

Original Research

Glucose dependent insulintropic polypeptide in impaired glucose tolerance and its association with insulin secretion and sensitivity

Marufa Akhter¹, Zebunnesa Zeba², Mamun Mia³, Salima Akter^{3,5*}, Rahelee Zinnat⁴, Liaquat Ali⁴

¹ Department of Biochemistry, Ad-din Sakina Women's Medical College, Jashore 7400, Bangladesh

² Department of Public Health and Informatics, Jahangirnagar University, Savar 1342, Bangladesh

³ Department of Medical Biotechnology, Bangladesh University of Health Sciences, Dhaka 1216, Bangladesh

⁴ Department of Biochemistry and Cell Biology, Bangladesh University of Health Sciences, Dhaka 1216, Bangladesh

⁵ Department of Biochemistry and Molecular Biology, School of Medicine, Kyung Hee University, Seoul, Republic of Korea

*Correspondence to: Salima Akter, Department of Medical Biotechnology, Bangladesh University of Health Sciences (BUHS). 125/1-Darus Salam Rd, Dhaka 1216, Bangladesh. Phone: (880)2-9010932, 9010952. Fax: [880]2-8055312 and Department of Biochemistry and Molecular Biology, School of Medicine, Kyung Hee University, 26 Kyungheedaero, Dongdaemun-gu, Seoul 02447, Republic of Korea. Phone: 82-2-961-0524. Fax: 82-2-959-8168. E-mail: salima_2015@buhs.ac.bd

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Abstract

Background and Aims: The abnormalities of incretin effects have been established as major determinants of insulin secretion and sensitivity which starts early in prediabetes. However, the pathophysiology of these states with incretin in Bangladeshi population has only been started to be investigated. The present study was undertaken to explore the association of glucose dependent insulintropic polypeptide (GIP) with glycemic and insulinemic status in impaired glucose tolerance (IGT). **Material and Methods:** The analytic observational study was conducted under a case-control design with age and body mass index (BMI) matched 51 IGT and 47 control subjects. Serum C-peptide and GIP were measured by enzyme linked immunosorbent assay (ELISA). Insulin secretory capacity (HOMA-%B) and insulin sensitivity (HOMA-%S) were calculated by homeostasis model assessment. **Results:** IGT subjects showed significantly higher serum fasting GIP (FGIP) level compared to controls [pgm/ml, 74.2 (10.0–190.0) vs. 49.6 (6.1–278.0), ($p=0.001$)]. There was no significant difference of post glucose GIP (PGIP) in the study subjects, however, the ratio analysis revealed reduced secretion of PGIP to FGIP ($p<0.001$), and fasting C-peptide (FC-peptide) to FGIP ($p<0.05$), respectively in IGT. In addition, serum PGIP and 2 h postload serum glucose (2 h PG) ratio was also significantly reduced in IGT compared to controls [7.00 (2.87–18.42) vs. 10.08 (1.88–23.25), ($p<0.01$)]. In multiple regression, a significant positive association between FC-peptide and FGIP ($p<0.01$) and negative association between FGIP and HOMA-%S ($p=0.05$) were demonstrated. **Conclusion:** The incretin effect of GIP is diminished in IGT and it is associated with insulin resistance in Bangladeshi type 2 diabetic population.

Keywords: Glucose dependent insulintropic polypeptide (GIP), Insulin secretion, IGT, Insulin sensitivity.

Background and Aims

Diabetes mellitus (DM) is a major health burden all over the world increasing to epidemic levels particularly in the developing countries [1]. The pathophysiology as well as risk factors of the disorder has shown considerable heterogeneity depending on

racial, environmental, demographic, socio-economic, and cultural factors [2]. GIP, one of the primary incretin hormone secreted from the intestine [3, 4], stimulates insulin secretion in a glucose-dependent manner [5–7]. Approximately 70% of the overall post-prandial insulin response to glucose is mediated by GIP with the help of glucagon-like peptide-1 (GLP-1), another



member of the incretin hormone family [8]. Moreover, GIP stimulates proinsulin gene transcription and translation [9, 10], and act as a β -cell mitogenic and anti-apoptotic factor [11]. Incretin defects have been found to be associated with type 2 diabetes mellitus (T2DM) from its early stage [12]; however, the causal relationship between incretin and T2DM has still remain controversial as the studies have shown variable results [12–15]. One of the approaches to resolve the issue is to investigate the incretin hormone profile in subjects with impaired fasting glucose (IFG) and (IGT) who are considered to have a prediabetes status with a progression rate of 5 to 10 percent to DM every annum [16].

