

# Establishing Reference Interval for Fasting Blood Glucose in Healthy Adults of Ambalapuzha, Kerala, India: A Cross-sectional Study

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## ABSTRACT

**Introduction:** Fasting blood glucose is one of the major tests in the diagnosis, treatment and prognosis of Diabetes Mellitus. Researchers have found the racial/ethnic differences in reference interval of common biochemical and haematological laboratory tests. Reference Intervals denotes normative values related to laboratory parameters/analytes used by diagnostic centres for clinical diagnosis. An appropriate reference value is essential for effective clinical decision making.

**Aim:** To establish reference intervals for fasting blood glucose in healthy adult population of Ambalapuzha, North Grama Panchayath, Kerala, India.

**Materials And Methods:** The present cross-sectional study was conducted in the Department of Biochemistry, Govt. T.D. Medical College, Ambalapuzha, Alappuzha, Kerala, India, from February 2018 to August 2018. A total of 420 healthy individuals were included in the study. After random selection of subjects, fasting blood samples were collected and all the samples were

analysed for fasting blood glucose levels. Data was analysed statistically by using Analysis of Variance (ANOVA) test. Mean, standard deviation, reference interval and comparison between both sexes and different age groups was done.

**Results:** Out of 420 individuals 166 (39.5%) were males and 254 (60.5%) were females. The mean level of glucose was  $80.97 \pm 10.113$  mg% and the standard error of means was 0.493. The study population were categorised into 20-29 years, 30-39 years, 40-49 years and 50-59 years age groups. When compared those age groups, the mean blood glucose level was highest (83.81 mg%) for the age group 50-59 years. Significant difference in mean was seen when 30-39 years and 40-49 years was compared with 50-59 years age category.

**Conclusion:** The study concluded that there was no difference in the reference range established between males and females. There was a significant difference in the mean fasting blood glucose among different age groups.

**Keywords:** Diabetes mellitus, Reference range, Blood sample

## INTRODUCTION

Laboratory test reports play a pivotal role in diagnosis and prognosis of many diseases. Nearly 80% of physicians' medical decisions are based on information provided by laboratory reports [1]. This invariably requires appropriate Reference Interval (RI) for effective clinical decision making.

An appropriate RI adds value to the report and without RI, report interpretation becomes compromised. RI as defined by Ceriotti is an interval that, when applied to the population serviced by the laboratory correctly includes most of the subjects with characteristics similar to the reference group and excludes the others [2]. As defined by International Federation of Clinical Chemistry (IFCC), reference sample group is an adequate number of reference individuals (an individual selected for comparison using defined criteria) taken to represent the reference population. Ideally, they should be randomly drawn from the reference population [3].

Most laboratories use RI from the manufacturer method sheets or standard books due to the tedious process and high expenses involved in the process of establishing laboratory RI. However, these RIs may not be applicable to the local population despite using similar methods and instruments. The reason for this could be due to differences in patient population, operating conditions, selection criteria for healthy individuals, geographic variations and many more. Also CLSI endorses that the best means to establish a reference interval is to collect samples from sufficient number of reference individuals [4]. This further emphasizes the need for every laboratory to establish their own RI for making laboratory reports more useful in clinical decision making.

Fasting plasma glucose continues to be one of the essential tests in the diagnosis, treatment and prognosis of Diabetes Mellitus

(DM) [5]. It detects hyperglycemia at prediabetic stage and with lifestyle, dietary modifications, prevent the development of overt DM. There is a need to establish the RI for fasting plasma glucose to make laboratory reports more useful in clinical decision making and for better health care management. Hence, present study was conducted to know the RI for Fasting Plasma Glucose (FPG) in the local population of Ambalapuzha, Kerala, India.

## MATERIALS AND METHODS

The present cross-sectional study was conducted in the Department of Biochemistry, Govt. T.D. Medical College, Ambalapuzha, Alappuzha, Kerala, India, from February 2018 to August 2018. The study was approved by the Institutional Ethical Committee and Institutional Research Board (EC27/2017 dated 23-03-2017). Study subjects were counselled separately about the study and written informed consent was obtained from them.

**Inclusion criteria:** Apparently healthy individuals, aged between 20-65 years, irrespective of gender were included in the study.

**Exclusion criteria:** Based on IFCC guidelines [6], subjects with renal failure, congestive heart disease, chronic respiratory disease, liver disease, malabsorption syndromes, nutritional anemias, infections, hypertension, diabetes mellitus, hyperthyroidism, pregnant women, psychological and mental disorders, obese {Body Mass Index (BMI)  $>30$  kg/m<sup>2</sup>} were excluded. Subjects with a history of alcohol, tobacco, OCP, replacement or supplementation therapy like insulin, nephrotoxic drugs like chronic NSAIDS usage, aminoglycoside antibiotics, anticancer drugs, antipsychotics, steroids and other hyperglycaemic agents were also excluded from the study.

