ORIGINAL ARTICLE

INCRETIN HORMONES, THYROID STIMULATING HORMONE AND HUMAN GROWTH HORMONE RESPONSES DURING OGTT IN NEWLY DIAGNOSED T2DM PATIENTS

KA Jhuma[1], ASM Giasuddin[2], MS Hossain[3], AMM Haq[4]

[1]Dept of Biochemistry, Medical College for Women and Hospital, Dhaka; [2]Medical Research Unit, Medical and Health Welfare Trust, Dhaka; [3]Dept of Biochemistry and Molecular Biology, Jahangirnagar University, Savar, Dhaka; [4]Dept of Medicine, Medical College for Women and Hospital, Dhaka

ABSTRACT

Several research groups have reported variable results about incretin effects of glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1(GLP-1), altered thyroid stimulating hormone (TSH) status, human growth hormone (hGH) deficiency and perturbed cytokines balances in type 2 diabetes mellitus (T2DM).The present case-control prospective interventional study was conducted investigating responses of incretin hormones (GIP, GLP-1), TSH and hGH to oral glucose tolerance test (OGTT) in newly diagnosed Bangladeshi T2DM patient. Blood samples were collected from 36 OGTT positive newly diagnosed T2DM patients as cases and 30 normal adults as controls at '0' minute (fasting) and at 2 hours after OGTT. Laboratory investigations were done and special parameters in serum, i.e. hGH, TSH, Insulin, GIP and GLP-1 were analyzed using enzyme immunoassay (EIA) kits. Statistical analyses were made by Student's 't' test using SPSS programm. T2DM patients (cases) had FBG and BG2Hr levels much higher than controls ($p<0.001$). No significant differences were observed between controls and cases for F-TSH ($p=0.927$), TSH2HrA ($p=0.413$), F-hGH ($p=0.532$) and hGH2HrA ($p=0.773$) levels. It was observed that F-GIP ($p=0.309$) and F-GLP-1 ($p=0.984$) levels were similar between cases and controls. Interestingly, control subjects responded to OGTT by increasing GIP2HA and GLP-1, 2HR levels about 3 times compared to F-GIP and F-GLP-1 ($p<0.001$). In cases, F-GIP and F-GLP-1 levels were also raised responding to OGTT but by about 1.5 times only compared to F-GIP and F-GLP-1 ($p<0.025$). Although no significant differences were observed for F-TSH, TSH2HrA, F-hGH and hGH2HrA between cases and controls, F-GIP and F-GLP-1 levels were raised responding to OGTT in cases by about 1.5 times only compared to about 3.0 times in controls subjects. But responses of GIP and GLP-1 to glucose load were lower leading to reduced insulin levels in these T2DM patients reported earlier. Further studies with a larger sample size including cytokines are warranted. □

Key Words: Diabetes Mellitus. T2DM, GIP, GLP-1, TSH, hGH

Introduction

Diabetes mellitus (DM) poses a major global health threat, both in the developed and developing countries. It is a serious global health issue with type 2 diabetes mellitus (T2DM) accounting for about 90-95% of all cases. It is estimated that about 7.8% of the world's population aged between 20 and 79 years will have T2DM by 2030[1-3].

Although glucose-tolerant individuals are capable of adjusting their insulin secretion to their actual insulin sensitivity, people with T2DM are incapable of doing so. Beta-cell failure is the hallmark of this disease, although failure may be precipitated by the development of insulin resistance. In healthy subjects, a considerable part of the postprandial insulin response is due to

the actions of the incretin hormones, i.e. glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) particularly. Together, these two hormones are mainly responsible for the so-called incretin effect[3]. A convenient way of describing the effect is to calculate the gastro-intestinally mediated glucose disposal (GIGD)[3,4].

The normal human body has a remarkable capacity to handle the intake of increasing amounts of glucose and is therefore capable of maintaining almost unchanged postprandial glucose excursions, regardless of the oral load. In people with T2DM, this ability is dramatically reduced as illustrated by calculation of the GIGD. The almost complete loss of GIGD is typically accompanied by a greatly reduced difference between the insulin responses to the oral and the intravenous glucose load, i.e. the incretin effect[5,6]. The loss of incretin effect is therefore likely to contribute importantly to the postprandial hyperglycemia in T2DM. Research carried out by several groups during the last decades has indicated that the incretin effect is mediated mainly by GIP and GLP-1 and no other gut hormones fulfill all criteria to act as incretin hormones[7-10].

