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Area under the curve during OGTT in first-degree relatives of diabetic patients as an efficient indicator of future risk of type 2 diabetes and prediabetes

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Summary

Objective: To establish whether the area under the curve of an OGTT has a predictive role in identifying prediabetic and diabetic subjects among first-degree relatives (FDR) of patients with diabetes mellitus type 2 (DM).

Design, patients and measurements: In a population-based cohort study, 766 FDR of diabetic patients with a normal glucose tolerance test (NGT) completed a 2-hour OGTT. They were followed up for 7 years and classified according to the American Diabetes Association criteria into: NGT, impaired fasting glucose (IFG), impaired glucose tolerance (IGT) and DM. Relative risk (RR) and 95% confidence intervals (95%CI) were calculated based on logistic regression. Receiver operator characteristic (ROC) analysis along with AUC at different intervals and at time points during the OGTT was used to evaluate the risk of prediabetes and diabetes.

Results: Twenty-three subjects (3%) developed type 2 DM, 118 (29.3%) IFG, 81 (11.5%) IGT and 544 (71%) subjects remained NGT. AUC and mean difference of glucose in all high-risk groups demonstrated significant differences in both intervals and time points when compared to the NGT group. The cut-off values during OGTT to predict prediabetes and diabetes was determined as blood glucose more than 7.2 and 7.8 mmol/L at 30 and 60 minutes, respectively. The time point 60 has the highest predictive role for the development of diabetes, alone, and improved the performance of a prediction model containing multiple important clinical risk factors.

Conclusion: The data suggest that the glycaemic response to an OGTT may predict the risk of development of diabetes in first-degree relatives of DM patients.

KEYWORDS

diabetes mellitus, first-degree relatives, oral glucose tolerance test, pre-diabetes

1 | INTRODUCTION

Type 2 diabetes mellitus (T2D) is a common chronic disease with major morbidities and social aspects.[1] A combination of genetic and environmental factors has an important role in the progression of the disease.[2] Family members of people with T2D are at higher risk of developing diabetes.[3-5] Having one-first-degree relative (FDR) who is suffering from diabetes increases the risk of becoming diabetic to 40%.[6] Identifying the "at-risk" population for prediabetes and DM, such as the offspring of patients with T2D, is important for medical and research purposes, so that appropriate prevention strategies could be employed. In genetically predisposed individuals, insulin sensitivity declines many years before the clinical onset of the disease and could be used to predict the progression to T2D.[7]

In 1997, the Expert Committee on the Diagnosis and Classification of DM[8] introduced impaired fasting glucose (IFG) and impaired glucose tolerance (IGT) as intermediate stages in the natural history of type 2 diabetes: the so-called IFG/IGT phase. During this asymptomatic period, abnormalities in carbohydrate metabolism could be discovered by measuring plasma glucose in the fasting state or after a challenge with an oral glucose load.[9] The oral glucose tolerance test (OGTT) has traditionally been used to classify participants to either "T2D" or to "those at risk of developing diabetes".[9] Recent studies have suggested that the shape of the glucose curve during an OGTT could identify metabolic dysregulation and predict the risk of future T2D.[10-13] In FDR of people with diabetes, body mass index (BMI)[14,15] and glucose during an OGTT[15] were significantly greater than in controls. Both plasma insulin and proinsulin levels during an OGTT[16] and insulin resistance[17] were higher in FDR of patients with T2D compared to participants without a family history of diabetes.[18]

Due to the increased risk of cardiovascular problems[2] and risk of diabetes progression over the following 5-10 years from the initial stage of IFG/IGT,[19-21] it is important to establish appropriate prevention strategies.

The prevalences of T2D, IGT and IFG were 10.3%, 19.5% and 17.3%, respectively, in a FDR Iranian population and were significantly higher than those reported for a control population of the same age.[2] In addition, the incidence of type 2 diabetes in an Iranian FDR population was 3.4 per 100 person/year in men and 4.9 in women.[22] FDR of T2D in Iran are at higher risk of IGT and T2D than other populations in the world.[2] For the first time, this study has investigated the predictive value of the area under the curve (AUC) of an OGTT and determined the best cut-off values at different times during the OGTT to predict various degrees of glucose intolerance. This will, theoretically, assist in identifying more individuals with IFG/IGT and T2D in the FDR of T2D before they develop the WHO and ADA criteria.

2 | MATERIALS AND METHODS

2.1 | Study design and population

The study was conducted in the out-patients' clinic of Isfahan Endocrine and Metabolism Research Centre. The clinic is affiliated to the Isfahan University of Medical Sciences.

While conducting the Isfahan diabetes prevention program, between 2007 and 2013, in a total population of 3420, 1544 siblings and children of patients with T2D were followed and attended this clinic.[14] Of this total, 1876 were lost to follow up due to moving geographically, withdrawing consent or changing contact details and being unavailable. The Ethics Committee of Isfahan University of Medical Sciences approved the study and informed consent was obtained from every participant according to the Declaration of Helsinki.

