



## Prevalence Of Diabetes And Association Of Fasting And Postprandial Glucose With Hba1c, 6-Year Study In Reference Laboratory

Sanjay Gohil\*, Preeti Singh, Megha Kambli, Reshma Haryan, Raj Jatale, Rakhi Bajpai Dixit, Kirti Chadha

Department of Haematology, Global Reference Laboratory, Metropolis Healthcare Limited, Vidyavihar, Mumbai, Maharashtra, India

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### Abstract

#### Background

Prevalence of diabetes is estimated in the Indian population using hemoglobin A1c (HbA1c) data collected over the period of 6 years, in the Metropolis Reference Laboratory Mumbai. HbA1c values with gender and age structure was correlated during fasting and postprandial glucose for the non-diabetic, pre-diabetic, and diabetic populations in terms of frequency and percentile.

#### Method

Retrospective analysis of HbA1c data obtained in a referral laboratory based in Mumbai over a span of 6 years from 2015 to 2020. A total of 2,94,264 cases were divided in six age groups i.e. 15-20Y, 21-30Y, 31-40, 41-50, 51-60, and >60Y. High-performance liquid chromatographic separation and quantification of HbA1c in blood with gender and age were done. HbA1c values were also correlated with glucose fasting and postprandial in the studied population.

#### Results

Prevalence of diabetes (HbA1C >6.5) was 57.60% in the studied population. The incidence and prevalence of HbA1c in the diabetic range were highest in the age group of >60 years. Males showed a higher prevalence of diabetes than females, while females are found to be more pre-diabetic. A significant ( $p > 0.001$ ) positive linear correlation was found between HbA1c with fasting and postprandial glucose, respectively.

#### Conclusion

The age group of >60Y showed a maximum diabetic prevalence. Gender disparity was recorded more in males than females having higher HbA1c. The sensitivity of HbA1c is comparable with fasting and postprandial glucose. However, HbA1c cannot replace plasma glucose estimation and could be used in conjunction with fasting and postprandial glucose for proper diagnosis and disease management.

#### Keywords:

Diabetes, HbA1C, Indian Population, HPLC

\*Corresponding Author:

Dr Sanjay Gohil

[sanjay.gohil@metropolisindia.com](mailto:sanjay.gohil@metropolisindia.com)

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## Introduction

Diabetes mellitus type 2 (T2DM) is becoming more common in emerging countries due to rapid socio-economic changes,

urbanization, dietary shift and physical inactivity [1]. India is the second biggest population living with diabetes mellitus in the world after China [2]. According to International Diabetes Federation (IDF) in the year 2021, 74.2 and 140.9 million people were estimated with diabetes in India and China, while the numbers are expected to drastically increase to 124.9 and 174.4 in India and China respectively [3]. It was estimated that almost half of all people (49.7%) living with diabetes are undiagnosed [4]. By the year 2025, >75% of people with diabetes will reside in developing countries, as compared with 62% in 1995. The countries with the largest number of people with diabetes are from China, India, Pakistan, USA, Indonesia, Brazil, Mexico, Bangladesh, Japan, and Egypt [3]. The prevalence of people with diabetes is increasing worldwide due to population growth, increase in proportion of people >65 years, urbanization, obesity and physical inactivity [1]. Even if the prevalence of obesity remains stable until 2030, it is anticipated that the number of Diabetics will more than double ~ 366 million, as a consequence of population aging and urbanization. Furthermore, increased survival rates may contribute to increasing prevalence of DM in the future. The association of DM with other disorders such as end-stage renal disease, retinopathy and cancer are less explored, while its association with infections and cardiovascular diseases are well known [5]. It is reported that the presence of physical and psychological problems may worsen the prognosis for cardiovascular events in patients with underlying DM [6,7]. DM is a worldwide endemic and the American Diabetes Association has recommended glycated haemoglobin (HbA1c) as a possible substitute for fasting blood glucose for diabetes diagnosis [8,9].