The concentrations of GIP have been reported to be elevated, decreased and unchanged in patients with T2DM. Toft-Nielsen *et al.* found slightly decreased postprandial GIP concentrations in a large group of T2DM patients compared with a matched control group of non-diabetic subjects[11]. A major secretory defect regarding GIP secretion did not seem to exist in T2DM. Toft-Nielsen *et al.* also found a pronounced impairment of the postprandial GLP-1 response, particularly during the later postprandial phase (after the first 60 min)[11,12]. Some other studies could not confirm decreased GLP-1 responses in T2DM patients and reported contradictory results[13,14].

DM and thyroid disorders are endocrine abnormalities that are interrelated to each other, subclinical hypothyroidism being the most common disorder in T2DM. Thyroid hormones are necessary for normal glucose metabolism. Hyper or hypo secretion of thyroid hormones and thyroid stimulating hormone (TSH) can alter glucose homeostasis. Thyroid disorders not only worsen the metabolic control but also affect the management of diabetes. Therefore, DM patients need to be screened for thyroid dysfunction. American Diabetic Association (ADA) has proposed that the people with DM to be checked for thyroid disorders[15-19]. The magnitude of health problems related to DM in Bangladesh has been increasing rapidly, although limited study on hypothyroidism among T2DM has been reported from Bangladesh[20].

In addition, human growth hormone (hGH) deficiency may result in an increased risk of developing T2DM along with other clinical symptoms and abnormalities as reported in some studies. The insulin and hGH/insulin-like growth factor-1 (IGF-1) axis are two endocrine systems that are interlinked at many levels. hGH is one of the glucose counter-regulatory hormones, rising in response to hypoglycemia which has both intrinsic hyperglycemic actions and causes insulin resistance. Growth is compromised in poorly controlled diabetic children; however, a causal link with altered hGH/IGF-1 levels has not been proven. In poorly controlled diabetics, hGH levels are invariably raised whilst normal or low levels of IGF-1 indicating dissociation between the two factors. Raised hGH levels could result from altered hypothalamic/pituitary control or reduced feedback inhibition. That hGH has an effect on glycemic control is most evident from the abnormal glucose tolerance seen in acromegalics. Altered hGH/IGF-1 levels have been implicated in the long-term complications associated with diabetes[21-23].

Literature survey has indicated that no studies were done or reported on incretin hormones, TSH

status and hGH level and their responses to glucose load in newly diagnosed Bangladeshi patients with T2DM. Thus, we investigated serum incretin hormones (GIP, GLP-1), TSH and hGH and insulin status and their responses to oral glucose load (OGTT) in Bangladeshi T2DM patients and healthy control subjects in the present case-control prospective interventional study. The results on incretin hormones and insulin responses in these patients have been published recently[24]. In the present article, we have therefore reported the results of our study on the incretin hormones (GIP, GLP-1), TSH status and hGH level and their responses to OGTT in our newly diagnosed Bangladeshi T2DM patients comparing with normal control subjects.

Materials and Methods

The methodologies of this case-control prospective interventional study were reported in our recently published article[24]. This study was conducted during the period from November 2013 to June 2014 at the Medical Research Unit (MRU), Medical and Health Welfare Trust (MHWT), Uttara, Dhaka, Bangladesh with a research grant obtained from the Ministry of Science and Technology (MOST), Dhaka, Bangladesh. Suspected patients with T2DM had undergone oral glucose tolerance test (OGTT) after fasting. Blood samples were collected from individual patients at 'O' min (Fasting) and at 2Hrs (120 min) after glucose load. Samples were aliquoted for routine analyses and special research investigations. Among the routine analysis, glucose levels (Fasting/FBG, 2HA) and other usual routine tests in blood, serum and urine were done. Serum aliquots were preserved for a short time at -20^{o}C to -30^{o}C for incretin hormones (GIP, GLP-1), TSH, hGH and other special investigations. A total of 36 OGTT positive adult patients (male: 15, female: 21), only newly diagnosed T2DM patients (cases) were included in this study. Those who had other diseases such as thyroid diseases, any endocrine disorders, renal diseases, hypertension were excluded. And 30 normal healthy adults (male: 12, female: 18) were also investigated in parallel as normal control subjects. Routine laboratory investigations such as blood glucose, CBC, HbA_{1c}, LFTs, TFTs, RFTs were made according to usual clinical laboratory methods as practiced in the hospital laboratory. The special investigations, i.e. serum insulin, TSH, hGH, GIP and GLP-I, were made by adopting enzyme immunoassay (EIA) methods using kits obtained from reputed commercial companies such as R&D Systems (USA), Calbiotech (USA), Novatech (Germany). Statistical analyses were performed by Student's 't' test using SPSS programm.

