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Bimodal distribution of fasting, one and two hour post load plasma glucose in Asian Indian pregnant women without pre-gestational diabetes: Gestational age related changes

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Introduction

Bimodality assessment is accepted as a useful statistical tool to segregate two overlapping distributions of plasma glucose in a population [1–3]. Among Pima Indians [1] & Micronesians in island of Nauru [2] with very high prevalence of diabetes mellitus, community based studies revealed unequivocal evidence of Bimodality of Plasma Glucose distribution (BPG). In them, the two modes of glucose distribution are widely separated and the cut point of the two normal glucose distribution curves of the fitted bimodal model, distinctly separated individuals with normal and abnormal glucose tolerance. The BPG in Pima Indians was accepted by World Health organization (WHO) as a tool to set diagnostic thresholds for Diabetes mellitus (DM) in non obstetric population [3]. Further studies demonstrated BPG in ethnic groups with lower DM prevalence like Mexican Americans [4]. Chinese[5], Malaysians[6], Asian Indians [7,8], Egyptians [9] and Caucasians [10]. But, unlike Pima Indians, in these racial groups, due to the overlap of two normal glucose distribution curves in bimodal model, cut points of two normal distribution curves, were of lesser 'biological' significance [8].

There is only limited research on the usefulness and reliability of a BPG based approach to identify diagnostic threshold values for gestational diabetes mellitus (GDM). In a recent retrospective study involving Asian Indian pregnant women undergoing universal GDM screening by oral glucose tolerance test (OGTT) during 2006–2016 period, we searched for evidence of BPG and its relevance in GDM diagnosis [11]. We observed statistically significant BPG for both fasting (FPG) and 2 h post load plasma glucose (2-h PG) values. The identified cut point for FPG and 2-h PG values were in close agreement with National Institute for Health and Care Excellence (NICE) [12] and International Association of Diabetes and Pregnancy Study Group (IADPSG) criteria [13] respectively. But these cut points were not in between the means of the two normal distribution curves of the bimodal model–a requirement for a cut off value to be considered 'biologically' significant [8]. The women with known pre-gestational diabetes mellitus were not candidates for GDM screening and were excluded from the above study. Hence the BPG observed in this study was contributed by (a) undiagnosed pre-gestational diabetes mellitus and (b) newly developed gestational glucose intolerance. These hyperglycemic states, being either 'first recognized' or had 'its onset' during pregnancy, were regarded as gestational diabetes mellitus (WHO 1999) [14]. But the time of onset, duration and severity of hyperglycemia in pregnancy are different for the above hyperglycemic states and they are likely to influence the expression of BPG differently.

In 2013, WHO revised the diagnostic criteria and classification of 'hyperglycemia first detected in pregnancy' [15]. The women with FPG ≥ 126 mg /dl, 2-h PG ≥ 200 mg/dl or random plasma glucose ≥ 200 mg/dl are classified as 'diabetes mellitus in pregnancy'. The

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diagnosis of 'gestational diabetes mellitus' is confined to women having one or more of plasma glucose values in 75 gm OGTT above IADPSG threshold values but below the diagnostic threshold for DM diagnosis i.e. FPG ≥ 92 to 125 mg/dl, 1-h PG > 180 mg /dl (no upper limit), 2-h PG ≥ 153 to 199 mg /dl. [13,14]. There are indications in the recommendation of the WHO expert committee (2013) that it regards 'Diabetes in pregnancy' as pre-gestational in origin. The diagnostic criteria is same as of DM in non-pregnant state [16]. The recommendation to screen for presence of long term complications like retinopathy and nephropathy, suggests that the hyperglycemic state is of long duration in these women.