The common laboratory methods to recognize HbA1c are High performance liquid chromatography (HPLC), Immunoassay (Antibody based immunoassay and Enzyme based assay), Capillary Electrophoresis. HbA1c measurement was compared using immunoturbidimetric and HPLC and it was found that both the methods are reliable and show a strong correlation. HPLC is found to be more sensitive and accurate than immunoturbidimetric method, while immunoturbidimetric method is low in specificity but more cost effective [10]. The relationship between HbA1c and blood glucose is documented in the literature denoting a straight relationship [11-14]. In 2009, HbA1c was defined as one of the diagnostic criteria for diabetes. HbA1c is recommended as an essential indicator for the monitoring of blood glucose control. Hemoglobin is made up of 2 globin dimers, each with an associated heme moiety. Adult hemoglobin comprises of 97% HbA ( $\alpha_2, \beta_2$ ) and 1.5– 3.5% HbA2 ( $\alpha_2, \delta_2$ ) whereas the fetal hemoglobin (HbF;  $\alpha_2, \gamma_2$ ) forms <2%. These percentages might modify with bound haemoglobinopathies [15]. HbF levels are enhanced in the presence of hereditary persistence of HbF,  $\beta$ -thalassemia, sickle cell disease, pregnancy, anemia, and certain leukemia's. Levels may additionally be increased in hospitalized patients. The components of HbA were known by charge separation on cation exchange resin and named in order of their elution as follows: A0, A1a, A1b, and A1C. A1C is that the hemoglobin element that is composed primarily of glycohemoglobin. Glycohemoglobin is made by the non-enzymatic glycation of the N-terminal essential amino acid on the chain of Hemoglobin. HbA1c levels could vary with patient's race/ethnicity [16-18]. Advantages are A1C measurement reflects the typical plasma glucose over last 8-12 weeks and diagnosis of chronic hyperglycaemia. It may be done at any time of the day and doesn't need fasting. It reflects the glycation of proteins and thus correlates with micro and macro vascular complications that are because of glycation of proteins [19-21]. It can pick up diabetes patients who are additionally prone to protein glycation and therefore complications [19]. A1C isn't affected by simultaneous stress, diet, exercise or smoking. Baseline A1C is mostly used for monitoring of diabetes treatment and glycaemic management along with fasting and postprandial glucose.

It is observed that HbA1c testing is not widely available on standardized platform and also need to identify people with prediabetes wherein early intervention can be done to prevent and prolong onset of diabetes related complications. This retrospective study analyzed HbA1c results from territories of Mumbai. The study included all cases irrespective of history of presence or absence of

DM. This study demonstrated the burden of this silent killer in urban population of Mumbai in terms of higher incidence of poor glycemic control. The study included wide age range also estimated the prevalence of prediabetes and diabetes in different age groups.

## Materials and Methods

### *Study design*

Retrospective study was conducted on 2,94,264 HbA1c test results obtained over a period of 6 years, from 2015-2020 in the Metropolis reference laboratory based in Mumbai, India. The studied population belonged to the city of Mumbai (Urban population). Three parameters measured are glycosylated hemoglobin (HbA1c), fasting glucose, postprandial glucose. The incidence and prevalence of diabetes using HbA1c in different age groups and gender was monitored. The correlation between HbA1c with fasting and postprandial glucose for the diagnostic sensitivity of each.

### *High performance liquid chromatographic measurement of HbA1c*

High performance liquid chromatography (HPLC) was used for separation of HbA1c. In this study, fully automated HPLC (HLC-723G8 Tosoh, Belgium) reagent system was used that rapidly and precisely separates hemoglobin found naturally in blood. Charged hemoglobin and other hemoglobin components are eluted at varying times depending on the net charge of the molecule in relation to gradient of increasing ion strength passed through a non-porous cationic exchange column (negatively charged beads). In the HbA1c variant analysis mode predetermined windows are set in the software to detect the presence of hemoglobin A1a, A1b, F, LA1c and SA1c (predominant) and A0. Also, H-V0, H-V1 and H-V2 as a first indication that there is a hemoglobinopathy present. Calibration is for the quantitation of the stable A1c fraction. All other windows are for presumptive identification for various hemoglobin. The reference range for HbA1c quantification that we followed was in accordance with American Diabetes Association 2021 guidelines [9].