Of the 1544 study participants at baseline, 766 (49.6%) had a normal glucose tolerance (NGT) test, 304 (19.7%) had IFG, 467 (30%) had IGT and seven (0.5%) already met the diagnostic criteria for type 2 DM. For the purposes of the study, analysis was limited to NGT participants at baseline and glucose tolerance status was determined with an OGTT. We followed NGT participants after 7 years and then classified them according to the American Diabetes Association criteria.[19]

Participants completed a demographic questionnaire including age, gender, family and personal and medical history at baseline. Anthropometric and clinical measurements, including BMI (by dividing weight [kg] to the square of height [m^2]), waist circumference and blood pressure (both systolic and diastolic) were recorded. Data collection was conducted at baseline and at follow-up, 7 years later, according to the standards of Medical Care in Diabetes.[23]

2.2 | Laboratory parameters

All participants underwent a 75-g OGTT following a 12-hour overnight fasting period. Plasma glucose was measured at 0, 30, 60 and 120 minutes. Fasting plasma glucose (FPG) (mmol/L) was measured by photometric method (Pars Azmon kit Lot number: 94011). Other blood parameters including HbA1c, cholesterol (LDL, HDL) and triglyceride were also measured. Pairwise comparison between the area under the glucose curve of four groups (NGT, IGT, IFG and DM) was performed at three intervals (0-30, 0-60, 0-120) during the OGTT as well as at 0, 30, 60 and 120 minutes.

Participants with FPG ≥11.1 mmol/L were considered diabetic. If FPG was ≥7 and <11.1 mmol/L, a second FPG was measured on another day. If the second FPG was also ≥7 mmol/L, participants were classified as diabetic. FPG ≥7 mmol/L or 2-hour PG ≥11.1 mmol/L also defined diabetes mellitus. IGT was interpreted as FPG <7 mmol/L, but with 2-hour PG concentration ≥7.8 and <11.1 mmol/L). If FPG was in the range of 5.5-7 mmol/L and 2-hour PG was <7.8 mmol/L, it was considered as IFG. If the FPG was below 5.5 mmol/L and 2-hour PG was less than 7.8 mmol/L, it was regarded as NGT.[2] In addition, all participants developing IFG, IGT, and T2D were pooled in a unique "incident dysglycaemia" (DG) group in the main analyses.

2.3 | Statistical analysis

Quantitative variables are presented as mean ± SD or median (IQR), while qualitative ones are shown as frequency (percentage). Depending on the normal or not normal distribution of data, one-way analysis of variance (ANOVA) or Kruskal-Wallis tests were used. Categorical data were compared by chi-square test. For OGTT analysis, the AUC was calculated using the trapezoidal method. The mean values of AUC as well as plasma glucose at different intervals and time points during the OGTT were compared between each two studied groups using independent sample *t*-test. Adjustment was made for multiple testing by Bonferroni's approach based on the number of conducted tests, and a *P* value <.01 was used as a significant level.

The diagnostic accuracy was expressed as AUC and 95% confidence interval (CI). Optimal sensitivity and specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy were calculated using Youden index, based on derived cut-off values at different time points of OGTT. We used binary logistic regression analysis for evaluating the predictive value of AUC for dysglycaemia status of participants in different models. In these analyses, after

TABLE 1 Characteristics of participants at baseline and at the end of follow-up

	NGT 544 (71%)		IFG 118 (29.3%)		IGT 81 (11.5%)		DM 23 (3%)		DG 222 (29%)		
Variables	**2007**	**2013**	**2007**	**2013**	**2007**	**2013**	**2007**	**2013**	**2007**	**2013**	***P* values**
Age (years)	41.9 ± 6.4	53.1 ± 6.33	42.3 ± 6.4	54.05 ± 6.5	44.1 ± 5.9	55.5 ± 6.1	42.2 ± 5.6	54.13 ± 5.32	42.9 ± 6.2	54.6 ± 6.24	0.033
Sex (Male/Female)	136/407		35/83		18/63		5/18		58/164		0.623
BMI (kg/m^2)	28.1 ± 4.1	28.5 ± 4.14*	29 ± 4.2	29 ± 4.24*	28.1 ± 3.7	28.9 ± 3.45*	31.6 ± 4	33.1 ± 4.8*	28.9 ± 4.1	29.7 ± 4.24*	<0.0001
Waist circumference (cm)	86.9 ± 9.3	89.13 ± 10.1*	89.2 ± 9.8	89.3 ± 9.85*	88.2 ± 7.9	92.5 ± 8.9*	93.9 ± 8.6	97.6 ± 9.5*	89.3 ± 9.2	92.9 ± 9.74*	0.001
Systolic blood pressure (cm Hg)	11 ± 1.5	11.5 ± 1.6*	11.7 ± 1.5	11.8 ± 1.65*	12 ± 1.8	12.2 ± 1,75	11.8 ± 2	12.4 ± 2.14	11.8 ± 1.6	12.01 ± 1.75*	<0.0001
Diastolic blood pressure (cm Hg)	7.2 ± 1.13	7.73 ± 1.1*	7.5 ± 1.2	7.9 ± 1.14*	7.64 ± 1.17	7.9 ± 1.25.3*	7.7 ± 1.3	8.35 ± 1.72	7.6 ± 1.2	8 ± 1.22*	0.008
HbA1c	4.9 ± 0.7	5.15 ± 0.64*	5.1 ± 0.7	5.4 ± 0.62	5.1 ± 0.7	5.3 ± 0.49	5.2 ± 0.98	5.8 ± 0.9	5.1 ± 0.73	5.4 ± 0.62*	<0.0001
Total cholesterol (mmol/L)	4.9 ± 0.99	5.1 ± 1.00*	4.8 ± 0.87	5.2 ± 1.05*	5.0 ± 0.89	5.1 ± 0.87	5.6 ± 1.52	5.91 ± 1.24	4.9 ± 0.99	5.2 ± 1.03*	0.005
LDL Cholesterol (mmol/L)	3.0 ± 0.88	3.14 ± 0.80*	2.9 ± 0.78	3.2 ± 0.92*	2.9 ± 0.84	3.2 ± 0.76*	3.6 ± 1.6	3.6 ± 1.1	2.94 ± 0.94	3.25 ± 0.89*	0.01
HDL cholesterol (mmol/L)	1.1 ± 0.29	1.2 ± 0.28	1.1 ± 0.25	1.15 ± 0.28*	1.1 ± 0.25	1.1 ± 0.23	1.1 ± 0.27	1.2 ± 0.30	1.12 ± 0.25	1.15 ± 0.26	0.26
Triglyceride (mmol/L)	1.7 ± 0.85	1.6 ± 0.91*	1.8 ± 1.04	1.7 ± 0.92*	2.1 ± 1.17	1.83 ± 1.1*	2.3 ± 1.13	2.2 ± 1.4	1.94 ± 1.1	1.8 ± 1.05*	<0.0001