The ultimate aim of our studies on bimodal distribution of glucose in pregnant women is to identify a reliable diagnostic threshold for GDM as was achieved earlier for diagnosis of diabetes in non obstetric population [3]. Our earlier study was conducted in a mixed obstetric population which included women with and without pre-gestational diabetes and it failed to provide biologically relevant cut off points. The present study searched whether the exclusion of women with pre-gestational diabetes improve or diminish the chances of identification of glucose threshold values for GDM diagnosis. Those women with history of diabetes prior to pregnancy or with glycated hemoglobin (Hb A1c) ≥ 6.5% (48 mmol/mol) at first antenatal visit [13] or have 'Diabetes in pregnancy', are considered to have pre-gestational DM and were excluded from the study. The research questions are (a) Does the exclusion of women with pre-gestational diabetes alter the bimodality in glucose distribution and cut off values observed in our previous study? b) Any evidence for bimodality in distribution of 1 h post load plasma glucose value (1-h PG) in pregnancy? c) Does the pattern of glucose distribution change with gestational age?

Methods

Study design and selection of participants

This retrospective study involved pregnant women of Asian Indian origin who attended routine antenatal clinics at St Stephen's Hospital, a 600 bedded tertiary care hospital in New Delhi, during 2011 January to 2016 December period. The hospital manages ~2500 pregnant women annually and all of them are of Indian ethnic background, residing in New Delhi. The 75 gm OGTT based universal GDM screening at our centre is generally scheduled at 24–28 weeks of gestation. But those with high risk for GDM (previous GDM, family history of DM, obesity etc) had OGTT at an earlier date. Those who presented to us for antenatal check up after 28 weeks of gestation, had OGTT at the earliest convenient date. We followed GDM diagnostic criteria proposed by IADPSG [13] ie. any one of the glucose value in 75 gm OGTT above the threshold level; FPG ≥ 92 mg/dl or 1-h PG ≥ 180 mg/dl or 2-h PG ≥ 153 mg/dl.

Our centre also practices a hemoglobinopathy screening programme in pregnancy at first antenatal visit by hemoglobin electrophoresis which concurrently estimates HbA1c level of all pregnant women. Those women with HbA1c ≥ 6.5% (48 mmol/mol) are diagnosed to have pre-gestational diabetes.

During Jan 2011 to Dec 2016 period, 13,568 pregnant women participated in the OGTT based universal GDM screening program in our centre. 249 women with known DM, as were not candidates for GDM screening, were self excluded from the study. From these 13,568 pregnant women with OGTT data, we excluded a. 145 (1.07%) women with either HbA1c ≥ 6.5% (48 mmol/mol) or FPG ≥ 126 mg/dl (7 mmol/l) or 2-h PG ≥ 200 mg/dl (11.1 mmol/L) (Diabetes in Pregnancy) b. 2152 (15.9%) women in whom reliable date of last menstrual period was not available. The FPG, 1-hr PG and 2-hr PG values of the remaining 11,271 OGTTs were used for BPG analysis. The OGTTs were done at < 13 gestational weeks (Gw), 13- < 24 Gw, 24–28 Gw and > 28 Gw for 524 (4.64%), 4242 (37.6%) 4305 (38.19%), 2200 (19.51%) women respectively. These gestational age stratified subgroups were analyzed separately for any alterations in plasma glucose distribution.

This study protocol is approved by the institutional ethics committee.

OGTT procedure:

After 10 h of overnight fast, standard protocol for the OGTT with ingestion of 75 gm glucose [D-Glucose powder (Glaxo) 75 gm dissolved in 200 ml distilled water consumed in 5 min] was followed in all women. Venous plasma glucose values were obtained at 0 h (FPG), 1 h (1-h PG) and 2 h (2-h PG) after oral glucose. The OGTTs were supervised by a diabetic educator nurse who ensured proper pre test preparation, fasting state, full consumption of oral glucose and proper timing of blood sampling.

Laboratory methods

Our laboratory is certified by the National Accreditation Board for testing and calibration Laboratories and uses Biorad laboratories for proficiency testing. The plasma glucose was estimated by the glucose oxidase method on Beckman AU 680. All the laboratory standards for glucose were met (i.e., imprecision < 2.9%, bias < 2.2% and total analytical error < 6.9%) [17]. HbA1c estimation was done by Ion exchange High performance liquid chromatography using Bio rad D 10TM machine (Bio rad laboratories. Hercules CA). The estimation is traceable to the reference methods of both the National Glycohemoglobin Standardization Program and the International Federation of clinical chemistry and laboratory medicine. The inter-assay coefficient of variation was 1.9%.