According to IFCC recommendations minimum sample size was 120 [7]. Authors have included 420 subjects in the study. A total

of 484 healthy volunteers who attended the free medical camp in Ambalapuzha north grama panchayath, Kerala, India, were randomly enrolled in the study. Out of 484 volunteers 64 were excluded from the study as outliers. Outliers were identified in Interquartile Ranges (IQR: Q3-Q1; Q1: lower quartile, Q3: upper quartile). At levels of <Q1-1.5 IQR and/or >Q3+1.5 IQR, the outliers were discarded [8,9].

All samples were analysed at Clinical Chemistry wing of Centralised Lab in Government Medical College and Hospital. Demographic details such as name, age, residence etc were collected from all the study participants.

### Study Procedure

Fasting blood samples of 5 mL were collected in dry disposable syringe under aseptic conditions from antecubital vein of the subjects, between 8 am and 9.30 am after overnight fasting for 10-12 hours. For testing plasma glucose, samples were collected in fluoride tubes under aseptic precautions. Cold chain was maintained throughout sample collection and analysis of samples. Samples were analysed on the same day of collection. Blood sugar was analysed in fully automated analyser (Beckmann Coulter Au 680 chemistry analyzer) with closed system pack, with trilevel QC (Bio-Rad) and calibrators. External Quality Assurance Scheme (EQAS) for the test was done periodically with Christian Medical College (CMC), Vellore, India. Method used in testing was IFCC recommended.

### STATISTICAL ANALYSIS

All statistical data was analysed using Statistical Package for the Social Sciences (SPSS) software version 25.0. Qualitative data was expressed as percentages. Continuous variables were expressed as mean±Standard Deviation (SD). Difference between the groups were analysed by simple Analysis of Variance (ANOVA) but the subgroup analysis in each variable was done by Tukey's Honest Significant Difference (HSD) analysis.

### RESULTS

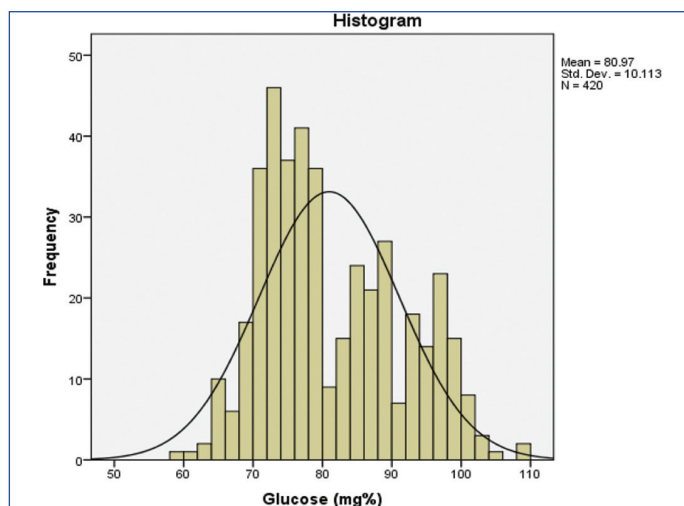
In the present study, out of 420 individuals, 166 (39.5%) were males and 254 (60.5%) were females. The study population were categorised into 20-29 years, 30-39 years, 40-49 years and 50-59 years age groups. Out of 420 subjects, majority 107 (25.48%) were aged between 50-59 years followed by the 105 (25%) in 30-39 years age group. Least number of subjects were 4 (0.95%) were aged between 60-65 years [Table/Fig-1].

Age group (years)	n	Percentage (%)
20-29	101	24.05
30-39	105	25
40-49	103	24.52
50-59	107	25.48
60-65	4	0.95
Total	420	100

[Table/Fig-1]: Age Distribution of the study population.

In present study, the mean level of glucose was 80.97±10.113 mg% and the standard error of means was 0.493 [Table/Fig-2,3]. Males had slightly higher glucose level than females (82.33 mg% as against 80.09 mg%), however it was statistically insignificant [Table/Fig-4]. The mean blood glucose level was highest (83.81 mg%) for the age group 50-59 years. There was no significant difference within group [Table/Fig-5].

