Diagnosis of Diabetes Mellitus and Pre-Diabetes with Fasting Plasma Glucose, Oral Glucose Tolerance Test and A1c Level: A1c Based Screening May Be A Better Diagnostic Tool For Diabetes Mellitus

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Abstract
The International Diabetes Federation estimates that 285 million people around the world have diabetes. The American Diabetes Association (ADA) has proposed hemoglobin A1C ≥ 6.5% (HbA1c) for the diagnosis of diabetes, and 5.7-6.4% as a risk factor for progression to diabetes. This new criterion's accuracy is controversial and has not yet been adopted internationally. We aimed to clarify the power of A1C in diagnosis of diabetes and pre-diabetes. In this retrospective study a total of 1814 patients (622 male, 1192 female) who had concurrent FPG, OGTT and A1C results and diabetes mellitus suspicion were included by using the hospital ProBel system. Diabetic subjects and patients who had been using drugs that may cause diabetes were excluded. According to ADA criteria; 760 of 1814 individuals had diabetes mellitus (41.8 %). With each of these tests (HbA1c, 2-h OGTT and FPG), diabetes was detected in 529 (69.6%), 488 (64.2%) and 328 (43.2%) subjects respectively. Differences between, FPG, 2-h OGTT; FPG and HbA1c; 2-h OGTT and HbA1c were statistically significant (p<0.0001, p<0.0001 and p=0.02, respectively). Among those 1814 subjects, 1094 (60.3%) had impaired FPG. Additionally, 511 (28.2%) and 980 (54.0%) of these subjects were glucose intolerant according to 2-h OGTT and A1C, respectively. Differences among these groups were also statistically significant (p<0.0001, p<0.0001 and p<0.0001, respectively). Diagnostic power of A1C criterion is greater than FPG and 2-h OGTT for the diagnosis of diabetes mellitus. In addition, A1C is more diagnostic than of 2-h OGTT. High diagnostic power A of A1C may lead to decrease in the number of undiagnosed patients.

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Introduction

The International Diabetes Federation estimates that 285 million people have diabetes internationally [1], with this number expected to rise to 438 million over the next 20 years, at a rate of a 7 million people annually; representing an unprecedented total. Thus, it is vitally important that national health policies focus on the early and accurate recognition of diabetes mellitus to prevent or delay adverse outcomes.

The current criteria for the diagnosis of diabetes require a fasting plasma glucose (FPG) and 75-g oral glucose tolerance test (OGTT); a method that is time-consuming, requires fasting, and effected by acute perturbations in glucose levels and short-term lifestyle changes [2]. Since fasting and post-challenge blood glucose levels were found to predict the risk of diabetic retinopathy, these tests have been the international standard for diagnosis [3]. On the other hand, there is not yet an accurate and reliable diagnostic method for the early detection of the undiagnosed diabetic patient. FPG and OGTT are commonly used as criteria to identify subjects at risk of type 2 diabetes, whereas, many diabetic subjects may be far from matching these criteria. Therefore, many diabetic or pre-diabetic subjects remained undiagnosed and may have chronic complications of diabetes mellitus at the time of diagnosis.

Impaired fasting glucose (IFG) and impaired glucose tolerance (IGT) are currently used for diagnosis of high-risk glucose levels below the diabetic range. In addition, assigning a type of diabetes to an individual often depends on the circumstances present at the time of diagnosis, and many diabetic individuals do not easily fit into a single class [4].

In 2009 International Expert Committee proposed new diagnostic criteria based on hemoglobin A1C (HbA1c); with HbA1c $\geq$ 6.5% for diabetes and 6.0-6.4% for "High Risk" of progression to diabetes [5]. Following this The American Diabetes Association (ADA) proposed A1C $\geq$ 6.5% for the diagnosis of diabetes and 5.7-6.4% for the highest risk to progress to diabetes [6]. The proposed diagnostic threshold of 6.5% was based on retinopathy risk at different levels of HbA1c [5].

HbA1c testing is highly standardized and exhibits low intra-individual variation. HbA1c samples can be obtained at any time, require no patient preparation, and are relatively stable at room temperature after collection. HbA1c is unaffected by acute effects of stress or illness

[7]. However, this new criteria's accuracy is controversial and has not yet been adopted internationally [8].