Statistical analysis

The data was analyzed by R - software 3.3.3.(R-core team, Vienna, Austria). The distribution of FPG, 1-h PG and 2- h PG values are generally skewed to the right. Log transformation was applied to remove the right skewness. A normal distribution and mixture of two normal distributions were fitted to log-transformed glucose data. The normal distribution was fitted using maximum likelihood method [18]. The mixture model of two normal components is $f(x) = \alpha f(x: \mu_1, \sigma_1) + (1 - \alpha) * f(x: \mu_2, \sigma_2)$ where f(x) = density function for a normal distribution; α, 1-α are the mixture proportions; μ_1 , μ_2 are the means and σ_1,σ_2 are the standard deviations and it was fitted through the expectation – maximization (EM). The normal mix EM function from the Mixtools in R was applied [19]. To assess the presence of bimodality the mixture model was compared with unimodal distribution using likelihood ratio test in the whole study group and in the gestational age stratified groups (< 13, 13- < 24, 24–28, > 28 Gw) [20]. The variance of two normal distributions were quite different, thus for finding the p values for significance of bimodal as compared to unimodal, χ^2 distribution with 6 degree of freedom was applied [18]. To overcome the regularity problems like identifiability of the mixture model, this comparison was further verified by bootstrapping method with 1000 bootstraps as follows [11,21].

a. A bootstrap sample was generated from the one-component normal distribution (H_0-null hypothesis) with mean and variance as estimated from our data. The sample size of the generated data was also the same as that of each corresponding gestational age group. The $-2 \log \lambda$ for the bootstrap sample was calculated ie $-2\log\lambda = -2[\log(L_0) - \log(L_1)]$ where L_0 = maximum likelihood estimates (MLE) under null hypothesis and L_1 = MLE of alternative hypothesis i.e. bimodal distribution.
   a. The above step was repeated 1000 times to obtain 1000 simulated $-2\log\lambda$.
   b. The $-2 \log \lambda$ for the observed data was calculated.

Table 1
Clinical Parameters.

Parameter	OGTT Whole group n = 11231(A)	OGTT 13–23 weeks n = 4242(B)	OGTT Whole group–13–23 weeks n = 6989(C = A-B)	Difference P ValueB vs C	OGTT < 13 wkn = 524(D)	OGTT Wholegroup - < 13 weeks n = 10707(E = A-D)	Difference P ValueD vs E
Age (years)	27.31 ± 3.86	27.69 ± 3.93	27.17 ± 3.82	< 0.001*	28.46 ± 3.97	27.26 ± 3.85	< 0.001*
BMI Kg/m2	25.16 ± 4.58	25.56 ± 4.26	25.06 ± 4.70	< 0.001*	26.24 ± 4.88	25.12 ± 4.65	< 0.001*
Multiparity n (%)	7048 (62.8)	2692 (63.4)	4356 (62.3)	< 0.228$	361(69)	6695(62.52)	0.003$
History of GDM n (%)	165 (1.4)	76 (1.8)	89 (1.3)	0.027$	26(4.96)	132(1.2)	< 0.001$
Family History DM n (%)	3167 (28.2)	1612 (38)	1555 (22.24)	< 0.001$	285 (54.3)	2882 (26.92)	< 0.001$
Family History Hypertensionn (%)	2130 (19.0)	925 (21.8)	1205 (17.2)	< 0.001$	163 (31.1)	1967 (18.37)	< 0.001$
GDM n (%) (IADPSG criteria)	2434 (21.7)	916 (21.6)	1539 (21.0)	0.596$	172(32.8)	2262 (21.12)	< 0.001$

BMI = Body Mass Index, IADPSG = International Association of Diabetes and Pregnancy Study Group
A = Whole study group, B = Women in gestational age from 13 to 23 weeks D = Women in gestational age < 13 weeks
* Unpaired student's *t*-test and the values are presented in Mean ± Standard Deviation
$ Chi-square test, OGTT = Oral Glucose Tolerance Test, GDM = Gestational Diabetes Mellitus, DM = Diabetes Mellitus,

c. m, which is the total number of simulated values of $-2 \log \lambda$ greater than or equal to the observed value, was counted and the p value = (m + 1)/1000, was determined.