In the present study, the Tukey's HSD shows that the mean difference between the 30-39 years age category and 50-59 years age category was statistically significant (p-value=0.030). The mean difference between the 40-49 years age category and 50-59 years age category was statistically significant (p value=0.005) [Table/Fig-6].



[Table/Fig-2]: Showing the gaussian distribution of the dispersed values.

Variable	N	Mean	Standard deviation	Std. Error Mean	95% Confidence Interval (CI) for mean		2.5 percentile	97.5 percentile
					Lower bound	Upper bound		
Glucose (mg %)	420	80.97	10.113	0.493	80.0	81.94	61	100.7

[Table/Fig-3]: Mean, standard deviation, standard error of mean fasting plasma glucose.

Variable	Sex	n	Mean	Standard deviation	Standard error of mean
Glucose (mg%)	Male	166	82.33	10.444	0.811
	Female	254	80.09	9.811	0.616

[Table/Fig-4]: Gender wise comparison of mean fasting plasma glucose level.

Age group (years)	n	Mean	Standard deviation	Standard error	95% Confidence interval for mean		2.5 percentile	97.5 percentile
					Lower bound	Upper bound		
20-29	101	81.21	9.912	0.986	79.25	83.16	61.79	100.63
30-39	105	79.81	9.781	0.955	77.92	81.70	60.64	98.98
40-49	103	79.05	9.435	0.930	77.20	80.89	60.55	97.55
50-59	107	83.81	10.723	1.037	81.76	85.87	62.8	104.82

[Table/Fig-5]: Fasting blood glucose level (in mg%) in four different age groups.

Multiple comparisons						
Dependent variable: Glucose (mg%) Tukey's HSD						
(I) Age category	(J) Age category	Mean difference (I-J)	Standard error	p-value	95% Confidence Interval	
					Lower bound	Upper bound
20-29	30-39	1.398	1.393	0.853	-2.42	5.21
	40-49	2.159	1.399	0.535	-1.67	5.99
	50-59	-2.605	1.386	0.330	-6.40	1.19
30-39	20-29	-1.398	1.393	0.853	-5.21	2.42
	40-49	0.761	1.386	0.982	-3.04	4.56
	50-59	-4.004*	1.373	0.030	-7.76	-0.24
40-49	20-29	-2.159	1.399	0.535	-5.99	1.67
	30-39	-0.761	1.386	0.982	-4.56	3.04
	50-59	-4.765*	1.379	0.005	-8.54	-0.99
50-59	20-29	2.605	1.386	0.330	-1.19	6.40
	30-39	4.004*	1.373	0.030	0.24	7.76
	40-49	4.765*	1.379	0.005	0.99	8.54

[Table/Fig-6]: Comparison of mean difference, standard error of mean of fasting plasma glucose among age categories.

\*The mean difference is significant at the 0.05 level. Since the number of subjects in 60-65 years category was only four, a comparison with other age groups having a size of nearly 100 might not be statistically sound

## DISCUSSION

In clinical management of patients, physicians depend on clinical chemistry analytes for accurate diagnosis, appropriate treatment and follow-up of patients. Correct interpretation of the results from these analytes is only possible if the physician has good reference interval information. Most of the available reference ranges are of the western population which differ from the study population by age, sex, genetics, diet, and altitude. In addition, the reference ranges in the kit inserts that most of the laboratory's follow are extrapolated from a smaller cohort, which warrants the need for each laboratory to establish its own reference range [10].

In present study mean fasting blood glucose level was 80.97 mg/dL. The mean fasting blood glucose level was similar to present study as demonstrated by Ousman et al., and Bhuvanendranath et al., [11,12]. The mean fasting glucose values was higher than the current study in an Ethiopian study by Fiseha T et al., [13].

The mean fasting glucose values in the present study was slightly on the higher side with that of the kit inserts that is followed in most of the Indian laboratories. Castillo O et al., has concluded that those residing in sea level can have a higher glucose value than those residing in higher altitude [14]. The present study was conducted in Alappuzha district in Kerala which is at sea level and the above-mentioned difference substantiates this fact. However, another study showed no significant difference between people living in high altitudes or at sea level [15].

Haggstrom M has stated that the standard reference range basically originates in what is most prevalent in a reference group taken from the population. He has added that the 95% prediction interval, is often estimated by assuming a normal distribution of the measured parameter, in which case it can alternatively be defined as the interval limited by 1.96 (often rounded up to 2) population standard deviations from either side of the population mean. He has added that reference ranges can also be established directly from the 2.5<sup>th</sup> and 97.5<sup>th</sup> percentile of the measurements in the reference group. Likewise the reference range established from this study is 61-100.7 mg/dL, which is on par with most of the studies from the western population [16].