In this study, we aimed to clarify the power and efficacy of HbA1c in the diagnosis of diabetes and pre-diabetes by comparing against the other ADA diagnostic criteria of FPG and OGTT.

Research Design and Methods

In this retrospective study, we screened 27,001 subjects attended to the internal medicine outpatient clinic for any problem between 2006 and 2010 years by the ProBel (Hospital Information Administration System / Oracle Partner Network). Only people with concurrent FPG, OGTT and A1C results and diabetes mellitus suspicion were included. OGTT is routinely obtained in our hospital if there is a suspicion of diabetes mellitus. Diabetic subjects and patients who had been using drugs associated with the development of diabetes were excluded. After these exclusions, 1814 subjects remained. Finally, we evaluated all of the 1814 subjects with diabetes mellitus suspicion that attended to our outpatient clinic. The study group consisted of 622 males (34.3%) and 1192 females (65.7%). Mean age of the subjects was 54.3 ± 13.6 years (male 54.4 ± 13.6, female 54.2 ± 13.7) (Table 1).

Table 1. Frequency of Individuals According to Age Groups

Age Groups	Gender		Total
	Male	Female	
< 45	143 (22.0 %)	243 (20.3 %)	386 (21.2 %)
45-54	169 (27.1 %)	353 (29.6 %)	522 (28.7 %)
55-64	171 (27.4 %)	332 (27.8 %)	503 (27.7 %)
65-74	90 (14.4 %)	172 (14.4 %)	262 (14.4 %)
≥ 75	49 (7.8 %)	92 (7.7 %)	141 (7.7 %)
	622 (100 %)	1192 (100 %)	1814 (100 %)

FPG, OGTT, A1C levels of subjects were obtained from Izmir Ataturk Training and Research Hospital's patient database by using the ProBel system. All subjects (n=1814) were grouped as diabetic patients, glucose intolerant (pre-diabetes) patients and non-diabetic patients according to new ADA criteria for the diagnosis of diabetes. The current diagnostic criteria proposed by ADA for diabetes are: A1C $\geq$ 6.5 percent, FPG $\geq$126 mg/dL (7.0 mmol/L), 2nd hour plasma glucose $\geq$200 mg/dL (11.1 mmol/L) during an OGTT an in a patient with classic symptoms of hyperglycaemia or hyperglycaemic crisis, a random plasma glucose $\geq$200 mg/dL (11.1 mmol/L). IFG was defined as FPG with 100 mg/dl [5.6 mmol/l] - 125 mg/dl [6.9 mmol/l]. IGT was defined as 2-h glucose with 140 mg/dl [7.8 mmol/l] - 199 mg/dl [11.0 mmol/l] or A1C values between 5.7% - 6.4%.

According to our hospital's biochemistry department's certified quality standards FPG, OGTT and A1C tests are performed with the following steps:

A) Fasting Plasma Glucose (FPG): After 12 hourly fasting period, blood samples were drawn by standard phlebotomy into regular blood (serum) test-tubes between 08:00-10:00 AM and serum glucose level was measured by an enzymatic method (hexokinase).

B) Oral Glucose Tolerance Test (OGTT): All subjects were informed to take at least 150 grams of carbohydrate each day, for at least three days before this test. After 12 hourly fasting period, 75 grams of glucose were given to each individual to ingest in the form of a cool drink. Blood samples were taken by standard phlebotomy into regular blood (serum) test tubes at time 0 and 120 minutes by a health care provider.

C) Glycated Hemoglobin (A1C): Blood samples were obtained by standard phlebotomy into EDTA-containing tubes following a 10 hour fast concurrently with FPG. High performance liquid chromatography method (HPLC) was used in analysis of HbA1c. The HbA1c result was calculated as a ratio to total hemoglobin by HPLC (A1C%).

Statistical analysis: All results were shown as mean $\pm$ standard deviation (SD). P values were based on two-sided tests with a cut off for statistical significance of 0.05. The Chi-square test, The Kolmogorov-Smirnov test and Analysis of Covariance test (ANOVA) were used to evaluate values. All statistical analyses were performed with The MedCalc Statistical Software Version 10.1.6.0 (Licensed to Medcalc Turkey 020931118117).