Bootstrapping method for hypothesis testing with 1000 bootstraps were done for FPG, 1 hr PG and 2-h PG values in all OGTT study groups. The 95% confidence intervals (CIs) of means of bimodal normal were estimated using bootstrapping with 1000 bootstraps [22]. Clinical variables were compared between the specific Gw groups with remaining data using the unpaired students *t*-test and Pearson chi-square test.

Results

The age, body mass index (BMI), parity, history of previous GDM, family history of hypertension, family history of diabetes and the prevalence of GDM, obtained by review of medical records, are available for 11,231 women in Table 1. Reliable pre gestational BMI of these women was not available, hence the BMI is calculated from the height and weight recorded at the time of their first antenatal visit before 20 weeks of gestation. Generally the prevalence of alcohol consumption [23] and smoking [24] during pregnancy is low among South Asian women and in our study population it was extremely rare. Those women in gestational age groups < 13 weeks and between 13 and 23 weeks, had higher GDM risk factors compared to the remaining pregnant women (Table 1).

The results of statistical tests of unimodal and bimodal models of log transformed FPG, 1 –h PG and 2 –h PG of the whole group are summarized in Table 2. The Log likelihood ratio statistics showed a significant difference between the unimodal and the normal bimodal distributions by chi square test for all glucose parameters ($p < 0.001$ for all). On detection of bimodality in likelihood ratio test for FPG, 1-h PG and 2-h PG values, the fitted bimodal distribution curves were superimposed on the histogram chart. There was marked overlap of the two normal distribution curves of bimodal distribution and no reliable cut points could be obtained in this study for any glucose parameters. (charts not shown).

The statistical analysis of FPG, 1-h PG and 2-h PG for gestational age stratified < 13, 13 to < 24, 24–28, > 28 weeks, is shown in Table 3. There was no difference between unimodal and normal bimodal distribution of any of the plasma glucose parameters, in < 13 Gw group (all p values > 0.05).

For > 28 Gw group, the p values for FPG, 1-h PG and 2-h PG were 0.0009, 0.002 and 0.012 respectively. For 24 to 28 Gw group, the p values for FPG, 1-h PG, 2-h PG were < 0.001, 0.036, and 0.033 respectively. Bootstrapping method for hypothesis testing with 1000 bootstraps also showed similar results except for 2- h PG in 24–28 Gw group (bootstrap p value 0.06 *vs* likelihood ratio test p value 0.033) . In the 13 to 23 Gw group, difference was significant for FPG ($p < 0.001$) and 2-h PG (p 0.016), but not for 1- h PG (p 0.301).

Discussion

The biological phenomenon of bimodality, which hints at unrecognized heterogeneity in a study population, is clinically relevant in several situations. It is useful in defining diagnostic cut points [3], identifying phenotypic [25] or genetic [26] types and eliciting seasonality [27] of a disease. Wilson et al in the context of screening for a disease in epidemiological surveys, stressed that "if the distribution is bimodal, the 'border-line' group (of a study population) comprises of a mixture of persons with and without a disease, while in a unimodal distribution the 'borderline group' represents homogenous population" [28]. Hence, bimodality signals the evolution of a new disease in a population. In the context of our study, the bimodality of glucose distribution signifies the emergence of gestational diabetes mellitus.

The earlier community based BPG studies were designed mainly to

Table 2
Statistical tests of unimodal and bimodal models of log transformed Plasma Glucose concentrations.

