Study of Phases of Insulin Secretion in Pre-Diabetes and Newly Diagnosed Type 2 Diabetes Mellitus

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Abstract:

Background: Insulin is released from the pancreas in a biphasic manner in response to arterial glucose concentration. The assumption has been generally made that the 30-minute response reflected first-phase insulin release, whereas the 120-minute response reflected second-phase insulin release. **Objectives:** The aim of this study was to identify the defect in first and second phases of insulin secretion in pre-diabetes and newly diagnosed T2DM. **Methods:** This case-control study was conducted in the department of Biochemistry, Bangabandhu Sheikh Mujib Medical University, Shahbag, Dhaka from March 2013 to June 2014. All the study subjects (n = 94) were collected from the one point centre, BSMMU as newly diagnosed T2DM, pre-diabetes and healthy normal glucose tolerant subjects according to fasting plasma glucose and 2 hour plasma glucose status. A total of 32 newly diagnosed T2DM and 32 pre-diabetes were included on the basis of inclusion criteria as cases. Another 30 healthy normal glucose tolerant subjects were enrolled as control. Fasting blood samples were collected from study subjects to estimate the plasma glucose and insulin level. Again blood samples were taken for measurement of plasma glucose and insulin level at 30 minute and 120 minute on OGTT. **Results:** Fasting plasma insulin was significantly higher in pre-diabetes than control and T2DM (p = 0.011). Plasma insulin at 30 minute and 120 minute of OGTT were significantly lower in T2DM than control and pre- diabetes (p = 0.001 & 0.016). The insulin secretion in first and second phases were significantly lower in T2DM patients than controls and pre-diabetes (p = 0.000). Beta-cell function was also significantly lower in T2DM than controls and pre-diabetes (p = 0.000). Median values of HOMA-IR were higher in pre-diabetes (1.68) and T2DM (1.53) than control (1.37), but not statistically significant (p = 0.153). There was significant positive correlation of both phases of insulin secretion with FPI, beta-cell function and insulin resistance in T2DM, pre-diabetes and controls. **Conclusions:** The study reveals that 1st and 2nd phase insulin secretory defect was detected in T2DM, but in pre-diabetes, we have failed to identify insulin secretory defects in both phases.

Key wards: Phases of Insulin Secretion, Pre-diabetes, Type 2 Diabetes Mellitus

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Introduction:

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels[1].

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The prevalence of diabetes mellitus (DM) continues to increase worldwide, especially in Asia. In 2010, the International Diabetes Federation estimated that 5.7 million (6.1%) and 6.7 million (7.1%) of people living in Bangladesh is suffering from diabetes and impaired glucose tolerance (IGT) respectively. By 2030, that number of diabetic population is expected to rise to 11.1 million. This explosion on diabetes prevalence will place Bangladesh among the top seven countries in terms of the number of people living with diabetes in 2030[2].

Insulin secretion is a highly dynamic process regulated by complex mechanisms. It is regulated by nutrient status, hormonal factors and neural factors[3]. In addition, the regulation of insulin secretion is a multi-tiered process, occurring at the level of the single β cell, the pancreatic islet, the whole pancreas, and the intact organism. Thus, in vivo, the dynamics of insulin secretion is the consequence of an integration of all of these systems[4].

It is well known that the insulin concentration in the blood oscillates even during post absorptive periods[5]. There are two major oscillatory secretion patterns, ultradian oscillations (pulsatile secretion), which have a period of 1–2 hours and may be due to a feedback loop between glucose production and insulin secretion[6] and more rapid oscillations, which have a period of 10–15 minutes[7]. Insulin secretion follows a characteristic biphasic time course: an initial component, which develops rapidly but only lasts a few minutes (1st phase), is followed by a slowly developing but sustained component (2nd phase)[8]. The assumption has been generally made that the 30-minute response after glucose ingestion reflected first-phase insulin release, whereas the 120-minute response reflected second-phase insulin release[9].