Results

Diabetes: According to new ADA criteria; we determined 760 diabetic patients among 1814 individuals (41.8 %). However, 190 diabetic patients (25.0 %) met all ADA criteria. All results are shown in Table 2 and Table 3. 529 diabetic patients (69.6%) were diagnosed by A1C alone, 488 diabetic patients (64.2%) with 2-h OGTT alone, and 328 (43.2%) diabetic patients were diagnosed with FPG alone (Table 3). Differences between FPG vs. 2-h OGTT, FPG vs. A1C, and OGTT vs. A1C were statistically significant ($p < 0.0001$, $p < 0.0001$ and $p = 0.02$, respectively). Diagnostic sensitivity of all diabetic criteria was 69.6% for A1C; 64.2% for OGTT and only 43.1% for FPG respectively.

Table-2. Distribution of mean FPG, 2-h OGTT and HbA1c Values of the Diabetic Patients and non-Diabetic Individuals According to Age Groups

Age Groups	Tests	All individuals Mean SD	Diabetic patients Mean SD	Non-diabetic individuals Mean SD
< 45	FPG	110 ± 35	144 ± 52	98 ± 13
	2-h-OGTT	148 ± 86	242 ± 111	113 ± 33
	HbA1c	6.15 ± 1.29	7.36 ± 1.96	5.71 ± 0.38
45 - 54	FPG	114 ± 26	129 ± 34	104 ± 10
	2-h-OGTT	159 ± 75	217 ± 82	120 ± 34
	HbA1c	6.29 ± 0.89	6.93 ± 1.06	5.87 ± 0.34
55 - 64	FPG	118 ± 32	133 ± 41	106 ± 10
	2-h-OGTT	175 ± 82	228 ± 92	131 ± 34
	HbA1c	6.46 ± 1.13	7.05 ± 1.44	5.98 ± 0.32
65 - 74	FPG	115 ± 22	124 ± 26	105 ± 11
	2-h-OGTT	176 ± 67	219 ± 61	129 ± 32
	HbA1c	6.32 ± 0.80	6.69 ± 0.90	5.92 ± 0.36
≥ 75	FPG	120 ± 26	131 ± 29	105 ± 10
	2-h-OGTT	202 ± 86	242 ± 87	144 ± 36
	HbA1c	6.51 ± 1.07	6.88 ± 1.24	5.96 ± 0.29

Table-3. Prediabetes and diabetes frequencies

	Diagnostic Criteria	Positive (%)	Negative (%)	Total	P value
	FPG	328 (43.2%)	432 (56.8%)	760	
1.	2-h OGTT	488 (64.2%)	272 (35.8%)	760	< 0.05
	HbA1c	529 (69.6%)	231 (30.4%)	760	
	IFG	386 (50.8%)	374 (49.2%)	760	
2.	IGT 2-h OGTT	167 (22.0%)	593 (78.0%)	760	< 0.05
	IGT HbA1c	197 (25.9%)	563 (74.0%)	760	
	IFG	1094 (60.3%)	720 (39.6%)	1814	
3.	IGT 2-h OGTT	511 (28.2%)	1303 (71.8%)	1814	< 0.05
	IGT HbA1c	980 (54.0%)	834 (45.9%)	1814	

1. Results according to new ADA criteria 2. Frequencies of impaired fasting glucose and impaired glucose tolerance (2-h OGTT and HbA1c). 3. Frequency of impaired fasting glucose and impaired glucose tolerance (2-h OGTT and A1C) among diabetic patients.

Impaired Fasting Glucose and Glucose Intolerance: According to new ADA criteria, of the 1814 subjects tested, 1094 (60,3 %) were classified as having impaired fasting glucose, 511 (28.2%) as having IGT following OGTT and 980 (54.0%) as having IGT by A1C. In terms of diagnostic ratio of glucose intolerance, difference between A1C and OGTT was statistically significant (p<0.0001). (Table 3, Table 4).