	Number of women	Log mean mmol/l	Unimodal		Bimodal				Mean* mmol/l 95% CI‡		P-value from Bootstrap $	Log likelihood value		
			SD(s)	Mean* mmol/l 95% CI‡	Log Plasma Glucose mmol/l							Unimodal	Bimodal	P-value
					m_1	S_1	M_2	s_2	$Mean_1$	$Mean_2$				
FPG	11,271	1.52	0.111	4.60(4.59–4.61)	1.516	0.079	1.524	0.133	4.60(4.54–4.65)	4.63(4.54–4.66)	< 0.001	8758	8839	< 0.001
I h PG	11,259	2.002	0.234	7.59(7.56–7.62)	1.914	0.202	2.152	0.174	6.92(5.89–9.28)	8.73(6.50–9.66)	< 0.001	873.6	892.4	< 0.001
2 h PG	11,259	1.803	0.211	6.20(6.18–6.23)	1.689	0.147	1.83	0.214	5.47(2.44–6.80)	6.38(2.50–9.94)	< 0.001	1563	1586	< 0.001

†P-value, log likelihood ratio test.

* $Mean_i = \exp(m_i + s_i^2/2)$; i = 1, 2 where m_i and s_i are log plasma glucose means and standard deviations of bimodal normal distribution respectively; exp = exponentiation.

‡ Bootstrap method using 1000 bootstrap and percentile (2.5%-97.5%) was used for 95% confidence interval.

$ P-value from bootstrap method described in the statistical section.

identify diagnostic cut off values for diagnosis of DM in non obstetric population. The factors favoring the expression of BPG and detection of a reliable diagnostic cut off point were; (a) ethnic group with high DM prevalence (b) large sample size (c) elderly population (d) inclusion of known diabetic patients in the study [1,2,6,7,8]. The Asian Indians have a high prevalence of diabetes mellitus, which is reflected as high prevalence of gestational diabetes as well, in their obstetric population [29,30]. The large sample size of our study favored the expression of BPG, while the younger age of these pregnant women might have negatively influenced its expression.

Even after exclusion of all women with pre-gestational diabetes, statistically significant BPG of Fasting, 1-h and 2-h PG values was evident in our study (Table 2). This observation suggests that mild glucose intolerance as observed in gestational diabetes, is sufficient to produce BPG in pregnant women. But it is not strong enough to produce useful cut off points for defining diagnostic threshold values for GDM. Hence the exclusion of pre-gestational diabetes weakens the expression of BPG in Asian Indian pregnant women. Therefore, the right approach to strengthen the BPG expression for identification of a biologically relevant cut point, seems to be the inclusion of pregnant women with marked hyperglycemia in the study population. The ideal study design for this purpose, is to include women with known pre-gestational diabetes along with those with newly diagnosed 'diabetes in pregnancy'. But, the inclusion of women with known pre-gestational diabetes raises certain practical problems. As a preparation for pregnancy, these women are often in a state of 'tight glycemic control' and the resultant normal plasma glucose values, defeat the purpose of their inclusion. On the contrary, withdrawal of anti hyperglycemic agents to obtain higher plasma glucose values in these women and to undertake OGTT in a hyperglycemic state, can have harmful effects on the foetus.

It is interesting to observe that a recent onset and transient glucose intolerance as occurs in gestational diabetes, could trigger BPG expression in pregnancy. Several researchers (Rushforth et al), stress that BPG develops when the 'diabetes epidemic' is established in a population [1,2]. But, Stern analyzed the implication of bimodality to the time course of diabetes development, in the context of transformation of impaired glucose tolerance to Type 2 diabetes [31]. He postulated that the chances to have BPG is more when the deterioration from impaired glucose tolerance to DM occurs rapidly rather than over years. We speculate that both of these prerequisites for BPG proposed by Rushforth et al and Stern, are operational in development of BPG in Asian Indian pregnant women. They belong to a background population with established diabetes epidemic and those women with pre-pregnancy predisposition for diabetes, deteriorate further in pregnancy at a rapid phase (in months) to exhibit bimodality of glucose distribution.