**P* value <.05 is considered statistically significant based on paired *t*-test.

Values are presented as mean ± SD.

NGT, normal glucose tolerance; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; DM, diabetes; DG, dysglycaemia group.

TABLE 2 Pairwise comparison of mean area under glucose curve in oral glucose tolerance test between study groups in three intervals (0-30, 0-60, 0-120 minutes)

	0-30 min		0-60 min		0-120 min	
Group	Mean ± SE[a]	AUC (95%CI)[b]	Mean ± SE[a]	AUC (95%CI)[b]	Mean ± SE[a]	AUC (95%CI)[b]
NGT	179.7 ± 1.09	0.679 (0.55-0.8)*	389.3 ± 2.87	0.716 (0.59-0.83)*	757.7 ± 5.68	0.721 (0.59-0.84)*
DM	195.4 ± 5.91*		444.5 ± 17.61*		874.6 ± 36.48*	
NGT	179.7 ± 1.09	0.59 (0.52-0.65)*	381.3 ± 2.87	0.644 (0.57-0.71)*	757.7 ± 5.68	0.7 (0.64-0.76)*
IGT	185.2 ± 2.43*		417.1 ± 6.54*		844 ± 12.49*	
NGT	179.7 ± 1.09	0.662 (0.6-0.71)*	389.3 ± 2.87	0.681 (0.62-0.73)*	757.7 ± 5.68	0.666 (0.61-0.72)*
IFG	193.29 ± 2.20*		428 ± 5.65*		830.9 ± 11.29*	
IGT	185.29 ± 2.43	0.622 (0.47-0.77)	417.17 ± 6.54	0.614 (0.46-0.76)	844.03 ± 12.49	0.561 (0.4-0.71)
DM	195.4 ± 5.9		444.5 ± 17.61		874.6 ± 36.48	
IFG	193.29 ± 220	0.534 (0.39-0.67)	428 ± 5.65	0.57 (0.42-0.71)	830.9 ± 11.29	0.576 (0.43-0.71)
DM	195.4 ± 5.91		444.5 ± 17.61		874.6 ± 36.48	
IGT	185.2 ± 2.43*	0.40 (0.32-0.49)	417.1 ± 6.54	0.459 (0.37-0.54)	844 ± 12.49	0.538 (0.45-0.62)
IFG	193.29 ± 2.20		428 ± 5.65		830.9 ± 11.29	
NGT	179.7 ± 1.09	0.64 (0.59-0.68)*	389.3 ± 2.87	0.67 (0.63-0.71)*	757.7 ± 5.68	0.68 (0.64-0.73)*
DG	190 ± 23.21		425.8 ± 61.4		840.1 ± 121.2	

AUC (95% CI), Area under the curve (95% confidence interval); IFG, impaired fasting glucose; IGT, impaired glucose tolerance; DM, diabetes; DG, dysglycaemia group;

**P* value <.01 resulted from Bonferroni's approach for covering multiple testing based on number of conducted tests.

[a]The reported values as AUC were calculated using the trapezoidal method and compared between groups using independent samples *t*-test.

[b]The AUC was calculated using ROC analysis based on obtained values from trapezoidal method.