### *Analysis of fasting and postprandial glucose*

Analysis of fasting and postprandial glucose was done using the plasma hexokinase method on automated Roche cobas 8000 modular analyzer series (cobas c 702, Roche, Switzerland) [22]. The reference range for glucose fasting and post-prandial quantification that we followed is also in accordance with American Diabetes Association 2021 guidelines [9].

### *Statistical analysis*

Statistical analysis was performed using R Studio (version 1.4.1103). The Shapiro-Wilks test was done to determine whether the data sets differed from a normal distribution. The differences in categorical variables were determined by the chi-square test. The differences between the three groups were examined using one-way ANOVA analysis. Pearson's correlation coefficient was used to examine the relationship between HbA1c and glucose fasting, glucose postprandial and age.

## Results

A total of 2,94,264 patients was included in the study, out of which 1,49,832 (50.92%) were females and 1,44,432 (49.08%) were males, with an age range of 15-101 years. Fasting blood sugar level varied from 36 mg/dl to 800 mg/dl with a mean level of 115.72±51.86 mg/dl. In a study population (15-101 Y), mean age of women and men tested for HbA1C was 49.3 ± 15.1 Y and 52.6±13.9 Y, respectively. Maximum data was collected from population aged above >60 years (26.74 %) followed by those in

the range of 51-60 years (24.90%) (Table 1).

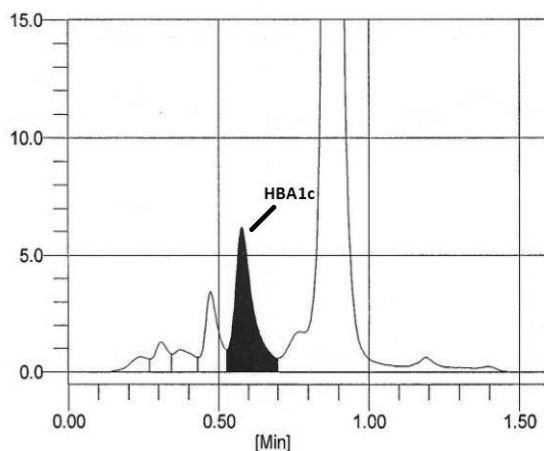
**Table 1 Age distribution in study group**

Age Group Year	Frequency	Percentage
15- 20 Years	3151	1.16
21-30 Years	22581	8.28
31-40 Years	45612	16.73
41-50 Years	60535	22.20
51-60 Years	67906	24.90
60-101	72926	26.74
Total	272711	100

Males had a higher prevalence of diabetes (52.55%) However, female predominance (53.73%) was found to be more in the pre-diabetic range (Table 2). HbA1c was detected at Rt of 0.58 min with a peak area of 6.2% (Fig.1). In the study population, prevalence of diabetes (HbA1c >6.5) was found to be 57.60%. It was observed that 25.25% of the total population had HbA1c falling in the pre-diabetic range, while 57.60 % in the diabetic range (Table 3). The population >60 years of age, 69.2% of the elderly falling in the diabetic (HbA1c>=6.5) range. Hence, the prevalence of diabetes was maximum in that age group. In the elderly, non-diabetics, comprised only 6.25 % of the population. The population age between 21-30 years had the least prevalence (17.82%) of diabetics, and a sharp increase to 38.4% was seen in the age group of 31-40 years. (Table-6). The incidence of pre-diabetics (HbA1c between 5.7-6.4) was almost similar and fell between 21-29%, in all the age groups studied (Table 4). It was found that increasing age has a significant correlation (p-value <0.0001) with incidence and prevalence of Diabetes (Table 4).