The references range for fasting blood glucose established in the current study was on lower side as compared to worldwide reference range as shown by Fiseha T et al., [13]. However, there was difference in ranges when compared with study by Chhatriwala et al., [17].

Racial/ethnic differences in reference interval of common biochemical and haematological laboratory tests in Asians, Blacks, Hispanics and Whites were analysed by Lim E et al., using their National Health and Nutrition Examination Survey data. Significant racial/ethnic differences were found in almost all laboratory tests including blood glucose levels. They have concluded that racial/ethnic subpopulations have unique distributions in the laboratory tests and race/ethnicity considerations must be incorporated in the development of reference intervals for more accurate disease diagnosis and appropriate treatment [18].

Likewise, Malati T has opined that considering enormous racial and ethnic diversity of Indian population, it is mandatory to establish reference intervals specific to Indian population [19]. The reference values of common blood chemistry analytes in healthy population, aged new-born to 80 years, of Rawalpindi Islamabad area were determined by Khan FA et al.,. In healthy adult males the reference values were of fasting plasma glucose was 3.6-6.0 mmol/L and the values in adult females and elderly subjects were slightly different than adult males. The reference values of their population showed mild to moderate differences from the other Asian, European and American populations. Finally, they concluded that reference values of different biochemical investigations should be established in various areas differently [20].

In the present study the mean fasting glucose was 82.21 mg/dL in males and 80.10 mg/dL among females and the difference was not statistically significant similar findings were noted in a similar study in kenyan population [21]. In contrast, a study by Cheneke W et al., in Ethiopia, has concluded that reference interval for RBS differ significantly among males and females in all age groups [22].

In the present study, though insignificant, the mean values of glucose levels in males were slightly higher than females. Soeters MR et al., has opined that the slightly higher mean values in males can be explained with the lower baseline insulin levels and higher among females [23]. However, in a study by Chhatriwala Mitul N et al., mean and SD values of random blood glucose and postprandial blood glucose was higher for males and fasting blood glucose and HbA1c was higher for females. Kutty VR et al., and TC Ko et al., has concluded that the increment of plasma glucose per decade was almost twice as high in women as in men. They accounted it to a rapid deterioration in insulin resistance in women after menopause, though concluded that the exact mechanism was elusive [24,25]. In a Multiple Regression Analysis (MRA) and nested ANOVA test by Shah SA et al., it was found out that both age and BMI were apparent sources of variation for many analytes in both sexes [26].

In the present study four age groups (20-29 years, 30-39 years, 40-49 years and 50-59 years) were compared. Significant difference was seen when 30-39 years and 40-49 years was compared with 50-59 years age category. And there was no significant difference in other categories. Likewise in a Chinese study, TC Ko et al, there were significant positive correlations between age and plasma glucose after the adjustment for body mass index, smoking and gender [25]. TC Ko et al., concluded that for every decade of increase in age, there was a 0.15 mmol/L increase in fasting and random plasma glucose level and 0.26 mmol/L increase in 2-hour postprandial plasma glucose level.

Similar to the present study, Kutty VR et al., reported lower plasma glucose levels in the young age group and higher plasma glucose level in the old age group [24]. TC Ko et al has opined that increase of plasma glucose with an increasing age is multifactorial. They have added that the age could affect the glycemic index values of foods. However, Wolever TM et al., have concluded that the glycemic index for lentils was not affected by age [27]. Chang AM and Halter JB stated that Insulin levels have been reported to be decreased with advancing age [28].

Further, insulin sensitivity has been reported to decrease with age explained by Basu R et al., [29]. Lee ZS et al., has opined that an increase in other stress hormones, such as plasma cortisol, may also play a role in the age-associated hyperglycaemia [30].

Likewise, based on Canadian health survey, Adeli K et al., has concluded there are fluctuations in biochemical markers over a wide age range, its understanding provides important insight into biological processes and clinical application as establishment of robust pediatric and adult reference intervals of biochemical markers to monitor manifestation of various disease states is essential for patient care [31].

### Limitation(s)

The present study was conducted with small sample size, so the results can not be generalised to whole population. A larger multicentric study would help derive a robust reference interval.

### CONCLUSION(S)

The reference range for fasting plasma glucose found to be on lower side in Ambalappuha compared to that of western population. Present study also demonstrated that there was no significant difference in the reference range established between male and female. However, there was significant difference in mean FPG in age group of 30-39 years and 50-59 years in same population.



Further, it accentuates, the need for every laboratory to establish their own Reference intervals for making laboratory reports more valuable in making clinical decision.

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