Bangladesh now ranks 10th in the total number of diabetic population with vast majority being of T2DM and the number is increasing very rapidly [1]. Studies on prediabetic subjects have shown that both the basic defects of T2DM, i.e. insulin secretory abnormality and insulin resistance, are present in Bangladeshi IFG and IGT subjects in variable degrees [17] with the secretory defect being highly predominant in the former group [18]. The association of incretin hormones with insulin secretion or insulin sensitivity has so far not been investigated in any of the prediabetic groups. Thus, the present study was undertaken to investigate whether basal and post stimulatory GIP profile has any relation with glycemic and insulinemic status.

Materials and Methods

Study design and subjects

An observational study with a case-control design was conducted in the biomedical research group, department of Biochemistry & Cell Biology, Bangladesh Institute of Research and Rehabilitation in Diabetes, Endocrine and Metabolic Disorders (BIRDEM), Dhaka, Bangladesh. Voluntarily agreed adult subjects with age ranging from 30 to 55 years, were included after taking informed consent and a total number of 51 IGT subjects and 47 control subjects were recruited in the study. The two groups were matched for age and BMI. Diabetes and

prediabetes were diagnosed following WHO Group Study criteria [19]. Subjects with serious co-morbid diseases like severe infection, stroke, myocardial infarction, major surgery, malabsorption, history of using drugs significantly affecting glucose metabolism (glucocorticoids, oral contraceptives containing levonorgestrel or high dose estrogen, phenytoin and high dose thiazide diuretics etc.) and pregnant women were excluded. All subjects underwent standard procedures of anthropometric measurements like body weight, height, waist and hip circumference (WC and HC).

Biochemical analysis

After overnight fasting (8–14 h), blood samples were collected by venipuncture to assess the biochemical tests including fasting and 2 h post-load (75 g glucose) glucose. All tests were measured by standard laboratory methods using a conventional automated analyzer (Dimension XL[®] clinical chemistry system, Siemens Healthcare Diagnostics Inc. USA). Serum C-peptide and serum GIP were measured by ELISA technique using commercial kits (DRG-International, Germany). For beta cell assessment, insulin secretory capacity (HOMA-%B) and insulin sensitivity (HOMA-%S) were estimated by homeostasis model assessment using HOMA-SIGMA software.

Statistical Analysis

Data were expressed as mean \pm standard deviation (SD) and/or median (range) wherever appropriate. Comparison of mean values between two groups was tested using either Student's 't' test (Unpaired) or Mann-Whitney 'U' test. Bivariate correlation analysis was done by using Spearman's correlation analysis. Univariate regression analysis was performed taking C-peptide and HOMA-%S as dependent variable and others as independent/confounding variables as appropriate. All statistical measures were performed using statistical package for social science (SPSS) for windows version 11.5.

Table 1: Anthropometric and clinical characteristics of the study subjects.

| Variables | Control (n = 47) | IGT (n = 51) | z/p value |
|----------------------------|--------------------|---------------------|-------------|
| Age (yrs) | 40±6 | 41±5 | 0.673/0.502 |
| BMI (kg/m ²) | 24.0±2.9 | 24.0±3.5 | 0.570/0.955 |
| WHR | 0.88±0.05 | 0.92±0.08 | 2.340/0.02 |
| FSG (mmol/l) | 5.2 (4.1–6.0) | 5.3 (4.4–6.0) | 0.502/0.615 |
| 2 h PG (mmol/l) | 6.3 (4.8–7.8) | 9.3 (7.9–11.0) | 8.502/0.001 |
| FC-pep (pmol/l) | 0.64 (0.18–1.62) | 0.68 (0.21–1.39) | 1.000/0.310 |
| HOMA-%B | 117.0 (58.4–461.0) | 117.0 (39.4–455.0) | 0.109/0.914 |
| HOMA-%S | 71.0 (27.0–247.0) | 65.0 (22.0–222.0) | 1.890/0.050 |
| FGIP (pgm/ml) | 49.6 (6.1–278.0) | 74.2 (10.0–189.9) | 3.30/0.001 |
| PGIP (pgm/ml) | 267.0 (37.8–700.4) | 255.0 (131.0–616.3) | 0.175/0.861 |
| z/p value of FGIP and PGIP | 5.958/0.001 | 6.205/0.001 | |

Results were expressed as mean ± SD or median (range). Unpaired Student's 't' test and Mann–Whitney U test were performed to compare between groups and the test of significance at 5% significance level. n, number of subjects; IGT, impaired glucose tolerance; BMI, body mass index; WHR, waist hip ratio; FSG, fasting serum glucose; 2 h PG, 2 hour post-load (75 g) glucose; serum glucose; FC-pep, fasting C-peptide; HOMA-%B, β cell secretory capacity; HOMA-%S, insulin sensitivity by homeostasis model assessment; FGIP, fasting serum glucose dependent insulintropic polypeptide; PGIP, post glucose serum glucose dependent insulintropic polypeptide.