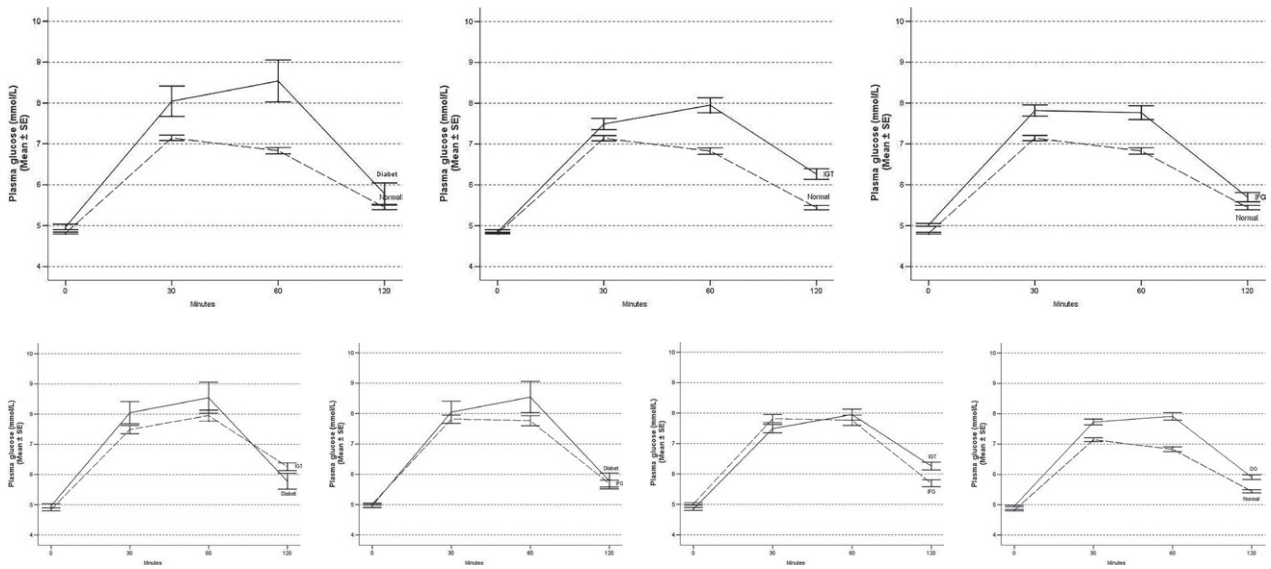

FIGURE 1 Area under glucose curve in oral glucose tolerance test based on trapezoidal method for diabetes (DM), impaired glucose tolerance (IGT), impaired fasting glucose (IFG), normal glucose tolerance (NGT) groups and dysglycaemia group (DG) at three intervals (0-30, 0-60, 0-120 minutes)

obtaining relative risk (RR) and 95% confidence interval (95%CI) in the crude model, adjustment was made for age and gender in the first model. Additional adjustment was made for BMI in the second model. Finally, adjustment was made for all mentioned variables, blood pressure and lipid profile in the third model. All statistical calculations were carried out with the SPSS15 for Windows (SPSS Inc., Chicago, IL, USA). Net reclassification improvement (NRI) analyses were conducted for evaluating the improvement due to each OGTT time point value when it was added to basic predictive logistic regression model for predicting the dysglycaemia status of participants. "HMISC package" in R free statistical software (Version R-3.4.0) was used for NRI analyses.

3 | RESULTS

Table 1 presents the anthropometric, laboratory and clinical characteristics of the study population. Over the 7 years' follow-up, from the 766 NGT participants at base line, 23 (3%) developed DM, 118 (29.3%) progressed to IFG and 81 (11.5%) developed IGT. Overall, 222 (29%) developed DG and 544 (71%) remained NGT.

During 4596 person-years of follow-up, the overall incidence of subsequent diabetes was 5 (95% CI: 3-7) per 1000 person-years. The incidences of IFG, IGT and DG were 28 (95% CI: 23-33), 19 (95% CI:15-23) and 183 (95% CI: 168-198) per 1000 person-years, respectively.

A significant difference was observed between the studied groups in almost all evaluated characteristics at the start of cohort study (Table 1). Laboratory evaluations indicated that participants who developed diabetes in 2013 exhibited significantly higher HbA1c, BMI and cholesterol and triglyceride than the other participants in the baseline assessment (Table 1). In addition, when comparing anthropometric and laboratory parameters between 2007 and 2013, a significant difference was observed in the majority of variables, particularly in IFG, DG and NGT groups, while the observed changes in the DM group were only notable for anthropometric measures (Table 1).

Table 2 and Figure 1 present the results of comparison of area under the curve (AUC) for plasma glucose between study groups. The results showed that mean AUC in all high-risk groups, including DM, IGT, IFG and DG, have significant differences in terms of plasma glucose when compared to NGT, at all three intervals, that is 0-30mins, 0-60 minutes and 0-120 minutes (*P* value <.05). As shown in Figure 1 and indicated in Table 2, in NGT participants, the glucose level at 60 minutes was lower than at 30 minutes, but in IGT and participants with diabetes, it was the opposite and in the IFG group a consistent level of glucose was observed between 30 to 60 minutes. The results of ROC analysis on the calculated values of AUC by trapezoidal method indicated their significant predictive role for differentiating DM, IFG, IGT and DG from NGT (AUC [95%CI]) (Table 2). In logistic regression analysis, after adjustment for confounding variables, the predictive value of AUC values (RR [95% CI]) for DM, IFG, IGT and DG remained statistically significant (Table 3). In addition, we evaluated the predictive values of OGTT at all time points for dysglycaemia by entering them simultaneously in a logistic regression model after adjustment for covariates in different models. Significant predictive roles were detected for time points 0, 60 and 120 (RR > 1; *P* < .05, Table 4).

