbA1c also has some limitations itself as to its testing on the average level of blood glucose within 2 to 3 months, diabetes less than 3 months will cause some problem in the test. In addition, the level of hemoglobin will affect the testing of HbA1c for which the illness (such as aciderosis, kidney diseases and hemoglobinopathy) resulting in the increasing or decreasing of hemoglobin will not be suitable with this testing method.

In addition, the threshold of HbA1c is also affected by many extrinsic elements, such as age, gender, areas, climate, and race etc. The areas and climate elements in our investigation and Shanghai's community investigation [18] indicated that blood glucose in the fasting state equaled approximately and the level of post-load plasma glucose and the average level of HbA1c for investigation of people in the Harbin area were obviously

higher than in Shanghai, which may be related to the high latitude and long, cold, and dry winter of Harbin. Studies have demonstrated that there was a seasonal variation in non-fasting serum C-peptide [20], so we can infer that the season can also affect insulin secretion and glucose regulation. A study from Japan directly through large-scale census calculated the monthly average of HbA1c values through the year. The results proved that HbA1c values were the highest in March and the lowest in October with a difference of 0.30%. There were also significant seasonal changes in diabetic patients, which were the highest in the spring and the lowest in the autumn. These effects may be caused by cold climate, decreased physical activity, and food intake and body weight gain in the winter [21]. We therefore concluded that Northeast China in determining the critical point of diabetes diagnosis with HbA1c may be a higher value than that of a southern city is consistent with the study findings.

According to the nation-wide American health and nutritional survey from 1999 to 2004, a threshold of HbAlc greater than or equal to 6.5% is defined as the best threshold for diabetes mellitus of Americans [18]. In Canada, a multi-ethnic country, the sensitivity is 73% and the specificity 98% under the best threshold of HbAlc of 7.1% [22]. A study on the diabetic screening method of Chinese people's fasting blood-glucose and HbA1c shows that the sensitivity is 80% and the specificity 89.8% under the best threshold of HbAlc of 6.0% [23]. Such results can also be found in Japanese people, the threshold of HbAlc is 6.1% (the sensitivity of 56.9% and the specificity 95.3%) which is considered as the suitable way to test diabetes mellitus and predict the likelihood of blood vessel complication [24].

Our research has certain limitations. The subjects of this study were diabetics in the high-risk groups, rather than the general population. So, there may be a deviation in HbA1c cutoff point selection, which then leads to the occurrence of misdiagnosis. But the subjects of this study were from the Endocrinology Department at high risk of diabetes. Therefore, we thought that 6.5% of HbA1c was more conducive to out-patient screening. Besides, this test was usually performed in hospital, where the experiment method was more advanced, therefore, more feasible and necessary. But as for the inadequacy of using HbA1c as the method to screen and diagnose diabetes, maybe we can consider the method combined the test of blood glucose and the test of HbA1c as recommended by British Ministry of Health to raise the diagnostic efficiency [25]. As for the lack of patients' height, weight and other physical data, for the group this may impact individual factors. In addition, our study was limited to the northern Chinese population, and not on behalf of China's overall HbA1c levels. Because the data we used was from the outpatients and inpatients of the Second Affiliated Hospital of Harbin Medical University on the study of people at high risk of diabetes, the investigation presented far higher probability of diabetes than that of general population. So the result of our study was more applicable to the diabetic diagnosis of people at high risk of diabetes among whom we thought the threshold of HbA1c for diabetic diagnosis was 6.5% [26], and the result of community research (6.0% [23], 6.1% [25,27,28], 6.2% [29], 6.3% [18]) were more applied to the screening of diabetes among general population. So, these two results had certain differences. Therefore, we could assume two thresholds for diabetic diagnosis, one as the threshold for screening people at high risk of diabetes, the other as the threshold for the diagnosis, which could also be called exclusion criterion and diagnosis criterion, respectively [30]. Or OGTT can be taken when HbA1c ranges from 6.0% to 6.5%, as recommended by British health departments [29], which will save many processes of diagnosis and treatment. In Figure 2, we also took more a detailed and objective evaluation for HbA1c 6.5% as the diagnosis of diabetes [31]. The result showed it was as diagnostic criterion of diabetes diagnosis effect and also could be seen the diagnosis range of different types of diabetes and abnormal glucose tolerance. At the same time, it suggested that it may not include groups of blood glucose abnormalities and proportions. The application for us to more fully and wisely use HbA1c and blood glucose to diagnose diabetes may have deep significance.