Type-2 diabetes is associated with complete loss of 1st phase secretion and strong reduction of 2nd phase release[10]. But there are very few studies on phases of insulin secretion in pre-diabetes and newly diagnosed T2DM worldwide as well as in our country. As pre-diabetic and diabetic patients are increasing day by day, the knowledge about phases of insulin secretion in our population need to be established. This will help to better understand the etiopathogenesis of T2DM in this population.

Methods:

This was a case control study conducted in the department of Biochemistry, Bangabandhu Sheikh Mujib Medical University during the period March 2013 to June 2014. All the study subjects were selected from the one point collection centre of BSMMU. According to non probability sampling technique a total 94 study subjects were included in this study of age range from 20 to 70 years. The study subjects were categorized on basis of OGTT findings into cases as newly diagnosed T2DM (n = 32) & pre-diabetes (n = 32) and healthy normal control (n = 30). According to WHO Type 2 Diabetes Mellitus was diagnosed, a single raised glucose reading with symptoms, otherwise raised values on two occasions, of either fasting plasma glucose $\geq$ 7.0 mmol/L or with a glucose tolerance test, two hours after the oral glucose load a plasma glucose $\geq$ 11.1 mmol/L. Pre-diabetes was the state in which some but not all of the diagnostic criteria were met and it includes IFG and IGT. IFG if fasting plasma glucose between 6.1 – 6.9 mmol/L and if measured on OGTT at two hour plasma glucose < 7.8 mmol/L. IGT if two hour after glucose load plasma glucose >7.8 and <11.1 mmol/L and fasting plasma glucose < 7.0 mmol. Insulin sensitivity was calculated by the original HOMA model M[11].

Fasting blood samples were collected from all the study subjects to estimate the plasma glucose and insulin level. Again blood samples were taken for plasma insulin level measurement at 30 minute and 120 minute on OGTT. All the biochemical tests were performed at the department of biochemistry, BSMMU. Plasma glucose concentrations were measured by Glucose oxidase method on Dead Behring (Bio-Trade, USA). Plasma insulin levels were estimated by microparticle enzyme immunoassay (MEIA) on AxSYM system (Abbott USA).

All the data were expressed as median (range). The statistical significance of differences was analyzed by non parametric Kruskal-Wallis test. Spearman's correlation co-efficient test was done for correlation. A p-value of <0.05 was considered statistically significant.

Results :

A total of 94 study subjects by doing OGTT were categorized 32 as newly diagnosed T2DM, 32 pre-diabetes and 30 normoglycemic control subjects.

During OGTT the median (range) value of fasting plasma insulin (FPI) were significantly lower in T2DM than pre-diabetes and controls (p = 0.011). Median (range) value of 30 min plasma insulin were significantly lower in

T2DM than pre-diabetes and non-diabetic controls (p=0.001). Median (range) value of 120 min plasma insulin level were significantly lower in T2DM than pre-diabetes and controls (p = 0.016) table-I.

Table-I

Insulinemic status of study subjects (N=94)

Variables	Control (n=30)	Pre-DM (n=32)	T2DM (n=32)	p values
FPI (μU/ml)	9.4 (6.95-14.15)	11.7 (9-18.15)	7.5 (5.1-14.9)	0.011
30 min PI (μU/ml)	65.6 (38.65-100.58)	79.4 (47.8-126.6)	40.5 (13.6-55.9)	0.001
120 min PI (μU/ml)	107.9 (31.15-163.1)	78.75 (39.8-167.8)	47.7 (17.9-87.4)	0.016

Non-parametric -Kruskal-Wallis test was done to measure the level of significance.
Data was expressed as mediam (range)

Phases of insulin secretion of study subjects were calculated from fasting and 30 minute plasma glucose and insulin. Both 1st and 2nd phase of insulin secretion were significantly lower in T2DM than controls and pre-diabetes (p = 0.000) table II.