Results

The results of the special laboratory investigations i.e. glucose (Fasting/FBG, 2HA), TSH, hGH, GIP, GLP-1 in normal controls and T2DM cases are stated in Table-I, Table-II, Table-III, Table-IV and Table -V respectively. T2DM patients (cases) had FBG and 2HA levels much higher than controls ($p<0.001$) (Table-I).No significant differences were observed between cases and controls for F-TSH ($p=0.927$), TSH2HA ($p=0.413$), F-hGH ($p=0.532$) and hGH2HA ($p=0.773$) levels (Table-II, Table-III). It was observed that F-GIP ($p=0.309$) and F-GLP-1 ($p=0.984$) levels were similar between cases and controls (Table-IV, Table V). Interestingly, control subjects responded to OGTT by increasing GIP2Hr and GLP-1, 2Hr levels about 3 times compared to F-GIP and F-GLP-1 ($p<0.001$) and in cases, F-GIP and F-GLP-1 levels were also raised responding to OGTT but by about 1.5 times only ($p<0.001$) (Table-IV, Table-V). In these T2DM patients (cases), F-Insulin ($p<0.001$) and Insulin 2Hr ($p<0.001$) were much lower than controls, although Insulin 2Hr level was higher than F-Insulin level in cases ($p<0.001$) which we reported previously[24].

Table-I: FBG and 2HABG levels in normal controls and T2DM cases

Parameter	Glucose (mmol/L)		Glucose (mmol/L)	
	FBG (control)	FBG (cases)	BG 2Hr (controls)	BG 2Hr (cases)
Number	30 (12M,18F)	36 (15M,21F)	30 (12M,18F)	36 (15M,21F)
Observed range	4.0-6.50	6.31-14.21	5.50-7.80	8.52-23.21
Mean±SD	5.19±0.71	10.09±1.94	6.47±0.66	14.56±4.77
Student's 't'-test (control vs cases)	p<0.001**		p<0.001**	
Paired 't'-test	controls (FBG vs BG 2Hr): P<0.025** cases (FBG vs BG2Hr): P<0.005**			

M: Male, F: Female; FBG: Fasting blood glucose, BG 2Hr: Blood glucose at 2hrs of OGTT.
***P ⩽ 0.05: Significant, P > 0.05: Not Significant.*

Table-II: F-TSH and TSH 2Hr levels in controls and T2DM cases

Parameter	TSH (μU/ml)		TSH (μU/ml)	
	F-TSH (control)	F-TSH (cases)	TSH2Hr (control)	TSH2Hr (cases)
Number	30 (12M,18F)	36 (15M,21F)	30 (12M,18F)	36 (15M,21F)
Observed range	0.30-11.10	0.42-13.72	0.40-13.70	3.32-11.12
Mean±SD	3.29±2.59	3.35±2.72	3.55±2.88	3.01±2.46
Student's 't'-test (control vs cases)	p=0.927		p=0.413	
Paired 't'-test	controls (F-TSH vs TSH2Hr): P<0.045** cases (F-TSH vs TSH2Hr): P<0.011**			

*M: Male, F: Female; F-TSH: Fasting thyroid stimulating hormone; TSH2Hr: Thyroid Stimulating hormone at 2hrs of OGTT. **P ⩽ 0.05: Significant, P > 0.05: Not Significant.*