Results

Characteristics of the study subjects

The clinical characteristics of the controls and subjects with isolated IGT are shown in Table 1. Two groups were matched for age and BMI ($p = 0.955$). The IGT group showed significantly higher WHR as compared to the control group [(0.92±0.08 vs. 0.88±0.05), $p = 0.02$]. IGT subjects had reduced HOMA-%S index compared to controls [65.0 (22.0–222.0) vs. 71.0 (27.0–247.0), $p = 0.050$], but HOMA-%B was not significantly different between the groups (Table 1). Fasting GIP was significantly higher in subjects with IGT compared to controls [pgm/ml; 74.2 (10.0–189.9) vs. 49.6 (6.1–278.0), $p = 0.001$]. In contrast, subjects with normal glucose tolerance and IGT did not show any significant difference in post glucose GIP levels ($p = 0.861$) (Table 1).

C-peptide, glucose and GIP ratios of the study subjects

Subjects with IGT exhibited significantly reduced serum fasting C-peptide and fasting GIP

ratio than the control subjects [0.037 (0.009–0.251) vs. 0.045 (0.009–0.205), $p = 0.050$] but fasting C-peptide-glucose ratio was comparable (Table 2). The fasting GIP/glucose ratio was significantly higher in IGT [3.53 (0.41–7.96) vs. 2.26 (0.29–11.26), $p=0.002$]; however, postglucose GIP/glucose was significantly lower in IGT compared to controls [7.0 (2.87–18.42) vs. 10.08 (1.88–23.25), $p<0.01$]. Similarly, the ratio of post glucose GIP with fasting GIP was significantly lower in IGT compared to controls [3.47 (0.98–22.0) vs. 5.14 (0.96–19.85), $p < 0.01$] (Table 2).

Multiple regression analysis of the association of C-peptide and HOMA-%S with variables of interest of the study subjects:

On regression analysis, a significant positive association was found between fasting C-peptide and fasting GIP ($p < 0.01$) (Table 3).

A significant negative association was found between fasting GIP and insulin sensitivity (HOMA-%S) ($p = 0.05$) (Table 4).

No significant association was found between fasting glucose and fasting GIP in both

Table 2: C-peptide, Glucose and GIP ratios of the study subjects.

| Variables | Control (n = 47) | IGT (n = 51) | z/p value |
|---------------|---------------------|---------------------|-------------|
| FC-pep : FSG | 0.120 (0.04–0.30) | 0.123 (0.03–0.29) | 0.639/0.523 |
| FC-pep : FGIP | 0.045 (0.009–0.205) | 0.037 (0.009–0.251) | 1.95/0.050 |
| FGIP : FSG | 2.26 (0.29–11.26) | 3.53 (0.41–7.96) | 3.07/0.002 |
| PGIP : PSG | 10.08 (1.88–23.25) | 7.00 (2.87–18.42) | 4.46/0.001 |
| PGIP : FGIP | 5.14 (0.96–19.85) | 3.47 (0.98–22.0) | 4.469/0.001 |

Results were expressed as median (range). Mann-Whitney U test was performed and the test of significance at 5% significance level. n number of subjects; IGT, impaired glucose tolerance; FC-pep, fasting C-peptide; FSG, fasting serum glucose. FGIP, fasting glucose dependent insulinotropic polypeptide; 2 h PG, 2-hour post-load serum glucose; PGIP, post glucose serum glucose dependent insulinotropic polypeptide.

Table 3: Multiple regression analysis of the association of fasting C-peptide with variables of interest of the study subjects.

| Variables | Model 1 | | Model 2 | | Model 3 | | Model 4 | |
|-------------------------|--------------|-------|--------------|-------|--------------|-------|--------------|-------|
| | β | p | β | p | β | p | β | p |
| Intercept | | 0.001 | | 0.215 | | 0.767 | | 0.882 |
| FGIP (pgm/ml) | 0.343 | 0.002 | 0.307 | 0.006 | 0.306 | 0.006 | 0.298 | 0.009 |
| FSG (mmol/l) | | | 0.177 | 0.106 | 0.147 | 0.189 | 0.136 | 0.242 |
| WHR | | | | | 0.124 | 0.258 | 0.113 | 0.327 |
| Group | | | | | | | -0.042 | 0.732 |
| Adjusted R ² | 0.106 | | 0.125 | | 0.129 | | 0.118 | |

Standardized regression coefficients (β) were given with the level of significance. R² for adjusted R square (Multiple coefficient of determination). FSG, fasting serum glucose; FGIP, fasting GIP; WHR, waist to hip ratio.