Table-II

Phases of insulin secretion of study subjects (N=94)

Variables	Control (n=30)	Pre-DM (n=32)	T2DM (n=32)	p value
1st phase (pmol/L)	1046 (697-1586)	1051 (738-1783)	338 (-406-739)	0.000
2nd phase (pmol/L)	280 (204-409)	293 (218-461)	140 (-3.52-223)	0.000

Non-parametric-Kruskal-Wallis test was done to measure the level of significance.
Data shown as mediam (range)

The values of HOMA-% B were significantly lower in T2DM compared to pre-diabetes and controls (p = 0.000). Insulin sensitivity (HOMA-% S) and Insulin resistance (HOMA-IR) showed no significant difference among T2DM, pre-diabetes and controls (p = 0.285 and p = 0.153) table III.

Table-III

Insulin secretory capacity, (%B) insulin sensitivity (%S) and insulin resistance (IR) in study subjects (N=94)

Variables	Control (n=30)	Pre-DM (n=32)	T2DM (n=32)	p values
HOMA %B	125 (97-194)	135 (107-168)	44 (22.4-90.4)*	0.000
HOMA %S	67.7 (46-98.7)	58.5 (38-75)	65.3 (35.5-92)	0.285
HOMA IR	1.37 (0.99-1.98)	1.68	1.53 (1.3-2.6)	0.153 (1-2.8)

Non-parametric-Kruskal-Wallis test was done to measure the level of significance.
Data shown as mediam (range)

FPI showed highly significant correlation with phase 1 where rs = 0.595, p = 0.001 and also with phase 2 where rs = 0.611, p = 0.001. HOMA-%B showed significant correlation with phase 1 where rs = 0.477, p = 0.021 and also with phase 2 where rs = 0.481, p = 0.020. HOMA-IR showed highly significant correlation with phase 1 (rs = 0.597, p = 0.004) and also with phase 2 (rs = 0.610, p = 0.003). FPG did not show any significant correlation with phase 1 and phase 2 insulin secretion table IV.

Table-IV

Correlation of phase 1 and phase 2 of insulin secretion with FPG, FPI, beta-cell function and insulin resistance in controls (n=30)

Variables	Phase 1		Phase 2	
	rs	p	rs	p
FPG (mmol/l)	0.019	0.928	0.050	0.809
FPI (μU/ml)	0.595	0.001**	0.611	0.001**
HOMA% B	0.477	0.021*	0.481	0.020*
HOMA IR	0.597	0.004**	0.610	0.003**

Spearman's correlation test was done for correlation. rs (rho) = spearman's correlation co-efficient.
*P<0.05 ** P< 0.01

FPI shows highly significant correlation with phase 1 where rs = 0.645, p = 0.000 and also with phase 2 where rs = 0.660, p = 0.000. HOMA% B shows highly significant correlation with phase 1 where rs =0.445, p=0.008 and also with phase 2 where rs = 0.433, p = 0.010. HOMA IR shows highly significant correlation with phase 1 (rs = 0.626, p = 0.000) and also with phase 2 (rs = 0.648, p = 0.000) table V.

Table-V

Correlation of phase 1 and phase 2 of insulin secretion with FPG, FPI, beta-cell function and insulin resistance in pre-diabetes (n=32)

Variables	Phase 1		Phase 2	
	rs	p	rs	p
FPG (mmol/l)	0.046	0.798	0.079	0.656
FPI (μU/ml)	0.645	0.000***	0.660	0.000***
HOMA% B	0.445	0.008**	0.433	0.010*
HOMA IR	0.626	0.000***	0.648	0.000***

Spearman's correlation test was done. rs (rho) = spearman's correlation co-efficient.
* P<0.05 ** P< 0.01 *** P<0.001

FPG shows highly significant correlation with phase 1 (rs = -0.576, p = 0.000) and also with phase 2 (rs = -0.589, p = 0.000). FPI showed highly significant correlation with phase 1 (rs = 0.560, p = 0.001) and also with phase 2 (rs = 0.601, p = 0.000). HOMA%B shows highly significant correlation with phase 1 (rs = 0.674, p = 0.000) and also with phase 2 (rs = 0.707, p = 0.000). HOMA IR shows significant correlation with phase 1 (rs = 0.370, p = 0.037) and also with phase 2 (rs = 0.413, p = 0.019) table VI.