Table 4. Distribution of All Diabetic Patients According to Fpg, 2-H Ogtt and Hba1c

	Fasting Blood Glucose mg/dL		Total	2-h OGTT mg/dL			Total
	< 126	≥ 126		< 140	140-200	≥ 200	
A1C < 6.5	1178	107	1285	750	387	148	1285
A1C ≥ 6.5	308	221	529	65	124	340	529
Total	1486	328	1814	815	511	488	1814

Discussion

An international committee of diabetes experts has recommended that the Hemoglobin A1C assay, now routinely used to monitor the course of the disease in patients with diabetes and signals the pending development of diabetic complications; should become the new "gold standard" for diagnosing diabetes [9]. A1C assay is more convenient than OGTT, because it has little inter-individual variation if there is not any hematologic disease, and easy to use in daily routine practice because it does not need any fasting and diet preparation. Recently, World Health Organization experts have also accepted the use of A1C for diagnosing diabetes [10]. We determined 760 diabetic patients according to new proposed ADA criteria. On the other hand, if FPG and OGTT were used as the sole diagnostic tool, we would diagnose only 328 and 488 of the diabetic patients. According to these results, diagnostic power of A1C criterion is higher than FPG and 2-h OGTT. FPG and OGTT have a lower sensitivity, failing to diagnose 56.0 % and 35.7 % of the diabetic patients respectively. In only 190 patients, all ADA criteria were positive (25 % of 760 diabetic patients) (Figure 1).

IFG and IGT are significant predictors of pre-diabetes. It is considerably important to detect subjects in pre-diabetic state for the purpose of taking preventative measures prior to the development of diabetic complications. According to our study, of the 1814 patients tested, 1094 (60.3%) were classified as having IFG, 511 (28.2%) as having IGT following OGTT and 980 (54.0%) as having IGT by A1C. Therefore, it is clear that the A1C criterion can result in a substantially lower prevalence of undiagnosed and total diabetes, and being at high

risk for diabetes, than prevalence estimated from fasting plasma glucose or 2-h glucose [7]. Within our population, we would have missed the diagnosis of pre-diabetes in 469 (25.8%) patients if we had relied only on 2-h OGTT rather than A1C.

As mentioned above, accurate and time appropriate diagnosis of diabetes is imperative, since chronic complications of diabetes may be prevented or delayed by early diagnosis and effective treatment. We indicate that the use of FPG or OGTT alone in the diagnosis of diabetes lead to a large number false negatives, potentially resulting in a greater diabetic complication rate. The epidemic of diabetes is a serious and growing public health problem that results in reduced life expectancy and increased morbidity [11]. Despite significant advances in hyperglycemia treatment, blood glucose monitoring and markers of glycemic control, debilitating vascular complications develop in most diabetic patients [12]. Furthermore, the results of the ADVANCE and the ACCORD trials raise questions about whether extremely tight glucose control is beneficial in all diabetic patients with ACCORD finding that tight glucose control resulted in increased mortality in high-risk type 2 diabetic patients [13,14]. However, the results of the United Kingdom Prospective Diabetes Study (UKPDS) were unable to show a significant effect of strict glycemic control on myocardial infarction [15]. A recent follow-up of the same study confirmed the utility of long-term hyperglycemic control in type 2 diabetes for preventing cardiovascular disease [16]. This apparent discrepancy between glycemic control and incidence and severity of diabetic complications has been termed as the "metabolic memory" [17]. Shown to be present in Type 2 diabetes mellitus, metabolic memory is the concept that early glycemic environment is remembered in the target organs (i.e. eye, kidney, heart, extremities). Follow up data from the UKPDS have shown that type 2 diabetic patients, like type 1 diabetic patients in the DCCT-EDIC, who were on the standard treatment regimen during the study still have a higher incidence of microvascular and cardiovascular complications compared with their counterparts receiving intensive therapy throughout the trial and the follow-up period [16]. This suggests that early metabolic control has enduring beneficial effects also in type 2 diabetes. We can say that recognition and effective treatment of at the earliest opportunity is paramount in preventing complications. We can speculate that high diagnostic power of A1C can lead to a decrease in undiagnosed patients and early detection of diabetes that may result in fewer long term diabetic complications.

As a conclusion; diagnostic power of A1C level is greater than FPG and 2-h OGTT regarding diagnosis of diabetes mellitus. High diagnostic power A of A1C may contribute to the decrease in the number of undiagnosed patients.

Acknowledgements

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