Table 4: Multiple regression analysis of the association of HOMA-%S with variables of interest of the study subjects.

| Variables | Model 1 | | Model 2 | | Model 3 | |
|-------------------------|--------------|-------|--------------|-------|--------------|-------|
| | β | p | β | p | β | p |
| Intercept | | 0.001 | | 0.109 | | 0.153 |
| FGIP (ng/ml) | -0.253 | 0.024 | -0.246 | 0.028 | -0.218 | 0.050 |
| WHR | | | -0.106 | 0.338 | -0.066 | 0.573 |
| Group | | | | | 0.121 | 0.319 |
| Adjusted R ² | 0.052 | | 0.051 | | 0.051 | |

The level of significance at p<0.05.

simple correlation and multiple regression (data not shown).

Discussion

This study investigated the association of GIP with insulin secretion and sensitivity in Bangladeshi IGT subjects. The present study reveals

that GIP secretion is up regulated in the fasting state. This is evident both in terms of absolute GIP and C-peptide to GIP ratio values at the fasting state when compared between control and IGT groups (Table 2). The importance of GIP has mostly been conceived in relation to nutrient intake [7, 20–25], but its role in the maintenance of glucose homeostasis in the fasting state has been discussed less. In the present study,

fasting serum glucose level does not differentiate between control and IGT groups with a minimal rise in fasting C-peptide levels in the later group. The secretory capacity of the pancreatic β cells is not significantly different between the two groups. However, data showed that insulin sensitivity, as assessed by HOMA-%S, is compromised in IGT subjects. Under these conditions it will be important to explore whether raise of serum GIP plays any role in preventing the rise of fasting serum glucose in the IGT group.

Our data is comparable with a previous study [26] that revealed hypersecretion of GIP at fasting state but normal secretion of plasma insulin in IGT subjects. Nonetheless, after 40 minutes of OGTT, plasma insulin level was increased with a later rise of C-peptide value. But they have reported normal plasma insulin concomitant with decreased GIP level in T2DM. The pathophysiology lies here that long term insulin receptor resistance leads to defective signaling of the glucose sensing genes both in β -cells and GIP secretory K cells of the duodenum resulting in β -cell failure and dysregulation of GIP, respectively.

Although the present work has demonstrated the significantly higher FGIP value in IGT compared to controls and both the groups showed acute rise in the GIP values in response to oral glucose load, the difference between two groups were totally lost at the postprandial state. There was a proportionately higher rise of GIP values (about 5 times) in the controls compared to a blunted response (about 3.5 times) in IGT subjects at the corresponding state (Table 2). The abnormality is also evident on ratio analysis where post glucose GIP to 2 h post-load serum glucose (PGIP : 2 h PG) is significantly lower ($p < 0.001$) in the IGT group compared to controls.

In support of the finding in univariate analysis, multiple regression analysis reveals a much stronger association of FC-pep with FGIP ($p < 0.001$) as compared to weaker association with HOMA-%S on adjusting the confounding variables of fasting glucose, waist-hip ratio (WHR) and presence of diabetes. Accordingly, it seems that FGIP has much more relevance to the insulin secretory pathway, as reported by some authors [14–15, 26], compared to the insulin action pathway. Nevertheless, further studies are required to clarify these issues.

Analysis of the anthropometric data in the present study shows that the IGT subjects do not have generalized obesity as evident by no difference in BMI between control and IGT groups. IGT, however, is associated with central obesity ($p=0.02$). The finding is comparable with the observations in a previous study conducted on the same as well as in other population [18, 27]. Central obesity is known to be more specifically related to the increased secretion of adipocytokines [28–30] (like resistin and adiponectin) and inflammatory markers (like hs-CRP) which, in turn, are associated with insulin resistance. Thus, consistent finding of central obesity in the IGT population can be a central issue in designing preventing campaigns for reducing abdominal fat through lifestyle and dietary modifications.

The limitations of the study were lack of various groups of impaired glucose regulation (such as IFG and combined IFG & IGT). Postglucose-load serum C-peptide was not analyzed, and glucagon like peptide-1 (GLP-1) and GIP were not studied together to estimate their relative contribution in insulin secretion and sensitivity.

Conclusion

In conclusion, the IGT subjects have insulin resistance but their pancreatic B cell function seems to be still uncompromised. GIP secretion in IGT is up regulated at the fasting state and it has a blunted response to oral glucose in this disorder. GIP does not have any association with insulin secretion in IGT, but it has an association with insulin resistance.

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Conflict of interests

The authors declare no conflict of interest.

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