It is noted that, in this research we applied the method of half-hour and 1-hour plasma glucose for diabetic diagnosis, and attempted to demonstrate that the threshold of half-hour and 1-hour plasma glucose for diabetic diagnosis were 10.6 mmol/L and 13.6 mmol/L with statistical results of the research data. In OGTT, we tested all venous blood samples collected at 0, 30, 60, and 120 minutes for determination of plasma glucose; however, none coinsided with the application of half-hour and 1-hour plasma glucose for diabetic diagnosis. As early as 2001, one study indicated that a 1-hour meal tolerance test could be used to determine the discontinuation of insulin therapy in insulin-treated patients with type 2 diabetes mellitus. The survey gave a reference index of 10.0 mmol/L [32]. The diagnostic criterion was for diabetes during pregnancy and gave the corresponding 1-hour plasma glucose (10.5 mmol/L) [33]. But no special type of diagnostic criteria have been mentioned in this. Some studies also proposed that 1-hour post-load plasma glucose is associated with the left ventricular diastolic dysfunction. Subjects with NGT $\geq$ 155 mg/dL (about 8.61 mmol/L) had significantly worse diastolic function [34]. Therefore, we believe that using the investigation of people, and data statistical analysis to determine the half-hour and 1-hour plasma glucose for diabetic diagnosis will make use of OGTT effectively and improve it reasonably, increase the assessment of the risk of diabetes complications, as well as the reliance and power of diabetic diagnosis.

In addition, 1-hour plasma glucose showed better compliance. We therefore focused on the recommendation to determine the cutoff point of 1-hour plasma glucose in OGTT for the diagnosis of diabetes in our study. As

mentioned in the results we have made it clear, that an area under the ROC greater than 0.9 had excellent diagnostic significance. The specificity for diagnosis of diabetes in decreasing order were 2-hour plasma glucose, 1-hour plasma glucose, FPG, HbA1c, and 1/2-hour plasma glucose. Here we could see, the diagnostic effectiveness of 1-hour plasma glucose ranked only second to 2-hour plasma glucose; moreover, it was higher than FPG. So, we had reason to believe that setting a boundary value point of 1-hour plasma glucose as the diagnostic standard of diabetes was very necessary. We selected half-hour and 1-hour plasma glucose as the criteria which is perhaps excessive because of selected IGT and thus requires a large sample of re-authentication.

Our results also suggest that postprandial glycemic excursions play a major role in the metabolic disequilibrium of patients suffering from mild or moderately high HbA1c. The result is consistent with previous research results [19]. On the contrary, fasting hyperglycemia appears to be a main contributor to the overall diurnal hyperglycemia in HbA1c of poorly controlled diabetic patients, whereas the role of postprandial glucose elevations decreases as patients' progress toward high HbA1c. Because this relationship was confirmed in the 2,857 patients' blood glucose monitoring, and the result was given by our four-point sampling model, which integrated markers during the four main periods of daytime (fasting, 1/2-hPG, 1-hPG, and 2-hPG). These results indicate that there exists a progressive shift in the respective contributions of fasting and postprandial hyperglycemia when patients progress from moderate to high HbA1c. The contribution of postprandial glucose becomes predominant in patients with mild or moderate HbA1c diabetes, whereas the contribution of fasting hyperglycemia increases with high HbA1c. Such observations seem to reconcile the different results that were observed in the literature because the shift in the respective contributions of fasting and postprandial hyperglycemia appears as a continuous spectrum of HbA1c from fairly to poorly controlled patients with type 2 diabetes. Then we put forward different solutions to control blood glucose for people with the different blood glucose levels according to the above trends. HbA1c as a regular monitor indicator for long-term control of blood glucose, of course, is necessary to guide the adjustment of the hypoglycemic scheme. In addition, we note that one-hour postprandial glucose showed a higher relative contribution compared to the fasting blood glucose when HbA1c < 6.6%. Therefore, we believe that in mild or moderate hyperglycemia the one hour postprandial glucose control should be noted. Then when HbA1c exceeds 7.2%, the contribution of fasting glucose was significantly higher than that of 1-hour postprandial glucose. At this point the focus should be transferred to the control of fasting blood glucose. When HbA1c > 9.3%, the relative contribution of fasting glucose even went beyond the contribution of 2-hours postprandial glucose, and became the main role to affect blood glucose levels over a period of time. The main strength of hypoglycemia should be placed on reducing fasting glucose. This study for refining the blood glucose control and formulating hypoglycemic strategy has a more definite guiding significance.