In all high-risk groups, when the mean glucose level and specific AUC of glucose at 0, 30, 60, 120 minutes were compared with the NGT group, significant differences were observed (*P* value <.05), except at 0 minute between IGT and NGT (Table 5). This difference was especially noticeable for plasma glucose at 30 and 60 minutes in all high-risk groups when compared to NGT. The difference for plasma glucose between IGT and IFG was only statistically significant at 0 and 120 minutes (Table 5).

Participants with IFG have an initial excessive increase in plasma glucose concentration (5.03 ± 0.35 mmol/L) that peaks at 1 hour compared to participants with IGT (7.83 ± 1.77 mmol/L), but the plasma glucose concentration returns to normal or near normal values after 2 hour (6.23 ± 1.11 mmol/L). Participants with IGT have a more gradual initial increase in plasma glucose concentration (4.86 ± 0.42 mmol/L) compared with IFG participants. However, their plasma glucose concentration continues to rise after 60 minutes (7.99 ± 1.55 mmol/L) and at 2 hours remains markedly higher compared to IFG participants (6.23 ± 1.12 mmol/L) (Table 5).

The best cut-off values at each of the four time points during the OGTT for differentiating DM, IGT, IFG, DG groups and combined IFG/IGT from NGT participants were chosen according to optimal test characteristics, sensitivity and specificity and have been illustrated in Table 6. According to ROC analysis, a blood glucose level more than 7.2 and 7.8 mmol/L during OGTT in FDR of participants with T2D at 30 and 60 minutes could predict IFG/IGT and diabetes, respectively. A basic predictive model based on logistic regression, included age, sex, BMI, blood pressure and lipid profile as predictors of participants' status in terms of glucose tolerance, was fitted, and net reclassification improvement for each OGTT time point value was calculated. Net reclassification improvement analyses reflected similar results to those obtained with ROC curve analyses (Table 7). The 60-minute plasma glucose achieved a high total NRI value of 0.48 (0.31-0.65) compared with other OGTT time points.

4 | DISCUSSION

In this study, we demonstrated that the mean and AUC of the plasma glucose response during an OGTT can predict high-risk groups in FDR of individuals with T2D. The AUC and the mean difference in plasma glucose show significant differences in all three time intervals in all high-risk groups compared with NGT. The time point 60 has the highest predictive value for the development of diabetes, alone, and improved the performance of a prediction model containing multiple important clinical risk factors.

It has previously been established by some studies that a positive family history of diabetes is a strong risk factor for T2D in both adults and younger people.[24,25] This is important in the Iranian population where it has been noted that FDR of patients with T2D have an increased prevalence of DM, IGT and IFG,[2] and this group is theoretically at risk of becoming diabetic. Undiagnosed T2D remains a concern, and early diagnosis could prevent development of most of the complications.[26,27]

To our knowledge, there are no "normal" reference values at time points 30 and 60 minutes during the OGTT in FDR of T2D. Previously, Zhou et al[28] have presented the cut-off values for IFG/IGT and diabetes, but this is the first study to examine ROC analysis during OGTT in regard to progressing to T2D or the IFG/IGT risk among the FDR population. We showed that a plasma glucose more than 7.2 and 7.8 mmol/L during an OGTT in FDR of participants with T2D at 30 and 60 minutes could predict IFG/IGT and diabetes, respectively.

The results of this study are consistent with a previous study[12,28,29] in which measurement of glucose during the OGTT showed superiority to fasting plasma glucose in predicting the future risk for T2D.

TABLE 3 Association of AUC (area under the curve) at three intervals (0-30, 0-60, 0-120 minute) with participants' dysglycaemia status (DM, IFG, IGT and DG groups)

	DM			IFG		
	0-30 RR %95(CI)	0-60 RR %95(CI)	0-120 RR %95(CI)	0-30 RR %95(CI)	0-60 RR %95(CI)	0-120 RR %95(CI)
Crude	1.02 (1.01-1.05)*	1.01 (1.01-1.01)*	1.01 (1-1.01)*	1.02 (1.01-1.03)*	1.01 (1.01-1.01)*	1 (1-1.01)*
Model 1	1.02 (1.01-1.04)*	1.01 (1.01-1.02)*	1.01 (1-1.01)*	1.02 (1.01-1.03)*	1.01 (1.01-1.01)*	1 (1-1.01)*
Model 2	1.02 (1.01-1.04)*	1.01 (1-1.02)*	1.01 (1-1.01)*	1.02 (1.01-1.03)*	1.01 (1-1.01)*	1 (1-1.01)*
Model 3	1.02 (1.01-1.05)*	1.01 (1-1.02)*	1.01 (1-1.01)*	1.02 (1.01-1.03)*	1.01 (1-1.01)*	1 (1-1.01)*

DM, diabetes; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; DG, dysglycaemia group.
Model 1: adjusted for age and sex, Model 2: adjusted model 1 plus BMI (body mass index) changes, Model 3: adjusted model 2 plus blood pressure and lipid profile.
*P<0.05 is considered statistically significant

TABLE 4 Association of AUC (area under the curve) at four time points (0, 30, 60, 120 minute) with dysglycaemia status (DM+IGT+IFG)