Table-VI

Correlation of phase 1 and phase 2 of insulin secretion with FPG, FPI, beta-cell function and insulin resistance in T2DM (n=32)

Variables	Phase 1		Phase 2	
	rs	p	rs	p
FPG (mmol/l)	-0.576	0.000***	-0.589	0.000***
FPI (μU/ml)	0.560	0.001**	0.601	0.000***
HOMA %B	0.674	0.000***	0.707	0.000***
HOMA IR	0.370	0.037	0.413	0.019*

Spearman's correlation test was done. rs (rho) spearman's correlation co-efficient.
* P<0.05 ** P<0.01 *** P<0.001

Discussion:

Phases of insulin secretion need to be studied specifically to better understanding the pathophysiology of T2DM, as the defect in phases were the earliest determinant of pre-diabetes and T2DM. The study was conducted to know the basic defects of phases of insulin secretion in pre-diabetes and newly diagnosed type 2 diabetes.

In this study, median value of first and second phase of insulin secretion was significantly lower in T2DM than controls and pre-diabetes. Matsuda and DeFronzo in 1999[12] found similar findings where they suggested that in T2DM subjects, the first phase insulin concentrations were significantly reduced compared with subjects with

both normal glucose tolerance and IGT. It is widely thought that diminution of first-phase insulin release is the earliest detectable defect of β-cell function. Prato, Marchetti and Bonadonna 2002[13] reported that first phase insulin release is almost invariably lost in the early stages of type 2 diabetes and it is quite common defect that may have a pathogenetic role in the development of postprandial hyperglycemia.

The present data indicate that the diabetic subjects have both insulin secretory dysfunction and insulin resistance. However, analysis of the data reveals that the pancreatic β-cell dysfunction is markedly predominant in these subjects compared to insulin resistance. This applies both for the fasting and stimulated states. In contrast to the insulin secretory defects the insulin sensitivity in the diabetic subjects did not show any significant difference from control. Our findings showed a markedly predominant presence of insulin secretory defect in these populations. This finding is similar with the observations of Seino, Shibski and Minami 2011[4]. They reported that insulin secretory capacity is the major factor contributing to the development of T2DM.

The insulin secretory capacity (HOMA-%B) were significantly lower in diabetic groups than pre-diabetes and controls ($p < 0.001$), whereas no statistical significant difference in insulin resistance ($p = 0.153$). The finding was in contrast with the usual notion that the insulin resistance is the earliest and predominant defect in T2DM and insulin secretory defect is secondary to the primary defect of insulin resistance showed by Defornzo & Tripathy 2009[14].

We measured insulin sensitivity by HOMA-%S and found no significant difference among groups ($p = 0.285$). This was consistent with the findings of the U.K. Prospective Diabetes Study 1995[15].

In this study highly significant correlation was found in phase 1 and phase 2 insulin secretion with fasting glucose, fasting insulin, beta-cell function and insulin resistance. This was consistent with the findings of Stumvoll et al. in 2000[16], where they found first and second phases were correlated to various degrees with numerous demographic and OGTT parameters. However, by using multiple linear regressions, only a few of the demographic and OGTT parameters contributed significantly in explaining variations in insulin sensitivity and β-cell function.

Conclusions:

In conclusion, the findings of this present study suggest that the 1st and 2nd phase insulin secretory defect was present in T2DM, but in pre-diabetes, we have failed to identify insulin secretory defects in both phases.

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