Table-III: F-hGH and hGH2Hr levels in controls and T2DM cases

Parameter	hGH (mmol/L)		hGH (mmol/L)	
	F-hGH (control)	F-hGH (cases)	hGH2Hr (control)	hGH2Hr (cases)
Number	30 (12M,18F)	36 (15M,21F)	30 (12M,18F)	36 (15M,21F)
Observed range	0.50-5.0	0.11-5.32	0.60-5.30	0.12-5.21
Mean±SD	1.26±0.96	1.11±1.01	1.24±1.04	1.17±0.94
Student's 't'-test (control vs cases)	p=0.0532		p=0.773	
Paired 't'-test	control (F-hGH vs hGH 2Hr): P<0.777** cases (F-hGH vs hGH 2Hr): P=0.349**			

*M: Male, F: Female; F-hGH: Fasting human growth hormone; hGH 2Hr: Human growth hormone at 2Hrs of OGTT. **P⩽0.05: significant, P > 0.05: Not significant.*

Table-IV: F-GIP and GIP2Hr levels in normal controls and T2DM cases Parameter GIP (pg/ml) GIP (pg/ml)

Parameter	GIP (pg/ml)		GIP (pg/ml)	
	FGIP (control)	F-GIP (cases)	GIP2Hr (control)	HGH2Hr (case)
Number	30 (12M,18F)	36 (15M,21F)	30 (12M,18F)	36 (15M,21F)
Observed range	42.0-85.40	42.11-81.12	105.1-213.5	84.21-162.21
Mean±SD	63.42±13.80	60.14±12.18	158.56±34.49	119.33±22.9
Student's 't'-test (control vs cases)	p<0.309**		p<0.001**	
Paired 't'-test	Controls (F-GIP vs GIP 2Hr): P<0.001** Cases (F-GIP vs GIP 2Hr): P<0.025**			

*M: Male, F: Female; F-GIP: Fasting glucose-dependent insulinotropic polypeptide; GIP2Hr: Glucose-dependent insulinotropic polypeptide at 2hrs of OGTT.**P ⩽ 0.05: Significant, P > 0.05: Not Significant*

Table-V: F-GLP-1 and GLP-1, 2Hr levels in normal controls (NC) and T2DM patients (Pt)

Parameter	GLP-1 (pg/ml)		GLP-1 (pg/ml)	
	F-GLP-1 (control)	F-GLP-1 (case)	F-GLP-1 (control)	GLP-1 2Hr (case)
Number	30 (12M,18F)	36 (15M,21F)	30 (12M,18F)	36 (15M,21F)
Observed range	32.40-75.10	33.22-78.12	97.20-225.30	42.51-111.02
Mean±SD	60.82±13.42	52.75±13.98	164.46±40.26	81.65±20.47
Student's 't'-test (control vs cases)	p=0.984**		p<0.001**	
Paired 't'-test	control (F-GLP-1 vs GLP-1, 2Hr): P<0.001** case (F-GLP-1 vs GLP-1, 2Hr): P<0.025**			

*M: Male, F: Female; F-GLP-1: Fasting glucagon-like peptide 1; GLP-1,2Hr: Glucagon -like peptide 1 at 2Hrs of OGTT.**P⩽0.05: Significant, P>0.05: Not Significant*

Discussion

Our T2DM patients (cases) had FBG (p<0.001) and BG2Hr (p<0.001) levels much higher than control subjects (p<0.001) which were expected (Table-I). No significant differences were observed between cases and controls for F-TSH (p=0.927), TSH 2Hr (p=0.413), F-hGH(p=0.532) and hGH 2Hr (p=0.773) levels (Table-II, Table-III). It was observed that F-GIP (p=0.309) and F-GLP-1 (p=0.984) levels were similar between cases and controls. Interestingly, control subjects responded to OGTT by increasing GIP 2Hr and GLP-1 2Hr levels about 3 times compared to F-GIP and F-GLP-1 (p<0.001) (Table-IV, Table-V). In cases, F-GIP and F-GLP-1 levels were also raised responding to OGTT but by about 1.5 times (p<0.001). We reported previously that in these T2DM patients (case), F-Insulin (p<0.001) and insulin 2HA (p<0.001) were much lower than control subjects, although insulin 2HrA level was higher than F-Insulin level in cases (p<0.001) which were reported previously[24].