	0 min RR %95 (CI)	30 min RR %95 (CI)	60 min RR %95 (CI)	120 min RR %95 (CI)
Crude	1.02 (1.001-1.05)*	1 (0.99-1.01)	1.01 (1.007-1.02)*	1.01 (1.001-1.01)*
Model 1	1.02 (1-1.05)*	1.00 (0.99-1.01)	1.01 (1.007-1.02)*	1.00 (1-1.01)*
Model 2	1.02 (1.01-1.05)*	1.00 (.99-1.01)	1.01 (1-1.01)*	1.00 (1-1.01)*
Model 3	1.02 (1.01-1.05)*	1.00 (.99-1.01)	1.01 (1.006-1.02)*	1.00 (0.99-1.01)

Model 1: adjusted for age and sex, Model 2: adjusted model 1 plus BMI (body mass index) changes, Model 3: adjusted model 2 plus blood pressure and lipid profile.
*P<0.05

TABLE 5 Pairwise comparison of mean glucose levels during the oral glucose tolerance test between study groups at four time points (0, 30, 60, 120 minute) and the predictive values of each time point for dysglycaemia status

	0 min		30 min		60 min		120 min	
Group	Mean ± SD[a]	AUC (95%CI)[b]	Mean ± SD[a]	AUC (95%CI)[b]	Mean ± SD[a]	AUC (95%CI)[b]	Mean ± SD[a]	AUC (95%CI)[b]
NGT	4.81 ± 0.44	0.625 (0.52-0.72)*	7.15 ± 1.46	0.67 (0.54-0.79)*	6.8 ± 1.79	0.744 (0.63-0.85)*	5.5 ± 1.18	0.6 (0.48-0.72)*
DM	5 ± 0.31*		8 ± 1.68*		8.6 ± 2.27*		5.8 ± 1.17*	
NGT	4.81 ± 0.44	0.528 (0.46-0.59)	7.15 ± 1.46	0.59 (0.52-0.65)*	6.8 ± 1.79	0.7 (0.64-0.75)*	5.5 ± 1.18	0.685 (0.62-0.74)*
IGT	4.8 ± 0.41		7.5 ± 1.18*		7.9 ± 1.55*		6.2 ± 1.11*	
NGT	4.81 ± 0.44	0.641 (0.58-0.69)*	7.15 ± 1.46	0.647 (0.59-0.7)*	6.8 ± 1.79	0.653 (0.59-0.7)*	5.5 ± 1.18	0.558 (0.5-0.61)*
IFG	5.02 ± 0.35*		7.8 ± 1.44*		7.8 ± 1.44*		5.7 ± 1.15*	
IGT	4.8 ± 0.41	0.593 (0.46-0.72)	7.5 ± 1.18	0.615 (0.46-0.76)	7.9 ± 1.55	0.587 (0.44-0.73)	6.2 ± 1.11	0.4 (0.27-0.52)
DM	5 ± 0.31		8 ± 1.68		8.6 ± 2.27		5.8 ± 1.17	
IFG	5.02 ± 0.35	0.458 (0.33-0.58)	7.8 ± 1.44	0.547 (0.4-0.68)	7.8 ± 1.44	0.6 (0.47-0.73)	5.7 ± 1.15	0.546 (0.41-0.67)
DM	5 ± 0.31		8 ± 1.68		8.6 ± 2.27		5.8 ± 1.17	
IGT	4.8 ± 0.41	0.379 (0.29-0.45)*	7.5 ± 1.18	0.428 (0.34-0.51)	7.9 ± 1.55	0.534 (0.45-.61)	6.2 ± 1.11	0.64 (0.56-0.71)*
IFG	5.02 ± 0.35*		7.8 ± 1.44		7.8 ± 1.44		5.7 ± 1.15*	
NGT	4.81 ± 0.44	0.593 (0.54-0.63)*	7.15 ± 1.46	0.631 (0.58-0.67)*	6.8 ± 1.79	0.673 (0.63-0.71)*	5.5 ± 1.18	0.613 (0.56-0.65)*
DG	4.9 ± 0.38*		7.7 ± 1.39*		7.9 ± 1.76*		5.9 ± 1.16*	

*P value <.01 resulted from Bonferroni's approach for covering multiple testing based on number of conducted tests.
[a]Independent samples t-test.
[b]The AUC was calculated using ROC analysis.
AUC (95% CI), Area under the curve (95% confidence interval); IFG, impaired fasting glucose; IGT, impaired glucose tolerance; DM, diabetes; DG, dysglycaemia group.

OGTT detects changes in postprandial glycaemia rather than changes in fasting glucose and is more sensitive for screening of impaired glycaemic conditions.