Here the four-point blood glucose profile model we used still has some flaws, this is because the four points we selected were fasting, 1/2-hPG, 1-hPG, and 2-hPG. Since the presence of insulin secretion is delayed and the peak is reduced in diabetic patients, the lack of 3-hour plasma glucose (3-hPG) meant post absorptive glucose, which may cause the missing area of a trapezoid in the area of computing the four-point model. Thus, we estimate that the relative contribution of fasting, 1/2-hPG, 1-hPG, and 2-hPG to HbA1c may be not estimated accurately. But the overall trend is still to determine it and the clinical significance has not changed. This may prompt us that we can focus on a 3-hPG testing in future related research, in order to further estimate its impact on the relative contribution of HbA1c. Thus we can give a more comprehensive guide in clinical glycemic control treatment. Moreover, using the trapezoidal integration method may tend to underestimate more than other methods.

CONCLUSION

This study found that an HbA1c threshold of 6.5% had higher sensitivity for detecting undiagnosed diabetes in China's northeast adults and a little lower specificity to that of the FPG threshold of 7.0 mmol/L with the highest Youden index of 64.8% among the HbA1c thresholds in Chinese adults, with a sensitivity similar to that of using a FPG threshold of 7.0 mmol/L. These findings suggest that the optimal HbA1c threshold of 6.5% as a screening criterion for diabetes and high-risk groups may be acceptable when FPG and OGTT are not available. This paper is suggesting that the diagnostic criteria of 1/2-hour plasma glucose and 1-hour plasma glucose, which were 10.6 mmol/L and 13.6 mmol/L, respectively, improved the application of the glucose tolerance test. The relative contribution of fasting hyperglycemia increased gradually with increasing levels of HbA1c, whereas the contribution of postprandial glucose decreased progressively.

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The authors declare that they have no conflict of interest.

References:

1. Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. Diabet Med 1998;15(7):539-53 (PMID: 9686693).

2. Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Report of the expert committee on the diagnosis and classification of diabetes mellitus. Diabetes Care 2003;26 Suppl 1:s5-20 (PMID: 12502614).

3. Saudek CD, Herman WH, Sacks DB, Bergenstal RM, Edelman D, Davidson MB. A new look at screening and diagnosing diabetes mellitus. J Clin Endocrinol Metab 2008;93(7):2447-53 (PMID: 18460560).

4. Bennett CM, Guo M, Dharmage SC. HbA(1c) as a screening tool for detection of Type 2 diabetes: a systematic review. Diabet Med 2007;24(4):333-43 (PMID: 17367307).

5. Gillett MJ. International Expert Committee report on the role of the A1c assay in the diagnosis of diabetes: Diabetes Care 2009; 32(7):1327-1334. Clin Biochem Rev. 2009 Nov;30(4):197-200 (PMID: 20011212).

6. Selvin E, Crainiceanu CM, Brancati FL, Coresh J. Short-term variability in measures of glycemia and implications for the classification of diabetes. Arch Intern Med 2007;167(14):1545-51 (PMID: 17646610).

7. American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care 2006;29 Suppl s43-s48 (PMID: 16373932).

8. American Diabetes Association. Diagnosis and classification of diabetes mellitus Diabetes Care 2011;34 Suppl s62-s69 (PMID: 21193628).

9. Diabetes Control and Complications Trial (DCCT): results of feasibility study. The DCCT Research Group. Diabetes Care 1987; 10(1):1-19 (PMID: 2882967).

10. Karsegard J, Wicky J, Mensi N, Caulfield A, Philippe J. Spurious glycohemoglobin values associated with hydroxyurea treatment. Diabetes Care 1997;20(7):1211-2 (PMID: 9203471).

11. Jian WX, Luo TH, Gu YY, et al. The visfatin gene is associated with glucose and lipid metabolism in a Chinese population. Diabet Med 2006;23(9):967-73 (PMID: 16922702).

12. Huisman TH, Henson JB, Wilson JB. A new high-performance liquid chromatographic procedure to quantitate hemoglobin A1c and other minor hemoglobins in blood of normal, diabetic, and alcoholic individuals. J Lab Clin Med 1983;102(2):163-73 (PMID: 6190972).

13. Genuth S, Alberti KG, Bennett P, et al.; The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Follow-up report on the diagnosis of diabetes mellitus; Diabetes Care 2003;26(11):3160-7 (PMID: 14578255).

14. Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Diabetes Care 1997;20:1183-97 (PMID: 9203460).

15. Hanley JA, McNeil BJ. The meaning and use of the area under a receiver operating characteristic (ROC) curve. Radiology 1982; 143(1):29-36 (PMID:7063747).

16. Hilden J, Glasziou P. Regret graphs, diagnostic uncertainty and Youden's Index. Stat Med 1996;15(10):969-86 (PMID: 87834 36).