Most of the GIGD in healthy subjects is accounted for by the actions of the incretin hormones, but inhibition of hepatic glucose production by suppression of glucagon secretion, hepatic uptake of glucose from the portal vein and gut vein or liver vein reflex activity may also play a role. There is no doubt that the incretin hormones play a major role in GIGD in healthy subjects and it can be concluded that the incretin effect plays a major role for normal glucose tolerance. In people with T2DM, this ability is dramatically reduced as shown in our results similar to some other reports. The loss of incretin effect is therefore likely to contribute importantly to the postprandial hyperglycemia in T2DM[5].

The concentrations of GIP have been reported to be elevated, decreased and unchanged in patients with T2DM. Toft-Nielsen *et al.* found slightly decreased postprandial GIP concentrations in a large group of T2DM patients compared with a matched control group of non-diabetic subjects[11]. A major secretory defect regarding GIP secretion did not seem to exist in T2DM. When GLP-1 was identified as the other important incretin hormone, it was relevant to evaluate GLP-1 secretion in T2DM also. Toft-Nielsen *et al.* found a pronounced impairment of the postprandial GLP-1 response in T2DM subjects, particularly during the later postprandial phase (after the first 60 min)[11,12]. Some other studies could not confirm decreased GLP-1 responses in T2DM patients[13,14]. However, presence of some other unidentified incretin hormones yet to be discovered cannot be ruled out.

Holst *et al.* concluded that the dramatic loss in patients with T2DM of the ability to dispose of orally ingested glucose i.e. GIGD is related to the inability of the incretin hormones to increase insulin secretion after meal or glucose load[25]. Several lines of evidence support that the loss of incretin effect is secondary to development of diabetes[25,26]. However, more recent findings suggest that the loss of incretin effects in T2DM

patients can only be explained by a specific loss of insulinotropic activity of the incretin hormones at physiological level[25,27,28]. In overt T2DM, the consequence of the impaired incretin effect is that the ability of the patients to efficiently dispose of orally as opposed to intravenously administered glucose is almost completely lost[25].

Subclinical hypothyroidism is a common disorder in T2DM. Thyroid hormones are necessary for normal glucose metabolism. Hyper or hypo secretion of thyroid hormones can alter glucose homeostasis. Thyroid disorders not only worsen the metabolic control but also affect the management of diabetes. Therefore, DM patients need to be screened for thyroid dysfunction. ADA has proposed that the people with DM to be checked for thyroid disorders[15-19]. Although the magnitude of health problems related to DM in Bangladesh has been increasing rapidly, limited studies on hypothyroidism among T2DM have been reported from Bangladesh[20].

Altered hGH/IGF-1 levels have been implicated in the long-term complications associated with diabetes. That hGH has an effect on glycemic control is most evident from the abnormal glucose tolerance seen in acromegalics. Raised hGH levels could result from altered hypothalamic/pituitary control or reduced feedback inhibition. In poorly controlled diabetics, hGH levels are invariably raised whilst normal or low levels of IGF-1 are found, indicating dissociation between the two factors[21-23].

We found that TSH and hGH levels were similar in controls and cases at fasting ($p=0.927$, $P=0.532$). However, TSH response to OGTT in patients was significantly lower ($p=0.011$), while hGH response was not significant ($p=0.349$). In controls, TSH response at 2 hrs was higher ($p=0.047$) while hGH response was not significant ($p=0.777$). The reason for normal TSH and hGH levels may be due to the fact that our patient group was small in number and were newly diagnosed T2DM with short duration. But responses of GIP and GLP-1 to glucose load were decreased leading to reduced insulin levels in these T2DM patients reported earlier[24].

Our observations of relatively reduced capacity of T2DM patients to produce GIP and GLP-1 seemed to be relevant and interesting. These incretin hormones responses may be linked to pro-inflammatory and anti-inflammatory cytokines status. These cytokines are thought to impair insulin signaling and abnormally high levels of them are associated with insulin resistance and T2DM[28-32]. Also, it has been reported that anti-inflammatory cytokines counteract the cytotoxic effects of pro-inflammatory cytokines in insulin-producing cells[29,30,32]. It is therefore evident that perturbation of this delicate balance in favor of pro-inflammatory cytokines is a strong possibility as the pathogenetic mechanism towards development of T2DM. Further studies with a larger sample size including cytokines are required to confirm and extend our findings.

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