Abul-Ghani[12] demonstrated that insulin resistance and beta cell dysfunction start in the NGT stage, and the relationship between plasma glucose concentration during the OGTT and

IGT			DG		
0-30 RR %95(CI)	0-60 RR %95(CI)	0-120 RR %95(CI)	0-30 RR %95(CI)	0-60 RR %95(CI)	0-120 RR %95(CI)
1.01 (0.99-1.01)	1.01 (1-1.01)*	1.01 (1-1.01)*	1.02 (1.01-1.02)*	1 (1-1.01)*	1 (1.004-1.006)*
1.01 (0.99-1.01)	1.01 (1-1.01)*	1.01 (1-1.01)*	1.02 (1.01-1.02)*	1 (1-1.01)*	1 (1.004-1.006)*
1.01 (0.99-1.02)	1.01 (1-1.01)*	1.01 (1-1.01)*	1.02 (1.01-1.02)*	1 (1-1.01)*	1 (1.003-1.006)*
1.01 (0.99-1.01)	1.01 (1-1.01)*	1.01 (1-1.01)*	1.02 (1.01-1.02)*	1 (1-1.01)*	1 (1.004-1.007)*

TABLE 6 Sensitivity, specificity and positive and negative predictive values of the different time points of OGTT in diabetes (DM), impaired glucose tolerance (IGT), impaired fasting glucose and DG (dysglycaemia group) when compared to normal glucose tolerance participants

Time (min)		DM	IGT	IFG	DG
0	Cut-off (mmol/L)	5.05	4.9	5.1	4.9
	AUC (CI 95%)	0.625 (0.52-0.72)*	0.528 (0.46-0.59)	0.641 (0.58-0.69)*	0.593 (0.54-0.63)*
	Sensitivity (%)	52	47	52	57
	Specificity (%)	68	53	68	52
	PPV (%)	6.5	13	26	33
	NPV (%)	97	87	87	75
	Accuracy (%)	67.2	52	65	54
30	Cut-off (mmol/L)	8	7.4	7.5	7.4
	AUC (CI 95%)	0.670 (0.54-0.79)*	0.591 (0.52-0.65)*	0.647 (0.59-0.70)*	0.631 (0.58-0.67)*
	Sensitivity (%)	62	58	62	62
	Specificity (%)	76	58	65	58
	PPV (%)	9.2	16.4	27.5	37
	NPV (%)	98	90	89	79
	Accuracy (%)	75	58	64.1	59
60	Cut-off (mmol/L)	7.9	7.7	7.2	7.2
	AUC (CI 95%)	0.740 (0.63-0.85)*	0.700 (0.64-0.75)*	0.653 (0.59-0.70)*	0.673 (0.63-0.71)*
	Sensitivity (%)	60	60	63	69
	Specificity (%)	76	72	62	60
	PPV (%)	9.7	24	27	42
	NPV (%)	98	92.3	88.2	83
	Accuracy (%)	74.5	70	62	62
120	Cut-off (mmol/L)	5.9	6	5.7	5.7
	AUC (CI 95%)	0.601 (0.48-0.72)	0.685 (0.62-0.74)*	0.558 (0.50-0.61)*	0.613 (0.56-0.65)*
	Sensitivity (%)	61	64	50	61
	Specificity (%)	61	66	58	55
	PPV (%)	6	22	20.2	35
	NPV (%)	97.2	92.4	84.2	78
	Accuracy (%)	60	66	56.3	57

AUC (95% CI), Area under the curve (95% confidence interval); PPV, positive predictive value; NPV, negative predictive value; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; DM, diabetes; DG, dysglycaemia group.
*$P<0.05$

fasting plasma glucose concentration provides a useful and simple tool to identify NGT people who are at increased risk for future T2D.

Several models for predicting the incidence of T2D based on clinical risk factors have also been suggested,[30,31] so the combination of a clinical prediction model with OGTT may potentially identify a larger

TABLE 7 Net reclassification improvement values for OGTT in different high-risk groups

Time (min)	NRI	DM AUC (CI 95%)	IGT AUC (CI 95%)	IFG AUC (CI 95%)	DG AUC (CI 95%)
0	Event	0.22 (−0.23 to 0.67)	0.09 (−0.15 to 0.33)	0.30 (0.11 to 0.49)*	0.18 (0.04 to 0.33)*
	Nonevent	0.1 (0.0186 to 0.195)*	−0.04 (−0.13 to 0.04)	0.07 (−0.01 to 0.16)	0.01 (−0.07 to 0.10)
	Total	0.32 (−0.13 to 0.788)	0.04 (−0.21 to 0.30)	0.37 (0.17 to 0.58)*	0.20 (0.03 to 0.37)*
30	Event	0.25 (−0.22 to 0.72)	−0.08 (−0.33 to 0.17)	0.19 (−0.008 to 0.38)	0.05 (−0.09 to 0.20)
	Nonevent	0.48 (0.40 to 0.562)*	0.38 (0.29 to 0.46)*	0.25 (0.12 to 0.29)*	0.25 (0.16 to 0.3)*
	Total	0.73 (0.25 to 1.21)*	0.29 (0.03 to 0.56)*	0.39 (0.18 to 0.61)*	0.30 (0.13 to 0.47)*
60	Event	0.22 (−0.23 to 0.67)	0.22 (−0.02 to 0.46)	0.15 (−0.04 to 0.35)	0.21 (0.07 to 0.359)*
	Nonevent	0.29 (0.21 to 0.38)*	0.32 (0.23 to 0.40)*	0.21 (0.12 to 0.29) *	0.27 (0.18 to 0.35)*
	Total	0.52 (0.06 to 0.97)*	0.53 (0.28 to 0.79)*	0.36 (0.15 to 0.58)*	0.48 (0.31 to 0.65)*
120	Event	0.22 (−0.23 to 0.67)	0.29 (0.06 to 0.52)*	0.08 (−0.11 to 0.28)	0.19 (0.05 to 0.33)*
	Nonevent	−0.029 (−0.12 to 0.06)	0.22 (0.13 to 0.31)*	0.11 (0.02 to 0.20)*	0.11 (0.02 to 0.20)*
	Total	0.19 (−0.27 to 0.65)	0.51 (0.26 to 0.76)*	0.19 (−0.02 to 0.40)	0.30 (0.14 to 0.47)*