The observation of BPG for 1-h PG in pregnancy is a novel finding. As 1-h PG estimation is not required for diagnosis of DM in non obstetric population, the pattern of its distribution is not evaluated in the recent community based studies [5,8]. In an earlier Pima Indian study, bimodality of 1-h PG distribution was demonstrable, but was found inferior to 2-h PG in separating normal and diabetic populations [32]. Of late, there is a renewed interest on the significance of 1-h PG value. In a retrospective study in general population, 1-h PG value of > 155 mg/dl (8.6 mmol/L) was more predictive than 2-h PG for future development of diabetes [33]. Bergman et al [34] reported a longitudinal association of elevated 1-h PG > 8.6 mmol/l (155 mg/dl) with and without impaired glucose tolerance with cumulative incidence of diabetes over 24 years in a non diabetic cohort. In pregnant women too, the need for insulin therapy is higher in those with high 1-h PG values in OGTT [35]. For GDM diagnosis, 1- h PG estimation is recommended by most professional organizations like IADPSG [13], WHO [14] and International Diabetes Federation [36], but has not been included in the NICE diagnostic criteria [12]. Our observation of BPG for 1-h PG in pregnancy, supports its strength in segregating women with and without glucose intolerance and favors its inclusion in the GDM diagnostic criteria.

In Asian Indian pregnant women without pre-gestational diabetes, BPG was absent in the first trimester but it was evident in the second trimester for FPG, 1-h PG and 2-h PG values. The bimodality attained highest level of statistical significance for all glucose parameters in the third trimester. These findings suggest that bimodality of glucose distribution emerges in mid gestation and it strengthens further with advancing gestational age. The bimodality of FPG and 2-h PG observed in pregnancy before 24 weeks (Table 3) in Asian Indian women, may be regarded as an evidence for the emergence of gestational diabetes earlier than the traditional GDM screening period of 24–28 Gw. But, the earlier detection of BPG in the present study, is attributable to our policy of undertaking OGTT for GDM screening before 24 Gw, only in women with high GDM risk factors. The ideal study design to time the emergence of BPG in pregnancy, is to undertake OGTT assessment in *all* pregnant women sequentially in different stages of gestation, irrespective of their GDM risk factors. An earlier study from South India, undertaking universal GDM screening in each trimester separately, revealed that 38.7% of the GDM women were diagnosed before 24 Gw [37]. In the present study too, 1088 GDM women were diagnosed before 24 Gw and they formed 44.7% of the total 2434 women with gestational diabetes (Table 1). These observations support the strong possibility of early emergence of GDM in Asian Indians. The right time for GDM screening remains controversial and there is growing evidence to support early screening strategies in high GDM risk populations [38]. A well designed prospective study to assess the benefits of early screening before 24 Gw in a low GDM risk pregnant population is ongoing in China, and its results may settle many controversial issues in this area [39].

To the best of our knowledge, there are no studies on the bimodality of glucose distribution in pregnancy in any ethnic groups other than Asian Indian women. The design of the present study and the interpretation of its findings are mainly guided by the conclusions of the

Table 3
Statistical tests of unimodal and bimodal models of log transformed Plasma Glucose (FPG, 1-h PG, 2-h PG) according to gestational age.