**Table 2 Prevalence of HbA1c based on gender**

Gender	HbA1C						Chi sq p value
	Non Diabetic		Pre Diabetic		Diabetic		
	Frequency	Percentage	Frequency	Percentage	Frequency	Percentage	
Female	26169	62.58%	33150	53.73%	90514	47.45%	<0.0001
Male	15647	37.42%	28547	46.27%	100239	52.55%	
Total	41816	100%	61697	100%	190753	100%	



**Figure 1 HPLC chromatograph showing HbA1c detection at Rt of 0.58 min**

**Table 3 Frequency of non-diabetics, pre-diabetics and diabetics based on HbA1c**

HbA1c	Frequency	Percentage
Non-Diabetic	50473	17.15
Pre-Diabetic	74306	25.25
Diabetic	169485	57.60
Total	294264	100

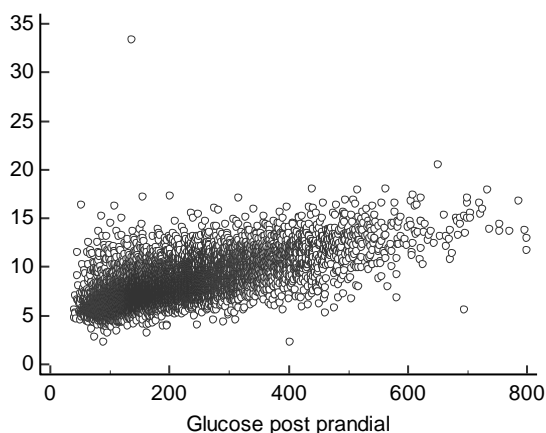
**Table 4 Age-wise distribution of HbA1c**

Age group (Years)	HbA1c						Chi sq p value
	Non-Diabetic		Pre-Diabetic		Diabetic		
	Frequency	Percentage	Frequency	Percentage	Frequency	Percentage	
15 – 20	1844	58.52	673	21.36	634	20.12	<0.0001
21 – 30	12994	57.54	5563	24.64	4024	17.82	
31 – 40	14532	31.86	13545	29.70	17535	38.44	
41 – 50	8937	14.76	16206	26.77	35392	58.47	
51 – 60	5034	7.41	16088	23.69	46784	68.90	
>60	4558	6.25	17876	24.51	50492	69.24	

HbA1c correlation with fasting glucose was available for 54,529 cases. (Table 5). The population falling in the pre-diabetic range of HbA1c had a fasting glucose ranging from 40-332 mg/dl, with a mean of 95+14mg/dl. The diabetic HbA1c population had the mean fasting glucose of 149+63 mg/dl. (Table 5). Scatter Plot below showed a linear relationship with fasting glucose levels and HbA1c levels (Fig. 2).

**Table 5 Association of fasting glucose with HbA1c in study population**

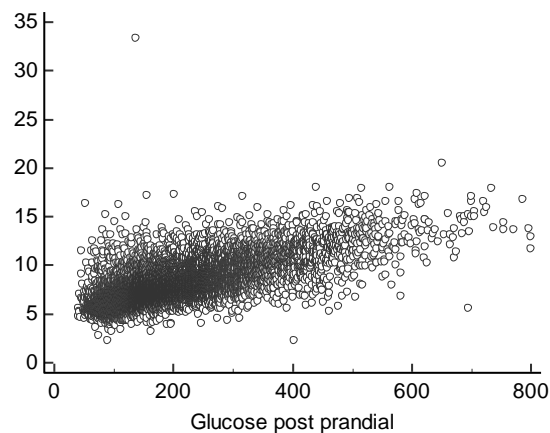
HbA1c	Glucose fasting (mg/dl)			One way Anova p value
	N	Mean + SD	Range	
Non Diabetic	12543	87 +10	36 – 229	0.0001
Pre Diabetic	17326	95+ 14	40 – 332	
Diabetic	24660	149 + 63	40 – 800	

**Figure 2 Scatter plot for fasting glucose and HbA1c**

HbA1c correlation with post-prandial glucose was available for 19,089 cases (Table 6). The population falling in the pre-diabetic range of HbA1c had a mean post-prandial glucose of 118+36 mg/dl with a range of 40-402 mg/dl. And the diabetic HbA1c population, had the mean post-prandial glucose of 211+98 mg/dl (Table 6). Scatter Plot below showed a linear relationship with post-prandial glucose levels and HbA1c levels (Fig. 3). Hence, our study concluded a significant positive linear correlation between HbA1c and age, fasting glucose and postprandial glucose (Table 7).