AUC (95% CI), Area under the curve (95% confidence interval); IFG, impaired fasting glucose; IGT, impaired glucose tolerance; DM, diabetes; DG, dysglycaemia group; NRI, Net reclassification improvement.
*$P<0.05$

percentage of high-risk people. During a 7- to 8-year follow-up period study, Abdul-Ghani et al[29,32-35] showed that OGTT 60 performed superiorly to OGTT 120 for identifying people at high risk of developing T2D. Alyass et al[36] suggested the superiority of 1-hour PG as a predictor of incident T2D when compared to both the traditionally used 120-minute glucose measurement, fasting glucose and AUC glucose. Interestingly, the recent article discusses the OGTT at 60 or 90 minutes providing better discrimination than BG at 120 minutes.[37] This is concordant with the findings in our study, where we have suggested that the 60-minute time point has the highest predictive value for diabetes in FDR of T2D patients based not only on the AUC method but also on the NRI method.

On the other hand, one study that was conducted in Iran, in the same population, found that the discriminating ability of OGTT60 and OGTT120 during a follow-up period of 3-5 years in FDR of patients with T2D[38] was almost equal. In all prediction models, the value of prediction decreased over time, and this was especially obvious for OGTT120.[37] Our study has a longer follow-up duration (7 years compared to 3-5 years in Jonghrbani study[38]), which allows for the OGTT120 to become less predictive and increases the significance of OGTT60.

Possible explanations for the heterogeneous results between studies could be the use of different diagnostic criteria forT2D, the use of clinical prediction models with a larger number of co-variables and finally different follow-up durations.

Furthermore, the generally superior predictive ability of BG obtained at earlier time points during the OGTT (30-60 minutes after the ingestion of a meal) may reflect the crucial role of the first phase insulin response in postprandial glucose homoeostasis.[39]

Several recent studies have suggested that the shape of the glucose curve during an OGTT can be used to identify metabolic dysregulation or the potential risk for future T2D.[10-13,40] Among nondiabetic participants, following an oral glucose challenge, an incretin response occurs which is directly related to the rate of gastric emptying and inversely linked with the postchallenge glucose and insulin concentrations.[41] However, the differences in gastric emptying and changes in the incretin response may be accompanied by different glucose responses.[13] In this study, we selected nondiabetic participants with genetic predisposition in order to predict future glucose dysregulation and observed a different glucose response. This difference in the shape of the curve between NGT, IFG, IGT and diabetic groups is consistent with findings from other studies.[28] This study also illustrates that, in the high-risk group, the glucose levels at 60 minutes are higher than those at 30 minutes. Abdul-Ghani et al[12] indicated that those participants whose plasma glucose concentration did not return to its baseline level within 60 min following an OGTT were more likely to progress to T2D compared to those whose plasma glucose returned to the fasting level within 60 minutes. This is also consistent with our findings.

Whether the shape of the glucose response is an inherent characteristic[13] and, hence, a reproducible biological process, warrants further investigation before it is referred to and used in longitudinal studies.[40] Ethnicity is one of the important factors responsible for the different contributions of insulin secretion and action to glucose intolerance.[42,43] Our study only included Iranian participants, and it remains to be established whether the results apply to other ethnic groups. The limitation of our study is the short follow-up period. It would be desirable to have a longer duration of follow-up to be able to allow for development of glucose intolerance and to add measurements of HbA1c.

To our knowledge, this is the first attempt to examine whether the OGTT AUC at different time points could predict future T2D or IFG/IGT in FDR of diabetic patients.

Perhaps one of the implications of this study that could be employed in future is in NGT participants who are FDR of people with diabetes and are candidates for kidney donation. Due to the increased risk of developing T2D in NGT participants who have a plasma glucose level more than 7.2 and 7.8 mmol/L at 30 and 60 minutes, it is recommended that they refrain from donating kidneys. More longitudinal

studies should be conducted to investigate whether glucose response phenotypes could predict high-risk groups in FDR of patients with diabetes. We conclude that the glycaemic response to an OGTT may predict the risk for progressing to IFG/IGT and diabetes in FDR of patients with T2D.

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CONFLICTS OF INTEREST

Nothing to declare.

AUTHORS' ROLE AND CONTRIBUTION

AF performed generating the idea and contributed in statistical analyses, revising it critically for important intellectual content.

RM drafted the work and revised it critically for important intellectual content in order for the final approval of the version to be published.

ME participated in most of the experiments and collected data.

MA conceived and designed the study, revising it critically for important intellectual content.

MN performed editing and solving grammatical errors in the article.

BI generated the idea and design of study, wrote a major part of the article and checked it for intellectual content and revised it.

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