Gestational Age Wks	BG g	Number of women	Log mean mmol/l	Unimodal		Bimodal					Mean* mmol /l 95% CI‡		P-value from the Bootstrap	Log likelihood value		
				SD(s)	Mean* mmol/l 95% CI‡	Log Plasma Glucose mmol/l								Uni-modal	Bi-modal	P value†
						m_1	s_1	m_2	s_2	$Mean_1$	$Mean_2$					
< 13	FPG	524	1.560	0.110	4.79(4.74–4.83)	1.554	0.123	1.574	0.058	*4.77(4.00–4.93)*	*4.83(4.53–5.12)*	0.122		412.3	417.53	0.1065
	1 h PG	519	2.006	0.228	7.63(7.46–7.77)	1.693	0.141	2.038	0.209	5.47(5.12–8.37)	7.84(6.22–9.97)	0.504		31.988	32.922	0.931
	2-h PG	524	1.822	0.224	6.34(6.22–6.46)	1.688	0.037	1.833	0.229	5.41(2.27–6.34)	6.42(2.55–9.86)	0.108		41.937	47.596	0.08
13–23	FPG	4242	1.518	0.108	4.59(4.58–4.60)	1.517	0.077	1.518	0.136	4.56(4.52–4.64)	4.61(4.54–4.64)	< 0.001		3391.8	3438.96	< 0.001
	1-h PG	4238	1.985	0.225	7.46(7.41–7.51)	1.984	0.215	2.181	0.163	7.18(5.54–8.62)	8.97(6.67–9.63)	0.600		318.33	321.94	0.301
	2-h PG	4242	1.795	0.209	6.15(6.12–6.19)	1.681	0.148	1.821	0.213	5.43(2.37–6.27)	6.32(2.39–9.46)	0.028		606.1	613.87	0.016
24–28	FPG	4305	1.516	0.111	4.58(4.57–4.60)	1.505	0.075	1.526	0.128	4.52(4.45–4.65)	4.63(4.48–4.92)	< 0.001		3360.58	3387.58	< 0.001
	1-h PG	4303	1.994	0.224	7.54(7.48–7.59)	1.715	0.147	2.030	0.205	5.62(5.24–8.50)	7.75(6.52–9.74)	0.040		331.66	338.40	0.036
	2-h PG	4305	1.793	0.207	6.14(6.10–6.18)	1.604	0.112	1.810	0.209	5.00(2.52–6.72)	6.24(2.51–9.74)	0.060		663.68	670.53	0.033
> 28	FPG	2200	1.523	0.115	4.62(4.60–4.64)	1.517	0.079	1.527	0.132	4.57(4.49–4.69)	4.64(4.53–6.00)	0.008		1636.17	1647.45	0.0009
	1-h PG	2199	2.049	0.215	7.94(7.87–8.01)	1.939	0.192	2.181	0.160	7.08(5.89–9.28)	8.97(6.50–9.66)	0.008		260.46	270.7	0.002
	2-h PG	2199	1.834	0.212	6.40(6.34–6.46)	1.685	0.139	1.872	0.211	5.44(5.06–8.74)	6.65(5.49–10.07)	0.022		287.13	295.27	0.012

$p-value from bootstrap method described in the statistical section *$Mean_i = \exp(m_i + s_i^2/2)$; i = 1,2 where m_i and s_i are log plasma glucose means and standard deviations of bimodal normal distribution respectively; exp = exponentiation FPG = Fasting Plasma Glucose, 1-h PG = 1 h Post load Plasma Glucose , 2-h PG = 2 h post load Plasma Glucose

† P-value, log likelihood ratio test

‡ Bootstrap method using 1000 bootstrap and percentile (2.5%-97.5%) was used for 95% confidence interval;

earlier BPG studies in non obstetric population. But the pathogenesis, glycemic targets and the natural history of glucose intolerance in pregnancy differ significantly from diabetes mellitus in general population [40]. Hence there can be limitations in comparison of the findings of our study with the conclusions of the earlier BPG studies in non pregnant population. The weakness in the design of the present study in assessing the time of emergence of BPG in pregnancy is highlighted in the previous paragraph. The large sample size, universal OGT Testing and single ethnic group of the study population are the major strengths of this study. The findings of this study, though do not translate to clinical decisions immediately, can aid future research to develop alternate strategies for GDM diagnosis.

Conclusions

The present study showed evidence for bimodality of FPG, 1-h PG and 2-h PG distribution in Asian Indian women, even after exclusion of women with pre-gestational diabetes. This finding suggests that mild glucose intolerance of pregnancy, even though of transient nature, can produce bimodality in plasma glucose distribution. But the exclusion of pre-gestational diabetes has weakened the expression of bimodality and a cut point for GDM diagnosis could not be identified. The finding of BPG of 1-h PG in pregnancy is a novel finding. The observation of emergence of BPG in 'high GDM risk' women prior to 24 weeks of gestation may be a prelude to further research to identify the time of emergence of BPG and gestational diabetes in pregnancy, in high risk populations.

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Author contributions

JP conceptualized the idea and prepared the manuscript, **RM** carried out statistical analysis and contributed to manuscript **KS, AM, AC** contributed to discussion and made constructive criticism to manuscript **AS & NC** helped in collecting data, data analysis and contributed to manuscript.

Disclosures

Nil.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jcte.2019.100195.

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