**Table 6 Association of postprandial glucose with HbA1c in study population**

HbA1c	Glucose post prandial (mg/dl)			One way Anova p value
	N	Mean + SD	Range	
Non Diabetic	2757	100+ 30	40 – 402	
Pre Diabetic	4949	118+36	49 – 433	0.0001
Diabetic	11383	211+98	43 – 800	



**Figure 3 Scatter plot for post-prandial glucose and HbA1c**

**Table 7 Relationship of HbA1c with glucose fasting, postprandial and age**

Variables	N	Correlation coefficient(r) (95% CI)	p value
HbA1C to Glucose fasting	54529	0.7985 (0.7955 – 0.8015)	<0.0001
HbA1C to Glucose post prandial	19089	0.7616 (0.7556 – 0.7675)	<0.0001
HbA1C to Age	272711	0.1790 (0.1753 – 0.1826)	<0.0001

## Discussion

Diabetes is the most prevalent worldwide in the age group 18-99Y [5]. The prevalence of diabetes is highest after 60Y and the population falling into the age group 51-60Y also showed a nearly similar rate. Compared to the elderly (>50Y), the prevalence rate is reduced to 15.2, 44.2, 74.1 and 70.8% in the age group 41-50, 31-40, 21-30, 15-20, respectively. Similar to our study, the global incidence rate for diabetes (194 countries) suggests that it is highest after age 55 [23]. Indian males showed a higher

prevalence (52.5 %) of diabetes than females (47.4 %), interestingly females are more pre-diabetic than males. A study conducted in China population showed the almost similar prevalence rate of diabetes in males and females, while in another study conducted in northern Sweden males showed 14.6% while females showed 9.1% prevalence rate [24,25]. A study conducted between 2008 and 2015 in 15 states of India showed the overall prevalence of diabetes and prediabetes to be 7.3% and 10.3%, respectively [26]. Prevalence of diabetes and prediabetes in the studied state Maharashtra was found to be 57.59% and 25.25 %, which is too high as compared to earlier study conducted by Anjana et al., it may be due to an increase in the socio-economic status of individuals and change in life style [26]. HbA1c level of 6.5% is relatively specific for the diagnosis of diabetes and in accordance with ADA recommendations [9].

The prevalence of diabetes and prediabetes among younger age groups was higher than reported by Nagarathna et al [27]. This study corroborates another study done by Feng L in China, which concluded that HbA1c and fasting glucose maintained significant correlations within the group with HbA1c < 6.5% and glucose <7.0 mmol/L and the group with HbA1c ≥ 6.5% and glucose ≥7.0 mmol/L in both males and females [14]. This study demonstrated a linear relationship ( $p > 0.001$ ) between HbA1c levels with a correlation coefficient 0.79 in fasting and 0.76 in post-prandial glucose levels. Similar to our study, it was reported that HbA1c has close relation to fasting plasma glucose then with postprandial plasma glucose level [28], while in other studies HbA1c is found to be more correlated with postprandial than fasting plasma glucose level [29].

## Conclusion

To conclude, in the study population, the prevalence of diabetes (HbA1c >6.5) was 57.60%, also the population >60 years of age, had maximum, 69.2% of the elderly falling in the diabetic (HbA1c ≥6.5) range. Hence, the prevalence of diabetes was maximum in the elderly. Males had a slightly higher prevalence of Diabetes than females, while more females than males had a pre diabetic HbA1c. Fasting and post-prandial glucose showed a significant correlation ( $p$  value < 0.0001) with HbA1c. Our study concludes that, age, fasting glucose and postprandial glucose, all these three parameters have a linear relationship with HbA1c. The sensitivity of HbA1c is comparable with fasting and post-prandial glucose. However, it cannot replace plasma glucose estimation, and should be used in conjunction with fasting and post-prandial glucose for proper diagnosis and management, as a number of factors (hemolytic disease, chronic liver disease, chronic liver failure etc.) can cause interference with HbA1c estimation.

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**Competing Interests:** The authors declare that they have no conflict of interest.

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