17. DeLong ER, DeLong DM, Clarke-Pearson DL. Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. Biometrics 1988;44(3):837-45 (PMID: 3203132).

18. Bao Y, Ma X, Li H, et al. Glycated haemoglobin A1c for diagnosing diabetes in Chinese population: cross sectional epidemiological survey. BMJ 2010;340:c2249 (PMID: 20478961).

19. Monnier L, Lapinski H, Colette C. Contributions of fasting and postprandial plasma glucose increments to the overall diurnal hyperglycemia of type 2 diabetic patients: variations with increasing levels of HbA(1c). Diabetes Care 2003;26(3):881-5 (PMID: 1261 0053).

20. Samuelsson U, Lindblad B, Carlsson A. Residual beta cell function at diagnosis of type 1 diabetes in children and adolescents varies with gender and season. Diabetes Metab Res Rev. 2013 Jan;29(1):85-9 (PMID: 23081842).

21. Furuta M, Tomisaka R, Yamana A, et al. [Evaluation of seasonal changes in hemoglobin A1c in diabetic patients] Rinsho Byori 2012 Jul; 60(7):599-604 (PMID: 22973717).

22. Rowley KG, Daniel M, O'Dea K. Screening for diabetes in Indigenous populations using glycated haemoglobin: sensitivity, specificity, post-test likelihood and risk of disease. Diabet Med 2005; 22(7):833-9 (PMID:15975095).

23. Zhou XH, Ji LN, Luo YY, Zhang XY, Han XY, Qiao Q. Performance of HbA(1c) for detecting newly diagnosed diabetes and pre-diabetes in Chinese communities living in Beijing. Diabet Med 2009 Dec;26(12):1262-8 (PMID: 20002479).

24. Takahashi Y, Noda M, Tsugane S, Kuzuya T, Ito C, Kadowaki T. Prevalence of diabetes estimated by plasma glucose criteria combined with standardized measurement of HbA1c among health checkup participants on Miyako Island, Japan. Diabetes Care. 2000;23(8):1092-6 (PMID: 10937503).

25. Cavagnolli G, Comerlato J, Comerlato C, Renz PB, Gross JL, Camargo JL. HbA(1c) measurement for the diagnosis of diabetes: is it enough? Diabet Med 2011;28(1):31-5 (PMID: 21210540).

26. Dong XL, Liu Y, Sun Y, et al. Comparison of HbA1c and OGTT criteria to diagnose diabetes among Chinese. Exp Clin Endocrinol Diabetes 2011;119(6):366-9 (PMID: 21104578).

27. Rohlfing CL, Little RR, Wiedmeyer HM, et al. Use of GHb (HbA1c) in screening for undiagnosed diabetes in the U.S. population. Diabetes Care 2000;23(2):187-91 (PMID: 10868829).

28. Herdzik E, Safranow K, Ciechanowski K. Diagnostic value of fasting capillary glucose, fructosamine and glycosylated hemoglobin in detecting diabetes and other glucose tolerance abnormalities compared to oral glucose tolerance test. Acta Diabetol 2002; 39(1):15-22 (PMID:12043934).

29. Jesudason DR, Dunstan K, Leong D, Wittert GA. Macrovascular risk and diagnostic criteria for type 2 diabetes: implications for the use of FPG and HbA(1c) for cost-effective screening. Diabetes Care 2003;26(2):485-90 (PMID: 12547886).

30. Mostafa SA, Khunti K, Kilpatrick ES, et al. Diagnostic performance of using one- or two-HbA1c cut-point strategies to detect undiagnosed type 2 diabetes and impaired glucose regulation within a multi-ethnic population. Diab Vasc Dis Res 2013;10(1): 84-92 (PMID: 22773521).

31. Mohan V, Vijayachandrika V, Gokulakrishnan K, et al. A1C cut points to define various glucose intolerance groups in Asian Indians. Diabetes Care 2010;33(3):515-9 (PMID: 19903752).

32. Kumar D, Luna-Lollie T, Madha S. One-hour meal tolerance test to assess withdrawal of insulin therapy in overweight patients with type 2 diabetes. Endocr Pract 2001;7(4):256-61 (PMID: 114 97476).

33. Emancipator K. Laboratory diagnosis and monitoring of diabetes mellitus. Am J Clin Pathol 1999 Nov;112(5):665-74 (PMID: 105 49254).

34. Sciacqua A1, Miceli S, Greco L, et al. One-hour postload plasma glucose levels and diastolic function in hypertensive patients. Diabetes Care 2011;34(10):2291-6 (PMID: 21911775).

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